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The role of polyphenols in causing cardiovascular disease

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ABSTRACT

Research on flavonoids has increased since the discovery of the French Paradox, the low cardiovascular mortality rate observed in Mediterranean population in association with red wine consumption and a high saturated fat intake. Plant polyphenol (flavonoid) occurs naturally in fruits, vegetables, and beverages such as tea and wine. Epidemiologic studies suggest that higher polyphenol intake from fruits and vegetables is associated with decreased risk for cardiovascular disease. The mechanisms explaining this observation remain unclear. The vascular endothelium is a critical regulator of vascular homeostasis, and endothelial dysfunction contributes to the pathogenesis and clinical expression of coronary artery disease. Platelet aggregation is a central mechanism in the pathogenesis of acute coronary syndromes, including myocardial infarction and unstable angina. There are numerous reports suggesting that plant polyphenols improve endothelial function and inhibit platelet aggregation in humans.

Keywords: Polyphenol, flavonoid, cardiovascular

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INTRODUCTION

Cardiovascular disease, especially ischemic heart disease, is the main cause of morbidity and mortality in many countries.[1] This disease is a multifactorial of complex etiology. A number of risk factors have been extensively studied and documented, as in the case of serum lipids and lipoproteins,[2] which are the cause of at least one-half the incidence of ischemic heart disease in Europe. Subsequent research has shown that cases of atherosclerosis may occur in spite of normal serum lipid and lipoprotein concentrations.[3] Other factors of interest to be discussed as causes of ischemic heart disease are endothelial dysfunction and platelet aggregation. The relationship between flavonoid intake and the risk for a number of chronic diseases has been investigated by Knekt et al.[4] This study shows that flavonoid intake is beneficial in decreasing the risk of chronic disease, such as ischemic heart disease. Flavonoids reputedly have a number of beneficial effects in controlling endothelial thrombosis, inflammatory processes, and vascular tone, i.e. factors which may cause occlusion of the arterial lumen, thus leading to acute coronary syndromes.

Pathogenesis of cardiovascular disease

Atherosclerosis is a chronic inflammatory disorder occurring at prone regions in medium-sized arteries. Atherosclerotic lesions may develop asymptomatically for a number of decades before becoming active and causing clinical conditions such as acute myocardial infarction, unstable angina, or sudden death. Above-mentioned events are in general frequently caused by acute rupture or erosion of a susceptible plaque causing exposure of the strongly thrombogenic subendothelium to the blood. The resulting disorder is an acute one, where platelet accumulation cause mural thrombus formation, leading to a total or partial occlusion of the arterial lumen, and thus influencing the occurrence of infarction or ischemia. To date the mechanisms explaining plaque susceptibility and rupture, are still incompletely understood, but available data point to local inflammation within the plaque and thinning of fibrotic peaks**,** while accumulation of fatty plaques may be a contributing factor. Once rupture of a plaque takes place, the extent of thrombus formation and the occurrence of acute changes in vascular tone will play a role in determining the extent of ischemia or infarction.[5]

The xanthine oxidase pathway also is implicated as an important pathway in the process of injury due to oxidation within the tissues. Both types of xanthine, i.e. xanthine dehydrogenase and xanthine oxidase, are involved in xanthine metabolism into uric acid. Xanthine dehydrogenase is the enzymic form present in physiological conditions, but in ischemic conditions the dehydrogenase will undergo configurational change into the oxidase. Xanthine oxidase is a source of oxygen free radicals. In the reperfusion phase, e.g. after reoxygenation, xanthine oxidase will react with molecular oxygen, releasing free radicals in the form of superoxide anions,[5] which may attract various mediators of inflammation, leading to inflammation and tissue damage.

Leukocyte immobilization and adhesion to the endothelial cell wall is another major mechanism responsible not only for the generation of reactive oxygen from free radicals, but also for the release of cytotoxic oxidants and mediators of inflammation, and the subsequent activation of the complement system. In normal conditions the leucocytes can freely pass through the endothelial cell wall. However, in ischemia and inflammation, various mediators, particularly those originating from the endothelium, and complement factors, may cause adhesion of leucocytes to the endothelial wall, thereby causing immobilisation and stimulation of neutrophil degranulation, resulting in tissue injury.[5]

Numerous studies undertaken recently, with a focus on vascular biology, provide the basis for a productive approach to developing the latest management programs and prevalence strategies for cardiovascular diseases. This strong interest is particularly aimed at anti-thrombotic and other therapies for improving endothelial cell fucntion as the key factor in cell regulation within the vascular wall.

