

# Wine Dilates the Brachial Artery but does not Increase Flow-mediated Dilatation over Two Hours

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**Epidemiological evidence suggests cardiovascular benefits from the consumption of alcoholic beverages, but corroboration by functional and outcome studies is still outstanding. Non-invasive functional tests on the brachial arteries of healthy volunteers were performed before and for two hours after consuming red wine. Brachial artery dimensions were determined by ultrasound at baseline, 30, 60 and 120 min. Flow-mediated dilatation (FMD) was similarly assessed. Putative minimum and maximum lumen diameters were determined after ice immersion and sublingual trinitroglycerine (TNG) at baseline and 60 min after wine consumption. Sixteen subjects had a mean resting brachial artery diameter of 3.84 mm, which significantly increased with wine to 4.44 mm at 30 min, 4.39 mm at 60 min and 4.49 mm at 120 min. The calculated blood flow rates during the study did not differ significantly over the measured intervals. The baseline mean diameter with TNG was not significantly different from the dilation with wine. The vasoconstrictor response varied, with a  $-2.6 \pm 2.9\%$  change with wine, compared with the baseline diameter. There was appreciable variation of the diameter relative to the range found with ice and TNG. The calculated FMDs (% changes) for the study were 10.8, 6.1, 5.6 and 7.5, indicating statistically significant effects of wine ( $p < 0.0001$ ). The authors conclude that red wine consumption leads to beneficial arterial effects that may relate to different doses or other mechanisms than FMD. Further studies need to be done to discriminate between the effects of alcohol and phenolic compounds on vascular function.**

The vascular endothelium is a cell layer that lines all blood vessels. It occurs between the lumen and the vascular smooth muscle and secretes potent vasorelaxing substances, such as nitric oxide (NO), and vasoconstricting substances, such as endothelin-1. Prior to the 1970s, the vascular endothelium was thought to be relatively inert. Since then, however, research has shown that it is capable of a number of important functions, including control over vascular growth and arterial tone (Celermajer, 1997). In 1986, Ludmer *et al.* (1986) reported that endothelial dysfunction was associated with atherosclerosis, and this has subsequently been confirmed (Gordon *et al.*, 1989; Healy, 1990). Endothelial dysfunction occurs early in atherogenesis (Healy, 1990), and has been demonstrated in asymptomatic children and young adults with risk factors for atherosclerosis, such as familial hypercholesterolaemia, lipoprotein (a) and smoking (Celermajer *et al.*, 1992; Sorensen *et al.*, 1994). An association between coronary risk factors (hyperlipidaemia, diabetes mellitus, hypertension and smoking) and endothelial dysfunction was described by Hashimoto *et al.* (2003). Endothelial dysfunction has also been shown to be reversible under certain conditions. Clarkson *et al.* (1996) found that oral treatment of young hypercholesterolaemic adults with L-arginine, a precursor of NO, improved endothelium-dependent dilatation significantly. It has been reported that oxidative stress may play a role in impairing endothelial function, from the action of some reactive oxygen species (ROS)

that have not been efficiently removed by endogenous antioxidant systems. These ROS might decrease the local production of NO by reacting with NO itself, thereby producing peroxynitrite. Alternatively, ROS might affect the expression levels of endothelial nitric oxide synthase (eNOS) (Praticò, 2005).

Research has shown that flow-mediated dilatation (FMD) is endothelium dependent (Green, 2005): in arteries with healthy endothelium, increased blood flow causes dilatation of the artery (Laurent *et al.*, 1990) via the release of NO (Pohl *et al.*, 1986; Corretti *et al.*, 2002). This does not occur during endothelial dysfunction, when there is an impaired vasodilatation response (Moens *et al.*, 2005). Determination of the FMD of the brachial artery is a non-invasive, ultrasonographic-imaging method for detecting endothelial dysfunction. The technique was first described by Celermajer *et al.* (1992). Arterial diameter is measured in response to an increase in shear stress caused by increased blood flow after occlusion of the artery, which causes endothelium-dependent dilatation, and to sublingual trinitroglycerine (TNG), an endothelium-independent donor of NO. FMD has been shown to be stable and reproducible and to correlate well with invasive intra-arterial testing of coronary endothelial function (Welsch *et al.*, 2002). Changes in arterial diameter of 0.1 to 0.2 mm can be detected accurately (Wendelhag *et al.*, 1992; Sorensen *et al.*, 1994). Guidelines for the technique have been published (Corretti *et al.*, 2002).

