Grape Extracts and Risk Factors for Cardiovascular Disease

a report by

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Introduction

In spite of advances in diagnosis and treatment, cardiovascular disease (CVD) continues to be the leading cause of mortality in the US.[1] Primary prevention offers new hope for combating the problem. Atherosclerosis, the underlying pathology of CVD, silently evolves starting when an individual is as young as 10-12 years old and potentially produces a serious clinical event by the age of 50 or 60. Thus, primary prevention must be built on a foundation of healthy dietary habits and lifestyle during early childhood. While the etiology of atherosclerosis is very complicated, hypercholesterolemia, oxidative modification of low density lipoproteins (LDL), endothelial dysfunction, and platelet hyperactivity are involved in the development of atherosclerosis and the precipitation of the fatal thrombotic event. Dietary habits that attenuate these risk factors are likely to be valuable for preventive cardiology.

Epidemiological studies indicate an inverse relationship between the consumption of fruits and vegetables, rich in polyphenolic compounds, and cardiovascular disease.[2] These findings offer an explanation for the 'French Paradox', the paradoxical observation that the French have a lower incidence of cardiovascular disease than Americans, despite having similar cardiac risk factors.[3] The French consume red wine, a rich source of grape polyphenolics, with their meals. Red wine polyphenolics have been shown to lower cholesterol in hypercholesterolemic animals,[4] and to improve endothelial function,[5,6] attenuate platelet activity,[7–9] and protect LDL from oxidation[10] in animals and humans. The beneficial effects of red wine have recently been reviewed.[11] Similarly, purple grape juice, another rich source of grape polyphenolics, has also been shown to lower cholesterol in animals,[12] and to improve endothelial function,[13] attenuate platelet activity,[14] and protect LDL from oxidation[13] in humans.

Despite the beneficial effects of red wine polyphenolics, the consumption of red wine cannot be recommended to the public because abuse of alcohol continues to plague society. Furthermore, alcohol has the potential problem of being pro-oxidant[15] and pro-inflammatory[16] which may outweigh the benefits offered by the grape polyphenolics in wine. While grape juice would be a good non-alcoholic alternative, its natural sugar content would be unwise for segments of the population combating diabetes and obesity. It is important to note that purple grape juice (containing 43g sugar/10oz) does not contain significantly higher amounts of sugar compared with other fruit juices (orange 35g/10oz, apple 38g/10oz, and cranberry cocktail 48g/10oz). In addition, we have shown that purple grape juice is a more effective platelet inhibitor when compared with orange juice or grapefruit juice.[14] Given the potential disadvantages of red wine and purple grape juice consumption, polyphenolic extracts of grapes may be a promising alternative. The polyphenolic compounds in grape beverages are obtained primarily from the seeds and skins of the grapes. Thus, extracts of grape seed (GSD) and grape skin (GSK) contain similar polyphenolics as red wine and purple grape juice but lack the alcohol and sugars. In this article, the effect of grape extracts on some of the risk factors for CVD is examined.

HyperCholesterolemia

Hypercholesterolemia is a sufficient, but not exclusive, cause for cardiovascular disease.[17] An increase in LDL in the blood results in a proportional increase in the LDL deposited in the arterial wall. The deposition of LDL within the arterial wall initiates a cascade of events, to be discussed later, that enhances the development of atherosclerosis. Thus, lowering of LDL cholesterol level is often one of the early targets of clinical therapy. Red wine[4] and grape juice[18] have been shown to reduce cholesterol levels in hypercholesterolemic animal models.[12] Surprisingly, several studies found that GSD extracts did not exhibit this cholesterol-lowering effect.[19–21] This may well depend on the source and content of the GSD extract. In addition, it is possible that the cholesterol-lowering properties of red wine and grape juice were due to the presence of polyphenolics in the grape skin (anthocyanins, hydroxycinamates and procyanidins) and not those in the grape seed (predominantly procyanidins). No studies with GSK have been done thus far to address this question. It is also possible that the presence of soluble fibers in red wine and grape juice may have been responsible for the observed cholesterol-lowering effect, as suggested in a study with grape pomace.[22]

LDL Oxidation

LDL in its native form contributes very little to the atherosclerotic process.[17] Reactive oxygen species (ROS) generated by normal and abnormal cellular metabolism, are thought to modify LDL and this modification is thought to be a key step in atherogenesis.[23] This modified LDL is taken up by macrophages at a faster, unregulated rate than native LDL.[24] The uptake eventually leads to the death of the cholesterol-engorged macrophages and their transformation into foam cells.[24] Progression of atherogenesis with further deposition of foam cells, the proliferation and migration of smooth muscle cells and the increased production of extracellular matrix lead to a thickening of the arterial wall and narrowing of the arterial lumen. If the initial modification of the LDL can be reduced, the rate of development of atherosclerosis could be attenuated.

