Grape Polyphenols Do Not Affect Vascular Function in Healthy Men

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Abstract

Data suggest that polyphenol-rich products may improve endothelial function and other cardiovascular health risk factors. Grape and wine contain high amounts of polyphenols, but effects of these polyphenols have hardly been investigated in isolation in randomized controlled studies. Our objective in this study was to test the chronic effect of polyphenol-rich solids derived from either a wine grape mix or grape seed on flow-mediated dilation (FMD). Blood pressure and other vascular function measures, platelet function, and blood lipids were secondary outcomes. Thirty-five healthy males were randomized in a double-blind, placebo-controlled crossover study consisting of three 2-wk intervention periods separated by 1-wk washout periods. The test products, containing 800 mg of polyphenols, were consumed as capsules. At the end of each intervention period, effects were measured after consumption of a low-fat breakfast (~751 kJ, 25% fat) and a high-fat lunch (~3136 kJ, 78% fat). After the low-fat breakfast, the treatments did not significantly affect FMD. The absolute difference after the wine grape solid treatment was −0.4% (95% CI = −1.8 to 0.9; \( P = 0.77 \)) and after grape seed solids, 0.2% (95% CI = −1.2 to 1.5; \( P = 0.94 \)) compared with after the placebo treatment. FMD effects after the high-fat lunch and effects on secondary outcomes also showed no consistent differences between both of the grape solids and placebo treatment. In conclusion, consumption of grape polyphenols has no major impact on FMD in healthy men. Future studies should address whether grape polyphenols can improve FMD and other cardiovascular health risk factors in populations with increased cardiovascular risk.

Introduction

Endothelial function has predictive value for future cardiovascular events (1–6). Noninvasive flow-mediated dilation (FMD)\(^4\) is a frequently used method to assess endothelial function in humans (7,8). FMD is the difference in brachial artery diameter measured by ultrasound before and after a period of induced ischemia of the forearm by inflation of a blood pressure (BP) cuff. FMD is expressed as the percentage increase in brachial artery diameter after release of the BP cuff. Because the dilation is mediated by nitric oxide, FMD is thought to reflect the bioavailability of nitric oxide (7).

Evidence for a role of dietary flavonoids in the prevention of cardiovascular diseases (CVD) from epidemiologic and intervention studies is increasing (9,10). Consumption of red wine has been proposed as explanation of the “French paradox” of relatively low coronary heart disease mortality rates in France despite high intake of saturated fat (10–12). This association has been attributed mainly to the high levels of grape polyphenols in wine, although other wine constituents and ethanol could also have beneficial effects (13). In vitro data suggest that red wine and its constituents induce vasodilation by increasing production of nitric oxide. Furthermore, although several studies investigated the acute effects on endothelial function of red wine and other grape products in humans (14), evidence from randomized, controlled intervention studies is still limited (9,10,15). In addition, most studies have been performed in participants with elevated CVD risk (14,16–23) rather than in healthy participants and in the majority of studies the contribution of grape polyphenols to the observed effects has not been addressed (14).

Our objective in this study was to assess the effect of polyphenol-rich grape solids on endothelial function and other cardiovascular health markers in healthy participants. To that end, we conducted a placebo-controlled, double-blind crossover study to measure whether daily intake of polyphenol-rich solids derived from either a wine-grape mix or grape seed provided at a dose of 800 mg of polyphenols would affect responses on endothelial function and other CVD risk markers compared with placebo after a 2-wk intervention. The wine grape mix represents a product with a broad range of polyphenols (monomeric anthocyanins, catechins, flavonols, procyanidins

1 Supported by Unilever Research and Development, Vlaardingen, The Netherlands.
3 Supplemental Tables 1 and 2, Supplemental Figure 1, and Supplemental Methods are available with the online posting of this paper at jn.nutrition.org.
4 Abbreviations used: BP, blood pressure; C1, large artery elasticity index; C2, small artery elasticity index; CVD, cardiovascular disease; FMD, flow-mediated dilation; LSMeans, Least-Squares Means.
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and stilbenes, and unidentified oligomers and polymers), whereas the grape seed primarily consists of monomeric catechins and unidentified oligomers and polymers (24). Both the red wine (Provinols) and the grape seed (Leucoselect) solids have been shown to affect vascular function in animal studies (25,26). We studied the effects after a low-fat breakfast and after a high-fat lunch, because it has been suggested that red wine might counteract endothelial dysfunction caused by a vascular function stressor, such as cigarette smoking or a high-fat meal (14,19,27–29).

