A High-Fat Diet Induces and Red Wine Counteracts Endothelial Dysfunction in Human Volunteers

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ABSTRACT: Endothelial dysfunction is associated with atherogenesis and oxidative stress in humans. In rat and rabbit blood vessels, wine polyphenol antioxidants induce vascular relaxation *in vitro* through the NO-cGMP pathway. To assess the effect of a regular high-fat diet (HFD) and moderate red wine consumption on endothelial function (EF), a study was performed in healthy male volunteers. EF was measured as flow-mediated dilatation of the brachial artery, employing high-resolution ultrasound after an overnight fast. Other clinical and biochemical parameters related to EF were also measured. Six volunteers received a control diet, rich in fruits and vegetables (27% calories as fat) and five volunteers received an HFD (39.5% calories as fat). Measurements were done twice on each volunteer: after a period of 30 d with diet plus 240 mL of red wine/d, and after a period of 30 d with diet, without wine. In the absence of wine, there is a reduction of EF with HFD when compared to the control diet ($P = 0.014$). This loss of EF is not seen when both diets are supplemented with wine for 30 d ($P = 0.001$). Plasma levels of *n*-3 fatty acids ($R^2 = 0.232$, $P = 0.023$) and lycopene ($R^2 = 0.223$, $P = 0.020$) show a positive correlation with individual EF measurements, but they do not account for the significant differences observed among dietary groups or after wine supplementation. These results help elucidate the deleterious effect of a high-fat diet and the protective role of wine, n-3 fatty acids and dietary antioxidants in cardiovascular disease.

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Normal endothelium-dependent vasomotor function or endothelial function (EF) is a physiological response, mediated by nitric oxide (NO), that appears to play a key role in the prevention or reduction of the risk of atherosclerosis (1,2). Endothelial dysfunction is associated with risk factors for coronary heart disease such as hypercholesterolemia, hypertension, cigarette smoking, hyperhomocysteinemia and diabetes mellitus and is detectable before anatomical vascular signs of atherosclerosis appear, supporting a pathogenic role of endothelial dysfunction in atherogenesis (3–5). Young healthy subjects with a family history of premature coronary disease and no other apparent risk factor also show impaired EF (6).

Hypercholesterolemia (7), genetic hyperlipidemias (8), or a single high-fat meal (9) decrease EF, suggesting that a regular high-fat diet (HFD) might lead to endothelial dysfunction. Ingestion of the antioxidant vitamins C and E (1,5,8,10,11) partially blocks the decrease in EF *in vivo*, a phenomenon attributed to protection of NO from inactivation by free radicals.

Vegetables and moderate wine consumption have been associated with a decreased risk of coronary disease (12,13), an effect related, at least in part, to their high content in flavonoids and other polyphenol antioxidants (14,15). Also, it has been shown that wine polyphenols exert vasorelaxing activity in rat and rabbit aortic rings and human coronary arteries rings (16–19). Plant polyphenols from other sources show a similar activity (20). Moderate wine consumption and a high intake of fruits and vegetables characterize Mediterranean diets (21,22). Therefore, plant polyphenol antioxidants could account, at least in part, for the cardiovascular health benefits associated with Mediterranean diets and moderate wine consumption.

The present study evaluates EF employing a noninvasive ultrasound detection method (23) in human volunteers on either an HFD or a control diet (CD) rich in fruits and vegetables, with and without moderate red wine consumption.

EXPERIMENTAL PROCEDURES

Study design. The experimental protocol employed was approved by the Ethics Committee of the Faculty of Medicine, Catholic University of Chile. Subjects were randomly selected from a larger group of volunteers participating in a diet and wine intervention study. For 90 d they received either an HFD ($n = 5$) or a CD ($n = 6$). From day 31 to 60 the diet was isocalorically supplemented with 240 mL/d of red wine (cabernet sauvignon). No other alcoholic beverage was allowed during the 3 mon of the study. EF, as well as the other measurements reported here, were performed at days 60 and 90.

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Abbreviations: ANOVA, analysis of variance; CD, control diet; cGMP, guanosine 3′:5′-cyclic monophosphate; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EDRF, endothelium-derived vascular relaxing factor; EF, endothelial function; γ-GT, γ-glutamyl transferase; HFD, high-fat diet; HPLC, high-performance liquid chromatography; NO, nitric oxide; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; VLCn-3, very long-chain n-3-fatty acids.

