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Flavanols: digestion, absorption and bioactivity

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Abstract Flavanols, or flavan-3-ols, are a family of bioactive compounds present in cocoa, red wine, green tea, red grapes, berries and apples. With a basic monomer unit of (−)-epicatechin or (+)-catechin, flavanols can be present in foods and beverages as monomers or oligomers (procyanidins). Most, but not all, procyanidins are degraded into monomer or dimer units prior to absorption. The bioavailability of flavanols can be influenced by multiple factors, including food processing, cooking, digestion, and biotransformation. Flavanols are potent antioxidants, scavenging free radicals in vitro and in vivo. While some of the actions of flavanols can be linked to antioxidant activities, other modes of action may also occur, including modulation of intracellular signaling, effects on membrane fluidity and regulation of cytokine release or action. Physiologically, flavanol-rich foods and beverages can affect platelet aggregation, vascular inflammation, endothelial nitric oxide metabolism, and may confer protective effects against neurodegeneration. Epidemiological data suggests that intake of cocoa, a rich source of flavanols, is inversely associated with 15-year cardiovascular and all-cause mortality in older males. (−)-Epicatechin and its metabolite, epicatechin-7-*O*-glucuronide, have been identified as independent predictors of some of the vascular effects associated with the consumption of a flavanol-rich beverage. Targeted dietary components and nutrition supplements that can influence the vascular system will be of great value in the prevention and treatment of chronic disease.

Keywords Flavanols · Epicatechin · Procyanidin · Vascular function · Cardiovascular disease

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Introduction

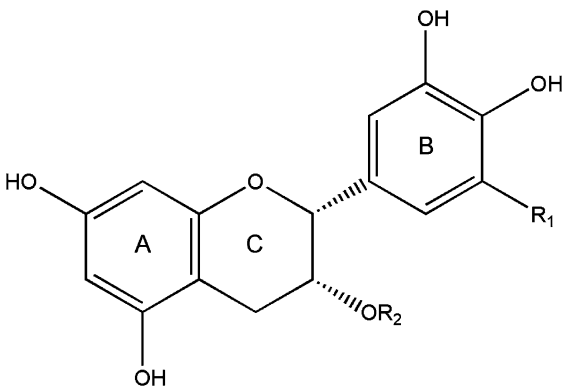
Grapes, cocoa and tea have a prominent place in food history, and extracts from the fruit, pods and leaves of these plants have been used in traditional medicine and nutrition in many cultures. The high flavonoid content of these plants may be one basis for their selection and use in ancient and modern times. Foods rich in flavonoids have been reported to augment oxidative defense, promote vascular health, protect the central nervous system and reduce the risk of certain cancers.

Flavonoids are a diverse family of polyphenolic plant compounds present in fruits, vegetables, nuts, seeds and leaves, and are the most abundant family of

polyphenols in the human diet. A double ring structure is common to all flavans, and six distinct subclasses of flavonoids exist based on the pattern of hydroxylation of the ring structure, degree of oxidation of the C-ring, and moieties in the 3 position (Haslam 1974; Haslam and Lilley 1988). These six subclasses include: flavanols and procyanidins in red wine, cocoa, tea and fruits; flavanones present in citrus fruit; flavonols in fruits and vegetables; flavones; isoflavones in soy; and anthocyanins in berries (Beecher 2003; Merken and Beecher 2000).

Flavanols, also known as flavan-3-ols, are present in a wide range of botanical sources as both monomers and oligomeric procyanidins. The richest sources of flavanols include cocoa, red wine, green tea, red grapes, berries and apples. Flavanol monomers are (−)-epicatechin and (+)-catechin, and procyanidins are oligomers of epicatechin and catechin. Unlike other classes of flavonoids, which exist in plants primarily in glucoside forms, flavanols are usually present in the aglycone form as monomers, oligomers or esterified with gallic acid to form epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (Fig. 1).