Methods

Participants. Apparently healthy males (n = 35) aged between 18 and 45 years were recruited. Participants were not included if they reported current or previous metabolic diseases, gastrointestinal disorders, or CVD, had a BMI (in kg/m²) < 18 or > 32, had blood markers (white blood cells, RBC, hemoglobin, hematocrit, and platelet count), plasma liver enzymes (ALAT, ASAT, and γ-GT), BP (systolic BP ≥ 160 mm Hg and/or diastolic BP ≥ 95 mm Hg), fasting serum lipids (total cholesterol > 8 mmol/L, total cholesterol:HDL cholesterol ratio > 8, and triglycerides > 4 mmol/L), urininary protein and glucose markers outside normal reference range, and/or lifestyle habits that could influence the primary outcome of the studies. All participants received both written and oral information about the study and gave their written consent. The study took place from November to December 2003 at Unilever Research and Development Vlaardingen, The Netherlands. The protocol was approved by the Medical Ethics Committee of Wageningen University, The Netherlands.

Design and randomization procedure. The study had a double-blind, placebo-controlled, randomized full crossover design with a 3-d run-in period, three 2-wk intervention periods, and two 1-wk washout periods. Participants were randomly divided into the 3 treatment orders according to a complete balanced design (30). The 3 treatments consisted of 2 different grape solids and a placebo. Measurements were performed following the run-in period (baseline assessment) and at the end of each of the intervention periods. The effect of the treatment under basal conditions and after a fat load was investigated by measurements after a low-fat breakfast and 3 h after a high-fat lunch, and these responses were compared with the placebo response (Supplemental Fig. 1). Staff and participants remained unaware of the treatment during the study and data analysis.

Test products. The placebo and grape solids (wine grape and grape seed) were consumed in 6 capsules/d each of 300 mg. The placebo capsules contained micro-crystalline cellulose. The total polyphenol content of both grape solids in the 6 daily capsules was 800 mg according to gallic acid equivalents; 1405 mg of the wine grape solids contained 550 mg polyphenols of Provinol (Seppic) and 250 mg polyphenols of MegaNatural Rubired grape juice (Polyphenolics); 2547 mg of the grape seed solids contained 800 mg polyphenols of Leucoselect Phytosome (Indena).

Identification by HPLC analysis of monomeric polyphenols revealed that the 550 mg of wine solids contained 18.8 mg anthocyanins, 6.9 mg phenolic acids, 4.0 mg catechins, 0.4 mg flavonols, and 0.1 mg stilbenes and that 250 mg of grape juice contained 118.5 mg anthocyanins, 2.7 mg phenolic acids, 0.2 mg catechins, 0.5 mg flavonols, and 0.1 mg stilbenes. However, the majority of polyphenols in these solids are oligomers and polymers that could not be identified by this analysis. According to analysis by Gabetta et al. (31), the polyphenolic profile of the grape seed consists of ~15% (±)-catechin and (±)-epicatechin and 85% (±)-epicatechin 3-O-gallate and unidentified oligomers and polymers.

During the 2-wk treatment periods, volunteers consumed 3 capsules/d with breakfast and 3 with dinner. On the test day after each treatment period, participants ingested 3 capsules with a low-fat breakfast and 3 capsules with a high-fat lunch. To assess compliance, participants were asked to register their intake of the capsules on each day in the intervention period.

Background diet and test meals. Volunteers were asked to refrain from vitamin supplementation from the day of the screening until the end of the study. On the days prior to a test day, standardized meals were provided to the participants. On the day prior to the test day, participants were asked to avoid eating fruit, drinks high in fruit, apple-sauce, alcoholic drinks, and chocolate and to limit their intake of tea to 2 cups. To assess compliance, participants were asked to record their food intake on the day prior to the first test day and to follow a similar dietary pattern on the day prior to the 3 remaining test days.