The vascular endothelium is an important key factor in the regulation of vascular homeostasis. There is evidence indicating that functional changes of the endothelium contribute to the pathogenesis and expression of clinical cardiovascular disease.[6] The endothelial cells regulate vascular homeostasis by producing factors acting locally in the vascular lumen and vascular wall, through formation of nitric oxide (NO). Originally NO was known as endothelium-derived vasodilator, but at present it is apparent that NO also regulates other important aspects of vascular homeostasis. For example, NO prevents leucocyte adhesion to the endothelial surface and inhibits expression of adherent leukocytes. NO also prevents platelet adhesion

and aggregation. Additionally, NO inhibits proliferation of vascular smooth muscle fibers and alters expression of noncellular components of the vascular wall matrix. These features of NO result in a correlation between NO and lesion formation, vascular wall hypertrophy, and vascular compliance. In other words, there is an interrelationship between endothelium-derived NO and all stages of the atherosclerotic process, due to its important properties as vasodilator, anti-inflammatory and anti-thrombotic agent, and supressor of vascular smooth muscle proliferation.[7]

Being endothelium-derived is not only a feature of NO, but also that of other products having the same characteristics in regulating vascular homeostasis, such as substances influencing the vascular tone (prostacyclins and endothelins), fibrinolytic factors (tissue plasminogen activator and plasminogen activator inhibitor-1), coagulation factors (tissue factors, heparan, and von Willebrand factor), and pro-inflammation factors (adhesion molecules and inflammatory cytokines).[8] In general, loss of NO in parallel with changes in the regulatory system mechanism, results in the development of a pathological endothelial phenotype. This observation shows that endothelial condition may be an indicator of vascular health, and that examination of endothelial vasomotor function may yield clinical benefits. Cardiovascular risk factors which may adversely affect the endothelium are such as dyslipidemias, hypertension, diabetes mellitus, smoking, ageing, physical inactivity, systemic inflammation, infection, and hyperchromocysteinemia; the postmenopausal condition is also linked to endothelial dysfunction. Genetic and environmental factors may influence the effect of risk factors on endothelial function. Here genetic variation influences the synthesis of NO and the activity of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase.[10] Food may also influence the effect of risk factors on endothelial function, thus polyphenol intake may be important in determining the risk for cardiovascular events.[5]

POLYPHENOLS

Polyphenols are a major constituent of vegetables and comprise the majority of antioxidants found in our food. Polyphenols have various properties, depending on the molecular structure. These substances are abundant in fruits, vegetables, nuts, barks, roots, stems, flowers, tea, and wine.[10] Most polyphenols are important in imparting attractive colors and flavors to plant foods and beverages, which is termed their organoleptic qualities.[11] The major pigment in plant polyphenols is anthocyanin, detectable by its red, violet, or blue color, while the less numerous yellow colors are due to flavonols and flavones. Some polyphenols, such as vanillin and eugenol (imparting the typical fragrance to cloves), have volatile properties, but the main characteristic of polyphenols is their bitter taste.[11]

Plant polyphenols are broadly divided into two main groups, i.e. flavonoids and nonflavonoids. Nonflavonoids have a simpler structure; examples of nonflavonoids are phenolic acids (subdivided into benzoic acid and hydroxycinnamic acid, based on the C1-C6 and C3-C6 chains, respectively), stilbene and derivatives, (such as stilbene oligomers, gallotannins, ellagitannins, and lignins). The flavonoids possess a core consisting of two phenolic rings and an oxygenated heterocyclic pyrane ring. They are the most important group, and are further subdivided into 13 classes (Figure 1), based on differences in oxidation state of the heterocyclic pyrane ring.[12]

