

Grapes and Cardiovascular Disease¹⁻³

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Abstract

Epidemiological studies suggest that consumption of wine, grape products, and other foods containing polyphenols is associated with decreased risk for cardiovascular disease. The benefits of wine consumption appear to be greater than other alcoholic beverages. Experimental studies indicate that grape polyphenols could reduce atherosclerosis by a number of mechanisms, including inhibition of oxidation of LDL and other favorable effects on cellular redox state, improvement of endothelial function, lowering blood pressure, inhibition of platelet aggregation, reducing inflammation, and activating novel proteins that prevent cell senescence, e.g. Sirtuin 1. Translational studies in humans support these beneficial effects. More clinical studies are needed to confirm these effects and formulate dietary guidelines. The available data, however, strongly support the recommendation that a diet rich in fruits and vegetables, including grapes, can decrease the risk for cardiovascular disease. *J. Nutr.* 139: 1788S–1793S, 2009.

Introduction

The medicinal value of the grapevine and its fruit, *Vitis vinifera*, has been recognized for over 6000 y (1). In ancient Egypt, sap from grapevines was made into an ointment to treat skin and eye conditions. The fruit was crushed into wine elixirs or ripened to serve as therapeutics for a multitude of conditions, including nausea, constipation, cholera, smallpox, liver disease, and cancers. In the past century, disease states such as hypertension, coronary heart disease, and stroke have become markedly more prevalent. Despite aggressive management of cardiovascular risk factors and improved outcomes, the societal burden from cardiovascular disease remains high, and in the past few de-

cares there has been increased interest in lifestyle and dietary approaches to reducing cardiovascular risk. Recent evidence suggests there are cardioprotective benefits from diets rich in natural fruits and vegetables, such as grapes. This review will outline the epidemiological evidence supporting the cardiovascular benefits of grape consumption. We will then consider experimental and translational studies that elucidate potential mechanisms of benefit.

Epidemiology

The "French Paradox." In 1979, St. Leger et al. (2) drew attention to the protective effects of wine against ischemic heart disease. Epidemiologic data collected by the WHO revealed a discord in cardiovascular mortality in a cohort of subjects from Toulouse, France, compared with other cohorts from 17 Western countries, including the United States and the United Kingdom (3–5). Interestingly, the French cohort had lower risk despite higher consumption of saturated fat (3). This counterintuitive finding, which was coined the "French Paradox," stimulated further analysis leading to the suggestion that increased consumption of wine in France and other Mediterranean countries might be the explanation.

More recent epidemiological studies provide further support for a beneficial effect of wine. Initially, reduced risk was attributed to its ethanol content (3,6–8). Other studies, however, indicated that wine might confer benefits above and beyond those of other alcoholic beverages, suggesting that nonalcoholic factors in wine may also play a protective role (9–11). For example, wine consumption was found to be cardioprotective, whereas beer intake was not, in a meta-analysis of 13 studies and 209,418 participants (9). Despite these data, it remains possible that ethanol and/or other aspects of the Mediterranean diet or lifestyle might better explain the French paradox.

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Grape polyphenols and cardiovascular risk. Grapes contain a wide variety of polyphenol compounds, including flavonoids, phenolic acids, and resveratrol. There is extensive epidemiological evidence suggesting that dietary intake of these compounds reduces cardiovascular mortality (12–17). Numerous studies in vitro as well as in animals and humans demonstrate beneficial effects of grape polyphenols on traditional cardiovascular risk factors (Table 1). Population-based studies have observed markedly lower cardiovascular disease mortality in cohorts with higher consumption of relevant flavonoids, including flavonols, flavones, and flavan-3-ols (13–15,18–20). In a study of 34,489 postmenopausal American women, dietary intake of foods containing flavanones and anthocyanidins was associated with decreased cardiovascular and all-cause mortality (21). In that study, consumption of red wine was specifically associated with decreased risk of coronary heart disease.

Further evidence that polyphenol content in wine accounts for cardiovascular benefits may be derived from studies comparing different types of wine. The highest concentrations of grape polyphenols are found in the skin, stems, and seeds. The longer contact with these components during the production of red wine increases polyphenolic content up to 10-fold compared with white wine (22). Investigators suggest that this difference in phytochemical content explains the reported additional health benefit of red wine over white wine or grape juice (23). In support of this possibility, numerous human studies suggest that red wine has greater antioxidant effects and more favorable effects on lipid metabolism than white wine (24). It must be acknowledged, however, that several observational studies from North American cohorts did not reveal differential effects of red and white wines (10,25).

