

Review

Dietary flavonoids: effects on endothelial function and blood pressure

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Abstract: Several population studies have found an inverse association between flavonoid intake and risk of cardiovascular disease. These studies have resulted in the hypothesis that dietary flavonoids protect against cardiovascular disease. Many *in vitro* studies, studies using animal models and human intervention trials have been carried out to investigate how flavonoids might provide protection. Emerging and largely consistent evidence suggests that flavonoids can improve endothelial function and may reduce blood pressure. *In vitro* studies show that a variety of flavonoids cause vasorelaxation of isolated arteries from rats. In human intervention trials, flavonoids derived from tea and cocoa or dark chocolate – both rich sources of catechins – have been found to improve endothelial function acutely and with regular ingestion. The evidence for benefits of flavonoids from other dietary sources is less clear. Improvements in endothelial function could contribute to lower blood pressure. Population studies have associated higher intake of tea and chocolate with lower blood pressure. Short-term intervention studies in humans have shown blood pressure lowering with cocoa or dark chocolate, but short-term regular ingestion of tea has not been found to lower blood pressure. The long-term effects of regular ingestion of a flavonoid-enriched diet on endothelial function and blood pressure have yet to be assessed. In addition, there is evidence that flavonoid metabolism is an important factor influencing the biological activity and effects of dietary flavonoids, but further studies are needed to investigate this area.

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INTRODUCTION

Over the past two decades there has been considerable research interest in the potential health benefits of flavonoids. Results of population studies suggest that dietary flavonoids provide modest protection against cardiovascular disease.^{1,2} There is also a growing body of evidence from controlled trials that dietary flavonoids can improve endothelial function and reduce blood pressure in humans,³ and inhibit the development of atherosclerosis in animal models.⁴ These effects may be at least partly responsible for any reduction in the risk of cardiovascular disease.

DEFINITIONS AND DIETARY SOURCES

Many thousands of polyphenolic compounds are produced as secondary plant metabolites. Within the plant they have a diverse range of functions. When ingested by humans they may provide health benefits. Flavonoids are a major class of polyphenols. They have a C₆–C₃–C₆ structure consisting of two aromatic rings that are linked together by a three-carbon unit to form an oxygenated heterocycle. The A ring is characteristically of the phloroglucinol or resorcinol hydroxylation pattern and the B ring usually 4-, 3,4-

or 3,4,5-hydroxylated (Fig. 1). The six major classes of flavonoids include flavonols, flavones, flavanols, flavanones, anthocyanins and isoflavones (Table 1). The structures of four of the flavonoids which have been of considerable research interest are presented in Fig. 1. A great deal of the available data on health effects of flavonoids relates primarily to foods and beverages rich in flavonols, flavanols or isoflavones.

CARDIOVASCULAR DISEASE IN POPULATION STUDIES

The relationship between flavonoid intake and risk of cardiovascular disease has been investigated in a number of epidemiological studies.^{2,3} There have now been 12 prospective studies on flavonoid intake and coronary heart disease and five prospective studies on flavonoid intake and stroke.² Seven studies suggest a protective effect on coronary events and or death: six for flavonols and/or flavones and one for flavanols. Two studies suggest a protective effect of flavonols and flavones against stroke. Huxley and Neil⁵ performed a meta-analysis of seven prospective studies of flavonoids in relation to coronary heart disease. The highest tertile of flavonoid intake was

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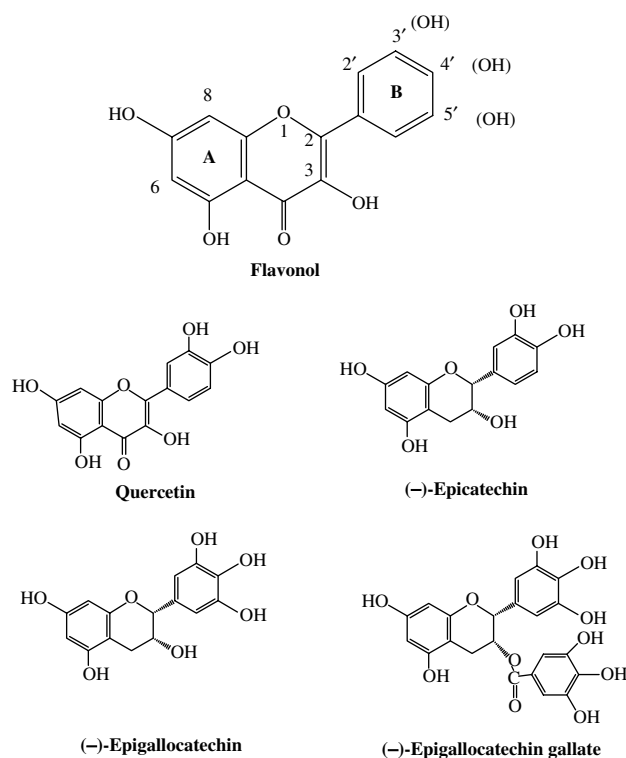
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Table 1. Flavonoid classes, representative flavonoids and food and beverages sources

