The red wine hypothesis: from concepts to protective signalling molecules

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We review evidence for and against the 'red wine hypothesis', whereby red wine is more likely to confer cardiovascular benefits than white. As background, there is a strong epidemiological and mechanistic evidence for J-shaped relation between alcohol intake and total mortality. However, epidemiological data favouring a specific benefit of red over white wine are not strong and the 'French paradox' could at least in part be explained by confounding factors. More convincing evidence is that human studies with de-alcoholized red but not white wine show short-term cardiovascular benefits. The specific components of the de-alcoholized wine that are active on cardiovascular endpoints, are the polyphenols found in red wine, especially resveratrol. The effects of resveratrol on isolated tissues or organs are well-described including molecular mechanisms leading to decreased arterial damage, decreased activity of angiotensin-II, increased nitric oxide, and decreased platelet aggregation. Anti-ischaemic effects include stimulation of prosurvival paths, decreased LDL-oxidation, atheroma, and on the ischaemic-beneficial metabolic changes. Most recently, the agonist effect of resveratrol on the anti-senescence factor sirtuin has lessened cell death in myocytes from failing hearts. Mechanistic feasibility strengthens the case for prospective therapeutic trials of alcohol vs. red wine vs. resveratrol, for example in those with heart failure.

KEYWORDS
Wine; Alcohol; Resveratrol; Molecular mechanisms; Sirtuin

Introduction

The harmful effects of risk factors for cardiovascular disease as often linear, as in the case of cigarette smoking and blood lipid abnormalities. For alcohol, however, the situation is different, with data for moderate-dose cardiovascular benefits and high-dose harm. This article will first review the increasing evidence for this dose-related protection, then evaluate the proposal that wine is more protective than other alcoholic drinks, and thereafter examine the hypothesis that red wine is more protective than white. Important arguments favouring this hypothesis derive from the role of specific protective chemical polyphenols found both in red wine and de-alcoholized red wine. Putative molecular mechanisms involve newly described signals and paths that may help to explain the proposed protective qualities of moderate amounts of red wine.

The J-shaped mortality curve: still controversial?

The major and consistent finding is that alcohol consumption, from whatever source, appears to have a J-shaped curve, whereby a modest intake is beneficial and either no intake or an increased intake is harmful. This relation has been established in a series of studies starting with the Framingham study, followed by the British Doctors Study, the Cancer Prevention study on about 490 000 persons, the Nurses Health Study, the Physicians' Health Study, the British Heart Study, the Multiple Risk Factor Intervention Trial, and the Copenhagen City Heart Trial. However, the issue of confounding factors has bedevilled these findings as shown by two opposing views. Of particular relevance is a meta-analysis of mortality in 34 outcome studies on more than 1 million subjects. Nearly 100 000 deaths were analysed to show a clear J-relationship with decreased total mortality associated with decreased alcohol intake compared to two to four daily drinks for men and one to two for women vs. abstinence or higher drinking rates. Of note in relation to the controversy regarding the significance of confounding factors, adjustment for social factors, and dietary markers made the J-shaped dip shallower and shorter, yet still evident. The J-shaped mortality curve might in part be explained by less myocardial infarction and in part by less incident heart failure, but not by decreased hypertension.

Regarding mechanistic studies, these are many and difficult to explain by the confounding hypothesis. One established mechanism of the cardiovascular benefit is through a consistently reported increase in plasma high-density lipoprotein (HDL)-cholesterol levels, thought to be the result of increased hepatic synthesis. The definitive study shows that...
a genetic variation in hepatic alcohol dehydrogenase, which slows the rate of ethanol metabolism, is associated with higher plasma HDL-levels and lower rates of myocardial infarction. This was a prospective study with a high rate of completeness of follow-up data, and part of the carefully monitored Physicians’ Health and Nurses’ Health studies. The benefits found in moderate drinkers were profoundly altered by the presence or absence of the ADH3 genotype, which is contrary to the confounding hypothesis. About half of the benefit of moderate drinking can be related to increased HDL-levels. Other possible protective mechanisms include anti-platelet, anti-coagulatory, improved glucose control, and anti-inflammatory effects as shown in the MONICA study. The latter were more evident in moderate drinkers than in abstainers or heavy drinkers. When given acutely for 8 weeks, ethanol reduced triglyceride levels, but achieved increased protection by moderate alcohol intake. When given acutely for 8 weeks, ethanol reduced triglyceride and insulin levels. Of these effects, those on HDL, fibrinogen, and insulin sensitivity have been established in randomized prospective studies, which are especially difficult to explain by the confounding hypothesis. Alcohol also has detrimental high-dose (0.6–0.9 g/Kg body weight) pro-oxidant effects in humans, which together with liver damage may contribute to the right-hand upward slope of the J-curve.

Is no alcohol really harmful?