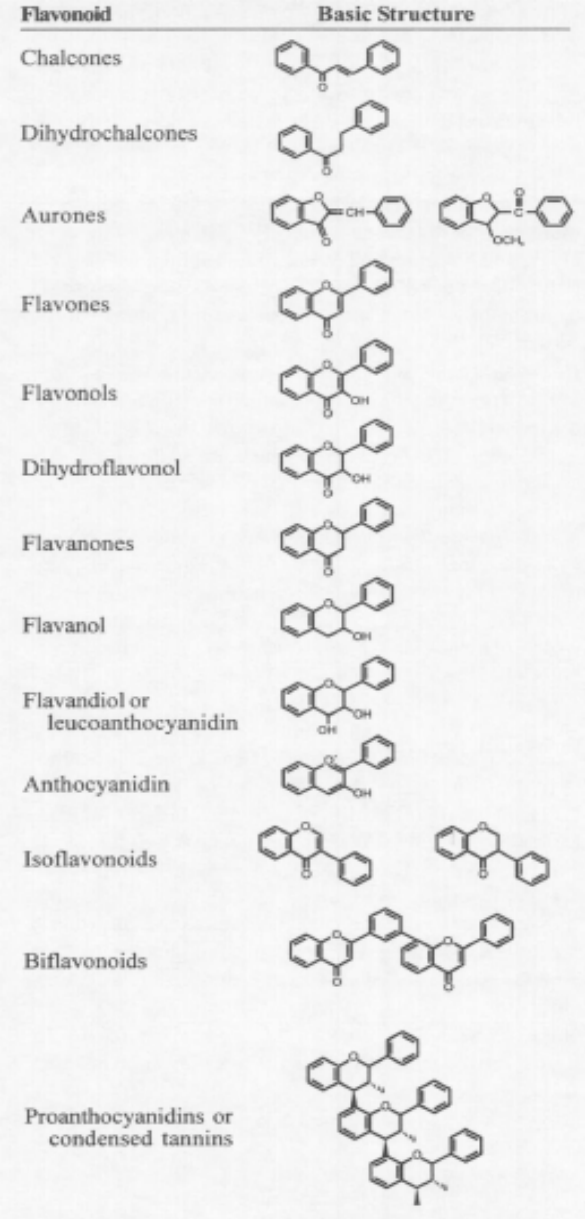

Figure 1. Group classes of flavonoid

To date, more than 4000 flavonoid variants have been successfully identified from plants.[13] The four main flavonoid groups together with their constituents and food sources are listed in Table 1. Flavones are described as planar structures marked by the presence of flavonoid double bonds at the center of the aromatic ring. Quercetin is one of the flavonoids found abundantly in onions, apples, broccoli, and berries. The second group are the flavanones, mainly found in citrus fruits. An example of flavonoids in this group is narigin. Catechin-containing flavonoids are mainly found in green and black tea, and in red wine. Anthocyanins are encountered in strawberries and other kinds of berry, grapes, red wine, and tea.[9] Flavonoids are thought to contribute to the potential protective effect against cardiovascular disease through their antioxidant,[14] anti-thrombogenic,[15] and anti-inflammatory properties. The most recent research results indicate that flavonoids may also improve vascular function.[16]

Numerous epidemiological studies are in existence,[17,18] indicating a decreased risk for cardiovascular disease in subjects consuming large amounts of flavonoids in tea or other food sources. One of these is the meta-analysis study by Peter at al,[17] showing a decrease in risk for cardiovascular disease of approximately 11% on daily consumption of three cups of tea. The results are supported by the study of Geleijnse et al,[15] indicating a lower relative risk (RR) for incidence of myocardial infarction in the group of subjects ingesting more than 375 ml of tea daily (RR: 0.57;95% CI: 0.33 - 0.98), as compared to non-users.

Mechanism of action of flavonoids

The cells of the body and various tissues are continually subject to injury due to free radicals and reactive oxygen produced during oxygen metabolism. The mechanisms and events by which free radicals disrupt cellular function are not fully understood. One of the events which appears to be most important in membrane damage is lipid peroxidation. The membrane damage caused may induce a shift in net electric charge of the cells and changes in osmotic pressure, leading to swelling of the cells and even cell death. The formation of free radicals may

Table 1. Main flavonoid groups, components, and food source [9]

Group	Component	Food source
Flavones	Apigenin	Apple peel
	Chrysin	Berries
	Kaempferol	Broccoli
	Luteolin	Celery
	Myricetin	Fruit peel
	Rutin	Cranberries
	Sibelin	Grapes
	Quercetin	Lettuce
		Olives
		Onions
		Parsley
Flavanones	Fisetin	Citrus peel and fruits
	Hesperetin	
	Narigin	
	Naringenin	
	Taxifolin	
Catechins	Catechin	Red wine
	Epicatechin	Tea
	Epigallocatechin gallate	
Anthocyanidins	Cyanidin	Cherries
	Berries	Grapes
	Delphinidin	Raspberries
	Malvidin	Red grapes
	Pelargonidin	Red wine
	Peonidin	Strawberries
	Petunidin	Tea
		Dark-pigmented fruit peel