To start a test day in a fasting state, participants had to refrain from foods for 10 h and drinks for 8 h, except (mineral) water. During the test days, all foods and drinks were provided, including breakfast (water and a maximum of 2 sandwiches with low-energy filling, 410–1092 kJ, 1–9 g fat, 2–15 g protein, and 16–32 g/d carbohydrates), and all products were low in antioxidants, flavonoids, fat, glucose, and caffeine. The high-fat lunch was similar to the low-fat breakfast but was consumed together with 143 mL liquid whipping cream (2385 kJ, 61 g fat, 3 g protein, and 4 g carbohydrates).

Measurements. BP was assessed at the left arm by a calibrated Omron IC device. The BP cuff around the left arm was inflated 3 times and BP values were recorded. FMD was assessed by echo-Doppler (Esaote) on each test day at baseline and 3 h after the fat challenge at the participant’s right arm in a recumbent position by using a 7.5-MHz transducer and automatic tracking vessel wall movement software (Wall Track System). The brachial artery was imaged in the upper arm (~5 cm above the antecubital crease) in B-mode. The baseline arterial diameter was measured in M-mode (motion-mode). Following this, a cuff placed around the upper arm was inflated to 200 mm Hg for 4 min. After deflation of the cuff, the arterial diameter was measured at 20-s intervals for 5 min. FMD was defined as the maximal percentage diameter change of the postocclusion arterial diameter measurement relative to the mean of the corresponding 3 baseline measurements. The device automatically calculated pulse wave transit time starting with the electrical signal reflecting the ventricular systole to the arterial wall movement of the brachial artery measured by the echo-transducer. The wall movement results from the pulse wave propagation initiated by each ventricular contraction and is a measure of arterial stiffness.

As an explorative vascular function tool, the HDI/PulseWave CR-2000 Research Cardio Vascular Profiling System (Hypertension Diagnostics) was included in the study protocol and was used according to the indications of the supplier. The device generates a radial artery waveform by tonometry and, by using an algorithm, calculates the large (C1) and small (C2) artery elasticity index. C1 reflects capitative arterial complianc and C2 oscillatory or reflective arterial compliance. Detailed information on measurement of platelet function and blood lipids is available in Supplemental Methods.

Additional measurements. Body weight was measured without shoes and heavy clothing at each visit on an electronic device. Height was measured during screening using a wall-mounted stadiometer. BMI was computed based on these 2 measurements. All adverse events experienced during the study were reported and coded by the study physician.

Statistical analysis. Data collected on paper were 100% verified and all data were archived at Unilever Research and Development Vlaardingen, The Netherlands. Data were analyzed according to the intention-to-treat principle. Values reported in the text and tables are Least-Squares Means (LSMeans) ± SEM or mean difference (95% CI), except for baseline data, which are reported as means ± SD. Results of the power calculation showed that 35 participants would be sufficient to detect a clinically relevant FMD improvement of 2.5% (power of 0.8, α = 0.05). Data were analyzed using an ANCOVA (Proc Mixed, SAS software V9.1) to compare the absolute differences after the intervention periods between the 2 treatments and the placebo. The model terms were treatment, period, and their interaction. Baseline values were used as covariables. For the FMD analyses, baseline diameter of the brachial artery was also included in the model. To test the differences of both interventions with the placebo treatment, a multiple comparison according to Dunnett was performed.
Results

Participants and compliance. Of the 53 volunteers who responded to advertisements and underwent screening, 11 were excluded based on their blood and urine results and 7 were excluded by lottery. The remaining 35 participants were randomized and completed the study. Compliance of the test products was 86% as indicated by registration of test product consumption by participants. The participants were young, healthy males with BMI, indicators of vascular function, and serum lipid concentrations in the normal range and not receiving medication that might affect outcome variables or bioavailability of the test products (Table 1).

Vascular function. Neither FMD after a low-fat breakfast nor FMD after a high-fat lunch were significantly affected by the treatments (Table 2). The mean difference in maximal response in diameter within 5 min of measurement after breakfast between the end of the intervention period and the end of the placebo period treatment was −0.4% (95% CI = −1.8 to 0.9; P = 0.77) for the wine grape solids and 0.2% (95% CI = −1.2 to 1.5; P = 0.94) for the grape seed solids. After the high-fat challenge, the mean differences in FMD compared with placebo were 0.7% (95% CI = −0.6 to 2.0; P = 0.49) after the wine grape solids intervention and −0.2% (95% CI = −1.5 to 1.1; P = 0.94) after the grape seed solids intervention.

