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Review Article

The Cardioprotective Effects of Flavonoids -

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ABSTRACT

Cardiovascular Disease (CVD) is the leading cause of death worldwide. Flavonoids derived from plants and fruit polyphenols offer cardioprotective benefits. Epidemiologic studies have demonstrated that flavonoid intake is inversely associated with risk of CVD. Flavonoids are phenolic compounds that possess antioxidant, anti-inflammatory, anti-thrombotic and vasodilatory properties that may enhance cardiovascular health. This review presents the main sources of flavonoids, their intake, metabolism, potential mechanisms of action and the evidence for an important preventive role of flavonoids in cardiovascular health.

Keywords: Endothelial function; Polyphenols; Vascular

ABBREVIATIONS

CVD: Cardiovascular Disease; LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; NO: Nitric Oxide; eNOS: Endothelial Nitric Oxide Synthase; FMD: Flow Mediated Dilation; NF- κ B: Nuclear Transcription Factor

INTRODUCTION

Cardiovascular Disease (CVD) remains the leading global cause of death [1]. It is predicted that by 2030, 44% of the population in the United States will have CVD [2]. Nutritional strategies with increasing intake of flavonoids may decrease the risk of CVD. Initial interest in the consumption of flavonoids was prompted by the “French Paradox” when cardiac death rates were found to be lower in populations with high saturated fat intake [3]. They also consumed wine with polyphenol resveratrol, which is considered a cardioprotective factor. Epidemiological evidence also suggests that the consumption of flavonoid-rich fruits, vegetables, wine, and tea is inversely associated with the risk of CVD [4-7]. In population-based studies, flavonoid subclasses including anthocyanins, flavanones, and flavanols, have also been associated with a reduction in the risk of hypertension, stroke, and CVD [8-11]. In this review, the evidence to support a cardioprotective role for flavonoids in cardiovascular health as well as their potential biological mechanisms will be presented.

FLAVONOID SOURCES OF INTAKE

Flavonoids are phenolic compounds with 15 carbons arranged in a 3-ring structure [12]. They comprise a ubiquitous and abundant group of polyphenols in the diet [13]. Rich sources of flavonoids include fruits, nuts, and vegetables, as well as, tea, cocoa and red wine [12]. The main dietary flavonoids are categorized in five subclasses including anthocyanins, flavanols, flavonols, flavones, and flavanones [14] (Table 1). It is estimated that the average flavonoid intake is between 20-200 mg daily with approximately 10-15 mg/day in anthocyanins [15,16]. Anthocyanin intake in Americans ages 51-70 years is on an average 4.2 mg/day and lower amounts of 2.6 mg/day are reported in those > 70 years old [17]. Compared to other flavonoids, anthocyanins have a higher antioxidant capacity [18]. Anthocyanins contribute to the food coloration of red, blue and purple hues found primarily in berries, red wine and vegetables (red cabbage, onions, beans) [12]. High anthocyanin intake can be incorporated in a typical diet by consuming 1-2 portions of berries or red grapes daily.

METABOLISM

Following ingestion and passage into the stomach, flavonoids undergo phase I metabolism in the small intestine with only 5-10 % reportedly absorbed [19]. From uptake in the epithelial cells of the small intestine, flavonoids are then transformed via the phase II metabolic pathways in the gastrointestinal tract and liver

Table 1: Types and sources of flavonoids.

Flavonoid Subclass	Dietary Sources	Flavonoids
Anthocyanins	Berries (red, blue, purple), red wine	Cyanidin, delphinidin
Flavanols	Tea, cocoa, chocolate	Catechin, epicatechin
Flavonols	Onions, broccoli, apples, kale	Quercetin, myricetin, kaempferol
Flavanones	Oranges, lemons, grapefruit	Hesperetin, naringenin
Flavones	Celery, parsley, hot peppers	Luteolin, apigenin, chrysin