In a large multinational study, no alcohol intake was a harmful risk factor for myocardial infarction, especially in women. The first and most obvious explanation would be the lack of the benefit of moderate alcohol intake on HDL-cholesterol and, as will be discussed, the absence of the postulated non-ethanolic benefits of red wine. Alcohol has beneficial effects on insulin and triglyceride levels, and abstinence is a risk factor for new type 2 diabetes. Alcohol gives experimental cardioprotection against ischaemic-reperfusion injury in isolated hearts, which is contrary to the confounding hypothesis. About half of the benefit of moderate drinking can be related to increased HDL-levels. Other possible protective mechanisms include anti-platelet, anti-coagulatory, improved glucose control, and anti-inflammatory effects as shown in the MONICA study. The latter were more evident in moderate drinkers than in abstainers or heavy drinkers. When given acutely for 8 weeks, ethanol reduced triglyceride and insulin levels. Of these effects, those on HDL, fibrinogen, and insulin sensitivity have been established in randomized prospective studies, which are especially difficult to explain by the confounding hypothesis. Alcohol also has detrimental high-dose (0.6–0.9 g/Kg body weight) pro-oxidant effects in humans, which together with liver damage may contribute to the right-hand upward slope of the J-curve.

Wine vs. other alcoholic drinks

As already mentioned, many studies have related alcohol to the J-shaped beneficial curve. An important claim is that wine may have benefits that, for example, beer or spirits lack. For example, Gronbaek found all-cause mortality reduced in wine but not in non-wine drinkers in his study on 24 523 Danes over 10 years. In a meta-analysis on 209 418 persons, consumption of wine but not that of beer was protective. Klatsky followed-up 128 934 adults in Northern California for up to 20 years. The J-shaped curve was once again confirmed. Drinkers of any type of wine had a lower mortality risk than did beer or liquor drinkers, in part because wine drinkers were the lighter drinkers. Renaud’s group studied 36 250 healthy French men, also to show that moderate wine but not other alcohol types reduced all-cause mortality over 12–18 years. These impressive data from three entirely different countries must be balanced against other studies that differ and the recurrent problem of confounding factors. Thus, the type of alcohol made no difference to its protective effect on myocardial infarction in the 38 077 participants in the US male health professions study over 12 years of follow-up. Moderate consumption of both wine and beer equally decreased inflammatory markers such as C-reactive protein. The real problem is that there are very few long-term prospective studies comparing the possible beneficial effects of the types of alcohol on cardiovascular outcomes or total mortality while fully allowing for confounding factors. According to such factors, wine drinkers have a more healthy diet than beer drinkers with increased intake of fruit, vegetables, and fibre.
Regional Heart study, 7735 men drawn at random were followed-up for an average of 16.8 years.\textsuperscript{47} Wine drinkers had a lower age-adjusted risk of coronary heart disease and all-cause mortality than did beer and spirits drinkers, but the wine drinkers also had a better life style (for example, much less smoking). Thus the wine vs. beer data suggest, but do not conclusively give the benefit to wine.

The red wine hypothesis

It is often thought that epidemiological data from France strongly suggests a protective effect of red wine despite a high-fat diet (the 'French paradox'). This idea goes back to St Leger \textit{et al.}\textsuperscript{48} who in 1979 found an inverse relation between coronary heart disease mortality and wine consumption, with France having the lowest mortality. The jump to the specific benefit of red wine was made in 1993 when Frankel \textit{et al.} showed that red wine diluted 1000-fold and contained 10 µmol/L of total phenols that inhibited the oxidation of LDL more than α-tocopherol, an established antioxidant.\textsuperscript{39} The antioxidant mechanisms involved are scavenging of peroxyl radicals as well as chelation of redox metal ions such as a copper.\textsuperscript{50} Indirect evidence favouring the ‘French paradox’ and the red wine hypothesis is that the French habitually drink wine with their meals (which are often fatty) and this wine is most often red. Furthermore, Alsace, a white-wine drinking region of France, has a much higher mortality (about 50% higher) than red wine-drinking Mediterranean areas,\textsuperscript{51} despite having a lower mean serum cholesterol level.\textsuperscript{20} However, such comparisons need to take into account the many confounding inter-regional differences in diet and life style.\textsuperscript{30} Furthermore, there are several other explanations for the French paradox, including the ‘time lag’ hypothesis which states that the French diet had low fat in the past and that it takes about 30 years for any dietary pattern to manifest itself in mortality data.\textsuperscript{52} A PubMed search could uncover no good epidemiological study firmly to establish any clinical relation between coronary heart disease mortality and wine consumption, with France having the lowest mortality. The jump to the specific benefit of red wine was made in 1993 when Frankel \textit{et al.} showed that red wine diluted 1000-fold and contained 10 µmol/L of total phenols that inhibited the oxidation of LDL more than α-tocopherol, an established antioxidant.\textsuperscript{39} The antioxidant mechanisms involved are scavenging of peroxyl radicals as well as chelation of redox metal ions such as a copper.\textsuperscript{50} Indirect evidence favouring the ‘French paradox’ and the red wine hypothesis is that the French habitually drink wine with their meals (which are often fatty) and this wine is most often red. Furthermore, Alsace, a white-wine drinking region of France, has a much higher mortality (about 50% higher) than red wine-drinking Mediterranean areas,\textsuperscript{51} despite having a lower mean serum cholesterol level.\textsuperscript{20} However, such comparisons need to take into account the many confounding inter-regional differences in diet and life style.\textsuperscript{30} Furthermore, there are several other explanations for the French paradox, including the ‘time lag’ hypothesis which states that the French diet had low fat in the past and that it takes about 30 years for any dietary pattern to manifest itself in mortality data.\textsuperscript{52} A PubMed search could uncover no good epidemiological study firmly to establish any clinical outcome benefit of red vs. white wine drinking in Europe. Most large-scale epidemiological data coming from North America suggest that there is no difference between white and red wine,\textsuperscript{43,44,53} thus concluding that alcohol intake rather than wine colour predicts the eventual cardiovascular outcome. Again there may be confounding factors. The pattern of wine drinking could be different, with less wine with meals and more prior to meals in America than in Europe. Furthermore, American and French diets are typically very different, for example with much more wine used in cooking in France. Marinating meat in red wine substantially decreases formation of potentially toxic heterocyclic amines\textsuperscript{54} and of lipid peroxides.\textsuperscript{55}

