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Does a glass of red wine improve endothelial function?

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Aims To examine the acute effect of red wine and de-alcoholized red wine on endothelial function.

Methods and Results High frequency ultrasound was used to measure blood flow and percentage brachial artery dilatation after reactive hyperaemia induced by forearm cuff occlusion in 12 healthy subjects, less than 40 years of age, without known cardiovascular risk factors. The subjects drank 250 ml of red wine with or without alcohol over 10 min according to a randomized procedure. Brachial artery dilatation was measured again 30 and 60 min after the subjects had finished drinking. The subjects were studied a second time within a week of the first study in a cross-over design. After the red wine with alcohol the resting brachial artery diameter, resting blood flow, heart rate and plasma–ethanol increased significantly. After the de-alcoholized red wine these parameters were unchanged. Flow-mediated dilatation of the brachial artery was significantly higher ($P<0{\cdot}05$) after drinking de-alcoholized red wine ($5{\cdot}6 \pm 3{\cdot}2\%$) than after drinking red wine with alcohol ($3{\cdot}6 \pm 2{\cdot}2\%$) and before drinking ($3{\cdot}9 \pm 2{\cdot}5\%$).

Conclusion After ingestion of red wine with alcohol the brachial artery dilated and the blood flow increased. These changes were not observed following the de-alcoholized red wine and were thus attributable to ethanol. These haemodynamic changes may have concealed an effect on flow-mediated brachial artery dilatation which did not increase after drinking red wine with alcohol. Flow-mediated dilatation of the brachial artery increased significantly after de-alcoholized red wine and this finding may support the hypothesis that antioxidant qualities of red wine, rather than ethanol in itself, may protect against cardiovascular disease.

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Key Words: Endothelial function, ethanol, ultrasound

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Introduction

Several studies have suggested that the relationship between alcohol consumption and mortality rates is characterized by a U-shaped or a J-shaped curve[1,2]. The downstroke of the U is probably explained by a beneficial effect of moderate alcohol consumption on the risk of ischaemic heart disease[3]. Both the drinking pattern and the type of alcohol consumed have been suggested as determinants of the shape of the curve. One study has shown steady drinking to be more protective than binge drinking against coronary heart disease[4]. Other studies have suggested that wine has a greater protective effect than beer and spirits[5,6]. Alcohol consumption may be associated with many favourable effects, including improved lipid-profile[7], reduced thrombocyte aggregation and increased fibrinolysis[8]. However, the underlying mechanism behind the relationship between alcohol consumption and coronary heart disease is not known.

Endothelial dysfunction has been proposed to play a pathogenic role in the initiation of vascular disease[9] and there are several studies[10–12] demonstrating that administration of antioxidants improves endothelial function. These observations may suggest that nitric oxide inactivation by oxygen free radicals contributes to endothelial dysfunction.

Polyphenols, which are important components of red wine, have been shown to possess antioxidant properties[13,14]. This antioxidant capacity may explain why red wine may have more pronounced cardioprotective effects than other alcoholic beverages. There are studies demonstrating increased antioxidant activity in

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blood following ingestion of red wines, but not with other beverages[15,16]. In a study in rabbits, acute exposure of aortic rings to red wine and polymeric phenols resulted in vasodilatation which was abolished by removal of the endothelium and by L-NMMA (an inhibitor of nitric oxide synthesis)[17]. Consequently, the vasodilatory effect was mediated by nitric oxide. In the same study pure ethanol had no such effect.

These data support the hypothesis that red wine has a beneficial effect on endothelial function. However, this has not been demonstrated in human studies despite considerable epidemiological data.

The aim of this study was to examine the acute effect of red wine (alcohol and antioxidants) and de-alcoholized red wine (antioxidants) on endothelial function.

Methods

Subjects

Twelve adult volunteers were recruited from our institution. All were normotensive, less than 40 years of age, non-smokers, non-diabetic, with normal serum cholesterol and on no medications.