Grape polyphenolics have been shown to inhibit LDL oxidation and the initial onset of atherosclerosis.[25,26] In an *in vitro* study, a flavonoid-rich grape extract dose-dependently inhibited several markers of Cu^{2+}-induced LDL oxidation including the lag time to formation of conjugated dienes and thiobarbituric acid reacting substances (TBARS).[27] Bagchi and colleagues demonstrated that a grape seed proanthocyanidin extract was more effective at scavenging free radicals *in vitro* and protecting against 12-tetradeca-noylphorbol-13-acetate (TPA)-induced hepatic and brain lipid peroxidation *in vitro* than vitamin E and C.[28] Grape extracts may also spare the endogenous enzymes responsible for protecting against oxidative damage. A single oral gavage of grape pomace extract prevented the decrease in the liver enzymes catalase, superoxide dismutase and peroxidase in rats administered CCl4, an *in vivo* model of oxidant stress. The extract also prevented the three-fold increase in liver lipid hydroperoxides produced by CCl4.[29] These *in vitro* and animal studies suggest that human consumption of grape extracts may offer protection against oxidative stress.

Consumption of red wine (375 mL/d), red wine extract (1g/d), or white wine with red wine extract added for two weeks increased lag time to LDL oxidation and decreased lipid peroxides and TBARS in human subjects. However, consumption of white wine alone failed to show any beneficial effects.[30] The polyphenolics present in the red wine and red wine extract, but not in the white wine, appeared to protect the LDL from oxidation. Grape extracts containing biologically active polyphenolic groups may be especially beneficial in combating increased oxidative stress.

An acute increase in oxidative stress is observed in the postprandial state.[31] Antioxidant protection from grape polyphenolics may be most helpful during this four to five hour-period. We believe that consumption of red wine by the French, especially with their meals, may help explain the French Paradox. Fuhrman demonstrated that the consumption of red wine with meals for two weeks reduced the susceptibility of LDL to oxidation and increased the antioxidant capacity of plasma in healthy volunteers.[10] Natella *et al*. showed that the consumption of 300mg GSD proanthocyanidins with a test meal prevented the postprandial increase in lipid hydroperoxides. Plasma ascorbate and (-tocopherol levels were also significantly increased after the test meal supplemented with GSD extract, but not after the control meal. In addition, the reduced form of glutathione, an endogenous antioxidant, was slightly increased, though not significantly, in the GSD extract supplemented meal, while it was significantly decreased after the control meal.[32] The preservation of these endogenous antioxidants in the plasma may be due to the antioxidant sparing effect of the polyphenolics in the GSD extract. Though antioxidants in the plasma likely play an important role in the protection of LDL, an association or attachment of the polyphenolics to the LDL may better protect it against oxidative modification once it enters the arterial wall. In an *in vitro* study, LDL was incubated with flavonoid sources then washed to remove unbound flavonoids. The LDL pre-treated with GSD extract retained greater protection against oxidation than those pre-treated with GSK extract, purple grape juice, red wine or dark beer.[33] This suggests greater association/attachment of GSD extract to LDL than the other flavonoid sources. The GSD extract used was comprised mostly of large polymers,[34] which may have more potential binding sites and may associate more strongly with the LDL to help protect against unwanted modification.

Endothelial Dysfunction

The release of nitric oxide (NO) by a healthy vascular endothelium, which consists of a single layer of endothelial cells, prevents the adherence of platelets and leukocytes to the arterial wall.[27] In addition to its anti-atherogenic properties, NO also stimulates the vascular smooth muscle to relax and produce vasodilation. However, the endothelium can be damaged by many of the known vascular disease risk factors such as hypercholesterolemia, hypertension, diabetes and cigarette smoking, thus reducing the bioavailability of NO.[35]

A number of studies have demonstrated that the polyphenolic (flavonoid) compounds derived from grape products can improve endothelial function and increase endothelial NO production.[36] Fitzpatrick and colleagues have shown, using isolated rings of arterial tissue, that red wine, grape juice, and extracts of GSD and GSK can produce improvements in endothelium-dependant vasodilation.[37] In a similar study, flavonoids extracted from

red wine produced arterial vasorelaxation through a Ca^{2+}-dependant increase in NO production.[38] Human studies measuring increases in flow-mediated dilation of the brachial artery have shown that the consumption of grape juice[13] and red wine[6] can improve endothelial function *in vivo*. Agewall and colleagues found similar improvement in brachial artery flow-mediated dilation by a red wine extract.[39] Fitzpatrick's group has recently shown that the larger polymeric polyphenolics in GSD extract were more vasoactive than the smaller ones.[40]