French vs. German reds: effects on nitric oxide

French red wine is better than German reds in the induction of human endothelial nitric oxide synthase (eNOS) in human umbilical vein endothelial cells.\textsuperscript{56} Presumably, this is a class effect although only six French and three German wines were studied. In view of the similar effects on nitric oxide production by two French reds, the one barrel-fermented and the other not,\textsuperscript{56} it is very unlikely that better French than German oak can explain these differences. While the human inclination is to add such basic observations to the known better aroma, palate, and after-taste of French vs. German reds, we still await a large comparative epidemiological study proving the proposed superior cardioprotective effects of French red wines.

Red vs. white wine or vodka: comparative studies

In normal human volunteers, drinking an unspecified amount of red wine but not white or vodka increased coronary flow-velocity reserve.\textsuperscript{57} Drinking red wine raised HDL-cholesterol and plasma antioxidant status more than did an equivalent dose of white wine when given to healthy volunteers.\textsuperscript{58} Furthermore, in vascular smooth muscle cells, pre-incubation with red wine but not white inhibited the platelet-derived growth factor beta-receptor (PDGF) that is crucial in the development of atherosclerosis.\textsuperscript{59} Conversely, incubating white wine with grape skins (that contain the polyphenols found in red wine) and alcohol endowed white wine with a similar power of inhibition of LDL-oxidation to that possessed by red wine.\textsuperscript{60} In human mononuclear cells incubated with VLDL (very low-density lipoproteins), drinking moderate doses of red wine but not vodka inhibited the activation of nuclear factor (NF)-kappa-B\textsuperscript{61} during post-prandial lipaemia, thus providing an anti-inflammatory mechanism.\textsuperscript{42} This effect was simulated by antioxidants contained in red wine. These comparative studies support the red wine hypothesis.

Red wine: protective effects beyond alcohol

The conclusive studies showing that red wine has qualities ‘beyond alcohol’ are those on de-alcoholized red wine, which has cardiovascular protective effects in short-term studies on humans.\textsuperscript{37,62} In patients with coronary artery disease, 250 mL of de-alcoholized Greek red wine decreased arterial stiffness and improved the augmentation index, as derived from arterial wave reflection patterns.\textsuperscript{37} A similar dose of de-alcoholized red wine decreased post-smoking arterial wave reflections and lessened the rise in systolic BP.\textsuperscript{62} Brachial artery flow-mediated vasodilation was improved by 250–500 mL of de-alcoholized red wine.\textsuperscript{63,64} Ingestion of purple grape juice in patients with coronary artery disease (about 640 mL/day for 14 days) similarly improved brachial flow.\textsuperscript{65} The purple grape juice also reduced LDL-cholesterol susceptibility to oxidation, with the benefits being attributed to flavonoids. Alcohol-free red but not white wine greatly increased plasma antioxidant capacity 50 min after ingestion.\textsuperscript{66} Red wine incubated with human plasma strongly inhibited LDL-oxidation and uptake of LDL by macrophages, and the active components were catechins and flavonols, whereas ethanol had no effect.\textsuperscript{56} All these human studies are among the most powerful arguing for the benefit of red (but not white) wine beyond alcohol. Supporting data comes from dogs with stenosed coronary arteries, in whom administration of French blended red wine, apparently verging on vintage in age, eliminated cyclic flow reductions caused by periodic acute platelet-mediated thrombus formation.\textsuperscript{67} This markedly beneficial effect was also achieved by purple grape juice, but about 2.5 times greater volume was required than in the case of red wine. White wine was not protective, so that the explanation must again lie in the effects of red wine beyond alcohol. Experimentally, the benefit of the red wine lies in the polyphenols, found in de-alcoholized red wine extracts.
which improve endothelial nitric oxide release and revert the prothrombotic effects of a cholesterol-rich diet. De-alcoholized red but not white wine decreased atherosclerosis in apolipoprotein E gene-deficient mice. However, there is no strict clinical proof that these 'beyond alcohol' effects operate in red wine drinkers to give clinically significant cardiovascular protection. Rather, there is only indirect observational evidence in a case-control study that links high dietary anthocyanidin intake to reduce the risk for acute myocardial infarction independent of alcohol intake.