Negative studies of polyphenol consumption and cardiovascular disease. Despite the large body of evidence supporting a link between polyphenol consumption and reduced

cardiovascular risk, some concerns are worth noting. A number of well-done observational studies have shown no relationship between polyphenol consumption and cardiovascular outcomes (26,27). These apparently discrepant results might be explained by a variety of factors. Dietary questionnaires are an imperfect method to assess polyphenol intake and the available studies have focused on only a few specific compounds. In addition, the overall levels and ranges of polyphenol intake within the studied cohorts and the confounding effects of socioeconomic class, other dietary factors, and concomitant risk factors might explain the lack of consistent findings (28). Despite these lingering questions, the accumulated data from multiple studies prompted the AHA to recommend a diet rich in fruits and vegetables, including grapes, as an approach to prevent cardiovascular disease (29).

Mechanistic studies

Antioxidant properties. Grape polyphenols have important antioxidant properties. According to the oxidative hypothesis, oxidative modification of LDL is a primary initiating event in atherosclerosis [reviewed by Diaz et al. (30)]. As a corollary, this hypothesis suggests that antioxidant treatment to limit LDL oxidation should prevent atherosclerosis and its complications. These concepts stimulated many studies that examined the antiatherosclerotic effects of antioxidant vitamins in animal models and surrogate endpoints in humans and ultimately prompted investigators to conduct large-scale clinical trials of antioxidant treatment for cardiovascular disease (31). Despite the failure of randomized trials with antioxidant vitamins, such as vitamin E, vitamin C, and β carotene, there remains strong evidence that an imbalance between production of reactive oxygen species and antioxidant defense mechanisms contributes to the pathogenesis of atherosclerosis (32). We now understand that redox signaling is important for normal cellular physiology and the response to environmental stress, possibly explaining

TABLE 1 Effects of grape polyphenols on traditional cardiovascular risk factors

Risk factor	Product	Study outcome/mechanisms	Year	Reference
Hypertension	Red wine	Decreased systemic blood pressure in rats	2001	(76)
	Flavanol-rich foods (wines), procyanidins	Inhibited angiotensin-converting enzyme activity	2003	(77)
	Red wine-derived polyphenol extract	Reduced systemic blood pressure and improved aortic elasticity in stroke-prone spontaneously hypertensive rats	1999	(78)
		Decreased blood pressure in NO-deficient hypertensive rats	2002	(79)
	Grape juice	Prevented angiotensin II-induced hypertension in rats	2006	(80)
		Reduced systolic blood pressure in hypertensive humans	2004	(81)
	Grape seed-derived proanthocyanidin extract	Decreased arterial pressure in estrogen-depleted female hypertensive rats	2005	(82)
	Diabetes	Grape seed-derived procyanidin extract	Antihyperglycemic effect in streptozotocin-induced diabetic rats	2004
Resveratrol		Enhanced insulin sensitivity in diabetic mice	2007	(84)
Hypercholesterolemia	Polyphenol-enriched white wine, red wine	Lowered cholesterol concentrations and increased ratio of apoA-1:apoB in hamsters ¹	2005	(85,86)
	Grape juice-derived polyphenol extract	Increased both the activity and cell surface expression of the LDL receptor	2006	(87)
	Dealcoholized red wine	Decreased expression and excretion of apoB in cultured human hepatic cells ¹	2003	(88)
	Grape juice	Reduced LDL cholesterol and apoB concentrations, decreased concentration of oxLDL, increased concentrations of HDL cholesterol and apoA-1 in healthy and hemodialysis-receiving humans ¹	2006	(89)
Smoking	Dealcoholized red wine	Reduced lipid peroxidation in human male smokers	2001	(90)
	Grape seed-derived polyphenol extract	Reduced susceptibility of LDL to oxidation in human male smokers	2003	(91)

¹ apo, Apolipoprotein.

why high-dose antioxidant supplementation does not show benefits. It remains clear, however, that “oxidative stress” has pathophysiological effects on enzyme function, cell signaling, and gene expression that contribute to disease development. Interventions that affect cellular redox status have the potential to reduce risk.

In this context, many studies investigating beneficial mechanisms of grape polyphenols have focused on their antioxidant properties. Flavonoids and other polyphenols found in grapes have the capacity to scavenge reactive oxygen species (33). When fed to animals and humans, such compounds have been shown to increase the radical scavenging capacity of plasma (34). In addition to scavenging radicals, polyphenols alter cellular redox status by other mechanisms, including chelating metals that promote lipid peroxidation and modulating the activity of antioxidant enzymes.