Subjects. Subjects were healthy males, aged 20–28 yr, undergraduate or graduate students from the Faculty of Medicine, who signed an informed consent form and were well informed about the project procedures and objectives. They were randomly selected from a larger group of 42 subjects. The smaller size of the groups for EF measurement was determined both by the chronogram of biological sampling defined for the larger study, and limitations in the access to the ultrasound imaging equipment, since the volunteers were required to fast overnight. Criteria for inclusion were absence of clinical disease, body mass index 20–25 kg/m^2, and normal values for serum lipids, blood pressure, and blood glucose. They were normally active, nonsmokers, and were not taking any medication or vitamin supplementation. Clinical interview, physical examination, and laboratory evaluation were done every 30 d. Blood pressure values correspond to the mean of two measurements, separated 5 min, with the subject in a sitting position.

Diets. HFD and CD were prepared by a catering company, with daily supervision by a nutritionist and a food microbiologist working for the project. Diets were delivered at the work place, or at home at night and weekends, in isolated personalized boxes. The average daily caloric supply was 2,565 kcal. Proteins supplied 17.6% of calories for both diets. Fats, as detailed in Table 1 supplied 27.3 or 39.9% of calories for CD and HFD, respectively. Diet composition was calculated employing Food Processor II (Esha Research, Salem, OR). Fruits and vegetables consumption was an average of 675 g/d for CD and 246 g/d for HFD. HFD used preferentially red meats and was low in fish; CD used predominantly fish and white meat. On the whole, HFD resembled a regular Western or U.S. diet from the last decades (24,25) and CD a Mediterranean-style diet (21,22).

Biochemical analysis. Plasma glucose, serum enzymes, total cholesterol, low density lipoprotein cholesterol, and triglycerides were measured following routine clinical laboratory procedures. Fatty acids were measured by gas chromatography as follows: plasma lipids were extracted according to Bligh and Dyer (26) using chloroform/methanol (2:1, vol:vol) containing butylated hydroxytoluene (0.01%). Total fatty acids were transesterified with methanolic-HCl (1 N) overnight at room temperature; after extraction with *n*-hexane, the methyl esters were quantified by gas–liquid chromatography employing a capillary column (50 m × 0.25 mm, SGE BPX-70) with hydrogen as carrier gas and a temperature program of 5°/min from 110 to 230°C. Vitamin E (as α-tocopherol), β-carotene, and lycopene were determined by high-performance liquid chromatography (HPLC) with electrochemical detection (LC-4C, Bioanalytical Systems Inc., W. Lafayette, IN) (27). Serum vitamin C was detected by a procedure based on the reduction of ferric chloride (28); the accuracy of these measurements was checked by HPLC. Folate and vitamin B_{12} in serum were determined by ion capture and microparticle immunoassay, respectively (IMX® Folate and AxSYM B_{12}; Abbott Labs, Abbott Park, IL). Plasma homocysteine was measured by HPLC with fluorometric detection, employing a commercial kit (Chromsystems, München, Germany).

TABLE 1
Daily Intake of Fats and Fatty Acids[a]

	High fat diet (g/d)	Control diet (g/day)
Total fat	112.7 ± 12.9	77.0 ± 2.6
SFA	35.8 ± 9.6	22.7 ± 2.3
MUFA	35.3 ± 4.2	37.9 ± 2.5
PUFA	32.0 ± 4.5	9.6 ± 1.4
VLCn-3	0.12 ± 0.04	0.38 ± 0.13
Cholesterol	0.61 ± 0.12	0.29 ± 0.09

[a]Mean values ± SD. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; VLCn-3, very long-chain n-3 fatty acids = eicosapentaenoic acid + docosapentaenoic acid + docosahexaenoic acid.

EF. EF was assessed twice for each volunteer: on day 60, at the end of the 30-d wine supplementation period; and at day 90, after a period of 30 d without wine, only with diet. Measurement were made at 8–9 A.M., with an overnight fast. The noninvasive procedure employs 7.5 MHz ultrasound imaging (GAIA 8800, Medison, Seoul, Korea) and has been validated by Celermajer *et al.* (23). The measurements were performed essentially as described by Plotnick *et al.* (10). The brachial artery was longitudinally imaged approximately 5 cm proximal to the antecubital crease, twice at baseline and then 1, 3, and 5 min after release of 5 min arterial occlusion with a blood pressure cuff, 12.5-cm wide, placed on the forearm, kept at 200 mm Hg. Results are expressed as percent dilation at minute 1 after flow reestablishment. Two independent investigators, unaware of subject identity and dietary status, performed the measurements of end-diastolic brachial artery diameter in the video recordings obtained. Following flow-mediated reactivity measurement, after 15 min rest, volunteers received 300 μg nitroglycerin sublingually to control the dilatation response 3 min after nitroglycerin administration.