Class	Representative flavonoids	Main food and beverage sources
Flavonols	Quercetin; kaempferol; myricetin	Tea; apples; onions
Flavones	Apigenin; luteolin	Herbs; vegetables
Flavanols	Catechins; procyanidins	Tea; red wine; red grapes; grape seeds; cocoa
Flavanones	Hesperidin; naringenin	Citrus fruits
Anthocyanins	Cyanidin	Berries
Isoflavones	Genistein; daidzein	Soy protein-containing foods

**Figure 1.** Structures of four flavonoids that have been of considerable research interest.

associated with a 20% reduction in the risk of fatal coronary heart disease in comparison the lowest tertile of intake. The lack of benefit observed in some studies could be explained by either a uniformly very low or high flavonoid intake within these populations.⁶

Cross-sectional and prospective population studies have also investigated the relationship of polyphenol-rich foods and beverages with cardiovascular disease. Results of more than ten prospective studies suggest that three cups of tea per day (rich in catechins) can reduce coronary heart disease risk by about 10%.⁷ Data from cross-sectional studies would also suggest a similar protective effect of tea.⁷ A recent cross-sectional study from the Zutphen Elderly Study suggests that cocoa (rich in catechins and procyanidins) intake is inversely associated with blood pressure and all-cause mortality.⁸ The available data from population studies on red wine (rich in procyanidins) consumption are more difficult to interpret because of the confounding effect of alcohol. There is some suggestion that light-to-moderate consumption of red wine may have

greater cardiovascular benefits than similar alcohol intake from beer.⁹ This may be related to the high flavonoid content of wine but could also be due to other confounding lifestyle factors such as diet, socioeconomic status or pattern of alcohol consumption.¹⁰ Isoflavone intake can be linked closely to the intake of foods containing soy protein. To date, one prospective study has investigated the link between soy protein intake and coronary heart disease events and found a protective relationship.¹¹

Although these studies do not establish a causative link, the overall evidence from population studies does suggest that a higher flavonoid intake may provide modest protection against cardiovascular disease.^{2,3}

ANIMAL MODELS OF ATHEROSCLEROSIS

The effects of flavonoid-rich foods or extracts on the development of atherosclerosis have been studied in the apoE deficient mouse and in hamsters. These studies are consistent in demonstrating favourable effects. In the apoE deficient mouse, which develops human-like atherosclerotic plaques, inhibition of lesion development has been demonstrated with tea,¹² red wine-derived polyphenols^{4,13-15} and isolated quercetin or catechin.¹³ Oral supplementation with a pure phenolic acid derivative from honey, caffeic acid phenethyl ester, also attenuates atherosclerosis in the apoE deficient mouse.¹⁶ Similar inhibition has been found with tea¹⁷ and red-grape extracts¹⁸ in the cholesterol-fed hamster. Some studies have suggested a dissociation of the anti-atherosclerosis activity with oxidative damage.^{4,15} This suggests that the bioactivity of the polyphenols may not be due to antioxidant activity *in vivo*.

FLAVONOIDS AND CARDIOVASCULAR DISEASE RISK FACTORS

Intervention studies in humans have explored the effects of flavonoids and flavonoid-rich foods on a range of cardiovascular disease-related endpoints.¹⁹ These have included processes involved in the pathogenesis of vascular disease such as oxidative damage and inflammation, cardiovascular disease risk factors such as blood lipids, homocysteine, blood pressure and body weight, and markers of vascular disease such as arterial compliance and endothelial function. For many of these endpoints, the results are mixed and a consistent picture has yet to emerge.

There has been considerable research interest in flavonoids as antioxidants. Flavonoids possess potent antioxidant activity *in vitro*. However, despite many investigations, there remains limited evidence to suggest that flavonoids can actually inhibit oxidative damage *in vivo*.^{4,20–22} Manach *et al.*²³ in a review of the most recent human intervention trials with antioxidant/oxidant endpoints highlight the large discrepancies in this area. In addition, studies showing inhibition of atherosclerosis in animal models point to mechanisms other than antioxidant effects.^{4,15,18}

Results of *in vitro* studies, studies in animal models and population studies suggest that flavonoids could reduce blood cholesterol concentrations. However, many human intervention studies have found little or no change in blood lipid and lipoproteins with increased flavonoid intake.^{23,24} Overall, the available data suggest that flavonoids may have a small beneficial effect on blood cholesterol concentrations in individuals with elevated blood cholesterol concentrations.