also attract various mediators of inflammation, which play a role in systemic inflammatory responses and tissue damage. The living organism is capable of protecting itself from reactive oxygen through a number of defense mechanisms. One of these is antioxidant formation, either through enzymatic activity, such as of superoxide dismutase, catalase, and glutathione peroxidase, or through the nonenzymatic activity of supplementary glutathione, ascorbic acid, and α-tocopherol.[9] Nearly all flavonoid groups have the capability of functioning as antioxidants. Flavones and catechins appear to be the flavonoids with the strongest effect in protecting the body against reactive oxygen.[9]

Flavonoids are also able to prevent injury caused by a number of free radicals. Oxidation of flavonoids by free radicals will result in more stable and less reactive radicals. In other words, flavonoids stabilize reactive oxygen by reacting with this component. The high hydroxyl group reactivity of flavonoids inactivates free radicals through the following reaction equation: Flavonoid (OH) + R* > flavonoid (O*) +RH, where R* is a free radical and O* is an oxygen free radical. Some flavonoids can scavenge highly reactive oxygen originating from radicals called peroxynitrites. Some flavonoid types such as epicatechin and rutin are potent radical scavengers. This scavenging ability is due to inhibition of xanthine oxidase activity. The

ability of flavonoids for scavenging free radicals causes flavonoids to be able to inhibit oxidation of low density lipoproteins (LDL). Oxidation of LDL by oxygen radicals will cause endothelial wall injury, thus increasing atherosclerotic changes. The ability of flavonoids for protecting the endothelial wall against injury due to LDL particles, has led to the statement that they have a preventive effect against atherosclerosis.[9] This was proven in a study conducted by Arai et al.[19] in Japan, reporting an inverse relationship between flavonoid intake and total plasma cholesterol concentration.

The anti-atherosclerotic effect of flavonoids takes place through synthesis of NO, which functions in maintaining vascular dilatation. The higher NO concentration, as a result of its synthesis in macrophages, apparently will cause oxidative damage. This is presumably linked to simultaneous activation of macrophages, which increases NO production concurrently with enhanced large-scale synthesis of superoxide anions. NO is produced by various cell types, including endothelial cells and macrophages. NO reacting with free radicals may possibly produce the strongly deleterious peroxynitrites. Injury arising from NO through peroxynitrites plays the main role, as peroxynitrites can directly react with oxidized LDL, resulting in irreversible membrane damage. The use of flavonoids as antioxidants leads to scavenging of free radicals, because these indirectly react with NO, such that the resulting damage is reduced. Interestingly, NO itself can behave as a radical, and it is reported that the NO molecule is directly scavenged by flavonoids.[9]

Cyclo-oxygenase and 5-lipoxygenase play an important role as mediators of inflammation. Involvement of both substances is preceded by arachidonic acid release, as the starting point for a systemic inflammatory response. Neutrophils produced by the lipoxygenase pathway synthesize chemotactic components from arachidonic acid, and also provoke cytokine release. Some phenolic components, particularly quercetin, may down-regulate both cyclooxygenase and 5-lipoxygenase pathways, thus inhibiting the formation of inflammatory metabolites, with resultant arachidonic acid release. The precise mechanism of enzyme inhibition by flavonoids is still unclear. The anti-inflammatory effects are also caused by the ability of flavonoids for inhibiting the biosynthesis of eicosanoids. Eicosanoids, such as prostaglandins, are involved in various immunological responses and are the end products of cyclooxygenase and 5-lipoxygenase. Flavonoids also inhibit tyrosine kinases in the cytosol and cell membrane. Integral membrane proteins, such as tyrosine 3-monooxygenase kinase, are involved in various functions, such as enzyme catalysis, cross-membrane transport, signal transduction for hormone receptors and growth factors, and energy transfer in adenosine triphosphate (ATP) synthesis. This protein-to-protein inhibition causes down-regulation of uncontrolled cell growth and proliferation. Tyrosine kinase is an important key factor in the signal pathway regulating cell proliferation. The anti-inflammatory effects of flavonoids are demonstrated by the capability of flavonoids for inhibiting neutrophil degranulation. This is a direct effect of flavonoids in decreasing arachidonic acid release by neutrophils and other immune cells.[9]