Statistical procedures Data are expressed as mean ± SD. The measurements within the same dietary group, at different times, were compared by analysis of variance (ANOVA) for repeated measures and paired *t* test with Bonferroni adjustment. To determine if wine supplementation to both dietary groups modifies the variables in the same direction, ANOVA for repeated measures with interaction between diet and wine was applied. For comparisons among the two dietary groups, the Student *t* test for independent samples was employed. Bivariant correlation analysis was performed according to Pearson. *P* values <0.05 were considered statistically significant.

RESULTS

The relative increase in brachial artery diameter that follows the reestablishment of circulation constitutes the basis of the noninvasive procedure to quantitate EF. The kinetics of the relative change in mean arterial diameter values for the two experimental conditions are shown in Figure 1. In the HFD maximal response occurs after 1 min of flow reestablishment, whereas for CD the vasodilation response apparently is slower. The difference observed with wine was maximal after 1 min for both dietary groups. In fact, this time interval is routinely em-

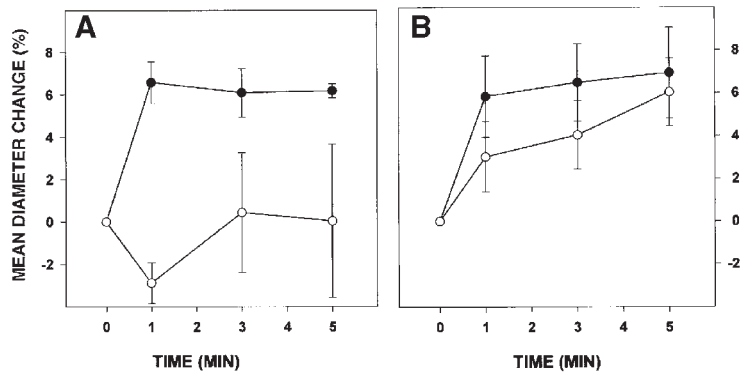

FIG. 1. Effect of diet and wine on endothelium-dependent brachial artery vasoactivity. Flow-mediated brachial artery vasoactivity, expressed as percent change in arterial diameter, was measured with high-resolution ultrasound, before and at various times after releasing the circulatory arrest imposed by an inflated cuff in the forearm. The measurements were made after 12 h without food and wine, and correspond to the mean values ± standard error, from (A) 5 subjects under a high-fat diet or (B) 6 subjects under a control diet rich in fruits and vegetables, with (●) or without (○) supplementation with red wine (240 mL/d). The change in diameter is expressed relative to the diameter before circulatory arrest.

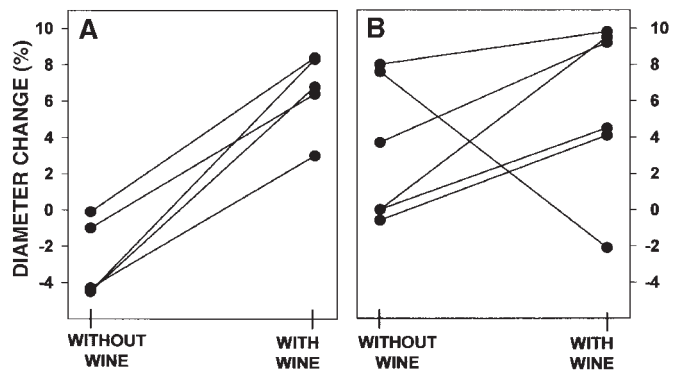

FIG. 2. Effect of wine supplementation on endothelium-dependent flow-mediated brachial artery vasoactivity, expressed as percent change in arterial diameter for each experimental subject consuming a high-fat (A) or a control (B) diet. Values represented correspond to individual measurements, 1 min after releasing the arterial flow, obtained during the observations summarized in Figure 1.

ployed in the standard procedure to evaluate EF. The individual EF values with and without wine, for the five HFD and the six CD volunteers, are shown in Figure 2. A striking result is the consistent increase of EF in the HFD subjects when wine is present in the diet. The average percent diameter change varies from –2.9 ± 2.1 without wine to 6.6 ± 2.2 with wine ($P = 0.001$ for paired data analysis). It can also be seen that in the absence of wine, HFD values are significantly lower than those for CD, average 3.1 ± 3.9 ($P = 0.014$). The effect of wine, clearly seen for HFD, is not evident with CD; 3.1 ± 3.9 is the average percent diameter change without wine supplementation and 5.8 ± 4.6 with wine supplementation ($P = 0.358$ for paired samples), yet ANOVA for repeated measurements with interaction among diet and wine shows a parallel behavior for HFD and CD, i.e., both groups change with wine in the same direction. Unexpectedly, one CD volunteer showed decreased EF in the wine supplementation period, a result for which we do not have a more precise explanation than biological variability.