Specific protective chemicals in red wine: molecular effects

Polyphenol extracts

Red wine polyphenol extracts have experimental cardioprotective properties, and may counter one of the mechanisms underlying atherosclerosis, namely thrombin-induced matrix invasion of vascular smooth muscle cells. An extract of Cabernet Sauvignon suppressed endothelin-1 synthesis and release in bovine aortic endothelial cells. Another polyphenol extract protected against angiotensin-II-induced hypertension in rats by blunting endothelial dysfunction and promoting formation of nitric oxide. Yet, another polyphenol extract increased eNOS promoter and nitric oxide release in human umbilical endothelial cells. Of the possible polyphenol components tested, the activity only resided in resveratrol which is a stilbene derivative found in grape skins, and hence found much more in red than white wine. Resveratrol also was an active component of a red wine polyphenol extract that transcriptionally inhibited the expression of endothelial vascular cell adhesion molecule, VCAM-1. Besides the polyphenols currently attracting most attention are resveratrol and the procyanidins (the latter are flavan-3-ols and hence part of the flavonoid family).

Resveratrol

Antioxidant effect

Dosing animals with resveratrol gives a variety of cardioprotective results (Table 1) of which the strong antioxidant properties are perhaps the best known, and could explain the inhibition of LDL oxidation. The antioxidant effect on LDL is also linked to increased expression of the paraoxonase-1 gene. Other antioxidant effects are widespread, including cardioprotection where it offsets the adverse pro-oxidant qualities of alcohol. Furthermore, resveratrol inhibits the inflammatory response by suppressing prostaglandin biosynthesis and by downregulating the gene expression of intercellular adhesion molecule-1 (ICAM-1) and NF-κ-B, the latter taken as an indirect biomarker of oxidative stress. The NF-κ-B signalling pathway can be evoked by oxidative stress and is in turn inhibited by resveratrol (Figure 1). Not surprisingly, TNF-α-induced activation of NF-κ-B is attenuated by resveratrol and this sequence is in part mediated by an antioxidant effect, which in turn reduces platelet activation. This sequence can also explain the resveratrol-induced inhibition of oxidative stress in platelets induced by TNF-α. Resveratrol inhibits angiotensin-II (A-II)-induced cardiomyocyte hypertrophy, which is clearly linked to its antioxidant effects, because it inhibited the A-II-induced production of reactive oxygen species (ROS).

As the cellular redox state influences so many reactions, and is sensitive to both free radicals and to resveratrol, it is likely but usually assumed rather than directly proven that the antioxidant effect of resveratrol could play a major role in at least some of the other protective mechanisms now to be discussed.

Proposed vascular protective mechanisms

Resveratrol acts through multiple mechanisms (Figure 1), including the antioxidant effects on platelet formation already discussed. Resveratrol vasodilates isolated human small arteries, in response to oestrogen-receptor stimulation with formation of nitric oxide. The vasodilation is absent in arteries from patients with coronary artery disease. The mechanism involves increased formation and release of nitric oxide associated with increased eNOS activity. Although there is no direct evidence linking such increased eNOS activity to platelets, there is a well-known effect of nitric oxide in inhibiting the activation of platelets. Links between resveratrol and reduced platelet aggregation could also be mediated by TNF-α. Resveratrol at a concentration of only 0.1 μmol/L increases the activity of NF-κ-B, which lies on the TNF-α signalling pathway, in human endothelial cells after overnight incubation, thereby providing the mechanism for a possible anti-inflammatory and anti-platelet-effect. Ingestion of 200 mg of resveratrol increased eNOS activity in the heart, lungs, and spleen, as well as protein synthesis and release of nitric oxide associated with increased eNOS activity.

Cardiac fibroblasts

Resveratrol (5–25 μmol/L) pretreatment inhibited growth pathways stimulated by angiotensin-II and epidermal growth factor signalling, as well as transforming growth factor-β, which are essential in fibroblast proliferation and differentiation. Thus resveratrol is potentially anti-fibrotic.