and they are eliminated quickly after consumption [20]. There is also variation in the bioavailability of different flavonoids. Large structured flavonoids continue into the large intestine where they are degraded into smaller low molecular weight metabolites followed by their absorption. In the circulation, the metabolites are delivered to various tissues where they influence cellular processes [21]. A recent study suggests that flavonoids may improve health by modulating intestinal microorganisms. Blueberry anthocyanins reportedly have a prebiotic effect and the ability to alter the amount and composition of human intestinal microbiota [22]. Given the rapid metabolism and degradation of flavonoids, it may be beneficial to consume a variety of flavonoid-rich foods such as fruits, vegetables, cocoa or tea throughout the day to maintain continuous circulating concentrations of flavonoids and their positive effects. For example, improvement in flow-mediated dilation occurs two hours after consumption of 46 grams of dark chocolate and is associated with increased blood levels of epicatechin, the major flavonoid in chocolate [23].

CARDIOVASCULAR EFFECTS OF FLAVONOIDS

The positive cardiovascular effects of flavonoids are attributed to their interference in many pathophysiological processes including oxidative stress and inflammation that are associated with atherosclerosis [4] (Figure 1). Oxidative stress is one proposed mechanism for the oxidation of low-density lipoprotein cholesterol (LDL-C) and the subsequent onset of age-related CVD. It is characterized by a cellular increase in free radicals which are cytotoxic to the vascular endothelial cells. Flavonoids exert antioxidant actions [7,24] by scavenging free radicals and reducing oxidative stress; thereby limiting cellular injury [25]. Moreover, flavonoids stabilize cell membranes by decreasing membrane fluidity [26].

Flavonoids also provide beneficial effects by interfering with cholesterol metabolism. Dyslipidemic subjects with increased levels of circulating lipids (total cholesterol, LDL-C, triglycerides) are at high risk for CVD. Hepatic LDL receptors are impaired in CVD which causes LDL-C to circulate longer making the body more

Vascular Effects of Flavonoids

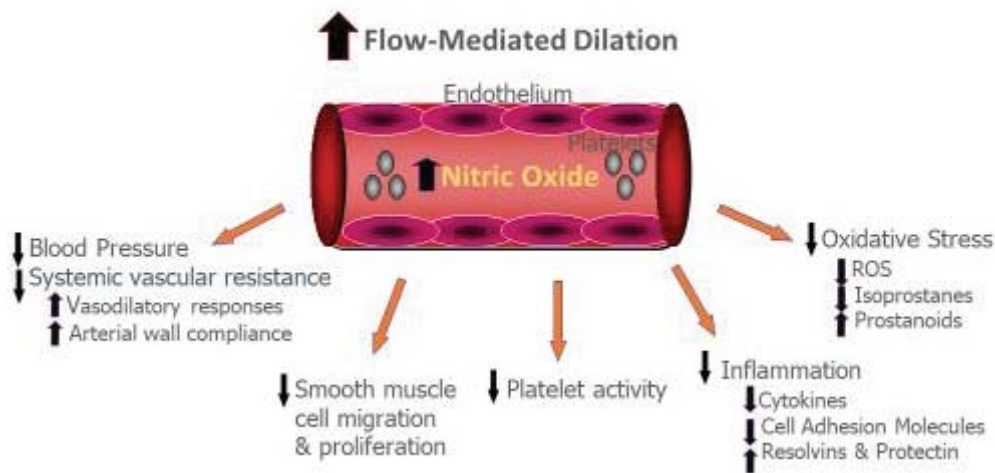


Figure 1: Schematic overview of vascular effects of flavonoids. Nitric oxide plays an important role in maintaining vascular homeostasis. Flavonoids stimulate release of endothelium and platelet-derived nitric oxide which increases flow-mediated dilation of the artery. This results in a reduction in blood pressure due to a decrease in systemic vascular resistance. Flavonoids inhibit cellular oxidative stress, inflammation, vascular smooth muscle cell proliferation, and platelet activity that limits thrombosis formation. Flavonoids also decrease the expression of cytokines and adhesion molecules involved in inflammatory responses. ROS: Reactive oxygen species.