BP was not affected by the treatments; the mean difference between the wine grape solids intervention and the placebo was −1.3 mm Hg (95% CI = −4.7 to 2.2; P = 0.70) in systolic BP and for diastolic BP −1.6 mm Hg (95% CI = −4.4 to 1.3; P = 0.44). For the grape seed solids treatment, differences were −0.6 mm Hg (95% CI = −4.0 to 2.1; P = 0.92) for systolic BP and −0.6 mm Hg (95% CI = −3.4 to 2.2; P = 0.88) for diastolic BP. In addition, heart rate, pulse wave transit time, and large and small arterial elasticity did not differ (Table 2). Results for all these vascular function measures were similar after the fat challenge (data not shown, except for FMD).

Platelet function and serum lipids. Measures of platelet function did not differ after the treatments compared with placebo, although platelet activation in whole blood induced by platelet function and serum lipids.

Table 2 Vascular function of men after 2-wk interventions with wine grape solids, grape seed solids, and placebo

<table>
<thead>
<tr>
<th>Difference compared with placebo</th>
<th>Placebo</th>
<th>Wine grape solids</th>
<th>Grape seed solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD max, %</td>
<td></td>
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<tr>
<td>After low-fat breakfast</td>
<td>3.9 ± 0.5</td>
<td>−0.4 (−1.8, 0.9)</td>
<td>0.2 (−1.2, 1.5)</td>
</tr>
<tr>
<td>After high-fat lunch</td>
<td>4.5 ± 0.5</td>
<td>0.7 (−0.6, 2.0)</td>
<td>−0.2 (−1.5, 1.1)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>118.6 ± 1.2</td>
<td>−1.3 (−4.7, 2.2)</td>
<td>−4.0 (−4.0, 2.9)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>70.8 ± 1.0</td>
<td>−1.6 (−4.4, 1.3)</td>
<td>−3.6 (−4.3, 2.4)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>61.4 ± 1.1</td>
<td>1.2 (−1.9, 4.3)</td>
<td>0.0 (−3.1, 3.1)</td>
</tr>
<tr>
<td>Pulse wave transit time, ms</td>
<td>168.7 ± 2.9</td>
<td>4.2 (−4.0, 12.5)</td>
<td>0.8 (−7.4, 9.1)</td>
</tr>
<tr>
<td>C1, mL/mm Hg x 10</td>
<td>18.3 ± 0.7</td>
<td>−0.7 (−1.5, 1.2)</td>
<td>−0.2 (−1.6, 1.1)</td>
</tr>
<tr>
<td>C2, mL/mm Hg x 100</td>
<td>10.3 ± 0.3</td>
<td>0.5 (−0.3, 1.3)</td>
<td>0.3 (−0.5, 1.1)</td>
</tr>
</tbody>
</table>

1 Values are LSmeans ± SEM or LSmeans (95% CI), n = 35. Data were analyzed by ANCOVA with the baseline value as the covariable or for FMD, baseline FMD and baseline brachial artery diameter as covariables.

Discussion

Possible protective effects of wine (constituents) on CVD risk factors have been suggested by in vitro and animal studies (15), whereas data from well-controlled human studies are scarce (9). The present hypothesis-testing study in 35 healthy males investigated the potential of the polyphenolic part of 2 different grape solids in a randomized, double-blind, placebo-controlled cross-over setting. We found no significant differences in FMD and other vascular function markers after both grape solids treatments compared with after placebo treatment, despite the wide range of indicators assessed (FMD, BP, heart rate, pulse treatment compared with the placebo [14.5 (95% CI = 1.3 to 27.6; P = 0.06)] (Supplemental Table 1). Platelet aggregation in platelet-rich plasma induced by ADP (10 μmol/L) after the fat challenge also tended to be reduced [−6% (95% CI = −12.1 to 0.1; P < 0.10) after the wine grape solids treatment compared with placebo (Supplemental Table 1).

After the wine grape solids intervention, serum total cholesterol tended to be lower after the low-fat breakfast [−0.16 mmol/L (95% CI = −0.31 to −0.01; P = 0.07)] and was lower after the high-fat lunch [−0.20 mmol/L (95% CI = −0.33 to −0.07; P < 0.01)] compared with after the placebo treatment. The serum total cholesterol concentration did not differ after the grape seed and placebo treatments. Compared with the placebo treatment, serum triglycerides were reduced by the wine grape solids treatment after the high-fat lunch [−0.29 mmol/L (95% CI = −0.54 to −0.04; P < 0.05)] and tended to be reduced by the grape seed treatment [−0.25 mmol/L (95% CI = −0.51 to 0.00; P = 0.10)]. Serum LDL- and HDL-cholesterol concentrations did not differ after the treatments compared with after the placebo (Supplemental Table 2).