Metabolic effects of resveratrol

Resveratrol in high doses shifts the physiology of middle-aged obese mice, with features resembling the metabolic syndrome in humans, towards those of non-obese mice on a standard diet. The metabolic changes included increased insulin sensitivity, decreased circulating free fatty acids, decreased insulin-like growth factor (IGF-1), increased activity of the energy-sensing enzyme, AMP-activated protein kinase (AMPK), increased activity of peroxisome proliferator-activated receptor-gamma coactivator-alpha (PGC-1α), and increased mitochondrial number (Figure 2). These diverse effects are mediated by sirtuin (see next paragraph). Whether similar trends can be achieved by the very much lower resveratrol dose associated with red wine drinking is a subject for future research.

Pharmacological preconditioning by resveratrol

In several studies, resveratrol decreases ischaemic-reperfusion damage (Table 1). A burst of short perfusion by resveratrol or pretreatment before the onset of ischaemic-reperfusion injury stimulates a plethora of preconditioning-like survival paths.
Table 1 Concentrations of resveratrol that have relevant cardiovascular effects on isolated tissues or organs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human coronary artery endothelial cells</td>
<td>0.1–10 μmol/L</td>
<td>Inhibits TNF-α-induced NF-κ-B activation and inflammatory markers</td>
<td>81</td>
</tr>
<tr>
<td>Human umbilical vein endothelial cells</td>
<td>0.0228–2.28 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human umbilical vein endothelial cells</td>
<td>0.1 μmol/L</td>
<td>Increased the inhibitor of TNF-α (NF-κ-B)</td>
<td>89</td>
</tr>
<tr>
<td>Human umbilical vein endothelial cells</td>
<td>1–10 μmol/L</td>
<td>Increased eNOS promoter and nitric oxide release</td>
<td>68</td>
</tr>
<tr>
<td>Human umbilical vein endothelial cells</td>
<td>10–100 μmol/L</td>
<td>Increased eNOS promoter activity and eNOS protein levels</td>
<td>86</td>
</tr>
<tr>
<td>Human umbilical vein endothelial cells</td>
<td>5–100 μmol/L</td>
<td>Inhibition of tissue factor activity</td>
<td>132</td>
</tr>
<tr>
<td>Human endothelial progenitor cells</td>
<td>1.0 μmol/L</td>
<td>Increased activity vs. decrease with 50 μM</td>
<td>96</td>
</tr>
<tr>
<td>Human hepatoma cells</td>
<td>5–10 μmol/L</td>
<td>Increased expression of paraoxonase-1 gene</td>
<td>77</td>
</tr>
<tr>
<td>Human platelets, ASA-resistant</td>
<td>10 μmol/L</td>
<td>Reduced platelet aggregation</td>
<td>87</td>
</tr>
<tr>
<td>Human small arteries, subcutaneous</td>
<td>0.3–30 μmol/L</td>
<td>Dilation but not in those with coronary heart disease</td>
<td>61</td>
</tr>
<tr>
<td>Rat neonatal ventricular myocytes</td>
<td>0.1–100 μmol/L</td>
<td>Inhibited A-II-induced phosphorylation of ERK and A-II-induced increase of ROS levels</td>
<td>84</td>
</tr>
<tr>
<td>Rat aortic smooth muscle cells</td>
<td>0.05–50 μmol/L</td>
<td>Inhibition of A-II and epidermal growth factor-mediated growth signalling path</td>
<td>91</td>
</tr>
<tr>
<td>Rat cardiac fibroblasts</td>
<td>5–25 μmol/L</td>
<td>Inhibition of signalling paths mediated by A-II, epidermal and transforming growth factors</td>
<td>94</td>
</tr>
<tr>
<td>Ischaemic-reperfused rat heart</td>
<td>10 μmol/L</td>
<td>Expression of anti-apoptotic Bcl-2</td>
<td>99</td>
</tr>
<tr>
<td>Ischaemic-reperfused rat heart</td>
<td>10 μmol/L</td>
<td>Preconditioning of heart via adenosine-3 receptor and survival paths</td>
<td>99</td>
</tr>
<tr>
<td>Ischaemic-reperfused rat heart, after pretreatment</td>
<td>Gavage 2.5 mg/kg</td>
<td>Less infarct size and apoptosis via survival paths</td>
<td>97</td>
</tr>
<tr>
<td>Ischaemic-reperfused rat heart, after pretreatment</td>
<td>18.5 μmol/L in blood after i.p injection</td>
<td>Less post-reperfusion depression function</td>
<td>133</td>
</tr>
<tr>
<td>Ischaemic-reperfused rat heart, after pretreatment</td>
<td>10 μmol/L</td>
<td>Decreased infarct size</td>
<td>26</td>
</tr>
<tr>
<td>Ischaemic-reperfused rat heart, after pretreatment</td>
<td>10 μmol/L</td>
<td>Less ischaemic-reperfusion injury</td>
<td>134</td>
</tr>
<tr>
<td>Myocytes from failing mice hearts</td>
<td>0.5 μmol/L–0.114 mg/L</td>
<td>Inhibits falling levels of sirtuin (inhibitor of cell death)</td>
<td>104</td>
</tr>
</tbody>
</table>

Note the wide range of concentrations used of resveratrol, always in trans form (molecular weight = 228 g/mol); for relation to circulating concentrations likely to be found after red wine intake in humans, see text. TNF, tumour necrosis factor; NF, nuclear factor; A-II, angiotensin-II; ERK, extracellular signal-regulated kinase; ROS, reactive oxygen species.