susceptible to oxidation in the vascular endothelium with progression to atherosclerosis. Supplementation with anthocyanins reportedly improves LDL-C and high density lipoprotein-cholesterol (HDL-C) concentrations and enhances cellular cholesterol efflux [27]. These benefits may be due to an inhibitory effect of anthocyanins on plasma cholesteryl ester transfer protein [27]. Moreover, healthy subjects who consumed 500 grams of strawberries daily for 1 month had similar improvements in lipid levels [28]. The subjects' levels of total cholesterol, LDL-C and triglycerides were all decreased while HDL-C levels were not affected. These findings are also consistent with a meta-analysis of 45 studies on the consumption of berries and purified anthocyanin extracts and the association of cardiovascular risk factors [29]. Overall, a reduction in LDL-C, triglycerides, blood pressure (systolic and diastolic) and inflammatory biomarkers were reported with a significant increase in HDL-C levels [29]. It has been proposed that flavonoids modulate, regulate or inhibit cell signaling pathways [30]. This results in less activation and attenuated signaling networks which are downstream associated with inflammation and CVD.

Flavonoids also have anti-thrombotic and anti-inflammatory properties which preserve endothelial function. Platelets are implicated in the atherosclerotic process by their cellular effects and platelet activation. They interact with platelets, as well as, leukocytes and endothelial cells to form platelet-leukocyte aggregates. This in turn promotes leukocyte adhesion and migration into the vascular sub endothelial space which enhances inflammation and atherogenesis [31]. By inhibiting the arachidonic acid cascade and production of thromboxane A_2 (TXA_2), flavonoids have anti-aggregatory effects on platelets [32]. Anthocyanin extracts inhibit platelet aggregation and early thrombi formation [33]. Investigators have also shown that healthy subjects who consumed a flavanol-rich cocoa beverage

(897 cocoa flavanols) had reduced platelet activation two hours after consumption [34]. Moreover, platelet-related primary hemostasis was reduced in healthy adults following consumption of semi-sweet chocolate bits (25 grams) [35].

Inflammation is another mechanistic target for flavonoids. The inflammatory process plays a pivotal role in the progression and destabilization of atherosclerotic plaque that precedes clinical events [36]. Atherosclerosis progresses in the coronary arteries over time with damage to the endothelium and development of arterial plaques. The endothelial injury causes proliferation and migration of monocytes, leukocytes and vascular smooth muscle cells into the endothelium. Monocytes are transformed into macrophages and then lipid-filled foam cells in the vascular intima [37]. Chemokines and pro-inflammatory cytokines regulate these cellular processes. These pathophysiological processes in atherogenesis may be clinically silent until there is an acute rupture of vulnerable plaque with subsequent exposure of the lipid-rich core to arterial blood flow [38]. Platelets accumulate and the resulting thrombus occludes the coronary artery lumen limiting blood flow to the myocardium causing ischemia or an infarction. These events disrupt vascular homeostasis and endothelial cell function which affect the extent of myocardial injury.

Flavonoids exert their anti-inflammatory effects by modulating pro-inflammatory gene expression of enzymes including cyclooxygenase, lipoxygenase, nitric oxide synthase (eNOS) and cytokines [39]. Furthermore, flavonoids limit the expression of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and E-selectin in the endothelium which recruits monocytes into the subendothelial layer. They also reduce production of inflammatory cytokines TNF- α , IL-6, and C-reactive protein [20] and decrease signaling through the nuclear transcription factor NF- κ B pathway which is important for inflammation [40].



Telomere length is also associated with CVD and biological aging. Telomeres are nucleotide sequences found at the end of chromosomes that stabilize the gene by allowing normal cell division [41]. After each cell division, telomere length decreases until they no longer function. A Mediterranean dietary intervention rich in flavonoids increased telomere length in the 5-year PREDIMED-NAVARRA trial [42]. In addition, various food groups, such as fruits and vegetables, as well as, individual flavonoid-rich nutrients including tea and grape seed are associated with longer telomere length [43]. The mechanisms for telomere lengthening may be related to increased enzymatic telomerase activity that adds telomeric repeats to the new DNA strands, enhanced DNA methylation or reduced oxidative stress [43]. These studies are promising and provide an added benefit of flavonoid-rich nutrients and components in CVD prevention.