Other outcomes. Body weights did not differ after the treatment periods compared with after placebo. Participants experienced a total of 39 adverse events, which were determined to be unrelated to any of the test products. Most of the adverse events were mild and all participants recovered. The 4 most frequent events were acute nasopharyngitis (n = 7), headache (n = 6), diarrhoea (n = 4), and flatulence (n = 4).

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wave transit time, and large and small arterial elasticity) and the considerably high polyphenol content of the solids.

The strength of this study is its randomized, double-blind, placebo-controlled design. In addition, all participants completed the study and compliance with consuming the test capsules was high. Furthermore, unlike most other studies in this field, encapsulation of the solids ensured blinding of the test products. To reduce variation, we measured FMD on all test days in each participant on the same day of the week at the same time point and by the same observer. We included only men in this study to decrease variation in FMD measurements due to hormonal fluctuations in women (32). Effects of the grape solids on FMD and other outcomes were measured after a low-fat breakfast and a high-fat lunch, because it has been suggested that a high-fat meal or other vascular function stressors, such as smoking, may acutely impair FMD and that this impairment may be prevented by intake of red wine constituents (14,19,27–29). For this proof of principle study, we selected extracts with a high polyphenols content (~80% of the dry weight) and with a wide range of polyphenols subtypes. The polyphenol dose (800 mg polyphenols/d) was similar to the dose tested in other intervention studies addressing the chronic effects (2–8 wk) of the polyphenolic fraction of grape consumption on endothelial function and/or BP (23,33–37).

The study design also had its limitations. The intervention period was relatively short, but we expected to show benefits of polyphenols within 2 wk, because previous studies investigated acute and several weeks’ effects on FMD with positive outcomes (16,28,29,33–35,37,38). The wash-out period was 1 wk, which was considered more than sufficient to prevent carry-over effects, because polyphenols are rapidly metabolized and their maximum plasma concentrations are usually reached within hours (24). Furthermore, only participants without elevated CVD risk factors were included, whereas in most other studies investigating the effects of grape polyphenols on vascular function, the focus was on participants with elevated CVD risk (14). Indeed, our study population appeared to be largely insensitive to the fat challenge, indicating a balanced and healthy vascular function. Consequently, grape solids may still protect vascular function in participants at increased risk for CVD. In addition, our study may have not been optimally designed to answer the secondary objectives. The trends we found in platelet function were not consistent across the different methods we applied. This may reflect differences in sensitivity of the applied methods toward specific platelet activation pathways (39). More consistent seem the total cholesterol-lowering effects after wine grape solids treatment in this normolipidemic population. Some human studies also found beneficial effects of grape products on lipid profile; however, the evidence is not consistent (10,13). Moreover, triglycerides were significantly lower after the high-fat challenge for the wine grape solids treatment compared with the placebo treatment and tended to be lower after grape seed consumption. This effect may be explained by inhibition of chylomicron secretion from intestinal cells by the grape polyphenols (40). It should be noted that the observed benefits could well be chance findings and require confirmation in a separate study, including multiple blood sampling in the postprandial phase. We investigated effects on blood lipids as secondary outcome with the same wine grape solids treatment in 30 hypertensive participants and did not confirm the lipid-lowering effects (our unpublished data). To directly test effects on blood lipids, it would require a cross-over study with ~60 subjects to detect differences of a similar magnitude as observed here.

In conclusion, this randomized controlled study does not support the hypothesis that grape polyphenolic compounds have important potential to improve endothelial function and related cardiovascular health markers in a healthy population. Future studies should focus on the potential of grape polyphenols for improving endothelial function and cardiovascular health in participants with an elevated cardiovascular risk profile with interventions of sufficient duration to accurately allow assessment of longer term effects.

Acknowledgments
R.D. and H.C.M.K. designed research; H.C.M.K. and L.A.J.M. analyzed data; L.A.J.M., R.D., and P.I.Z. wrote the paper; and L.A.J.M. and R.D. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


