Original Research

Acute Smoking Induces Endothelial Dysfunction in Healthy Smokers. Is This Reversible by Red Wine's Antioxidant Constituents?

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Objective: Acute smoking causes endothelial dysfunction through impairment of nitric oxide (NO) production, or increased oxidative stress, but the exact mechanism still needs to be elucidated. In healthy non-smokers acute endothelial dysfunction caused by smoking one cigarette was counterbalanced by red wine's antioxidants. The aim of the present study is to investigate whether red wine's antioxidant substances could counteract the acute endothelial dysfunction induced by acute cigarette smoking in healthy smokers as well.

Methods: Twenty healthy volunteers (12 males) participated in a double-blind, cross-over study, comprised of three study days. All subjects either smoked one cigarette, or smoked and drank 250 ml of red wine, or smoked and drank 250 ml of dealcoholized red wine in each one of the study days. Flow mediated dilatation (FMD) was measured at fast and 30, 60 and 90 minutes after each trial.

Results: Smoking one cigarette induced a significant decrease in FMD ($p < 0.001$), which remained significant 30 ($p < 0.001$), and 60 ($p = 0.003$) minutes after the end of smoking. FMD remained statistically unchanged after consumption of either regular red wine, or dealcoholized red wine together with smoking.

Conclusions: The observed endothelial dysfunction following smoking of one cigarette was counterbalanced by consumption of either red wine or dealcoholized red wine in healthy smokers. It is possible that acute endothelial dysfunction caused by smoking could be attributed to increased oxidative stress and red wine's antioxidants counteract these acute effects of smoke on endothelium.

INTRODUCTION

Endothelial dysfunction is a well known premature indication of atherosclerosis and vessel damage [1]. Detection and modification of the factors that influence endothelial function is of great interest, especially when related with life style habits.

Cigarette smoking, a significant risk factor for atherosclerosis and coronary artery disease [2], is found to provoke endothelial dysfunction both in chronic use and in the acute phase [3,4]. In addition, endothelial dysfunction has been observed in healthy chronic [5], but also in passive smokers [6], as well as in healthy non-smokers when they smoked one cigarette [7,8].

Impairment of NO production [9] and increase in oxidative stress by a large number of free radicals known to exist in smoke [4], are some of the possible mechanisms responsible for acute endothelial dysfunction observed after smoking one cigarette. In contrast, supplementation of antioxidant vitamin C in healthy smokers acutely improves endothelial function suggesting that increased oxidative stress plays a crucial role in the effects of acute smoking on endothelium [10]. It is known that red wine contains powerful antioxidant substances [11] which

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Abbreviations: NO = nitric oxide, FMD = flow mediated dilatation, BMI = body mass index, CAD = coronary artery disease.

seem to have significant favorable effects on endothelial function when consumed chronically [12–14]. In addition, there are some recent studies concerning the possible acute effects of regular and dealcoholized red wine in healthy subjects and coronary artery disease patients [15–17]. These studies concluded that probably the antioxidant substances found in red wine are responsible for the favorable acute effects observed in endothelial function, expressed as endothelium dependent flow mediated dilation (FMD).

Previously it has been described that simultaneous consumption of either regular red wine or dealcoholized red wine with smoking one cigarette, counterbalances the acute deleterious effects of smoke on FMD in a group of healthy nonsmokers [18].

Since there are no data concerning the possible effects of wine and smoking on smokers, which is a high-risk and a very large population, the aim of the present study was to investigate whether regular red wine or dealcoholized red wine could counteract endothelial dysfunction induced by acute cigarette smoking in healthy smokers.