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The main objectives of this study were:

- To determine the blood pressure, brachial artery diameter, blood flow and FMD after acute consumption of a moderate amount of red wine.
- To determine the effect of ice and TNG on the brachial artery diameter.

## MATERIALS AND METHODS

### Study design

Sixteen healthy, non-smoking volunteers, who were not taking any medication, were recruited for the study. Eleven were male (aged 19 to 43) and five were female (aged 23 to 48). Informed consent for the study was obtained from the subjects. They refrained from exercise within 6 to 9 h before the study. Testing was performed in a quiet room in the morning after a 12-h fast. An Acuson 128 ultrasound machine equipped with a 7.5 MHz Acuson 7 transducer was used to generate images of the artery, which were recorded on a Sony VHS video recorder. The blood pressure (BP) was recorded at baseline and at 30, 60 and 120 min by means of an Accutorr Plus Datascope electronic BP recorder.

Each subject consumed the same cultivar and vintage of red wine as 0.35 g ethanol/kg body mass (ranging from 175 mL to 320 mL in the cohort) over 5 min. This was considered a moderate volume of wine.

### FMD assessment

The standardised protocol of Celermajer *et al.* (1992) was adapted for the study, and the same sonographer assessed the FMD throughout the study.

The brachial artery was imaged at the cubital fossa in the longitudinal plane. For the assessment of FMD, a blood pressure cuff was placed distally around the forearm and inflated to 50 mm Hg above systolic pressure for 5 min to cause ischemia and subsequent dilatation of the downstream vessels. When the cuff was released, there was a brief high flow state through the brachial artery into the dilated vessels. This increased shear stress, which in turn caused the brachial artery to dilate in normal individuals. Additional manoeuvres were used to evaluate the range of arterial size. While the left arm was used for FMD measurements, the right arm was used for the determination of BP, as well as for the determination of minimum arterial diameter by immersion of the right hand in ice for 30 s. This latter response may be absent in subjects with autonomic neuropathy, but none of the study subjects had neuropathy. Trinitroglycerine was sprayed sublingually, at a total dose of 0.8 mg, in order to determine the maximum vasodilator response. Images were recorded continuously during this time.

The B (brightness) mode displays a longitudinal image of the artery, allowing the measurement of diameter, while the spectral Doppler allows the measurement of velocity in m/s. The conventional blood flow in mL/min can then be calculated.

The brachial artery diameter and FMD were measured at baseline, and then 30, 60 and 120 min after consumption of the wine. Minimum and maximum lumen diameters were measured at baseline and then again at 60 min after consumption of the wine.

### Oxygen radical absorbance capacity (ORAC) analysis

The antioxidant capacity of the red wine consumed by the volunteers was determined by the modified method of Ou *et al.* (2001).

The ORAC analyses were performed on a fluorescence spectrophotometer in white, flat-bottomed, 96-well microtitre plates and the wine was diluted to 1:1600 in 75 mmol/L phosphate buffer, pH = 7.4. Fluorescence readings were taken at time 0, 2 min, 5 min and every 5 min until 30 min; after which readings were taken every 15 min until 3 h. The reactions were compared with a standard of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), representing a water-soluble form of tocopherol. The excitation and emission wavelengths were 485 and 520 nm respectively. The area under the curve (AUC) was determined from a fluorescence/time plot with PRISM software (statistical analysis). Values were measured as mmol/L Trolox equivalents (TE). The inter-assay CV (coefficient of variation) was < 8%.

### Statistical analysis

All data are expressed as mean  $\pm$  SD. All statistical analyses were performed with the use of Graphpad PRISM software (version 3, San Diego, USA). Unpaired t-tests were used to compare the results from the 11 males and five females. Comparisons of groups at the four time points were analysed by repeated measures of analysis of variance (ANOVA). Student's t-test was used to compare the vasoconstrictory and vasodilatory changes at baseline and at 60 min. Statistical significance was accepted at  $p < 0.05$ . PRISM software was also used to determine the AUC values for the ORAC analysis, and to generate the box and whiskers plots.

## RESULTS

There were no significant differences in the age, baseline systolic blood pressure, baseline diastolic blood pressure, arterial diameters, FMD percent change or blood flow between males and females (unpaired t-tests  $p = 0.074, 0.684, 0.223, 0.378, 0.201$  and  $0.098$  respectively), and therefore the data for males and females were analysed together. The trends for differences in age and blood flow were not interpreted as being meaningful in altering the parameters of interest for the study. A summary of all the results is shown in Table 1.