Identification of polyphenol compounds in tea has been pursued for over 30 years, and this research provides, in part, a strong foundation upon which current flavanol research is based. Tea production involves slightly steaming the raw leaves (green tea), partially oxidizing the leaves (oolong tea), or fully oxidizing the leaves (black tea). The oxidation process is termed "fermentation" in Asia. Over 60 polyphenols have been identified from tea, and have been classified as flavanols, proanthocyanidins (Nonaka et al. 1984), chalcan-flavan dimers, oolonghomobisflavans (Hashimoto et al. 1989), hydrolyzable tannins, theasinensins (Nonaka et al. 1983), and theaflavins (Hashimoto et al. 2003). Green tea is rich in gallated flavanols, and oolong tea contains novel catechins with galloyl moieties not identified in green tea (Ikeda et al. 2005). Chinese pu-er tea, made through a postoxidation fermentation process, contains 8-C substituted flavanol monomers not reported in other teas (Zhou et al. 2005).



Name	R1	R2
(-)-Epicatechin (EC)	H	H
Epigallocatechin (EGC)	OH	H
Epicatechin gallate (ECG)	H	gallate
Epigallocatechin gallate	OH	gallate

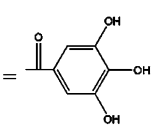


Fig. 1 Flavanol monomers in foods and tea

In addition to monomers, a number of dimers have been identified, such as theaflavin in oolong and black (fully oxidized) tea (Tanaka et al. 2001), dimer A1 in peanut skins, and dimer B2 in cocoa beans (Verstraeten et al. 2005) (Fig. 2). Larger oligomers with chain lengths of three to ten epicatechin units and longer exist in a variety of foods (Prior and Gu 2005). Daily dietary flavanol intakes have been estimated to be around 60 mg/day, though this can vary widely depending on fruit, vegetable, cocoa, tea and red wine intakes (Gu et al. 2004). Current flavonoid and flavanol databases exist (US Department of Agriculture, 2003, 2004), but the actual content in foods and beverages can vary widely.

Ripening and other environmental factors prior to harvest can influence flavanol content. Two varieties of grapes analyzed for catechin, dimer and total procyanidin content of seeds and skins over a two year period showed differences between two seasons to be the most significant factor in determining levels, while variations between content in seeds and skins also existed (Freitas and Glories 1999). Since red wine is produced by maturing grape juice with the seeds and skins, while white wine is made by separating the juice from the seeds and skins, flavanol levels of red wine may be significantly higher than levels in white wine.

Food processing can significantly influence the total content of flavanols, and the profile of monomers and oligomers, both favorably and unfavorably. Oxidation (fermentation) of green tea leaves promotes epimerization of catechins, creating compounds such as theaflavins and thearubigin, and