MATERIALS AND METHODS

Subjects

Twenty healthy smokers (12 males) with mean age 29.0 ± 5.4 years (mean ± SD) and body mass index (BMI) 23.7 ± 4.6 kg/m^2, were recruited for the study. All volunteers had normal blood pressure (<140/90 mmHg), no history of diabetes mellitus, obesity, no family history of coronary artery disease (CAD), absence of liver and endocrine diseases and cholesterol levels <240 mg/dl. The amount of cigarettes smoked per day by each individual and the duration of smoking were measured in pack/years, ranging from 1 to 20 (10 of them had <10 pack years and 10 had >10 pack years). Exclusion criteria were supplementation of antioxidant vitamins, dieting at the time of the study, supplementation of any medication, use of contraceptives by female volunteers and alcohol consumption more than the recommended (20–30 gr alcohol/day). The study was approved by the institutional committee on human research and all subjects gave their informed consent before entering the study.

Experimental Protocol

The study protocol was of a double-blind, cross-over design. At each study day, subjects were instructed to attend the Vascular Laboratory of Alexandra Hospital, Athens, Greece, after a 12 hour fast and 12 hour abstinence from smoking. On the first day, an initial ultrasound assessment took place at fasting. Afterwards, subjects were asked to smoke a regular cigarette (with 12 mg tar and 0.9 mg nicotine). Ultrasound measurements were repeated 30, 60 and 90 minutes following completion of smoking. On the second and on the third study day, an identical set of measurements took place, but the volunteers were asked to smoke the same type of cigarette and simultaneously drink either a glass of 250 ml of red wine, or 250 ml of dealcoholized red wine. Together with either smoking or smoking and drinking each of the two types of red wine, subjects were also consuming 1 slice of white bread (30 gr) and 30 gr of cottage-cheese (4% fat). All the procedure (wine ingestion, meal consumption and smoking) lasted 15 minutes. All participants were encouraged not to change their diet during the study and to consume the same menu the day before each study day. 24-hour recalls were reviewed to assure compliance to dietary instructions.

Wine Preparation

Red wine used in this study (Rapsani, Tsantalis, Greece), contained 12% of ethanol. The same type of red wine was also used for the preparation of dealcoholized red wine (containing 1% of ethanol) with partial lyophilization. Regular red wine was partially lyophilized at −50°C and at 1 mb pressure for 6 hours, using Cryodos 45 (Telstar, Spain) and the concentrated red wine (~50%) was then diluted with distilled water until it reached the initial volume, in order to produce dealcoholized red wine. Total antioxidant estimation in both types of red wine was performed by the Folin-Ciocalteau procedure [19], using a UVIKON, spectrophotometer, 931, Kontron Instruments (Milan, Italy) and expressing results as micrograms of caffeic acid per 100 milliliter. A Hewlett-Packard Model 1050 UV-DAD/FLD with Autosampler (California, USA), and a Hewlett-Packard Model 6890 with MS detector and Autosampler (California, USA), was used for High Pressure Liquid Chromatography (HPLC) [20] and Gas Chromatography (GC/MSD) respectively, for the quality control of specific antioxidants in both types of experiment's red wine (regular and dealcoholized). Finally, sensory characteristics of both red wines and the capacity of subjects to distinguish the two beverages were examined through use of duo—trio tests and a hedonic scale.

Flow Mediated Dilatation Measurement

A B-Mode high-resolution ultrasound imaging (Acuson 128xp California, USA) was used for assessment of FMD. The method to assess endothelial function, has been previously described [21,22]. All measurements were performed in a quiet room with a temperature controlled at 20–25°C, at supine position and after a 10 minutes resting period. A 7.0 MHz linear array transducer was used to obtain measurements from the right brachial artery in a specific anatomic point. The measurement of the artery diameter was performed by two independent observers who were unaware of the study phase, at end-diastole, by using electronic calipers and all procedure was guided by electrocardiographic assessment. After the initial measurement at resting conditions, a cuff fitted 8 cm distal to the brachial artery and near the wrist was inflated at 250–300

mmHg, altering arterial flow for 4.5 minutes. Then it was deflated, increasing arterial flow (reactive hyperemia).