Nitroglycerin-induced percent dilation values for HFD were 19.1 ± 10.3 and 26.5 ± 2.9 without and with wine supplementation; and for CD 21.4 ± 5.3 and 20.9 ± 3.5, without and with wine supplementation. These values suggest an increase in the nitroglycerin-induced dilatation for the HFD group after wine supplementation, but the difference is not statistically significant for the HFD group ($P = 0.262$ for paired samples).

The diets employed had either 39.5 (HFD) or 27% (CD) of total calories as fat. As shown in Table 1, both provided almost the same amount of monounsaturated fatty acids; in contrast, HFD supplied three times more polyunsaturated fatty acids (PUFA) and 1.6 times the saturated fatty acids (SFA) of CD. For CD, SFA amount to 8.7% of total energy consumption. For fruits and vegetables the supply was almost three times higher in CD.

The average values for some plasma biochemical parameters in both dietary groups, with and without wine, are shown in Table 2. The plasma fatty acid profiles apparently reflect the dietary pattern; nevertheless, statistically significant differences among diets are seen only in PUFA and very long chain n-3 fatty acids (VLCn-3) values after wine supplementation. Total and low density lipoprotein cholesterol values as well as triglycerides apparently reflect the diet composition, but the changes are not statistically significant, most probably as a consequence of the relatively small number of subjects evaluated. Other biochemical parameters known to influence EF do not show variations that could account for the changes in EF observed with diet and wine: homocysteine and vitamin B_{12} remain constant, and folic acid increases with the diet rich in fruits and vegetables, but without correlation with homocysteine levels. A consistent change observed with both diets is a statistically significant 14% decrease in plasma vitamin E (α-tocopherol) at the end of the wine supplementation period. Lycopene mean values do not show statistically significant variations within or among diets, yet there is a positive correlation among all individual lycopene values and EF values expressed as percent dilation of the brachial artery ($R^2 = 0.223$, $P = 0.020$). A similar correlation is seen among VLCn-3 mass plasma levels and EF ($R^2 = 0.232$, $P = 0.023$). This positive and statistically significant correlation of EF with n-3 fatty acids is mainly due to docosapentaenoic acid (DPA) ($R^2 = 0.504$, $P = 0.017$) and docosahexaenoic acid (DHA) ($R^2 = 0.437$, $P = 0.042$). A significant increase in VLCn-3 plasma concentration, in the presence of wine supplementation, is seen only in CD; in contrast, the most striking change in EF is seen with the HFD, when wine is added. In HFD, VLCn-3 plasma levels do not change with wine, in contrast to the striking effect of wine on EF. This probably means that lycopene and VLCn-3 plasma levels influence the absolute value for each EF measurement, while the relative changes observed with wine apparently are independent of lycopene or VLCn-3 concentration changes.

As part of the biochemical monitoring procedure, (γ-glutamyl transferase), serum glutamic-oxalacetic transaminase, and alkaline phosphatase levels in serum were measured (Table 3). The main change observed, a 40% increase in γ-GT in HFD

TABLE 2
Biochemical Measurements in Plasma[a]

Plasma variable	High-fat diet		Control diet	
	Without wine	With wine	Without wine	With wine
Glucose (mg/dL)	82.0 ± 3.3	83.0 ± 5.5	81.2 ± 5.5	87.3 ± 6.4
Total cholesterol (mg/dL)	177 ± 33	185 ± 33	155 ± 24	150 ± 19
LDL (mg/dL)	102 ± 24	111 ± 28	89 ± 21	88 ± 18
Triglycerides (mg/dL)	137 ± 128	127 ± 89	88 ± 41	68 ± 31
SFA (μg/mL)	622 ± 136	737 ± 180	559 ± 60	525 ± 14
MUFA (μg/mL)	404 ± 182	537 ± 214[a]	377 ± 81	421 ± 83
PUFA (μg/mL)	896 ± 116	1,166 ± 252[a]	776 ± 176	756 ± 90[b]
VLCn-3 (μg/mL)	80.2 ± 36.2	91.0 ± 16.0	80.8 ± 25.7	137 ± 18.9[a,b]
Vitamin E (μmol/L)	27.4 ± 10.5	23.6 ± 9.5[a]	19.9 ± 4.7	17.0 ± 3.9[a]
Vitamin C (μmol/L)	32.5 ± 23.4	25.8 ± 6.8	54.1 ± 7.2	58.3 ± 11.7[b]
Lycopene (nmol/L)	156 ± 63	318 ± 130	167 ± 62	245 ± 105
Folic acid (ng/mL)	3.96 ± 1.29	3.88 ± 0.62	5.82 ± 2.03	6.35 ± 2.19[b]
Vitamin B_{12} (pg/mL)	376 ± 177	334 ± 164	423 ± 173	381 ± 142
Homocysteine (μmol/L)	16.5 ± 6.1	15.3 ± 4.9	14.7 ± 2.4	11.8 ± 2.2