Figure 1 Proposed multiple vascular protective mechanisms of resveratrol. ROS, reactive oxygen species; NF-κ-B, nuclear factor kappa B; eNOS, endothelial nitric oxide synthase; TNFα, tumour necrosis factor alpha; Ang-II, angiotensin II; VSMC, vascular smooth muscle cells.

The red wine hypothesis

**Effects on sirtuin (anti-senescence) proteins**

These effects of resveratrol are the focus of current research (Figure 3). A high resveratrol diet opposes the effects of a high fat diet in mice, increasing insulin sensitivity, decreasing myocardial and aortic damage, and prolonging lifespan. Mechanistically, at least some of these effects can be related to the recent discovery that resveratrol is an agonist of the sirtuins (also called SIRT proteins, Silent Information Regulator Two) which belong to the histone deacetylase family. By deacetylating they inactivate the histone senescence factor p53. Conversely, overexpression of sirtuins limits premature cellular senescence. Activation of sirtuins by resveratrol can experimentally prolong the life of myocytes from failing hearts and decrease myocyte death induced by angiotensin-II. Furthermore, resveratrol protects against Alzheimer-like neurodegeneration by increasing sirtuin activity and, thereby, inhibiting adverse NF-κ-B signalling, thus, experimentally protecting both heart and brain via sirtuins in high doses.

**Resveratrol: male vs. female differences**

Resveratrol stimulates the oestrogen E-beta receptors in vascular endothelial cells, and functions as an agonist for insulin, may explain the insulin-like effect of resveratrol in diabetic rats.
It would be intriguing to imagine that women could offset this gender disadvantage of alcohol by drinking red rather than white wine, but there are no supporting data.

**Resveratrol pharmacokinetics**

Are the protective concentrations used in such studies within the range that could be expected after drinking red wine? (Table 1 and 2). Resveratrol itself exists in two forms, cis- and trans-forms that are in equilibrium and have similar biological activities, so that the total resveratrol concentration is relevant. However, it varies substantially between wines (Table 2). Resveratrol is only one of several stilbenes in red wine although the best studied. In Brazilian red wine, the total stilbene consumption from drinking half a bottle of some types of red wine per day is about 11 mg,106 which if all were absorbed could give a peak total stilbene concentration of about 10 μmol/L, or about 4 μmol/L of resveratrol. This potential peak value compares well with the lowest in vitro resveratrol concentration having an in vitro biological effect (0.1–0.3 μmol/L; Table 1), and raises the theoretical possibility that therapeutic resveratrol levels could be reached by drinking high-resveratrol red wines. Proof of this hypothesis requires studies in humans of red wine dosage and related resveratrol blood levels. In one study, after 25 mg of resveratrol, as would be found in 2.5 L of some red wines (Table 2), peak total (free and conjugated) resveratrol was about 450 μg/L or about 2 μmol/L,107 20-fold more than the lowest resveratrol concentrations experimentally active (Table 1). Thus half a bottle of such wine should still give enough resveratrol to be active. However, the calculation assumes that resveratrol conjugates are active, which has not yet been studied. The peak free circulating unconjugated resveratrol was just about 5 mol/L,108 which is 20-fold below the lowest effective concentration reported. In another study, a dose of 1 g as in 10 L of some red wines, gave higher peak free

![Figure 2](https://academic.oup.com/eurheartj/article-abstract/28/14/1683/2887413)  
**Figure 2** Proposed anti-ischaemic protective mechanisms of resveratrol. Those myocardial signalling paths involved in resveratrol pharmacological preconditioning are shown on the left, and those giving metabolic protection on the right. Note multiple antioxidant effects. P-I-3 kinase, phosphatidylinositol-3 phosphate-kinase; Akt, protein kinase B; Bcl-2, mitochondrial anti-apoptotic protein; NF-κ-B, nuclear factor kappa-B; LDL, low-density lipoprotein; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator-alpha; IGF-1, insulin-like growth factor; AMPK, AMP-activated protein kinase; mito, mitochondria. Outlines of heart and atheroma modified from Opie LH Heart Physiology, from Cell to Circulation, Lippincott, Williams and Wilkins, Philadelphia, 2004, with permission.