BLOOD PRESSURE AND VASCULAR FUNCTION

Blood pressure is an important indicator of cardiovascular health. A meta-analysis showed that lowering blood pressure significantly decreased the risk of CVD and death in different patient populations [44]. In an experimental study, a blood pressure lowering effect has been observed following flavonoid intake. Hypertensive rats fed a diet rich in blackcurrant oil for 7 weeks have a significant 30 mmHg reduction in blood pressure compared to controls [45]. Systolic and diastolic blood pressure is reduced by 4.4 and 3.9 mmHg, respectively, in healthy individuals consuming a flavanol-rich cocoa drink (900 mg) daily for 1 month [46]. Drinking black tea, another good source of catechins, is also effective and has been shown to lower systolic and diastolic blood pressure by approximately 2-3 mmHg in healthy and hypertensive subjects [47,48]. A meta-analysis of 99 randomized controlled trials demonstrated that intake of food products rich in anthocyanins such as berries, red grapes and wines, lowered both systolic and diastolic blood pressure in subjects independent of their health status [49].

Flavonoids exert direct effects on vascular tone. In vitro studies suggest that flavonoid subclasses can improve vascular function by increasing the bioavailability of nitric oxide (NO), a potent endothelial-derived vasodilator [50,51]. Blackcurrant fruit is a rich source of anthocyanins which has potent vasorelaxant activity in vessels from human and animal models [52,53]. Recent evidence also suggests that high anthocyanin intake reduces the risk of myocardial infarction by 32% [54] and is inversely associated with decreased arterial stiffness [55].

There is mounting evidence demonstrating the beneficial effects of flavonoids on endothelial function. The endothelium consists of a layer of endothelial cells that line the arterial wall. It provides an interface between the circulating blood and arterial wall. The endothelium produces and releases vasoactive molecules that constrict or dilate the artery. It regulates vascular homeostasis through changes in blood flow, arterial vasomotor tone and platelet activity [56]. Endothelial dysfunction is the earliest stage of atherosclerosis development [36] and correlates with future CVD events [57]. It is characterized by a reduction in NO which leads to a vasoconstrictive feature, pro-inflammatory and pro-thrombotic state in the endothelium [58,59]. Reduced NO also results in the loss of inhibition of parentheses which binds to promoter regions of genes that code for proinflammatory proteins such as cell adhesion molecules and cytokines [60]. NF κ B mediates inflammatory responses and if its activation is attenuated, the inflammatory state is altered. NO inhibits the synthesis and

expression of the pro-inflammatory proteins that are involved in atherogenesis. Flavonoids may increase NO bioavailability by enhancing the expression and activity of eNOS [61]. This is evidenced by a study with flavonoid-rich red wine that produced increases in eNOS mRNA expression in endothelial cells [62].

Experimental studies have also demonstrated that wine extract rich in flavonoids increases NO production in the endothelium of isolated arteries and causes endothelium-dependent relaxation [63,64]. Moreover, we found daily consumption of dark chocolate for 2 weeks significantly improves endothelial function in healthy adults with concomitant increases in the flavanol, epicatechin in plasma [23]. Blackcurrant extract rich in polyphenolic compounds induces a NO- and endothelium-dependent vasorelaxation in rat thoracic aorta [52]. Another study demonstrated that supplementation with purified anthocyanins improves endothelial function in hypercholesterolemic individuals [65]. Collectively, these studies provide strong evidence of the important role of flavonoids in improving blood pressure and vascular function.

As described, flavonoids have a wide spectrum of positive effects on the human body. An overall summary of the health benefits of flavonoids is depicted in Figure 2.