### Blood pressure determination

The systolic blood pressure did not vary significantly from baseline to 2 h after wine consumption ( $p = 0.397$ ), and the trend for the diastolic blood pressure to decrease was not significant ( $p = 0.054$ ).

### Brachial artery diameter, calculated blood flow and FMD assessment

The diameter of the artery increased significantly from baseline to 120 min after consumption of wine ( $p < 0.0001$ ). The mean baseline diameter of 3.8 mm increased to 4.4 mm, 4.4 mm and 4.5 mm respectively at 30, 60 and 120 min (Fig. 3). At the same time, the FMD decreased significantly: 10.8% at baseline, 6.1% at 30 min, 5.6% at 60 min, and 7.5% at 120 min after wine consumption ( $p < 0.0001$ ) (Fig. 4). The conventional blood flow in mL/min was calculated from the measured diameter of the artery and the measured blood velocity (mean; see Fig. 1), and did not change significantly from baseline ( $p = 0.6$ ).

There was a statistically significant difference between the ice response before and after wine consumption ( $p < 0.0001$ ), but no significant difference between the TNG responses ( $p = 0.07$ ) (Fig. 5).

### ORAC analysis

The red wines were analysed for their total antioxidant potential and showed a range of results with a mean of  $10.25 \pm 9.33$  mmol/L TE.

### DISCUSSION

Brachial artery FMD is a non-invasive technique for assessing vascular (endothelial) function and dysfunction. As indicated previously, studies have shown that abnormal endothelial function may be associated with atherosclerosis. Thus, nutritional products that improve FMD may be viewed as potentially beneficial to the functioning of the endothelium, and possibly also for atherosclerosis. It was therefore of interest to evaluate the effect of wine on endothelial function.

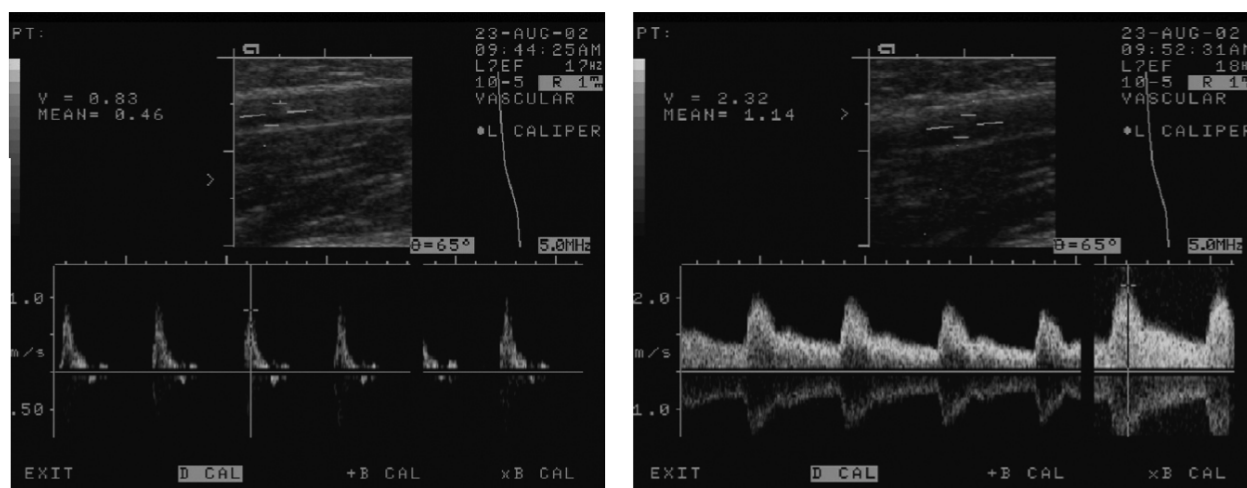
This study found that the diameter of the brachial artery increased significantly up to 2 h after consumption of red wine. The systolic and diastolic blood pressure and blood flow did not change significantly. The trend for lower diastolic pressure may relate to vasodilatation and reduced peripheral vascular resistance, compatible with the increase in diameter noted during the study period. Flow-mediated dilatation decreased significantly, relative to the baseline, over 2 h. The minimum diameter on cold provocation was significantly affected by wine consumption, but not the maximum physiologic diameter. This study confirms the ability of ethanolic beverages to cause vasodilatation. What has not been seen in the

TABLE 1

Comparison of FMD and related measurements from 16 subjects before and after wine consumption.