Given the oxidative hypothesis of atherosclerosis, a potentially important property of grape-derived polyphenols is the ability to inhibit LDL oxidation. In the key initial step in atherogenesis, oxidized LDL (oxLDL) is taken up by macrophages in an unregulated manner to form foam cells. OxLDL also promotes atherosclerosis by altering endothelial function, stimulating platelet activation, and inducing a proinflammatory state in the vascular wall (30). In vitro studies have demonstrated that grape-derived flavonoids and resveratrol limit ex vivo LDL oxidation (35). Red wine has been found to be more potent than white wine or pure ethanol in this regard (36).

There is evidence that these effects are relevant to atherogenesis in animals. Hayek et al. (37) observed that hypercholesterolemic mice consuming wine polyphenols for 6 wk had markedly less atherosclerosis than control animals. These treatments were associated with protection against LDL oxidation. Vinson et al. (38) demonstrated reduced aortic atherosclerosis in hamsters supplemented with polyphenol-rich beverages. Zern et al. (39) observed a reduction in cholesterol accumulation in the aortas of ovariectomized guinea pigs fed a lyophilized grape preparation. Interestingly, Stocker et al. (40) observed reduced atherosclerosis but no decrease in LDL oxidation within the arterial wall following treatment with dealcoholized red wine in mice, suggesting that mechanisms other than LDL protection may also be important.

To translate these mechanistic findings to humans, Stein et al. (41) demonstrated a reduction in the susceptibility of LDL to copper-mediated oxidation following consumption of purple grape juice for 2 wk in patients with coronary artery disease. Red wine consumption in healthy subjects also reduced urinary levels of prostoglandin F₂- α , a marker of systemic lipid peroxidation (42). A similar effect on urinary isoprostane concentrations were observed in pre- and postmenopausal women following treatment with lyophilized grape powder for 4 wk (43). At the present time, no study, to our knowledge, has shown a relation between polyphenol consumption and reduced atherosclerosis in humans. It remains to be determined whether inhibition of LDL oxidation is a clinically relevant mechanism in humans.

Endothelial function. The vascular endothelium plays a central role in the regulation of vascular tone, thrombosis, local inflammation, and cell proliferation by producing paracrine factors that act on the arterial wall and blood cells (44). Endothelium-derived nitric oxide (NO) is a vasodilator and inhibits platelets, leukocyte adhesion to the endothelial surface, and proliferation of vascular smooth muscle cells. When healthy, the endothelium promotes a vasodilator, antithrombotic, and

antiinflammatory state. However, cardiovascular disease risk factors alter endothelial phenotype in a manner that promotes atherogenesis, lesion progression, and plaque vulnerability.

In humans, endothelial dysfunction is associated with traditional risk factors and established atherosclerosis (45). Furthermore, endothelial dysfunction predicts progression of atherosclerosis and incidence of cardiovascular events (46,47). Furthermore, endothelial dysfunction is reversed by a number of interventions proven to reduce cardiovascular risk, such as lipid-lowering therapy, angiotensin-converting inhibitors, smoking cessation, and weight loss (44). Failure of the endothelium to respond to such therapy identifies patients at higher risk (48).

In vitro studies have demonstrated favorable effects of grape products on endothelial function. In cultured endothelial cells, wine, grape juice, grape seed extract, and specific polyphenols increase the activity of the endothelial isoform of NO synthase and stimulate NO production (49,50). In the short term, polyphenols stimulate endothelial NO synthase phosphorylation via phosphatidylinositol-3-hydroxy kinase and Akt (51). Longer term exposure to red wine extracts or resveratrol stimulates an increase in enzyme expression and activity (49,50). The effects of resveratrol on endothelial function may be mediated through an effect on Sirtuin-1, which regulates the expression of genes related to cell survival and the stress response (6,52). Furthermore, activation of Sirtuin-1 decreases the activity of p53, a regulator of apoptosis and the cell cycle, and activates AMP-dependent protein kinase, a regulator of cellular energy status (53).

Human studies support a benefit of grape beverages on endothelial function (54). Stein et al. (41) observed that consumption of purple grape juice for 2 or 4 wk improved endothelium-dependent brachial artery flow-mediated dilation in patients with coronary artery disease. Dealcoholized wine also improved brachial artery flow-mediated dilation in healthy subjects (55). Interestingly, red wine consumption prevents the acute impairment of endothelial function that occurs following cigarette smoking (56) or consumption of a high-fat meal (57).