Some flavonoids are actively involved in inhibiting platelet aggregation. Certain flavonols have an antithrombotic effect because they directly scavenge free radicals and thereby maintain endothelial prostacyclin and NO concentrations. Flavonoids show antithrombotic effects in vitro and in vivo, due to their capability in inhibiting cyclooxygenase and 5-lipoxygenase activation. Currently it is well known that arachidonic acid released in inflammation is

metabolized by platelets to form prostaglandins, endoperoxides, and thromboxane A2, causing platelet activation and aggregation. The anti-aggregation effect of flavonoids is thought to take place through inhibition of thromboxane A2 formation. The effects of flavonoids on other enzyme systems is through inhibition of arachidonic acid metabolism, thus conferring anti-inflammatory and antithrombogenic properties on flavonoids.[9]

Effects of polyphenols on endothelial function

Endothelial dysfunction is an early pathophysiologic abnormality occurring in atherosclerosis and is an independent predictor of adverse prognosis in nearly all cardiovascular diseases. Most studies on the effects of tea (*Camellia sinensis* L) on endothelial function utilize the noninvasive ultrasound method, which tests endothelium-dependent flow-mediated dilation in the brachial artery. A study conducted by Modena et al.[20] succeeded in proving that impaired flow-mediated dilation in the brachial artery can identify patients at high risk for cardiovascular disease.

Preliminary studies on the effects of the water-soluble antioxidant, ascorbic acid, and its relationship to increased endothelial NO synthesis, has promoted research on tea in connection with endothelial function, as tea also contains water-soluble flavonoid antioxidants.[21] Tea is very rich in polyphenols; with a concentration in dry tea leaves of 30%. Tea contains various water-soluble flavonoid antioxidants, including catechin, quercetin, kaempferol, and other polyphenols. The majority of flavanols are subsequently converted into a number of products, including thearubigin and theaflavin, which impart a black or dark brown color to tea, and a smaller number is converted into theaflavic acid and bisflavanol, also known as theasinensis.[11]

A number of studies[16,22] have been undertaken to search for a correlation between tea consumption and endothelial function. A cross-sectional placebo-controlled study on 50 subjects with coronary artery disease (confirmed by angiography), was conducted by Duffy et al.[16] with the objective of detecting an effect of short- and long-term tea consumption on flow-mediated dilation of the brachial artery. The results of this study indicate that both short-term and long-term tea consumption has been proven to be able to enhance endothelial function. These results are also corroborated by the finding that tea consumption has no effect on nitroglycerine-mediated vascular dilatation, basal arterial diameter, or extent of reactive hyperemia. This further strengthens the belief that tea consumption influences endothelial function . A study done by Hodgson et al.[22] has investigated the effect of consumption of 5 cups of black tea daily for 5 weeks in healthy subjects with moderate hypercholesterolemia, and this also showed improvement in flow-mediated dilation of the brachial artery. Interestingly, tea consumption was also associated with enhanced nitroglycerine-mediated vascular dilation, indicating that tea improves the bioactivity of endothelium-derived NO or that tea also has an effect on vascular smooth muscle function.

Other flavonoid-containing beverages are mainly derived from wine products. Products derived from purple grapes, including red wine and purple grape juice, contain flavonoids. The flavonoid content of red wine is approximately 10 times greater than that of white wine. Consumption of moderate amounts of red wine (5ml/kg or 1-2 glasses a day), or of 5-10 ml of purple grape juice, may reduce the incidence of cardiovascular disease. This decrease in cardiovascular disease incidence is due to the ability of this substance to protect LDL against oxidative processes.[23] Red wine contains flavonoids in the form of resveratrol. Because

red wine also contains alcohol, there is some question as to whether its cardioprotective effect might be due to interaction of alcohol and resveratrol. A study comparing the effects of red wine, dealcoholized red wine, and resveratrol, indicated that atherosclerotic plaque size and thickening of the tunica intima decrease on administration of those three substances. The results of this study show that dealcoholized red wine has a cardioprotective effect comparable to that in subjects given resveratrol only.[24] The study by Guarda et al.[25] attempted to find an association between red wine consumption in moderate doses with endothelial function and oxidative stress in patients with acute coronary syndrome. Twenty individuals who had undergone percutaneous coronary intervention subsequent to acute coronary syndrome, were divided at random into two groups. For two months, the group on red wine (n=9) ingested Cabernet Sauvignon 250 ml daily, while the control group (n=11) was free from alcoholic beverages**.** The results of the study indicate that red wine is effective in improving endothelial function and in reducing oxidative stress in patients with acute coronary syndromes.