![Figure 3](https://academic.oup.com/eurheartj/article-abstract/28/14/1683/2887413)  
**Figure 3** Proposed effect of resveratrol in countering heart failure by acting as an agonist to sirtuin, considered as a longevity factor. Hypothetically, heart failure induces overactivity of poly(ADP-ribose) polymerase (PARP), a multifunctional DNA-bound nuclear enzyme, that inhibits NAD-dependent paths thereby decreasing the activity of sirtuin. The proposal is that resveratrol, by increasing the activity of sirtuin, deacetylates and inhibits the pro-apoptotic effector p53 (a transcription factor). The result is that resveratrol protects cells from failing hearts against PARP-mediated cell death. For concepts and data, see Pillai et al.104 and Smith,131 NAD, nicotinamide adenine dinucleotide. Figure of heart from Drugs for the Heart, 6th edn., (Eds) Opie LH and Gersh B, Elsevier Saunders, Philadelphia, 2005, with permission.
The red wine hypothesis

Resveratrol levels of 1–2 μmol/L.108 Thus, 1 L of such red wine would give levels of 100–200 nmol/L, just within the bottom end of the therapeutic range (Table 2). Oral administration of resveratrol to rats in doses similar to a ‘reasonable’ wine intake gives plasma resveratrol levels of 0.1 μmol/L,109 about one-third of the lowest concentration that can relax isolated human small arteries,41 and one-fifth of the lowest concentration that protects myocytes from failing hearts,104 but about twice the lowest level that interferes with signalling by angiotensin-II.91

There are two further relevant issues. First, the plasma concentrations of the conjugated glucoside form, piceid, can be about six times higher than that of free resveratrol.110 The biological activity of these conjugated compounds is not known. Secondly, with prolonged daily dosing of rats with an amount of resveratrol found in 250 mL of some red wines, there is substantial accumulation in the liver, and further metabolic fates are unknown.109 Overall, resveratrol concentrations reached by drinking within the safe limits of some red wines might be just within or below the therapeutic range, judged by isolated tissue experiments.

Procyanidins

Resveratrol is not the only protective agent in red wine. Red wine contains a large number of other potentially active flavonoids, especially the flavan-3-ols which include procyanidins and proanthocyanidins (Table 3). Proanthocyanidin, like resveratrol, is a strong antioxidant and both equally reduce experimental myocardial infarct size and apoptosis in isolated rat hearts.26 The mechanisms include a preconditioning-like effect, shown by induction of heat shock protein (HSP) 70. Taking inhibition of endothelin-1 production by aortic endothelial cells as another endpoint, oligomeric procyanidins inhibit ET-1 synthesis at concentrations similar to those in some red wines, with some correlation between the procyanidin activity of the wine varietal and the longevity.111 Oligomeric procyanidins are also inhibitors of ACE activity, in concentrations similar to those found in red wines.112 The antioxidant effect of red wine on LDL oxidation is mediated chiefly by catechins but also by the flavonol and polymeric anthocyanidins.50 However, there are few strict studies relating post-ingestion blood levels found in humans to cardioprotective concentrations in isolated tissues. In this regard, flavonoids of the catechin family inhibited the PDGF beta-receptor when used in concentrations similar to those found in the blood after drinking red wine.59

Other active ingredients of red wine

Of the many other polyphenolic components of red wine, the less well-studied flavonoids include quercetin and catechin,75 which also have properties of potential cardiovascular importance. In a dietary study in over 10,000 persons for 1 year, those with a higher flavonoid intake, particularly quercetin, had less coronary heart disease.113 Both quercitin and catechin given orally can retard progression of atherosclerosis in apolipoprotein E-deficient mice, acting by reduction of the susceptibility of LDL to oxidation and aggregation.114 In humans, plasma catechin rises to about 75 nmol/L soon after the equivalent of one to two glasses of red wine.115 These levels contrast with those experimentally protective, such as 50 μmol/L.114 De-alcoholized red wine decreased atherosclerosis in apolipoprotein E gene-deficient mice, acting by phenolic acids not listed in Table 3.70 The mechanism involved decreased LDL oxidation associated with decreased alpha-tocopherol radicals, the oxidation product of vitamin E. Thus protective effects of vitamin E could be facilitated, overcoming the otherwise adverse excess pro-oxidant effects. In mice, a daily dose of Cabernet Sauvignon similar in dose to half a bottle of wine per day for humans, and with a long list of potentially active polyphenols, was neuroprotective against experimental Alzheimer’s disease.116 Multiple mechanisms of benefit of various ingredients seem probable. What is still missing are human data on the pharmacokinetics and bioavailability of the polyphenols found in red wine.117 Nonetheless of note, at least one laboratory effect of polyphenols, namely inhibition of thrombin-induced matrix invasion of vascular smooth muscle cells, can be found in concentrations similar to those achieved after moderate red wine ingestion.24

Proposed protective components in white wine and beer

White wine may have different protective qualities

Red and white wine have equal effects on fibrinolytic factors in a short-term cross-over study.118 Equal effects on collagen-induced platelet aggregation are also reported.119 In addition, white wines may contain anti-inflammatory protective substances that lessen cytokine release, as also found in extra virgin olive oil.120 Thereby, the inflammatory reaction that promotes atherosclerosis could be lessened.