Protocol

The subjects were served a standardized light lunch at noon. The lunch consisted of a baguette filled with 5 g of margarine, 2 leaves of lettuce, half of a sliced tomato, a slice of lean cheese and a little pepper, a low-fat yoghurt (150 g) and a banana. At 1400 h the subjects returned to the laboratory. Height and weight were recorded and body mass index was derived. After the subjects had rested in the recumbent position for 5 min blood pressure was measured using a mercury sphygmomanometer. Diastolic pressure was determined as Korotkoff phase V and the mean of two recordings was used. A butterfly needle was inserted into a superficial vein through which blood samples were drawn for determination of plasma glucose, plasma–ethanol and serum concentrations of cholesterol using established methods. The subject's left arm was comfortably immobilized in the extended position to allow consistent imaging of the brachial artery. Brachial artery diameter was imaged[18] using a 10 MHz compact linear ultrasound transducer (ATL HDI 3000, Bothell, WA, U.S.A.). The brachial artery segment 1–6 cm above the antecubital crease was located and imaged in the longitudinal plane, ensuring the lumen diameter was maximized and the gain optimized to provide clear arterial wall interfaces. The distance from the antecubital crease was noted and the following examinations were all performed in the same position. B-mode ultrasound images were obtained with gating from the R wave of the electrocardiogram.

Arterial flow velocity measurements were obtained using a pulsed Doppler signal at 60° to the vessel with the range gate (1·5 mm) in the centre of the artery. Images were recorded on videotape for subsequent off-line analysis on the same instrument.

After an initial 15 min rest period, baseline recordings of brachial artery diameter and flow velocity were performed. A standard sphygmomanometer cuff, placed around the forearm distal to the imaged brachial artery segment, was inflated to a pressure of 200 mmHg for 4·5 min. Blood flow was recorded prior to and immediately after cuff release for 20 s. Continuous B-mode images were collected for 2 min after cuff release.

Subsequently the subjects were randomly allocated to drink over 10 min either 250 ml of a Cabernet Sauvignon/Merlot red wine containing 12·5% alcohol by volume or 250 ml of a de-alcoholized red wine containing less than 0·5% alcohol by volume. After 30 min a blood sample for determination of plasma alcohol was drawn and another flow-mediated brachial artery vasodilatation examination was performed. Sixty minutes after the subjects has finished the glass of wine, another blood sample for determination of plasma alcohol was drawn and a third flow-mediated brachial artery vasodilatation examination was performed. The mean of the flow-mediated dilatation 30 and 60 min after drinking was used in the calculations.

All subjects returned for a second examination within one week. The study procedures were repeated according to the same protocol except that the subjects who at the first examination were randomized to red wine received de-alcoholized red wine and vice versa.

The polyphenol level of the wines was analysed from all the bottles[19]. If not brilliantly clear the wine sample was clarified by centrifugation in a closed tube or by filtration through Celite. One hundred microlitres of wine (or 200 µl for lightly coloured wines) was added to 10·0 ml 1 M-HCl. After 3–4 h the absorbance in a 10 mm cell at a wavelength of 280 nm in a UV spectrophotometer (Ultraspec 2000) with a cell block maintained at 25 °C was measured. The absorbance was corrected for the dilution used i.e. × 101 or × 51. The total phenolics were initially calculated in absorbance units = A − 4. (The factor 4 corrects for UV absorbing non phenolic wine components, this necessary correction factor has been derived from studies of many V.vinifera wines and juices.) The total phenolics are reported in Gallic Acid Equivalents (GAE). The units are mg . 1^{-1}.

Data analyses

Images were digitally acquired from the videotape and measured in random order by a single observer blinded to the phase of the study and randomization code. The brachial artery diameter was measured using the leading edge of the near wall to the leading edge of the far wall of the artery along a line perpendicular to the artery's long axis using an electronic calliper. The baseline

Table 1 Baseline characteristics of subjects (n=12)

Age (years)	31 (4)
Gender male/female	8/4
Body mass index (kg . m^{-2})	24·6 (3·4)
Systolic blood pressure (mmHg)	121 (6)
Diastolic blood pressure (mmHg)	79 (4)
Heart rate (beats . min^{-1})	61 (7)
Serum cholesterol (mmol . l^{-1})	4·3 (0·9)
Plasma glucose (mmol . l^{-1})	4·7 (0·9)

Means (SD)

brachial artery diameter was derived from the mean of eight measurements (two on each of four images).Two measurements were made of three individual images, providing six diameter measurements each at 45, 60 and 75 s after cuff deflation. There was no difference between the brachial artery diameter at 45, 60 and 75 s after cuff deflation. The mean diameter of the measurements after 45, 60 and 75 s was divided by the average diameter of the resting scan. Diameter changes were expressed as the percentage change relative to the mean baseline scan (100%). Volume flow was calculated by multiplying the velocity time integral of the Doppler flow signal for a single pulse wave by the heart rate and vessel cross-sectional area. Volume flow was measured during rest and at the peak response of the maximum flow in a single cardiac cycle during the 10–20 s period after cuff release.