The release of ROS is increased in states of oxidative stress and neutralizes the beneficial effects of NO by converting it to peroxynitrite. Release of NO and superoxide simultaneously results in the generation of the cytotoxic oxidant peroxynitrite. It has been demonstrated that procyanidins from GSD can protect endothelial cells from peroxynitrite damage, and enhance endothelium-dependant relaxation of isolated human arteries.[41] Damaged endothelial cells also produce endothelin 1 (Et-1), a potent endothelium-derived vasoconstrictor, which exacerbates the atherosclerotic process. Khan *et al.* showed that a red wine extract significantly inhibited the production of Et-1 by cultured bovine endothelial cells.[42]

Platelet Hyperactivity

Activated platelets are known to release growth factors that stimulate the proliferation and migration of vascular smooth muscle cells, which contribute to the developing atherosclerotic plaque.[43] Activated platelets also release ROS and increase oxidative stress. Thus, platelets can contribute to oxidation of LDL and impair endothelial function, which further aggravates the atherosclerotic process described above. Finally, when a vulnerable plaque ruptures, aggregating platelets initiate the acute fatal thrombosis. Therefore, reducing platelet activity could reduce athero-thrombotic events. Grape products have been shown to reduce platelet activity.[14,44] A red wine and red wine extract inhibited ADP-induced platelet rich plasma (PRP) aggregation in a dose-dependant manner.[45] Vitseva *et al.* demonstrated that platelets incubated with GSD and GSK extracts reduced thrombin receptor agonist peptide (TRAP)-induced platelet aggregation, increased NO and decreased superoxide release from the activated platelets.[46] The increase in NO and decrease in superoxide attenuate multiple mechanisms that are central to the development and progression of atherosclerosis.

Umar and colleagues demonstrated that a two-week consumption of an extract of armagnac, a wine aged in oak barrels, reduced thrombus size in an *in vivo* rat shunt model. *In vitro* incubations of the extract with human blood inhibited ADP-, but not collagen-induced PRP aggregation in a dose-dependant manner. The extract inhibited platelet aggregation more significantly with the presence of hypoxanthine-xanthine oxidase, used to simulate increased oxidative stress,[47] suggesting that grape polyphenolics may inhibit platelets in part by an antioxidant mechanism. As the grape wines and juices contain flavonoids from both the seeds and skins, it may be beneficial to combine extracts of each. Our group showed that feeding a combination of GSD and GSK extracts was more effective in decreasing collagen-induced *ex vivo* platelet aggregation in dogs than giving either one alone (see *Figure 1*). This effect was also seen *in vitro* when the GSD and GSK extracts were incubated together with human blood.[34] As the types of flavonoids contained in GSD and GSK are different, the two may complement each other in their action. Thus, a combination of GSD and GSK extracts may provide better protection from heart disease than either one alone.

Human consumption of GSD and GSK extracts may also have a platelet-inhibitory effect. However, the effect may be time- and dose-dependent. We have shown that a four-week consumption of GSD and GSK extracts by healthy humans produced a moderate, but statistically insignificant, reduction in collagen-induced platelet aggregation. However, an eight-week consumption produced a greater, statistically significant reduction in platelet activity (unpublished data; see *Figure 2*). This decrease in platelet aggregation over time may have been due to an increase in tissue-accumulation of polyphenolics.

The widely-used Folts *in vivo* model of coronary artery stenosis with periodic platelet-mediated thrombosis is thought to mimic the problem occurring in patients with unstable angina and acute coronary syndromes.[48] In this model, aspirin has been shown to protect the animals from acute coronary thrombosis. However, aspirin treatment does not prevent the renewal of coronary thrombosis when plasma epinephrine is elevated in these animals.[49] Humans taking aspirin to protect against platelet mediated thrombosis are not always protected either.[50] However, in the Folts model, administrating GSD and GSK extract treatment protected against the renewal of thrombosis when plasma epinephrine levels were elevated.[51] Thus, the flavonoids in GSD and GSK extracts appear to inhibit platelet activation by a different mechanism than aspirin.

Conclusion

Red wine and purple grape juice attenuate several risk factors for CVD. The potential for grape extracts to similarly inhibit multiple risk factors may also be useful in slowing the initiation and progression of atherosclerosis. However, further studies with grape extracts are needed to determine the best source of seed and skin, the best method of extraction and the proper dose and duration of administration for a desired favorable biological effect. In addition, more comprehensive chemical analysis of the

extracts is needed to determine which of the many groups of polyphenolics in grape seeds and skins are responsible for their potential health benefits. Combinations of the GSD and GSK extracts, containing a wider variety of potentially active components, may better target multiple mechanisms of atherosclerosis and thus, more effectively protect against CVD than a single source. Prospective double blind, placebo controlled studies of the grape extracts over longer periods of time are greatly needed to confirm efficacy. ■

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