n = 16	Fasting		Wine		P value
	0 min	30 min	60 min	120 min	
<b>Systolic BP</b> mm Hg	114 ± 6	114 ± 10	112 ± 12	110 ± 15	0.40
<b>Diastolic BP</b> mm Hg	67 ± 7	67 ± 14	64 ± 11	61 ± 10	0.05
<b>Diameter</b> mm	3.8 ± 0.6	4.4 ± 0.6	4.4 ± 0.6	4.5 ± 0.6	< 0.0001
<b>Flow</b> mL/min	249 ± 122	258 ± 114	301 ± 132	303 ± 166	0.60
<b>FMD</b> % change	10.82 ± 4.61	6.08 ± 2.91	5.59 ± 4.24	7.46 ± 2.67	< 0.0001
<b>Diameter after ice,</b> mm	3.8 ± 0.6		4.3 ± 0.6		< 0.0001
<b>Diameter after TNG,</b> mm	4.7 ± 0.6		4.8 ± 0.6		0.07

$$\text{Flow} = \pi (D/2)^2 * \text{mean velocity} * 60$$



	<u>Baseline</u>	<u>Post-ischemia</u>
diameter	0.35 cm	0.38 cm
mean velocity	46 cm/s	114 cm/s
flow	265.65 mL/min	776.05 mL/min

FIGURE 1

Ultrasonographic images of the brachial artery before and after ischemia.

Minutes	baseline	0	30	60	120
		← wine →			
BP	x	x	x	x	x
Rest flow	x	x	x	x	x
Rest diameter	x	x	x	x	x
FMD diameter	x	x	x	x	x
Ice diameter	x			x	
TNG diameter	x			x	

FIGURE 2

The protocol for the FMD study.

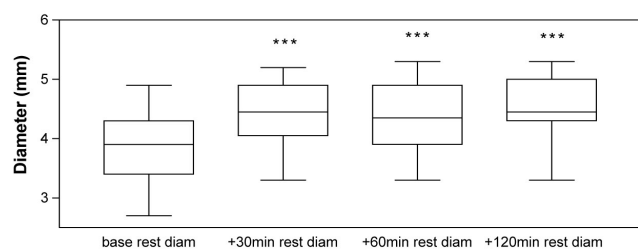


FIGURE 3

The resting brachial artery diameter in subjects consuming red wine, and the diameter at baseline and over two hours after wine consumption. \*\*\* denotes a significance of  $p < 0.0001$ , relative to the baseline.

review of the literature is that the minimum diameter is indeed altered as a result of cold provocation. This effect aggravates the heat loss after alcohol exposure and may contribute to hypothermia, because vascular dilatation cannot be countered effectively. The mechanism for vasodilatation by alcoholic beverages is not directly declared in the literature, but may be due to alcohol and/or its metabolism, or could be due to other compounds. The alcoholic beverage did not dilate the artery as much as the TNG, although its effect was powerful and persisted for up to 2 h. The increase over the baseline for alcohol was 9% and the corresponding increase for TNG was 24%. This study did not define the duration of dilatation or any effects that may be attributed to habituation.

Previous studies have found that several factors may interfere with FMD. Some studies found that FMD is inversely related to baseline arterial diameter (Hashimoto *et al.*, 1995; Hashimoto *et al.*, 2000). It is not clear whether it is appropriate to compare the FMD calculated for wider arteries with that calculated for undilated arteries. Certainly, when the diameter approaches the maximum possible, no further FMD is possible. The only practical approach for resolving the appropriateness of the response is to warm subjects or use some alternative vasodilator to describe similar dilatation for the evaluation of FMD. Ageing is associated with decreased FMD (Franzoni *et al.*, 2005), but our limited number of subjects did not show a correlation between FMD and age. None of our subjects suffered from sleep apnoea (Ip *et al.*, 2004) or hypertension (Lauer *et al.*, 2005). Circadian variations (Gaenger

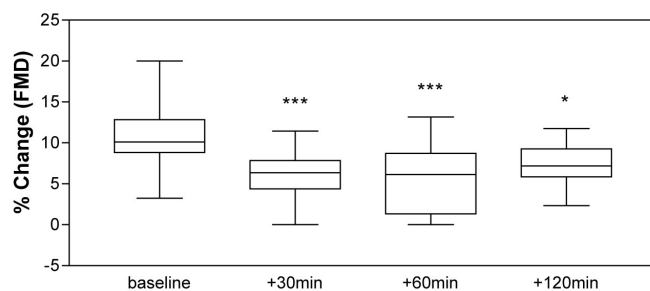


FIGURE 4

The FMD (percent change) in subjects consuming red wine at baseline and over two hours after wine consumption. \*\*\* denotes a significance of  $p < 0.0001$  and \* a significance of  $p < 0.05$ , relative to the baseline.