De-alcoholized beer may also give some protection

Some epidemiological studies have not shown less protection by beer than wine drinking.121 De-alcoholized beer inhibits platelet and thrombogenic activity in young adults.122 However, the dose was high (3 L) so that if translated into the amount of alcohol in standard beer, such intake would be harmful.
Overall perspective
There are several sequential proposals leading from observational population studies. Thus (i) only modest alcohol intake gives a J-shaped pattern of protection; (ii) wine is more protective than beer; and (iii) red wine is more protective than white. Of these, the J-shaped curve is best established, but in every case confounding factors are difficult to exclude. However, in the case of the J-shaped curve, the genetic links between alcohol dehydrogenase activity, increased HDL-cholesterol, and decreased myocardial infarction, and the prospective trial data on HDL, fibrinogen, and insulin, are powerful arguments against confounding. Moderate red wine drinking could be, at least in part, a surrogate for a healthy life style. In this case the cardiovascular data with de-alcoholized red but not white wine and studies on isolated tissues or organs give decisive mechanistic insights that strengthen the case already made for randomized prospective controlled trials with cardiovascular endpoints. The first trial should simply compare alcohol with red wine with abstinence, for example in post-infarct patients. Similar prospective studies would be needed conclusively to prove the red-better-than-white wine hypothesis.

As these studies may never be done, attention has shifted to the beneficial components of red wine. Resveratrol remains as the most powerful of the polyphenols and one of the most likely to give biological protection. The recent discovery that resveratrol is an agonist of the sirtuin (SIRT) proteins may lead to a dose-response trial of resveratrol and red wine in heart failure patients. However, pharmacokinetic data remain incomplete. Experimentally, other polyphenolic components of red wine also play a protective role. One hypothesis would be that no single component of red wine confers all the benefit, but rather several components contribute by different mechanisms. For example, resveratrol and procyanidins are both antioxidants, but resveratrol interacts with SIRT, whereas the procyanidins inhibit endothelin-1 synthesis.

Given that there will always be wine drinkers, do current data help in choice of wine? First, all drinkers of any alcohol should avoid overdosing and binge-drinking. Then they should consider the incomplete but emerging evidence that red wine is more cardioprotective (and possibly, neuroprotective) than white. Furthermore, as red wine-drinkers can choose a specific varietal, pinot noir is high both in resveratrol and in procyanidins. The price of pinot is high enough to ensure drinking only in moderate amounts and preferably with meals. Some may be influenced by the neuroprotective qualities of Cabernet Sauvignon given in low doses to rodents. And others, especially those influenced by American epidemiological data, will settle for ethanol as the major cardioprotective agent in wine. Teetotallers will be consoled by the substantial data linking de-alcoholized red wine to experimental cardiovascular benefits and turn to purple grape juice. Hopefully, in the future, low alcohol yet still protective wines will make their debut.

Special patient groups who need abstinence or specific limitations
Those with gout are traditionally warned against red wine, which increases blood uric acid levels. Those with alcoholic cardiomyopathy may not take any alcohol, despite the 'ambiguous' effects of ethanol on myocardial function. Those with atrial fibrillation should be safer with less than 35 drinks/week. Hypertensive males may need to limit drinks to not more than two/day with, presumably, even fewer for hypertensive females. However, as alcohol only elevates BP by a few millimetres of mercury, a reasonable alternative would be to monitor by BP measurements the contrasting policies of (i) allowing the normal moderate amounts of alcohol while watching the BP and, if needed, adjusting doses of anti-hypertensive drugs; and (ii) keeping the medication unchanged but cutting the alcohol. Post-myocardial infarction, moderate alcohol does not impose a greater risk of overt heart failure on those already having systolic dysfunction. Also, it is important to remember that alcohol interacts with many drugs such as nitroglycerin, anxiolytics, neuroleptics, and oral contraceptives.

Summary
There are strong epidemiological data favouring the view that modest alcohol but neither zero nor more than modest intake beneficially reduces total mortality and cardiovascular risk. As there is only a narrow window of opportunity for optimal intake, it becomes important to consider which is the most beneficial type of alcohol. The 'red-better-than-white' hypothesis rests on human studies showing short-term cardiovascular benefits achieved by de-alcoholized red but not white wine; and many studies on isolated tissues or organs assessing the effects of polyphenols in red wine, especially resveratrol. Thus, red wine potentially has beneficial effects beyond alcohol. Molecular mechanisms involved are many and complex as revealed by studies on isolated tissues or organs. Besides, the well known antioxidant effects of polyphenols, resveratrol effects include activation of preconditioning-like survival signalling paths similar to those responding to insulin, anti-apoptotic protection mediated by the senescence factor sirtuin, and inhibition of adverse signalling by TNF-α, NF-κB, angiotensin-II, and growth factors (epidermal and transforming growth factor-beta). Mechanistic feasibility strengthens the case for prospective therapeutic trials.

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