Numerous studies have also been conducted on chocolate, one of the food sources rich in flavonoids, in relation to endothelial function. One of such studies is that undertaken by Perez-Viscaino et al.[26] Intake of chocolate and chocolate-based foodstuffs in high doses (176 mg/dL) for two weeks may improve endothelial function.[27] Other studies were conducted on postmenopausal women at high risk for cardiovascular disease, yielding a correlation with endothelial dysfunction. The subjects in this study were 32 postmenopausal women with hypercholesterolemia, who were randomly assigned to a group on high-dose chocolate (440 mg total flavanols) and a group on low-dose chocolate (43 mg total flavanols) for six weeks. In both groups there was a significant increase in flow-mediated dilation of the brachial artery, amounting to 76% in the high-dose group and 32% in the group on low-dose chocolate. The results of this study show that chronic consumption of chocolate may improve endothelial function and decrease vascular cell adhesion in postmenopausal women with hypercholesterolemia.[28]

Polyphenols and platelet function

Platelet aggregation contributes to formation of atherosclerosis and acute platelet thrombi, followed by embolization of the stenotic arteries. Activation of platelets adhering to the vascular endothelium induces the formation of lipid peroxidation and oxygen free radicals, which will inhibit synthesis of endothelial prostacyclins and NO. It is apparent here that platelet aggregation plays a critical role in the pathogenesis of acute coronary syndromes. This is supported by extensive evidence that antiplatelet therapy can reduce the risk of cardiovascular disease.[29] The effect of polyphenols in decreasing platelet activity has a strong impact on cardiovascular disease and can explain the epidemiological data on polyphenol function in cardiovascular disease. Based on the potential mechanism of flavonoids on platelet function, Freedman et al[30] investigated the effect of purple grape juice on platelet function. The observational results indicate that the addition of purple grape juice to platelets ex vivo causes a decrease in platelet aggregation and superoxide anion generation, and an increase in nitric oxide production by platelets. These beneficial effects appear to be related to the ability of purple grape juice for inhibiting protein-C kinase activity. This study is important due to the reproducibility of its results, when repeated with platelets isolated from healthy volunteers consuming purple grape juice for two weeks.[5]

There are numerous data on the effect of tea consumption on platelet function. Among

these are those from a study undertaken by Duffy et al,[31] on the effect of short-term and long-term tea consumption on platelet aggregation ex vivo in response to ADP (adenosine diphosphate) or thrombin-related activated peptides in patients with coronary artery disease. This study did not show any effects of tea consumption on platelet function, although when the tea was administered concurrently with aspirin therapy, it did have an influence on the research results. Other studies indicate that tea consumption may decrease the plasma concentration of P-selectin, an in vivo platelet aggregation marker.[32]

Although polyphenols show many beneficial effects, especially in improving endothelial function and inhibiting platelet aggregation, further study is required on the ability of polyphenols in preventing cardiovascular disease.

CONCLUSIONS

Consumption of food rich in polyphenols, such as fruits and vegetables, and beverages derived from plants, such as tea, red wine and chocolate, has a protective effect on the risks for cardiovascular disease. Most acute coronary syndromes are due to adhesion, aggregation, and thrombus formation in ruptured atheromatous plaques. Here flavonoids will demonstrate their ability for reducing platelet aggregation, enhancing platelet NO release and decreasing superoxide anion production. Although these findings should not be interpreted as recommending flavonoid consumption in the prevention or treatment of cardiovascular disease, nevertheless the beneficial effects of flavonoid consumption are apparent. Furthermore, the results of these studies are in accord with the American Heart Association recommendation for increased consumption of vegetables and fruits, including plant-derived beverages**,** such as tea, for prevention of cardiovascular disease.