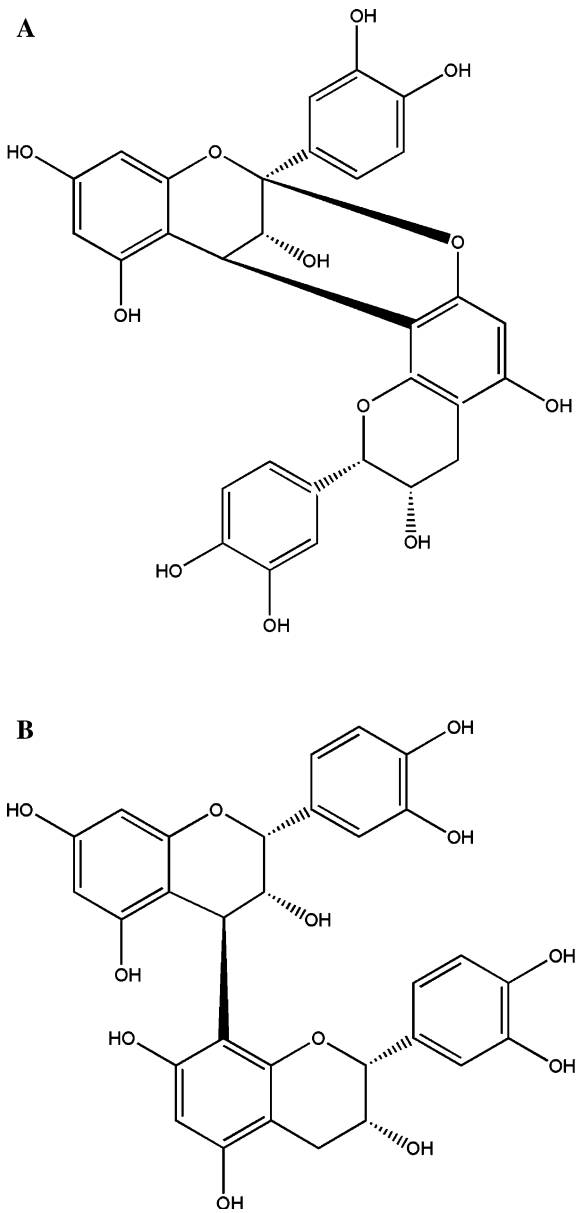


Fig. 2 Flavanol dimers from peanut skins (*Arachis hypogea* L.) and cocoa beans (*Theobroma cacao*). (**A**) Dimer A1 from peanut skins; (**B**) Dimer B2 from cocoa beans

longer chained oligomers than those present in the starting (green tea) material. Teas served in bottled or canned drinks can have widely varying catechin contents, based on the pH of the drink and the time between packaging and consumption. When standardized mixtures of catechins derived from longjing green tea were autoclaved for 20 min in distilled water or solutions of pH 4, 4.5 or 5 and stored, the percent of green tea catechins remaining after six months was 80, 50, 35 and 10, respectively (Chen et al. 2001).

The flavanol content of cocoa can be dramatically altered by processing. Alkalinization of the cleaned centers (nibs) of the cocoa beans (Dutch processing) was discovered roughly 200 years ago as a method to reduce bitterness in cocoa. Unfortunately, flavanols in the cocoa beans produce the bitter taste, so by changing the flavors of the cocoa, the beneficial properties of flavanols are reduced (Fisher and Hollenberg 2005). Since milk chocolate is typically produced from a Dutch processed cocoa, the flavanol content can be much lower than the amounts often present in dark chocolate, where Dutch processed powder is used less frequently. Procyanidin levels in defatted samples of cocoa beans can decline significantly as the beans are fermented (Table 1). Approximately 60% of monomers, pentamers and total flavanols in a cocoa sample were lost after five days of fermentation (Kealey et al. 1998 as reported in Wollgast and Anklam 2000). Temperature can also influence the flavanol content of cocoa and chocolate. When under-fermented nibs were roasted at low (127°C), medium (159°C) or high (181°C) temperatures and then milled into chocolate liquor, the loss of total procyanidins compared to the low temperature processed nibs was 30% during medium temperature and 50% during high temperature roasting. Utilizing a patented extraction process based upon the concepts above, a cocoa powder (CocoaPro®, Mars, Inc.) has

Table 1 Effects of processing of cocoa beans on procyanidin content

Sample & treatment	Monomer (mg/g)	Pentamer (mg/g)	Total procyanidin (mg/g)
Beans, hours of fermentation			
0	21.9	5.3	60.8
48	20.9	4.9	58.2
96	9.6	2.1	27.9
120	8.6	1.6	23.0
Liquor from under fermented nibs, roasted at			
127°C	n/a	1.9	23.7
159°C	n/a	0.7	16.8
181°C	n/a	0.4	11.7

n/a—data not available

been developed which has a flavanol content that is markedly higher than most other commercial cocoa powders (Fisher and Hollenberg 2005).

Another processing technique has produced an extract that contains higher than normal levels of monomers and intermediate chain oligomers (Oligonol®, Amino Up Chemical Co., Ltd.) relative to typical foods, beverages or supplements. To demonstrate this principle, grape seed extract (GSE) was depolymerized and the resulting monomers and low molecular weight oligomers were stabilized, resulting in a final product that contained five times more monomers, 50 times more dimers and seven times more trimers than the originating GSE (Table 2).

Digestion and metabolism

After ingestion, naturally occurring flavanols can undergo significant modifications that can result in a diverse family of bioactive molecules. Flavanols and procyanidins are relatively stable in stomach acid and during gastric transit (Rios et al. 2002). During digestion and transfer across the small intestine, and in the liver, flavanols are rapidly metabolized in phase I and II biotransformations to various *O*-sulfated, *O*-glucuronidated and *O*-methylated forms (Kuhnle et al. 2000; Spencer et al. 1999; Vaidyanathan and Walle 2002) (Fig. 3). In humans consuming cocoa, plasma levels of nonmethylated epicatechins such as epicatechin-7-sulfate and methylated metabolites such as 3′-*O*-methylepicatechin have been reported to occur in micromolar concentrations within 1 h after intake (Baba et al. 2000). Additionally, the metabolites 4′-*O*-methylepicatechin, and epicatechin-5-*O*-beta-D-glucuronide have been identified in human blood (Spencer et al. 2004) (Fig. 3). In humans, transport of metabolites into the blood occurs quickly, in a dose-dependent manner, with peak plasma concentrations occurring one to 2.5 h after ingestion of a flavanol-rich food (Wang et al. 2000), followed by a decline to near baseline levels within 8 h (Schramm et al. 2003b). Flavanols not absorbed in the small intestine are mostly metabolized by colonic microflora to a variety of derivatives of phenolic acid and valerolactone, which can be absorbed (Spencer et al. 2004). Flavanol metabolites are rapidly excreted in the bile and urine (Rechner et al. 2002).

Table 2 Effects of processing of grape seed extract on procyanidin content

Material	Monomer (mg/g)	Dimer (mg/g)	Trimer (mg/g)	Total procyanidin (mg/g)
Grape seed extract	36	2	10	968
Oligonol®	201	101	71	925

Bioavailability, defined here as the fraction of intake that reaches the systemic circulation, can be relatively low for flavanols. In rats, it has been estimated that less than 5% of ECG is absorbed when doses of 12.5 to 50 mg/kg are given (intakes that are in the upper range of typical human intakes) (Chen et al. 1997). ECG exhibits minimal uptake in the human intestinal cell Caco-2 model (Vaidyanathan and Walle 2003), while EGCG is poorly taken up by HT-29 human colon adenocarcinoma cells (Hong et al. 2002).

Certain monomers may be better absorbed than others. For example, in humans consuming a cocoa beverage containing equal amounts of epicatechin and catechin, epicatechin was identified to be the predominant plasma flavanol absorbed, with plasma catechin levels reaching less than 10% of epicatechin concentrations (Holt et al. 2002a). Similarly, plasma concentrations of EGC were two- to three-fold greater than concentrations of epigallocatechin-3-gallate following consumption of a green tea drink that contained equal levels of these two compounds (Lee et al. 2002). It is important to note that while the above may reflect differences in the bioavailability of these nutrients, it may also reflect differences in the systemic metabolism of these compounds.

Most, but not all, procyanidins are degraded into monomer or dimer units prior to absorption. Radiolabeled monomers, dimers and trimers were transported across a layer of Caco-2 cells, in contrast to oligomers of six units, which were transported approximately 10-fold less (Deprez et al. 2001). Procyanidins with an average chain length of six units were also degraded into low molecular weight aromatic acids after 48 h of incubation with human colonic microflora (Deprez et al. 2000). A 50 μmol/l dimer solution from cocoa, perfused for 90 min through the small intestine of rats, showed over 95% of the total passing flavanols to be unconjugated epicatechin (Spencer et al. 2001). In humans fed