In addition to the effects on NO, grapes have important effects on other molecular aspects of vascular function. For example, flavonoid-containing beverages increase endothelial production of prostacyclin and suppress production of endothelin-1, a potent endothelium-derived vasoconstrictor (58,59). In regard to regulation of fibrinolysis, catechins and resveratrol increase protein levels and activity of tissue plasminogen activator, an effect that is likely to be cardioprotective (60). Finally, there is increasing evidence that polyphenols affect endothelial regulation of inflammation. Red wine constituents reduce adhesion of monocytes to the endothelial surface and block cytokine-induced expression of endothelial adhesion molecules (61). Thus, grape polyphenols induce multiple favorable changes in endothelial cell phenotype that could reduce cardiovascular risk.

Antiplatelet effects. Platelets play a critical role in all phases of atherosclerosis. Antiplatelet drugs, particularly aspirin, have proven beneficial effects on cardiovascular risk. Because polyphenols have been shown to have platelet inhibitory effects, there is great interest in the possibility that grape consumption might provide similar protection. In vitro studies have shown that grape-derived polyphenols inhibit platelet activity and elucidated a number of potential mechanisms. Flavonoids inhibit cyclooxygenase and reduce production of thromboxane A₂. Red wine polyphenols also decrease platelet production of hydrogen peroxide and inhibit activation of phospholipase C and protein kinase C (62). Dilute grape juice inhibits platelet

aggregation and this effect is associated with decreased production of superoxide anion and increased platelet NO production (34).

Feeding grape juice to animals also has important antiplatelet effects. Demrow et al. (63) used a coronary artery platelet aggregation model (Folts model) that mimics acute coronary syndromes to demonstrate platelet inhibition following oral administration of red wine to dogs. Similar effects were observed in monkeys (64) and these effects have been shown to depend on NO production (65).

Human studies have also demonstrated antiplatelet effects of grape-derived beverages. Freedman et al. (34) demonstrated that grape juice consumption for 14 d decreased platelet aggregation and superoxide production and increased NO production in healthy volunteers. In that study, grape juice also inhibited protein kinase C and spared cellular antioxidants. Red wine has more potent antiplatelet effects than white wine (66) and these effects are not seen with other beverages, such as orange juice and grapefruit juice, which contain other antioxidants (67).

Antiinflammatory and other mechanisms. The importance of inflammation for all stages of atherosclerosis is increasingly recognized and there are data suggesting that grape polyphenols have antiinflammatory effects. For example, red wine and polyphenols inhibit activation of nuclear factor- κ B and production of proinflammatory factors in endothelial cells and inflammatory cells (61,68). Incubation of monocytes with catechin decreased their adhesion to endothelial cells (69). Relevant polyphenols also inhibit activation of nuclear factor- κ B in T lymphocyte cell lines (70). Resveratrol has also been shown to have antiinflammatory effects, including inhibition of adhesion molecule expression (6,71). In humans, treatment with lyophilized grape powder for 4 wk was associated with a reduction in tissue necrosis factor- α , but not C-reactive protein or interleukin-6 (43). Wine and gin consumption for 4 wk also reduced systemic markers of inflammation in healthy men and the effect was more marked following wine consumption (72). Thus, antiinflammatory effects might be a contributing mechanism for the benefits of grape polyphenols against cardiovascular disease.

Clinical implications

As reviewed in the preceding sections, there is strong epidemiological evidence linking reduced cardiovascular risk with consumption of grapes and other polyphenol-rich foods. Experimental and translational studies suggest several important mechanisms that might account for such an effect. An important remaining question is how to incorporate these data into specific recommendations for dietary intake for the general public. A detailed discussion of these issues is beyond the scope of this review, but a number of factors complicate the formulation of such recommendations at the population level. For example, obtaining an accurate estimate of current intake is confounded by structural diversity, wide distribution in foods, and variations in content of polyphenols (73). Polyphenol bioactivity is further clouded by variable bioavailability due in part to differences in food matrix and human gut absorption (74,75). Ultimately, such efforts are limited, because it remains uncertain which components of polyphenol-rich foods actually confer benefit.

In addition to developing better dietary recommendations, emerging studies showing cardiovascular benefits of polyphenol-rich foods have also prompted interest by the pharmaceutical industry. It is possible that purified formulations of specific grape constituents, e.g. resveratrol, might be efficacious as drugs for cardiovascular disease treatment or prevention. Indeed, such promise has led to identifying synthetic polyphenol analogs that

might have greater potency and therapeutic potential. Such compounds will require large-scale trials before they can be approved for clinical use. In the meantime, the available evidence supports a diet rich in fruits and vegetables, including grapes, as an appropriate strategy to reduce the risk of cardiovascular disease.

Other articles in this supplement include references (92–98).

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