The subjects had given informed consent to participate in the study, which was approved by the Ethical Committee of the Faculty of Medicine, Auckland University.

Statistical methods

Results are presented as means and standard deviations. A paired t-test was used to compare continuous variables. All test were two-sided and $P<0·05$ was regarded as statistically significant.

Results

Twelve subjects participated in the study (eight male and four female), mean age 31 years, serum cholesterol and glucose were within normal limits (Table 1).

The results from the first and second baseline examinations before wine digestion did not differ from each other and a mean of these results are presented; similarly the results of the examinations 30 and 60 min after ingestion of the red wine and the de-alcoholized red wine were the same and thus the mean of the results from these two time periods was used.

Red wine was associated with a significant increase in the resting brachial artery diameter, resting blood flow, heart rate and plasma–ethanol 30–60 min after drinking. After de-alcoholized red wine these parameters were unchanged (Table 2).

The peak blood flow after the cuff had been deflated during flow-mediated dilatation examination was significantly increased after both red wine and de-alcoholized red wine compared with peak blood flow before drinking (Table 3). The peak flow after red wine and de-alcoholized red wine was approximately the same, 637 ± 129 ml . min^{-1} and 608 ± 214 ml . min^{-1}, respectively. However the percentage increase in blood

Table 2 Resting conditions before and 30–60 min after oral ingestion of red wine and de-alcoholized red wine (n=12)

	Before drinking	After red wine	After de-alcoholized red wind
Baseline vessel diameter (mm)	4·43 (0·38)	4·77 (0·44)*	4·46 (0·33)†
Baseline blood flow (ml . min^{-1})	102 (56)	150 (93)*	93 (56)†
Heart rate (beats . min^{-1})	61 (6)	64 (7)*	60 (7)
Plasma–ethanol (mmol . l^{-1})	<3	9 (3)*	<3†

Means (SD)
$P<0·05$;*vs before drinking, †vs after red wine.

Table 3 Results of the flow-mediated dilatation examinations before and after oral ingestion of red wine and de-alcoholized red wine (n=12)

	Before drinking	After red wine	After de-alcoholized red wine
Peak blood flow (ml . min^{-1})	516 (181)	637 (129)*	608 (214)*
Blood flow increase (%)	618 (412)	446 (230)	743 (410)†
Flow-mediated dilatation (%)	3·9 (2·5)	3·6 (2·2)	5·6 (3·2)*†

Means (SD)
$P<0·05$ *vs before drinking, †vs after red wine.

flow during the flow-mediated dilatation examination was lower after red wine than after de-alcoholized red wine (Table 3) due to the increased basal blood flow after ingestion of red wine (Table 2). Flow-mediated dilatation was significantly higher after drinking de-alcoholized red wine (5·6 ± 3·2%) than after drinking red wine (3·6 ± 2·2%) and before drinking (3·9 ± 2·5%) (Table 3).

The subjects tolerated the flow-mediated dilatation examinations well and the heart rate was unchanged during the periods when the blood pressure cuff was inflated. The phenol content of the red wine and the de-alcoholized red wine were 1949 ± 50 mg . l^{-1} and 1110 ± 25 mg . l^{-1}, respectively.

Discussion

This study has demonstrated that a single dose of de-alcoholized red wine increases endothelium-dependent vasodilatation, in response to hyperaemia, while red wine ingestion resulted in a vasodilatation without affecting the percentage increase of the artery diameter during the flow-mediated vasodilatation examination.

Epidemiological studies have shown that moderate alcohol consumption is associated with a lower risk of coronary heart disease. The so-called 'French paradox' has arisen from the observation that, despite a diet rich in saturated fat, consumption of red wine by the French appears to afford some protection against coronary heart disease[5,6]. The underlying mechanism behind this beneficial effect of alcohol, or more specifically red wine, is unclear. In vitro studies have demonstrated that exposure of certain wines, grape juices and grape skin extracts to rat and rabbit aortic rings, causes vasodilatation which is abolished by removal of the endothelium and by a nitric oxide synthesis inhibitor (L-NMMA)[17,20,21]. Whereas the grape skin extract had a pronounced relaxation effect on the aortic ring, the grape pulp extract had no such effect[20]. It was also observed in these studies that red wines caused significant vasodilatation whereas white wines only had minor effects on vessel diameter. The vasoactive substance was probably derived from the grape skin and the superior effect of red wine relates to the fact that grape skins are removed before fermentation in the preparation of many white wines. Exposure to ethanol in these in-vitro studies did not produce relaxation of the precontracted vascular rings[17,20].