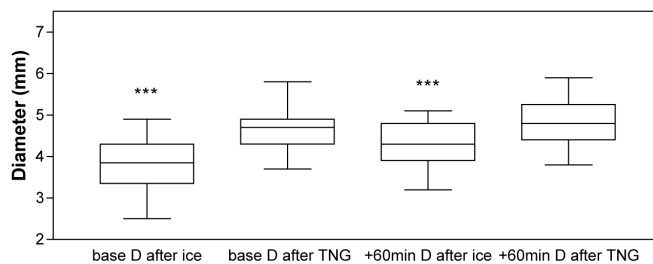


FIGURE 5

The effects of ice and TNG on the brachial artery diameter in subjects consuming red wine at baseline and over two hours after wine consumption. D denote diameter. \*\*\* denotes a significance of  $p < 0.001$ , relative to the baseline.

*et al.*, 2000) were minimised by performing the tests at the same time of day. The subjects had not been subjected to mental stress (Gottdiener *et al.*, 2003). Other factors that may impede FMD, such as hypercholesterolaemia, diabetes mellitus, smoking (active and passive) and obesity (Moens *et al.*, 2005), as well as high-fat meals (Vogel *et al.*, 1997; Cuevas *et al.*, 2003), were avoided, although some have found that the latter did not influence endothelial function (Djousse *et al.*, 1999). Meals rich in thermally-stressed fats have also been shown to reduce FMD (Williams *et al.*, 1999). Levenson *et al.* (2001) found significant gender differences, with women showing more pronounced shear-mediated vasoconstriction and vasodilatation than men. Flow-mediated dilatation was also found to be significantly different between men and women after a high-fat meal, with men showing a greater reduction in FMD than women (Schillaci *et al.*, 2001). The current study did not show significant gender differences.

Burns *et al.* (2000) found that the capacity of different red wines to act as vasodilators varied widely, and this capacity was associated with concentrations of specific phenolic compounds in the wine, in particular resveratrol and catechins. Red wine has been found to inhibit endothelin-1 synthesis, possibly by modifying tyrosine kinase-signalling in the endothelial cells (Corder *et al.*, 2001). The antioxidant activity of the red wines used in this study showed wide variation, as do South African red wines in general, as described by De Beer *et al.* (2005). It is possible that wine with more antioxidant activity might have resulted in an

improved FMD response in this study, or it is possible that habituation is required.

A study described by Hashimoto *et al.* (2001) found no significant change in systolic blood pressure, a significant increase in resting brachial artery diameter and, contrary to this study, a significant improvement in FMD by 120 minutes after red wine consumption. No obvious technical differences were found between this study and the one described by Hashimoto *et al.* They, however, administered more ethanol compared with this study: 0.8 g/kg body mass compared with 0.35 g/kg body mass. It is possible that a dose-related response would reveal a volume of ethanol that would result in improved FMD if ethnic differences do not explain the different results.

This study was undertaken on normal subjects. It is possible that responses may differ in subjects with CHD (coronary heart disease). The prevalence of abnormal vascular function in subjects with CHD has not yet been fully established. It would be of interest, therefore, to compare individuals with and without CHD for their responses to alcoholic beverages. Whilst it might initially be thought that peripheral arterial dilatation is desirable in subjects with CHD, as a result of after-load reduction, it is possible that a coronary steal syndrome may aggravate the CHD, unless similar responses were to sustain coronary perfusion. It may thus be necessary to evaluate exercise tolerance in such studies.

This study did not attempt to separate the different effects of the phenols and the ethanol on vascular function. Wine is a complex beverage made up of a large number of different polyphenols, as well as ethanol. Some studies have found that it is the phenols, particularly the flavonoids in wine, that improve endothelial function (Duffy and Vita, 2003), and not the ethanol in the wine (Agewall *et al.*, 2000; Hashimoto *et al.*, 2001). Red grape juice has been found to improve FMD (Stein *et al.*, 1999). Ideally, individual compounds should be studied to determine their concentration in plasma and urinary excretion, as there may be individual differences in the metabolism of antioxidant substances.

In conclusion, this study found that arterial diameter increased after the acute consumption of red wine, but that FMD did not. Further studies should be undertaken to characterise the appropriateness of FMD for vasodilatation and the duration of the vasodilatory response. Ideally, alcohol-free local wines should be compared. When these phenomena are understood, vascular function studies may be undertaken in CHD subjects.

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