For a variety of other cardiovascular disease-related endpoints, the data are limited to only a few investigations. Additional studies are needed to confirm suggested benefits of flavonoids on platelet function,²⁵ inflammation,²⁶ body fatness²⁷ and homocysteine.²⁸

FLAVONOIDS AND ENDOTHELIAL FUNCTION

In vitro studies

Isolated vessels from animals can be used to assess the effects of potentially vasoactive substances *in vitro*. The effects of individual flavonoids on the relaxation of isolated arteries from rats have been investigated in many studies. The relaxation responses of flavanols, flavones, flavanols, flavanones, anthocyanins and isoflavones have all been assessed in this model. These studies show that flavonoids can cause vasorelaxation at physiological concentrations. The relaxation observed is largely endothelium-dependent and nitric oxide (NO)-dependent, although other mechanisms also appear to be involved.^{29–34}

Human intervention trials

Consistent benefits of flavonoids from some dietary sources (catechin-rich foods and beverages) have been observed on endothelial function. In humans, one of the main methods to investigate endothelial function has been to use ultrasonography focusing on conduit vessels, such as the brachial artery.³⁵ This is a non-invasive technique that measures vasodilation of the artery in response to shear stress induced by increased blood flow. This is known as flow-mediated dilatation and is largely NO-dependent.³⁶ The validity of this technique is supported by observations showing that abnormalities in the peripheral circulation are associated with a range of cardiovascular risk factors,³⁷ with abnormal vasotonic responses in the coronary circulation³⁸ and with increased risk of coronary events.³⁹

The effect of dietary flavonoids on endothelial function in humans has been an area of major interest in recent years.^{3,23} Accumulating data suggest that flavonoids can improve endothelial function.^{40–53} These studies can be broadly categorised based on the dietary source of flavonoids used into tea, cocoa and dark chocolate, red wine and related sources, and soy isoflavones.¹⁹ In controlled intervention trials chronic^{40,41} and acute^{41–43} ingestion of tea has been shown to improve endothelial function. Similarly, improved endothelial function has been demonstrated in several studies with flavonoid-rich cocoa or dark chocolate.^{44–48} The results of Schroeter *et al.*⁴⁸ suggest that epicatechin in cocoa is primarily responsible for the vascular effects. Oral administration of pure (–)-epicatechin showed similar acute vascular effects as flavanol-rich cocoa. These effects were likely due to augmentation of NO synthesis by flavanols. Tea also contains epicatechin, but at lower concentrations, and contains high concentrations of other catechins with a similar structure.⁵⁴ The data on red wine and related sources is mixed. A controlled trial with very small numbers by Cuevas *et al.*⁴⁹ did suggest benefit of red wine, whereas other controlled trials have shown no effect.^{55–58} Several uncontrolled trials using de-alcoholised red wine or purple grape juice have shown a significant improvement in endothelial function.^{50–52} Many randomised controlled trials have now investigated the effect of isolated isoflavones^{59–63} or soy protein containing isoflavones^{53,64–66} on endothelial function in the brachial artery. The results of these studies suggest that isoflavones do not significantly improve endothelial function. However, studies with endothelial or vascular function outcomes other than flow-mediated dilatation are suggestive of benefits,^{67–69} indicating that different mechanisms could be involved.

FLAVONOIDS AND BLOOD PRESSURE

Effects of flavonoids to improve endothelial function may be at least partially responsible for any reduction in risk of cardiovascular disease. Acute and sustained improvement in endothelial function could contribute to lower blood pressure. Dietary flavonoids can reduce blood pressure in the spontaneously hypertensive rat.⁷⁰ Studies of the effects on blood pressure of short-term regular ingestion of flavonoids in humans provide mixed results.^{47,57,58,71–73} Three short-term studies have shown blood pressure lowering with flavonoid-rich cocoa or dark chocolate.^{47,72,73} In contrast, short-term regular ingestion of tea for up to 4 weeks appears not to significantly alter blood pressure.^{40,71} Studies showing that soy protein containing isoflavones can reduce blood pressure^{74,75} are likely to be confounded by an effect of protein *per se* to reduce blood pressure.⁷⁶ Isolated isoflavones appear to have little or no effect on blood pressure, at least in the short term.⁷⁷

Investigations of the possible longer-term effects of regular ingestion of flavonoids on blood pressure are limited to cross-sectional studies assessing

tea intake^{78–81} and chocolate intake.⁸ Inverse relationships of tea intake^{78,79} and a marker of tea flavonoid exposure⁷⁹ with blood pressure, and of tea intake with prevalence of hypertension^{80,81} have been reported. Recent cross-sectional data from the Zutphen Elderly Study suggest an inverse association between cocoa intake and blood pressure and cardiovascular mortality.⁸ Relationships of red wine intake with blood pressure are influenced by the more potent effect of alcohol to raise blood pressure.⁵⁸