The observation from the present study, that de-alcoholized red wine enhanced the endothelium function was in line with previous in-vitro studies. Previous data have demonstrated that diet influences endothelial function. An impaired endothelium function can be detected several hours after a high fat meal in healthy normocholesterolaemic subjects; effects which are not seen after a low-fat meal and which can be attenuated by pre-treatment with an antioxidant[22]. Several other studies have reported a positive effect of antioxidants on endothelial function[10,12], suggesting that oxygen free radicals contribute to endothelial dysfunction which at least partly can be reversed by dietary antioxidants. Oral ingestion of red wine[15,16] increases blood antioxidant activity. Polyphenols, which are important components of grape skins, and possess antioxidant properties[13,14] may be responsible for the enhanced flow-mediated brachial artery dilatation observed in the present study.

Interestingly, in this study, flow-mediated dilatation of the brachial artery did not increase after red wine.

Following administration of red wine, resting brachial artery blood flow, brachial artery diameter and heart rate increased. These changes were not observed following the de-alcoholized red wine, and are thus attributable to ethanol. Similar changes have previously been described after acute ethanol consumption in clinical trials[23]. The brachial artery dilatation was more pronounced after ingestion of red wine than the dilatation mediated with our flow-mediated dilatation examinations. During the flow-mediated dilatation examination after ingestion of red wine the percentage increase in the artery diameter was of the same magnitude as before the red wine. However, this increase occurred in an already dilated artery. From the present study it is uncertain whether this brachial artery dilatation induced by ethanol was endothelium-dependent or not. The brachial artery dilatation and the increased resting blood flow may have concealed an effect of wine on flow-mediated brachial artery dilatation. The results from in-vitro studies regarding the effects of ethanol on vasodilatation are variable. In a study of the pulmonary artery in rats, ethanol induced vasodilatation which was attenuated with rubbed endothelium cells and by L-NMMA, suggesting that vasodilatation of ethanol, at least partly, is endothelium dependent[24]. In other studies, ethanol infusion and chronic ethanol exposure suppressed endothelium-dependent relaxation[25,26]. Other in vitro studies found no vasodilatation effects of ethanol[17,20]. It is of course possible that the acute and chronic effect of ethanol on the endothelium are quite different. For example, acute alcohol consumption may result in a reduction of blood pressure via the above mentioned mechanisms, whereas long-term abuse of alcohol is associated with hypertension[27]. The present study only addresses the acute effect of red wine and the long-term effect remains to be determined.

The subjects in this study were healthy without cardiovascular risk factors. More research is needed to confirm our results in subjects with coronary heart disease and to study the long-term effect of red wine consumption on endothelial function.

The method used in this study to examine endothelial function relies on increased blood flow causing dilatation of the vessel locally[28]. This dilatation can be blocked by an antagonist to EDRF/NO produced by the endothelium[29], and the dilatation appears dependent on endogenous NO released in response to increased flow.

The degree of brachial arterial dilatation following forearm occlusion is now commonly used in clinical studies as a measure of endothelial function. Non-invasive measurement of flow mediated dilatation of the brachial artery has been shown to correlate with invasive assessment of coronary artery endothelial function using acetylcholine (an endothelium-dependent vasodilator) infusion[30]. It is also shown that acetylcholine infusion into the brachial artery results in brachial artery dilatation[31] and increased forearm blood flow[11].

In conclusion, the present study demonstrated that endothelium-dependent vasodilatation increased after a single oral dose of 250 ml of de-alcoholized red wine. This finding may support the hypothesis that anti-oxidant qualities of red wine, rather than ethanol in itself, may protect against cardiovascular disease. After ingestion of red wine the brachial artery dilated and the blood flow increased, whereas the flow-mediated dilatation remained unchanged. These haemodynamic changes were associated with ethanol as they were not observed after ingestion of de-alcoholized red wine. The brachial artery dilatation and the increased resting blood flow may have concealed an effect on flow-mediated brachial artery dilatation. It cannot be determined from the present study whether the brachial artery dilatation induced by ethanol was endothelium-dependent or not. More research is needed to confirm our results in subjects with coronary heart disease and to study the long-term effect of red wine consumption on endothelium function.

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