Afterwards, there was a continuous scan for 90 sec after cuff deflation, and vessel's maximal diameter at the same point with resting measurement, was defined again (diameter during reactive hyperemia). This procedure was repeated 30, 60 and 90 min after smoking and simultaneous smoking and ingestion of either dealcoholized, or regular red wine. FMD is defined as the percent change of artery's diameter (endothelial dependent vasodilation) and hyperemia refers to the percent increase of flow, from flow at rest (at each time point, of the study). The inter and intra observer variability for brachial diameter measurements in our laboratory is 0.1 ± 0.12 and 0.08 ± 0.19 mm respectively, while FMD variability measured on 2 different days was $1.1 \pm 1\%$.

Statistical Analysis

Analysis of variance for repeated measures was used to evaluate changes, following all three interventions. A further ANOVA designs with 2 interventions $\times$ 4 time periods (fast, 30, 60, 90 minutes post-consumption) was applied to assess the comparative effects of the interventions on the measured variables. Multiple comparisons were performed by Bonferroni corrected Student's t-test, two-tailed, paired Student t-test was applied for the evaluation of differences of baseline conditions among the three study sessions. All variables were tested for homogeneity of variance and normal distribution, before any statistical analysis was applied. All data are expressed as mean $\pm$ SD unless otherwise stated and a value of $p < 0.05$ was regarded as statistically significant. All statistical analyses were performed using statistical software SPSS 11 for Windows.

RESULTS

All subjects completed the study without any dissatisfaction and no acute alcohol related problems.

Analysis of the two types of red wine (regular and dealcoholized) showed no significant difference either in their total phenol content, or the types of their antioxidants. The phenol content of the original red wine and red wine after alcohol extraction was similar (658 μg/100 ml of caffeic acid vs 650 μg/100 ml of caffeic acid respectively). In addition, the polyphenol profiles of the two beverages, according to HPLC and GC analysis, were very similar. Finally, from the duo-trio test evaluation 12 out of 15 panelists could not distinguish beverages. The hedonic scale evaluation, showed no significant difference of color, flavor and taste (sweet, bitter and sour) between the two wines. These results prove that the two wines used for the study were similar in taste, colour, flavour and antioxidant content and their only difference was their alcohol concentration (12% for regular vs 1% for dealcoholized).

Baseline artery diameter, blood flow at rest, heart rate and FMD were similar and the increase in blood flow after at the reactive hyperemia was not statistically different among the three study days and at every specific time-point (Table 1).

FMD after smoking one cigarette was significantly decreased ($p < 0.001$). The reduction in FMD was significant 30 and 60 minutes after the end of smoking, compared to baseline

Table 1. Artery Diameter, Flow at Rest, Flow in Hyperemia and FMD of the Three Study Days (Smoking, Smoking + Dealcoholized Red Wine and Smoking + Regular Red Wine).

	Baseline	30 min	60 min	90 min
		Smoke		
Vessel size (mm)	3.6 ± 0.1	3.6 ± 0.2	3.7 ± 0.1	3.6 ± 0.2
Flow at rest (ml/min)	99.9 ± 16.6	134.2 ± 47.6	130.3 ± 38.8	98.3 ± 18.1
Hyperemia (%)	186.7 ± 61.2	201.2 ± 61.5	151.0 ± 49.1	131.7 ± 42.3
Heart rate (beats/min)	63.5 ± 1.6	69.7 ± 1.9*	66.1 ± 1.7	64.8 ± 1.9
FMD (%)	5.65 ± 0.77	2.07 ± 0.70*	2.25 ± 0.52*	5.33 ± 0.90
		Smoke + Dealcoholized red wine		
Vessel size (mm)	3.6 ± 0.1	3.7 ± 0.2	3.7 ± 0.1	3.7 ± 0.1
Flow at rest (ml/min)	91.1 ± 16.7	150.5 ± 39.1	133.1 ± 27.8	152.4 ± 34.1
Hyperemia (%)	174.2 ± 64.0	109.5 ± 62.9	143.8 ± 69.9	118.9 ± 63.4
Heart rate (beats/min)	64.1 ± 2.5	67.1 ± 2.7	66.7 ± 2.4	67.5 ± 2.9
FMD (%)	4.94 ± 0.8	6.93 ± 1.2	6.24 ± 0.7	5.81 ± 0.6
		Smoke + regular red wine		
Vessel size (mm)	3.7 ± 0.2	4.1 ± 0.1*	3.8 ± 0.2	3.9 ± 0.2
Flow at rest (ml/min)	104.0 ± 15.4	118.0 ± 14.9	107.4 ± 18.4	136.1 ± 24.1
Hyperemia (%)	177.7 ± 41.2	199.6 ± 49.1	179.0 ± 56.1	180.7 ± 95.8
Heart rate (beats/min)	62.5 ± 2.2	68.8 ± 2.0	64.3 ± 2.5	66.0 ± 2.0
FMD (%)	5.11 ± 0.67	4.09 ± 0.75	6.14 ± 0.58	5.53 ± 0.49