CLINICAL SIGNIFICANCE OF ENDOTHELIAL FUNCTION ASSESSED BY FLOW-MEDIATED DILATION

Flow-mediated dilation (FMD) of the brachial artery provides a non-invasive assessment of endothelial function. It is reflective of the bioavailability of NO. Attenuated FMD of the brachial artery is predictive of endothelial dysfunction in the coronary arteries [66] and occurs decades before the onset of symptomatic CVD [67]. FMD is reportedly decreased in the presence of atherosclerosis and risk factors [68]. In evaluating the FMD response following the consumption of various supplements, an increase in FMD indicates that there is enhanced activity and levels of eNOS in the endothelium producing NO [69]. In our previous investigations in hyperlipidemic children ages 9-20, we demonstrated a 2% increase in FMD after supplementation with ω -3 fatty acid, docosahexaenoic acid [70] and a 3.2% increase following antioxidant vitamins C and E for 6 weeks [71]. This is physiologically and clinically significant because it reflects the increase in blood flow from NO. A study in 12 hypercholesterolemic adults given oral berry anthocyanins had an increase of 2.7% in FMD two hours after the dose [65]. Another study in 14 adults reported a 3.7% increase in FMD after green tea consumption [72]. There is substantial evidence for the clinically relevant prognostic value of a 2% change in FMD. A meta-analysis suggests that for every 1% increase in FMD of the brachial artery, there is a 13% decrease in future risk of cardiovascular events [57]. Moreover, another meta-analysis was highly predictive of future CV events. They found a 1% increase in FMD was associated with a 9% (95% CI: 4%-13%) reduction in the future risk of CV events [73]. Based on these studies, a 2% increase in FMD would result in an 18%-26% reduction in future risk of CV events. Therefore, by improving FMD, flavonoids may slow or inhibit the progression of CVD.

CLINICAL CONSIDERATIONS FOR FLAVONOID USE

Flavonoids are generally considered quite safe and well tolerated possibly due to their low bioavailability and rapid metabolism.

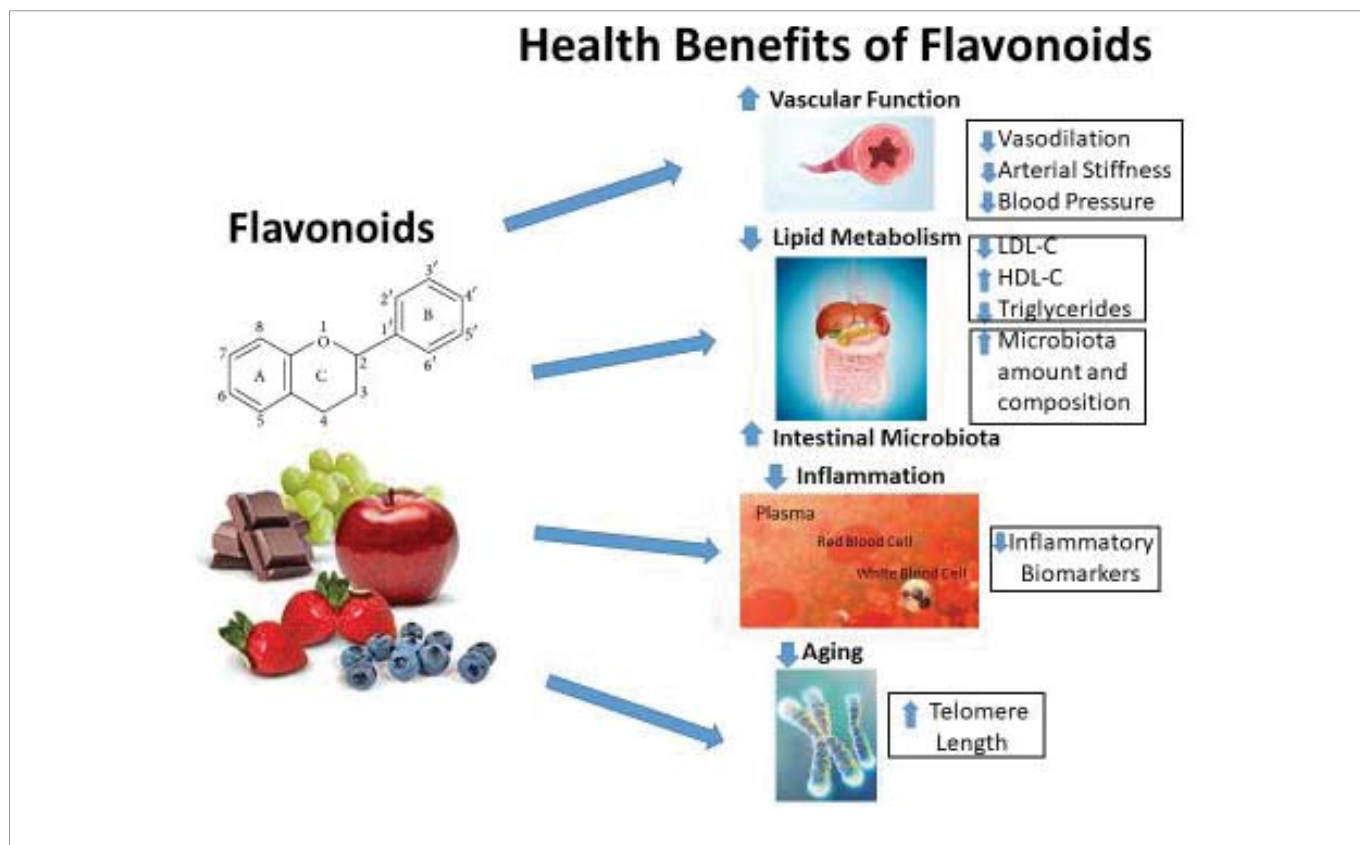


Figure 2: Broad array of health benefits of flavonoids. These include an improvement in vascular function with vasodilation and a decrease in arterial stiffness and blood pressure. Flavonoids improve the lipid profile with a decrease in LDL-C and triglycerides and an increase in HDL-C levels. In the gastrointestinal tract, flavonoids improve the amount, composition and function of the microbiota that colonize the intestines. Flavonoids reduce inflammation as reflected in a decrease in circulating inflammatory biomarkers such as TNF- α , IL-6, and C-reactive protein. Flavonoids may also decrease biological aging by increasing telomere length. LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol.

However, adverse side effects could occur. Flavonoids have potent effects but they are not regulated like prescription or over-the-counter medications. The use of pharmaceutical grade or NSF international certified nutritional supplements is recommended to ensure the quality of products such as flavonoids offering health benefits. The efficacy and safety of supplement use has been charged to the Food and Drug Administration with the development of a surveillance system [74].

Careful consideration should be given to the potential risks of flavonoids such as allergic reactions or interactions with other supplements or medications. For example, anthocyanins may compete for drug-metabolizing enzymes and transporters [75]. Therefore, there may be an increased propensity for drug-supplement interactions leading to adverse effects. There is no evidence for adverse effects with the consumption of typical intake of dietary anthocyanins. However, high amounts of bilberry anthocyanins (>100 mg/day) should be cautioned in patients on Coumadin[®] (Warfarin) or other anti-platelet drugs [76].

CONCLUSION

Epidemiological and experimental evidence supports an important cardioprotective role for flavonoids. These phenolic compounds derived from plant, fruit and vegetable sources are ubiquitous. Flavonoids affect several atherogenic mechanisms linked to CVD including oxidative stress, inflammation, endothelial

dysfunction and platelet aggregation. Flow-mediated dilation of the brachial artery provides a non-invasive assessment of endothelial function with flavonoid consumption that correlates with endothelial function in the coronary arteries. It is reflective of NO bioavailability and an increase in FMD also represents a reduction in future risk of CVD. This is a targeted area for future research that warrants well-controlled, randomized clinical trials on specific flavonoids to define optimal types and doses for cardiovascular health and CVD prevention.

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