REFERENCES

1. American Heart Association. Heart and stroke statistical update. Dallas: American Heart Association, 2000.
2. Expert Panel. Executive summary of the third report of the National Cholesterol Education Program (NCEP). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-97.
3. Kullo IJ, Gau GT, Tajik AJ. Novel risk factors for atherosclerosis. Mayo Clin Proc 2000; 75: 369-80.
4. Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Reunanen A, Hakulinen T, et al. Flavonoid intake and risk of chronic disease. Am J Clin Nutr 2002; 76: 560-8.
5. Vita JA. Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am J Clin Nutr 2005; 8(Suppl): 292S-7S.
6. Vita JA, Keaney JF Jr. Endothelial function: a barometer for cardiovascular risk? Circulation 2002; 106: 640-2.
7. Gokce N, Vita JA. Clinical manifestations of endothelial dysfunction. In: Loscalzo J, Schafer AI, eds. Thrombosis and hemorrhage. Philadelphia: Lippincott Williams & Wilkins; 2002. p. 685-706.
8. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol 2003; 42: 1149-60.
9. Leeson CPM, Hingorani AD, Mullen MJ. Glu298Asp endothelial nitric oxide synthase gene polymorphism interacts with environmental and dietary factors to influence endothelial function. Circ Res 2002; 90: 1153-8.
10. Nijveldt RB, van Nood E, van Hoorn DEC, Boelens PG, van Norren K, van Leeuwen PAM. Flavonoid: a review of probable mechanism of action and potential applications. Am J Clin Nutr 2001; 74: 418-25.
11. Cheynier V. Polyphenol in foods are more complex than often thought. Am J Clin Nutr 2005; 81(Suppl): 223S-9S.
12. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998; 56: 317-33.
13. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry 2005; 55: 481-504.

14. Leenen R, Roodenburg AJ, Tijburg LB, Wiseman SA. A single dose tea with or without milk increases plasma antioxidant activity in humans. Eur J Clin Nutr 2000; 54: 87-92.
15. Pignatelli P, Pulcinelli FM, Celestini A. The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. Am J Clin Nutr 2000; 72:1150-5.
16. Duffy SJ, Keaney JF, Holbrook M. Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. Circulation 2001; 104: 151-6.
17. Peter J, Poole C, Arab L. Does tea affect cardiovascular disease? A meta-analysis. Am J Epidemiol 2001; 154: 495-503.
18. Geleijnse JM, Launer LJ, van der Kuip DAM, Hofman A, Witterman JCM. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. Am J Clin Nutr 2002; 75: 880-6.
19. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. Dietary intakes of flavonols, flavone dan isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J Nutr 2000; 130: 2243-50.
20. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. J Am Coll Cardiol 2002; 40: 505-10.
21. Huang A, Vita JA, Venema RC, Keaney J. Ascorbic acid enhances endothelial nitric oxide synthetase activity by increasing intracellular tetrahydrobiopterin. J Biol Chem 2000; 275: 399-406.
22. Hodgson JM, Puddey IB, Burke V, Watts GF, Beilin LJ. Regular ingestion of black tea improves brachial artery vasodilator function. Clin Sci 2002; 102: 195-201.
23. Folts D. Potential health benefits from the flavonoids in grape products on vascular disease. Adv Exp Med Bio 2002; 505: 95-111.
24. Wang Z, Zou J, Cao K, Hsieh TC, Huang Y, Wu JM. Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid level. Int J Mol Med 2005; 16: 533-40.
25. Guarda E, Godoy I, Foncea R, Perez DD, Romero C, Venegas R, et al. Red wine reduces oxidative stress in patients with acute coronary syndrome. Int J Cardiol 2005; 104: 35-8.
26. Perez-Vizcaino F, Duerte J, Andriantsitohaina R. Endothelial function and cardiovascular disease: effects of quercetin and wine polyphenols. Free Radic Res 2006; 40: 1054-65.
27. Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M. Vascular effects of cocoa rich in flavan-3-ols. JAMA 2003; 290: 1030-1.
28. Wang-Polagruto JF, Villablanca AC, Polagruto JA, Lee L, Holt RR, Schrader HR, et al. Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecules in hypercholesterolemic postmenopausal women. J Cardiovasc Pharmacol 2006; 46: 177-86.
29. Awtry EH, Loscalzp J. Aspirin. Circulation 2000; 101: 1206-18.
30. Freedman JE, Parker III C, Li L, Perlman JA, Frei B, Ivanav V, et al. Selected flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation 2001; 103: 2792-8.
31. Duffy SJ, Vita JA, Holbrook M, Swerdloff PL, Keaney JF Jr. Effect of acute and chronic tea consumption on platelet aggregation in patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2001; 21: 1084-9.
32. Hodgson JM, Puddey JB, Mori TA, Burke V, Barker RI, Beilin LJ. Effects of regular ingestion of black tea on haemostasis and cell adhesion molecules in humans. Eur J Clin Nutr 2001; 55: 881-6.