FMD, flow mediated dilatation, min, minutes.

Values are expressed as Mean ± SEM.

* Indicates significant difference from baseline values.

Baseline values did not differ significantly among the three study sessions.

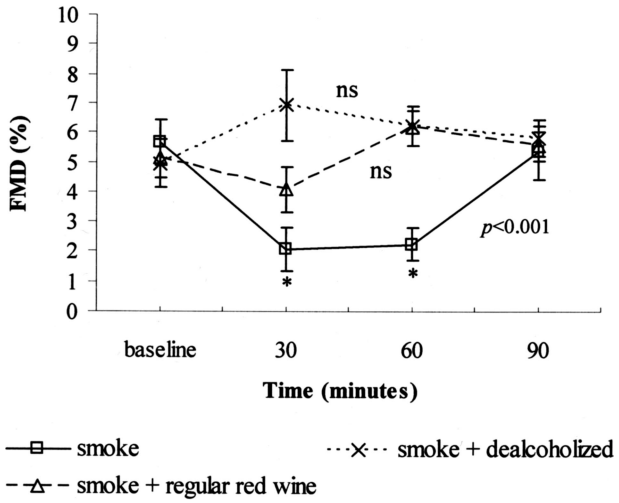

Fig. 1. FMD responses after smoking, or smoking and drinking either regular or dealcoholized red wine (n = 20). *p* indicates overall significant changes (ANOVA for repeated measures, observed power 99%). * indicates significant difference ($p < 0.01$) from baseline (Bonferroni corrected Student t-test for multiple comparisons). Bars represent standard error (SEM) of mean values.

FMD measurement ($p < 0.001$ and $p = 0.003$, respectively). On the other hand, overall FMD response after smoking was significantly different compared to FMD response after smoking + dealcoholized and smoking + regular red wine, as estimated by the significance level of the interaction term of two separate 2 × 4 general linear models for repeated measures; $p < 0.001$ and $p = 0.007$ respectively. FMD response between smoke + dealcoholized and smoke + regular red wine sessions was not statistically different (Fig. 1).

Baseline artery diameter increased significantly after smoking and drinking regular red wine, 30 minutes post-ingestion and it returned to its fasting levels 60 and 90 minutes after the end of the meal (Table 1).

DISCUSSION

In the present study which was performed in a group of smokers, it was shown that smoking one cigarette induces a significant decrease in FMD, which is maintained for 30 to 60 minutes, but simultaneous consumption of red wine with or without alcohol, counterbalances this unfavourable effect on endothelial function.

The observed effects of smoking on FMD are in accordance with previous studies [7,8,18], which also found that acute smoking induces a significant deterioration of endothelial function, which lasts for 30 to 60 minutes after the end of smoking. This could be explained by the fact that smoking elevates levels of endothelin-1, reduces NO levels and increases oxidative stress as shown by several in vitro, as well as in vivo studies [9,23–25]. It is of great interest that a habitual heavy smoker, like the volunteers that participated in the present study, usually lights the next cigarette in less than 30 minutes after they have finished the previous one. This suggests that a habitual smoker might have a prolonged endothelial dysfunction, during almost all day.