[a]Mean values ± SD. [a]Significant difference, within the same diet, with wine supplementation; Student *t* test for paired samples. [b]Significant difference among different diets; Student *t* test for independent samples. LDL, low density lipoprotein; for other abbreviations, see Table 1.

when compared to CD, was apparently due to the diet and not to wine supplementation. Wine supplementation induced only a slight, statistically significant, reduction in alkaline phosphatase in the CD group.

DISCUSSION

The results presented indicate that normal subjects experience a marked decrease or impairment of EF, under a usual western-style diet rich in fats. Strikingly, after moderate wine consumption vascular reactivity or EF is preserved. The EF measurements were done after an overnight fast, 12 h without food and wine; therefore, the changes induced by wine and diet do not correspond to a transient or acute postprandial response but rather to a stable change associated with the specific dietary condition.

The noninvasive procedure employed to measure EF has been validated (29–31). It has been applied to detect the consequences of an acute overload with fat (8) and the effect of antioxidant vitamins (1,5,8,10,11). The mechanism through which fats interfere with EF is apparently linked to free radical generation, since antioxidant vitamins prevent the effect. In the presence of free radicals NO is inactivated (32) and the reaction with superoxide leads to the formation of peroxynitrite. Therefore, as much as fats can lead to increased levels of free radicals and reactive oxygen species, it is not surprising that NO-mediated processes will be slowed, unless an adequate antioxidant defense is present. The initial *in vitro* observations with wine polyphenols (16) and the present work in humans suggest that wine polyphenols constitute an adequate source of antioxidants, readily available *in vivo*, capable of protecting NO function.

The finding that VLCn-3 plasma levels correlate with the degree of flow-mediated vasodilation is in agreement with previous findings. Dietary supplementation with fish oil, not necessarily with purified n-3 fatty acids, has been shown to augment endothelium-dependent vasodilation in human peripheral and coronary arteries, in a process apparently mediated by increased NO production (33,34). This effect of n-3 fatty acids is an example of the difficulties experienced when attributing to fats or fatty acids in general a specific biological effect: elevated lipid levels will favor free radical generation and oxidative stress, yet n-3 fatty acids protect EF and are antiatherogenic (35,36) in spite of their high degree of unsaturation, which makes them most susceptible to oxidation. The reduced EF observed with HFD might correlate with the three fold increase in PUFA, mostly 18:2n-6, when compared with CD. In fact, a diet rich in linoleic acid is expected to cause oxidative stress, and the mass or amount of linoleic acid supplied by the HFD is 100-fold or more higher than the amount of VLCn-3.

The present results most likely are the consequence of the

TABLE 3
Serum Enzyme Levels: Glutamate-Oxaloacetate Transaminase (SGOT), γ-Glutamyl Transferase (γ-GT), and Alkaline Phosphatase (ALP)[a]

Enzyme	High-fat diet		Control diet	
	Without wine	With wine	Without wine	With wine
SGOT (U/L)	23.3 ± 4.2	24.2 ± 4.1	18.5 ± 3.4	20.8 ± 6.4
γ-GT (U/L)	26.6 ± 5.6	27.0 ± 7.8	16.0 ± 4.7	19.3 ± 6.3[a]
ALP (U/L)	92.2 ± 25.2	87.0 ± 27.9	95.8 ± 12.7	85.3 ± 11.6[b]

[a]Mean value ± SD. [a]Significant among different diets; Student *t* test for independent samples. [b]Significant difference, within the same diet, with wine supplementation; Student *t* test for paired samples.

antioxidant properties of wine and the prooxidant effect of a HFD (37). We found no evidence in favor of other mechanisms. It has been observed that homocysteine plasma levels inversely correlate with EF values, and that the administration of antioxidants counteracts the homocysteine-induced inhibition of EF (5); however, in the present observations homocysteine levels did not correlate with EF changes. The supply of arginine in the diet (data not shown) was also unrelated to the changes observed in EF.

In our observations, among established plasma antioxidants, only lycopene levels correlate with vascular reactivity. This probably means that lycopene is particularly effective at this level, or that it is a marker for other antioxidants present in tomatoes (38), or that wine polyphenols from the diet exert a sparing or protective effect on plasma lycopene.

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