FLAVONOID METABOLISM: BIOLOGICAL ACTIVITY AND EFFECTS

Absorbed flavonoids are often rapidly metabolised. This may be relevant to biological activity and effects of dietary flavonoids. An important pathway of flavonoid metabolism following absorption is *O*-methylation by catechol-*O*-methyltransferase (*COMT*).⁸² It appears that much of the absorbed flavonoids are methylated, but the degree of flavonoid metabolism may vary between individuals. Flavonoids can act as acceptors of methyl groups, becoming *O*-methylated via the action of *COMT* during metabolism of methionine to homocysteine. Therefore, dietary polyphenols have the potential to raise total plasma homocysteine concentrations (tHcy).²⁸ In addition, the *O*-methylation of flavonoids reduces endothelial exposure to unaltered compounds and may alter vasodilator activity. Therefore, differences in flavonoid metabolism have the potential to influence biological activities and effects. The activity of *COMT* can vary as much as three-fold between individuals,⁸³ which could contribute to the variability in observed flavonoid methylation.⁸⁴ The *COMT* activity is related to genetic variation in the *COMT* gene. There are two main genotypes: low activity *COMT* and high activity *COMT*.⁸³ This is likely to be a major factor, but not the only factor influencing flavonoid methylation.

Biological effects

We have previously investigated the potential importance of flavonoid methylation in intervention trials.^{28,85} In individuals drinking five cups per day of black tea for 4 weeks, we assessed the relationship between degree of *O*-methylation of the tea-derived polyphenols and the change in tHcy. Overall, regular ingestion of black tea did not alter tHcy. However, the degree to which individuals *O*-methylated tea-derived flavonoids was positively associated with change in tHcy.²⁸ These results suggest that individual differences in *O*-methylation may influence the ultimate effects of black tea on tHcy. More recently we have investigated whether changes in endothelial function following chronic and acute ingestion of tea were related to the *O*-methylation of tea-derived flavonoids.⁸⁵ During chronic ingestion of tea, the degree to which individuals *O*-methylated tea-derived flavonoids was negatively associated with change in flow-mediated dilatation responses. Those individuals

who methylated fewer of the flavonoids had more of an improvement in endothelial function. There were similar findings during acute tea consumption. That is, any improvement in flow-mediated dilatation following ingestion of tea may be enhanced in individuals who *O*-methylate fewer of the absorbed flavonoids.⁸⁵ Thus, differences in flavonoid metabolism could be related to the level of benefit of dietary flavonoids on the risk of cardiovascular disease, but additional studies are needed to investigate this hypothesis.

In vitro activities of flavonoid metabolites

The available data on the effects of *O*-methylation of flavonoids on *in vitro* activities are limited.^{86–88} Most data from the many hundreds of *in vitro* studies are based on the use of flavonoids present in foods, and not as they exist in the circulation. This may limit the validity of the studies in extrapolating the data to human physiology.⁸⁹ We have shown that *O*-methylation of a phenolic compound can significantly reduce its antioxidant activity.⁸⁶ Koga and Meydani⁸⁷ found that catechin metabolites were more effective than catechin in inhibiting monocyte adhesion to endothelial cells. Spencer *et al.*⁸⁸ found that the protection against cell death induced by hydrogen peroxide elicited by 3'-*O*-methyl epicatechin is not significantly different from that of epicatechin. Further studies are clearly needed to investigate *in vitro* activities of flavonoids as they occur in the circulation.

CONCLUSIONS AND FUTURE RESEARCH

Population and intervention studies indicate that a higher intake of flavonoids protects against cardiovascular disease. Accumulating evidence suggests that flavonoid-rich foods and beverages can improve endothelial function and may reduce blood pressure. This may at least partly explain any benefit on cardiovascular disease risk. However, several questions have yet to be adequately addressed. The effects on endothelial function, blood pressure and other cardiovascular disease risk factors of long-term regular ingestion of a high flavonoid diet have not been assessed in a controlled trial. Long-term studies are important to confirm the benefits suggested by results of acute and short-term intervention studies, and for the translation of findings into appropriate public health advice. It is also uncertain whether changes in blood pressure follow improvements in endothelial function, and the extent to which any changes in blood pressure are due to acute vasoactive effects. Another unanswered question relates to whether or not a range of flavonoids of similar structure elicit the same vascular effects. The recent studies with cocoa suggest that epicatechin is primarily responsible for the effects of cocoa/dark chocolate to improve endothelial function. It remains uncertain whether a range of catechins as well as other classes of flavonoids have the same activity. These investigations will help to establish if cardiovascular benefits are likely to be limited to a

small number or a wider range of foods and beverages. Finally, results of recent trials with tea suggest that flavonoid metabolism could be an important determinant of biological effects. The importance of flavonoid O-methylation requires further investigation.

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