On the other hand, it seems that supplementation of antioxidant vitamins, especially vitamin C and E in smokers improve endothelial function, which in this case is already impaired [10,26,27]. This favourable effect of antioxidant vitamins has been observed both in chronic and in short term use and it implies that possibly increased oxidative stress is the key mechanism for smoke's endothelial dysfunction.

In this study red wine's constituents were used as a way of reversing acute unfavourable effects of smoke on endothelial function. Several studies investigating red wine's acute endothelial effects have concluded that the antioxidant substances found in red wine are very effective in increasing FMD both in healthy subjects, as well as in patients with coronary artery disease (CAD), within 30–120 minutes following its ingestion [15–17]. This could be explained by the fact that red wine's antioxidants induce vasorelaxation through increased production of NO, as well as an increased protection of NO from damage by free radicals [13,28,29]. Unfortunately, it is not possible from the present study to come to a conclusion as to which antioxidant substance found in red wine is responsible for the observed effects. There are many red wine antioxidants that have beneficial effects on the cardiovascular system and in specific on endothelial function, as an increase in eNOS is observed in response to the effects of trans-resveratrol, cinnamic and hydroxycinnamic acids, cyanidin and some phenolic acids [30]. However, resveratrol is possibly the most capable vasoactive antioxidant substance in red wine, as it was found to provoke endothelium-dependent and nitric oxide-mediated vasodilation in human internal mammary artery [31], to protect blood vessels by oxidative damage by upregulating endogenous antioxidants and phase 2 enzymes [32] and to inhibit endothelin-1 expression [33].

On the other hand, results from another recent study suggest that in the acute phase alcohol and not antioxidant substances of wine is responsible for the improvement observed in FMD postprandially [34], which is in accordance with studies which concluded that alcohol is a vasodilator substance, as it modulates NO synthase and promotes NO secretion [35]. This effect of alcohol is also observed in the present study, as it was found that in subjects who consumed red wine containing alcohol, there was a significant increase in baseline vessel diameter 30 minutes later. This phenomenon is explained by alcohol's vasodilatory effects, it has been observed previously and inevitably it could influence FMD [16,17]. Nevertheless, in the present study the observed increase in baseline vessel diameter by alcohol, didn't lead to a significant reduction in FMD as expected and therefore it didn't influence the observed results.

It should be mentioned that we have investigated previously the possible interaction of acute smoking and simultaneous

ingestion of red wine with or without alcohol in healthy non-smokers and we found that although smoking one cigarette deteriorates FMD, ingestion of either regular or dealcoholized red wine counteracts these harmful effects of smoke [18]. In the present study we tried to investigate for the first time whether the same interaction of smoke and wine could provoke the same effects on a group of healthy smokers that usually drink and smoke at the same time. Our recent findings are in accordance with the previous ones suggesting that the response of smokers in smoking one cigarette and in smoking and drinking red wine is the same as in non-smokers. In addition, since both regular and dealcoholized red wine counterbalanced the harmful acute effects of smoke on endothelial function, it is possible that red wine's antioxidants and not only alcohol, are responsible for these favourable effects.

The fact that antioxidant capacity and oxidative stress were not measured, could be considered as the main study limitation. It is likely that future designs with additional study of oxidative status at every time-point will possibly confirm our findings and clarify the mechanisms implicated.

CONCLUSION

Smoking one cigarette induced a decrease in FMD, and simultaneous ingestion of regular or dealcoholized red wine with smoking reversed the effects of smoke in a group of healthy smokers. Therefore, it is suggested that in smokers as well as in non-smokers, it is possible that smoke increases oxidative stress or decreases NO levels and red wine's antioxidants counteract these effects and reverse the unfavourable influence of smoke on FMD. It is probable that smoking and consumption of red wine act on endothelial function in the same way in smokers and non-smokers and that the favourable effects observed through use of red wine could be attributed to its antioxidant substances and not solely to its alcohol content.

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