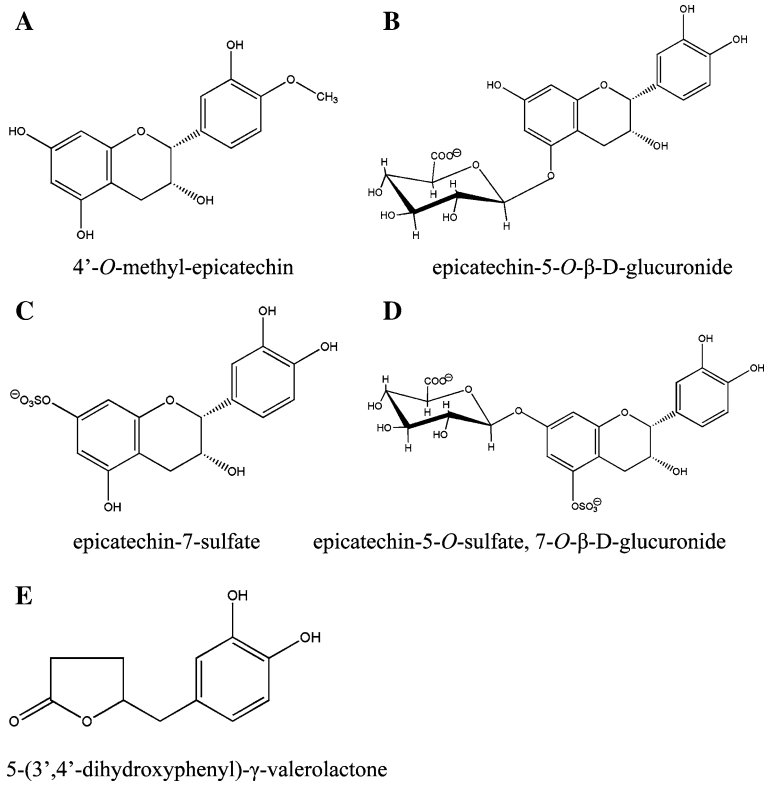


Fig. 3 Circulating metabolites of epicatechin

either Oligonol® or grape seed extract, the appearance of total plasma phenolics was substantially greater for those taking the supplement with high levels of monomers and dimers (Oligonol®) compared to those taking GSE (Fig. 4), supporting the concept that absorption of flavanols is enhanced when they are partially degraded to lower weight oligomers prior to consumption.

Small amounts of dimer B2 [epicatechin-(4-β-8)-epicatechin] and dimer B5 [epicatechin-(4-β-6)-epicatechin] can be absorbed intact. Dimers B2 and B5 have been detected in nanomolar levels in the plasma of rats given cocoa extracts (Baba et al. 2002; Zhu et al. 2002b). In humans consuming approximately 323 mg of monomers plus 256 mg of dimers in cocoa, dimer B2 was detected in plasma 2 h later at a concentration of 41 nmol/l (Holt et al. 2002a). The average plasma epicatechin concentration was 5.9 μmol/l at the same time point; thus, the B2 dimer concentration was less than 1% of the circulating plasma flavanol level. Subsequent studies in humans consuming 0.375 g/kg flavanol-rich cocoa illustrated dimer B2 in plasma within 1 h after consumption (Zhu et al. 2005). In contrast, dimer B3 [catechin (4-α-8)-catechin] and trimer C2 [catechin-(4-α-8)-catechin-(4-α-8)-catechin] given to rats were not detected in the plasma of rats given purified compounds (Gonthier et al. 2003).

Absorbed flavanols are widely distributed and they can be detected in many organs. [3H](−)-epigallocatechin gallate, intubated directly into the stomach

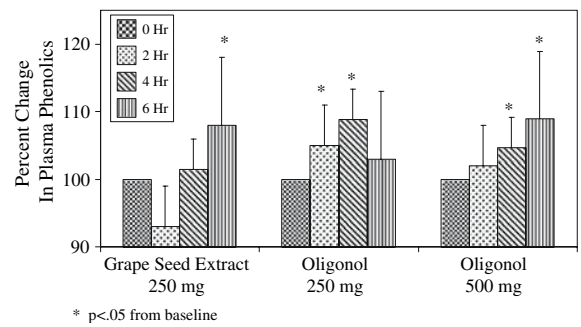


Fig. 4 Influence of grape seed extract and Oligonol® on plasma phenolics in humans

of male and female rats, was detected in the digestive tract, liver, lung, pancreas, mammary gland, skin, brain, kidney, uterus, ovaries, and testes (Suganuma et al. 1998). Twenty-four hours following administration, approximately 6.5% of the dose was recovered in the urine, and approximately 35% in the feces. