Daily Red Wine Consumption Improves Vascular Function by a Soluble Guanylyl Cyclase-Dependent Pathway

Ilse P.G. Botden1, Janneke G. Langendonk1, Marcel E. Meima1, Frans Boomsma1, Ann L.B. Seynhaeve2, Timo L.M. ten Hagen2, A.H. Jan Danser1 and Eric J.G. Sijbrands1

BACKGROUND
Polyphenols in red wine are supposed to improve endothelial function. We investigated whether daily red wine consumption improves in-vivo vascular function by reducing endothelin-1 (ET-1). Additional pathways mediating this effect were studied using porcine coronary arteries (PCAs).

METHODS
Eighteen young healthy women drank red wine daily for 3 weeks. Vascular function was evaluated by determining forearm blood flow (FBF) responses to endothelium-dependent (acetylcholine (ACh)) and endothelium-independent (sodium nitroprusside (SNP)) vasodilators. PCAs were suspended in organ baths and exposed to the endothelium-dependent vasodilator bradykinin, the nitric oxide (NO) donor S-nitroso-N-acetyl-l-arginine (SNAP) and/or red wine extract (RWE).

RESULTS
ACh-induced and SNP-induced FBF increases were equally enhanced after 3 weeks of red wine consumption, but an immediate enhancement (i.e., after drinking the first glass) was not observed. Vice versa, plasma ET-1 levels were not decreased after 3 weeks, but we observed an acute drop after drinking one glass of wine. RWE relaxed preconstricted PCAs in an endothelium-, NO-, and soluble guanylyl cyclase (sGC)/guanosine-3’5’-cyclic monophosphate (cGMP)-dependent manner. Short RWE exposure reduced the response to bradykinin and SNAP by inactivating sGC. This effect disappeared upon prolonged RWE exposure.

CONCLUSIONS
The enhanced FBF response following 3 weeks of red wine consumption, but not after one glass, reflects a change in smooth muscle sensitivity. Alterations in sGC responsiveness/activity, rather than changes in ET-1, appear to underlie this phenomenon.

Keywords: alcohol; blood pressure; endothelial function; endothelin-1; hypertension; nitric oxide; polyphenols; red wine; soluble guanylyl cyclase; venous occlusion plethysmography

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Polyphenols, which are abundantly present in red wine, are assumed to improve endothelial function, thus leading to cardioprotection.1 They enhance the production of vasodilating factors, like nitric oxide (NO) and endothelium-derived hyperpolarizing factor.2 In addition, red wine polyphenols reduce the synthesis of the vasoconstrictor endothelin-1 (ET-1) in bovine aortic endothelial cells,3 but their effect in humans after daily red wine consumption is unknown. Resveratrol, a red wine polyphenol that activates the protein deacetylase SIRT1 known from longevity studies, has attracted particular interest as cardioprotective compound.4

A number of studies have been performed to investigate the in-vivo effect of red wine on endothelial function,5–9 but the issue whether drinking of red wine is beneficial for the healthy population is still a matter of debate.

The purpose of the present study was to investigate the effect of daily red wine consumption on forearm vasodilation in healthy volunteers by venous occlusion plethysmography. Endothelium-dependent and endothelium-independent functions were evaluated by infusing acetylcholine (ACh) and sodium nitroprusside (SNP), respectively. In addition, we measured plasma ET-1 levels before and during red wine consumption and investigated the effect of red wine extract (RWE) on ET-1 release in cultured human endothelial cells. To determine through which pathways red wine constituents influence vascular function, we quantified the in-vitro effects of RWE and resveratrol on vascular function, using porcine coronary arteries (PCAs).

METHODS
Subjects. Nineteen young, healthy, nonsmoking women, whose age was <30 years, were enrolled in the study. The inclusion criteria were: maximal moderate consumption of alcoholic beverages, defined as at least three consumptions per week.
and less than two daily, and use of a hormonal contraceptive. The study complies with the Declaration of Helsinki. The Institutional Review Board of the Erasmus MC, Rotterdam, the Netherlands approved the study protocol. All subjects gave written informed consent before the start of the study.

Protocol of the in-vivo study. The subjects were asked to fully abstain from alcohol in the 3 weeks preceding the basal evaluation of vascular function. Subjects started with 112 ml of red wine (corresponding with 10 g of alcohol) in the morning at the study center and thereafter, with daily ingestion of 224 ml of red wine with each dinner at home. After 1 week, a fasting blood sample was taken and the daily red wine consumption was increased to 336 ml (30 g alcohol) for another 2 weeks. Additional alcoholic beverages, red grape juice, and red grapes were not allowed during the study. The wine used was Toques et Clochers, Cabernet Sauvignon, Vin de Pays d’Oc, 2002, France. The total phenol content of this wine was 2 g/l, determined as gallic acid equivalents using Polin Ciocalteu reagent.9

Forearm blood flow (FBF) was studied at baseline, 1 h after the intake of the first 112 ml of red wine, and after 3 weeks of daily red wine consumption, following an overnight fast. FBF was measured with venous occlusion plethysmography (Periflow; Janssen Scientific Instruments, Beerse, Belgium). This technique has been described previously.10 All experiments were performed in a quiet, air-conditioned room (22–24°C). The brachial artery of the nondominant arm was cannulated with a catheter, followed by a 60-min rest. A mercury-in-silastic strain gauge was fixed at the widest circumference of both forearms.

Venous occlusion was achieved with blood pressure cuffs around the upper arms with rapid cuff inflator to 40 mm Hg. Bilateral wrist cuffs were inflated to above-systolic blood pressure to exclude hand circulation. Intra-arterial blood pressure and heart rate were monitored continuously. The FBF response was measured to intra-arterially cumulative doses of the endothelium-dependent vasodilator ACh (10, 20, 30 μg/min) and the endothelium-independent vasodilator SNP (2, 4, 10 μg/min). The flow applied to the brachial artery was kept constant. The different infusion blocks were separated by a 30-min rest period. FBF was calculated from the rate of increase in forearm volume, whereas venous return from the forearm was prevented by inflating the upper arm cuff. FBF was expressed in ml per min per 100 ml of forearm tissue volume. Measurements during 2 min (steady state) were averaged to determine FBF.

Blood sampling. Blood samples were obtained after a 12-h overnight fast at baseline, and after 1 and 3 weeks of daily wine ingestion. Additional blood samples were drawn 1 h after the first glass of red wine. After centrifugation, plasma was collected and stored at −80°C until ET-1 and high-density lipoprotein (HDL) were assessed.

Cell culture studies. Alcohol-free RWE (Provinols, Seppic, France) was used for the cell culture studies and organ bath studies. This RWE contained 632 mg polyphenols/g, determined as gallic acid equivalents using Polin Ciocalteu reagent.9 Human umbilical vein endothelial cells (HUVECs) were isolated by collagenase digestion as described by Jaffe et al.11 HUVECs (passage 5) were cultured in HUVEC medium containing human endothelial-serum free medium (Invitrogen, Breda, the Netherlands), 20% heat-inactivated newborn calf serum (Lonza, Verviers, Belgium), 10% heat-inactivated human serum (Lonza), 20 ng/ml human recombinant basic fibroblast growth factor (Peprotech, London, UK), and 100 ng/ml human recombinant epidermal growth factor (Peprotech) in a humidified incubator at 37°C and 5% CO2.

HUVECs were plated at a density of 6 × 10⁴ cells/ml. After 24 h of culturing, medium was replaced by serum-free Dulbecco’s modified Eagle’s medium to secure low basal stimulation for an additional 24 h. Subsequently, the cells were incubated in RWE in different concentrations (0, 6.25, 12.50, 25, and 50 μg/ml), dissolved in serum-free Dulbecco’s modified Eagle’s medium for 6 h. The supernatant was removed and immediately stored at −80°C until the ET-1 assay was performed.

Growth of endothelial cells of the 48-well multiplates was measured using the sulforhodamine B protein stain assay, according to the method of Skehan.12

ET-1 and HDL measurements. ET-1 was measured in plasma samples and HUVEC supernatant using a chemiluminescent immunoassay ELISA (QuantiGlo; R&D Systems, Abingdon, UK) according to the manufacturer’s protocol. At mean levels of 1.8 pg/ml the intra-assay coefficient of variation was 3.4% and inter-assay coefficient of variation was 8.9%. All plasma samples were measured within one assay plate.

HDL cholesterol was measured by a direct enzymatic HDL cholesterol method (Roche modular P800).

Organ bath studies and cGMP measurement. Methods for organ bath studies and guanosine-3′,5′-cyclic monophosphate (cGMP) measurement were set up as previously described.13 Briefly, PCAs were obtained from 45 slaughterhouse pigs. The PCAs were removed, cut into segments, and then stored in the absence or presence of RWE (30 μg/ml) for 16 h in cold, oxygenated Krebs bicarbonate solution. In some segments, the endothelium was removed. The segments were suspended in 15-ml organ baths containing Krebs bicarbonate solution, aerated with 95% O₂/5% CO₂ and maintained at 37°C. The vessel segments were exposed to 30 mmol/KCI twice, and, subsequently, to 100 mmol/KCl to determine the maximal contractile response. The segments were then allowed to equilibrate in fresh organ bath fluid for 30 min or 2 h in the absence or presence of one or more of the following compounds: the NOS inhibitor N’-nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich, Zwijndrecht, The Netherlands; 100 μmol/l), the guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one (ODQ; Sigma-Aldrich; 10 μmol/l), or RWE (30 μg/ml). Vessels were then preconstricted with 9,11-dideoxy-11α,9α-epoxy-methano-prostaglandin F₂α (U46619; Sigma-Aldrich, 0.1–1 μmol/l) to ~80% of the maximal constriction, and concentration–response curves were constructed to bradykinin, S-nitroso-N-acetyl-L-τ-penicillamine (SNAP), resveratrol (all from Sigma-Aldrich) or RWE (0.01–100 μg/l).
To quantify cGMP production, vessel segments were exposed to bradykinin (1 µmol/l) or RWE (100 µg/ml) for 10 min in 15-ml oxygenated Krebs bicarbonate solution at 37 °C in the presence of the phosphodiesterase inhibitor IBMX (100 µmol/l). The response to bradykinin was also quantified after incubating the vessel segments for 120 min with 30 µg/ml RWE. Tissues were immediately frozen in liquid nitrogen and stored at −80 °C until cGMP was determined by ELISA following acetylation (ITK Diagnostics, Uithoorn, the Netherlands). Results were analyzed as pmol/mg protein and are expressed as content cGMP, fold over baseline. The lower limit of detection was 0.1 pmol/mg protein.

**Statistical analysis.** All data are expressed as mean ± s.e.m. A probability value of P < 0.05 was considered statistically significant.

Statistical analysis of FBF measurements for individual subjects was performed by two-way ANOVA for repeated measures. One subject did not complete the study. We performed analyses with and without her FBF data. We did not find different results with these two methods and decided to present the FBF data of the 18 participants whose measurements were fully available.

ET-1 levels in cell culture supernatant were corrected for cell growth. Statistically significant differences compared with basal levels were determined by one-way ANOVA with Dunnett’s post hoc correction for multiple comparisons.

Peak relaxant responses of the organ bath studies are expressed as percentage of the concentration to U46619. Statistical analysis was obtained by two-way ANOVA with Bonferroni’s post hoc comparisons or by one-way ANOVA of the mean pEC₅₀ and maximum relaxation values, where appropriate. cGMP data were analyzed by one-way ANOVA with Bonferroni’s post hoc comparisons.

**RESULTS**

The average age of the 18 females was 22.4 years, ranging from 19 to 28 years. Their average body mass index was 22.7 kg/m², ranging from 19 to 28 kg/m² (Table 1). Systolic and diastolic blood pressure did not decrease significantly after 3 weeks of daily red wine consumption, nor directly after one unit of red wine, and no significant changes in HDL levels were observed (Table 1). We studied the effects 1 h after drinking one unit of red wine to enable comparison with previous studies.⁵,⁶,⁸ Resting FBF at that time had increased from 3.3 ± 0.4 to 5.6 ± 0.7 ml/min/100 ml of FBF (P < 0.01). We corrected for this increase in FBF by subtracting the resting FBF values. Figure 1 shows that the ACh- and SNP-induced increases in FBF after drinking one unit of red wine were identical to those before wine drinking.

After 3 weeks of daily red wine consumption, when resting FBF had returned to prewine drinking level (3.5 ± 0.5 ml/min/100 ml of FBF), the mean delta FBF responses to ACh and SNP were equally increased (by 2.6 ± 1.3 (Figure 1a, P = 0.06) and 1.9 ± 0.6 (Figure 1b, P < 0.01) ml/min/100 ml of FBF, respectively).

Plasma ET-1 levels tended to decrease after one unit of red wine, from 1.7 ± 0.1 to 1.4 ± 0.7 pg/ml (P = 0.08). After 1 week of daily red wine consumption ET-1 levels decreased further to 1.1 ± 0.1 pg/ml (P < 0.01 vs. baseline). After 3 weeks of daily red wine consumption (n = 19), ET-1 levels were back to 1.5 ± 0.1 pg/ml (P = 0.25 vs. baseline; data not shown).

In vitro, incubating endothelial cells in RWE resulted in a concentration-dependent decrease (P < 0.05 vs. basal) of ET-1 release (Figure 2; n = 6).

To study the pathways via which red wine consumption might cause vasorelaxation, the direct effect of addition of

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**Table 1 | Parameters of the subjects, at baseline, and after red wine consumption**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After 1 glass</th>
<th>After 3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.4 ± 0.5</td>
<td>22.3 ± 0.5</td>
<td>22.4 ± 0.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.7 ± 0.6</td>
<td>22.7 ± 0.6</td>
<td>22.7 ± 0.6</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>121.6 ± 2.5</td>
<td>118.2 ± 2.3</td>
<td>118.7 ± 2.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>69.3 ± 1.3</td>
<td>67.3 ± 1.4</td>
<td>67.8 ± 1.6</td>
</tr>
<tr>
<td>Plasma HDL (mmol/l)</td>
<td>1.31 ± 0.07</td>
<td>1.34 ± 0.06</td>
<td>1.34 ± 0.06</td>
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</table>

Parameters at baseline, after one glass of red wine, and after 3 weeks of daily red wine consumption. N = 18. Values represent mean ± SEM. BMI, body mass index; HDL, high-density lipoprotein.
Porcine coronary arteries to cumulative doses of red wine extract (RWE), in the absence and presence of 100 μmol/l Nω-nitro-l-arginine methyl ester (l-NAME) and/or 10 μmol/l 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). In some vessels, the endothelium was denuded before the concentration–response curve was generated. Data (mean ± s.e.m. of 5–17 experiments) are expressed as percentage of the contraction induced by U46619. *P < 0.05 vs. RWE or RWE + endothelium denuded.

Figure 3 | Concentration–response curves of U46619-precontracted porcine coronary arteries to cumulative doses of red wine extract (RWE), in the absence or presence of 100 μmol/l Nω-nitro-l-arginine methyl ester (l-NAME) and/or 10 μmol/l 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). In some vessels, the endothelium was denuded before the concentration–response curve was generated. Data (mean ± s.e.m. of 5–17 experiments) are expressed as percentage of the contraction induced by U46619. *P < 0.05 vs. RWE or RWE + endothelium denuded.

Figure 4 | Porcine coronary artery cGMP content following exposure to 1 μmol/l bradykinin (BK) for 1 min, or 100 μg/ml red wine extract (RWE) for 10 min. Experiments were also performed after preincubation of the vessel segments for 120 min in 30 μg/ml RWE. All experiments were performed in the presence of IM8X (100 μmol/l). Data are mean ± s.e.m. of 2–11 experiments. *P < 0.05 vs. baseline, #P < 0.05 vs. bradykinin. cGMP, guanosine-3′,5′-cyclic monophosphate.

increasing concentrations of resveratrol or RWE to U46619-preconstricted PCAs was studied. Resveratrol, up to a concentration of 100 μmol/l was without effect (n = 15, data not shown). In contrast, RWE relaxed preconstricted vessels by maximally 73 ± 7.3% (n = 16).

l-NAME and ODQ greatly reduced this effect (P < 0.001), whereas endothelium denudation totally abolished it (Figure 3). This indicates that the effect of RWE predominantly depends on de novo formation of NO by endothelial NO synthase (eNOS) and subsequent soluble guanylyl cyclase (sGC) activation. In agreement with this concept, RWE, like bradykinin, increased the vascular cGMP content (Figure 4).

Figure 5 | Concentration–response curves of U46619-precontracted porcine coronary arteries to cumulative doses of bradykinin (BK) in the absence or presence of 100 μmol/l Nω-nitro-l-arginine methyl ester (l-NAME) after incubation with red wine extract (RWE, 30 μg/ml) for the indicated time periods. Data (mean ± s.e.m. of 6–12 experiments) are expressed as a percentage of the contraction induced by U46619. *P < 0.05 vs. control or 2 h RWE. #P < 0.05 vs. no l-NAME.

The endothelium-dependent vasodilator bradykinin relaxed PCAs by maximally 83 ± 5.9%. Preincubation of the vessels with RWE (30 μg/ml) for 0.5 or 2 h greatly diminished the vasorelaxing effect of bradykinin (P < 0.05; Figure 5), and abolished the bradykinin-induced increase in cGMP content (Figure 4). When prolonging the incubation with RWE to 16 h, the relaxant effect of bradykinin tended to return to normal. In agreement with previous studies,14 l-NAME modestly reduced the effect of bradykinin (P < 0.05; Figure 5). l-NAME, however, did not prevent the short-term effect of RWE on bradykinin-induced relaxation. Yet, with l-NAME, the effect of RWE after 16 h was no longer significant. These data indicate that the blocking effect of RWE toward bradykinin involves both an eNOS-dependent and an eNOS-independent phenomenon.

To investigate the latter, the endothelium-independent vasodilator SNAP was used. SNAP relaxed PCAs by maximally 94 ± 2.7% (Figure 6). Short, but not long preincubation of the vessels with RWE diminished this effect, as evidenced by a significant (P < 0.001) rightward shift of the concentration–response curve. ODQ largely blocked the effect of
This study is among the few that investigated both the endothelium-dependent and endothelium-independent effects of daily red wine consumption during a prolonged period (3 weeks) by healthy subjects, using the robust and precise method of plethysmography.\textsuperscript{15} It therefore differs from studies that investigated the endothelium-dependent component only.\textsuperscript{16} Individuals with proven endothelial dysfunction seem to profit from polyphenols of grape products without alcohol, both acutely and during follow-up.\textsuperscript{6,17–19} Red wine consumption during a maximum of 2 months however, did not affect vascular reactivity in patients with type 2 diabetes or after an acute coronary syndrome.\textsuperscript{7,20} This suggests that either the alcohol has diminished the beneficial effect of the polyphenols, or that the endothelial dysfunction in patients with type 2 diabetes and acute coronary syndrome was in an irreversible state.

We assessed the effect of red wine in healthy, young women to enable a study of the normal physiology, which is essential for understanding the preventive potential of red wine.

Our \textit{in-vivo} study has a number of limitations. First, the number of participants was relatively small. However, the technique used to measure vascular function is very sensitive and reproducible\textsuperscript{15,21} and each subject was used as its own control. Venous occlusion plethysmography allows us to evaluate the response to stepwise increased infusion of vasoactive substances, which is not common in flow-mediated dilation, the technique that has been used in a number of other studies.\textsuperscript{6,16–20} Secondly, it could be argued that a period of 3 weeks is too short to investigate the effect of daily red wine consumption, although our follow-up was longer than most other studies. Furthermore, only women were studied. This was done to exclude known hormonal differences between men and women, especially since red wine polyphenols are known to stimulate the estrogen-\alpha receptor in vascular endothelial cells.\textsuperscript{22} It is unlikely that hormonal status influenced our results, because we restricted our studies during the periods on hormonal contraceptives, and this does not affect endothelial function in the forearm microcirculation of young healthy women, as investigated before.\textsuperscript{23}

We assessed the vascular function before and after 3 weeks of red wine consumption, consumed during dinner. In addition, we tested the participants after drinking a single unit of red wine, to enable comparison with a number of studies in which the direct effect on healthy endothelium of red wine consumption had been analyzed.\textsuperscript{5,8,16} Although baseline FBF had increased at that time, the delta FBF induced by either ACh or SNAP was identical to the delta FBF induced by these agonists before the start of wine drinking. An acute effect of red wine on resting (saline) FBF has been observed before,\textsuperscript{5,16} and could relate to the direct effects of alcohol. Indeed, Bau et al.\textsuperscript{24} showed an increased diameter of the brachial artery with flow-mediated dilation immediately after intake of alcohol, but this was absent after 13 h. Flow-mediated dilation and FBF cannot be compared directly, but adjustment for the resting values would reduce the endothelium-dependent effect in the flow-mediated dilation study to a similar extent as the resting response did in our setting. The effect after 3 weeks in our study, determined after an overnight fasting, may therefore not be caused by the sensitivity of the vascular smooth muscle to NO instead of an increase in cGMP formation, whereas resveratrol exerted no such effect. Simultaneously however, preincubation in RWE diminished the bradykinin- and SNAP-induced relaxations.

SNAP ($P < 0.01$) and in the presence of this sGC inhibitor, the inhibiting effect of RWE did no longer occur (Figure 6). This indicates that the inhibiting effect of RWE toward SNAP involves sGC.

**DISCUSSION**

We found that daily red wine consumption during 3 weeks improved vascular function of young healthy women. FBF increased in response to ACh but also to SNP, and both increases were of similar magnitude. This suggests an increased sensitivity of the vascular smooth muscle to NO instead of an amelioration of endothelial function. Drinking a single unit of red wine reduced plasma ET-1. In agreement, RWE reduced endothelial ET-1 release \textit{in vitro}. Clearly, this effect was not long lasting \textit{in vivo}, since the ET-1 plasma levels returned to normal after 3 weeks of red wine consumption. Finally, RWE relaxed PCAs in an endothelium-dependent manner, involving NO generation by eNOS and subsequent sGC activation and cGMP formation, whereas resveratrol exerted no such effect. Simultaneously however, preincubation in RWE diminished the bradykinin- and SNAP-induced relaxations.

**Figure 6** Concentration–response curves of U46619-precontracted porcine coronary arteries to cumulative doses of S-nitroso-N-acetyl-\textit{l}-penicillamine (SNAP) in the absence or presence of 10 $\mu$mol/l 1H(1,2,4)oxadiazolo[4,3,\textit{a}]quinoxalin-1-one (ODQ) after incubation in red wine extract (RWE, 30 $\mu$g/ml) for the indicated time periods. Data (mean ± s.e.m. of 7–8 experiments) are expressed as a percentage of the contraction induced by U46619. *$P < 0.05$ vs. control or 2 h RWE, $P < 0.05$ vs. no ODQ.
alcohol content of red wine. The unaltered resting FBF levels combined with the increased responses to ACh and SNP at 3 weeks suggest that compensatory mechanisms (e.g., a decrease in the baseline levels of various endothelium-dependent agonists, including ACh) had occurred to overcome the consequences of the increased smooth muscle responsiveness. Under such circumstances, only the infusion of ACh or a NO donor allowed us to observe the enhanced sGC responsiveness.

In order to delineate the mechanisms that might underlie the in-vivo observations, in-vitro studies in PCAs and human endothelial cells were performed. Obviously, this approach cannot entirely mimic the in-vivo conditions, as such studies cannot last for several weeks, and rely on the use of a RWE that does not necessarily yield the same polyphenol concentrations reached in blood following the intake of red wine. It is important to note that previous findings from our laboratory regarding the (endothelium-dependent) relaxant effects of bradykinin and the constrictor effects of angiotensin II in PCAs fully resembled those in human coronary arteries. Since RWE contained 632 mg polyphenols/g, the RWE concentration of 30 µg/ml that we used in most organ bath experiments will yield polyphenol concentrations in the range expected in blood after drinking 336 ml red wine/day for 2 weeks.

In vitro, RWE caused endothelium-dependent, NO-mediated and sGC/cGMP-mediated relaxations of coronary arteries, in full agreement with previous studies. L-NAME and ODQ, alone or in combination, did not fully prevent the vasorelaxant effects of RWE. Thus, the relaxant response to RWE may also involve a non-NO, “endothelium-derived hyperpolarizing factor”-like response. The lack of effect of resveratrol in vitro, as opposed to RWE, suggests that the relaxant, NO-mediated effect of RWE does not involve this polyphenol, despite earlier studies showing that resveratrol increases eNOS activity through deacetylation by SIRT1. This latter phenomenon, however, has recently been disputed.

A short preincubation (0.5–2 h) in RWE diminished the responses to both the endothelium-dependent vasodilator bradykinin and the endothelium-independent vasodilator SNAP, an effect that tended to disappear upon prolonged (16 h) preincubation. The effect of RWE on bradykinin was greatly diminished upon prolonged (16 h) preincubation, whereas its effect on SNAP was no longer present after a 16-h preincubation. The eNOS inhibitor L-NAME fully prevented the (modest) prolonged effect of RWE toward bradykinin, but not its effects after short preincubation, whereas the sGC inhibitor ODQ fully prevented the effects of short RWE preincubation toward SNAP. These data indicate that the blocking effect of RWE involves sGC inactivation and NO depletion. The inability of bradykinin to increase cGMP following RWE exposure supports this view. The reduced sGC responsiveness following short RWE exposure resembles the cross-tolerance known to occur following nitrergic treatment. This reduced responsiveness to NO seems to be a temporary effect, because it had almost disappeared after a 16-h preincubation with RWE. Apparently a restoration of sGC responsiveness had occurred during longer exposure to RWE, e.g., via overexpression of sGC in vascular smooth muscle cells.

With regard to the effect of red wine on ET-1, we showed an acute reduction of ET-1 release after treating human endothelial cells with different concentrations of RWE. These in-vitro results are in line with Corder et al., who showed an inhibition of ET-1 release in bovine aortic endothelial cells over 6 h during incubation in RWE. In vivo, red wine reduced plasma ET-1 levels directly after the intake of one glass, in line with a recently published study. Unfortunately, the effect of longer-term red wine intake on ET-1 plasma levels was not determined in the latter study. In conclusion, we observed that the ET-1 reducing effect disappeared following longer exposure to red wine.

In conclusion, our study demonstrates a positive in-vivo vasodilatory effect of prolonged red wine consumption in young, healthy women. Constituents of red wine other than alcohol and resveratrol increased the vascular NO sensitivity. In the presented in-vitro work, we identified a number of mechanisms underlying these observations in young, healthy volunteers. The lack of an acute effect of red wine may be attributed to a reduced responsiveness of sGC to NO, whereas the improved effect after prolonged wine exposure may reflect sGC upregulation. These changes are unlikely to be mediated by ET-1. Red wine consumption can contribute to primary cardiovascular prevention by the above pathways. Further in-vivo and ex-vivo research, regarding the vascular and metabolic effects of daily red wine consumption on healthy endothelium, but clearly also on vascular smooth muscle, are needed to obtain a full understanding of the protective vascular effects of red wine.

Disclosure: The authors declared no conflict of interest.
Red Wine Consumption Improves Vascular Function


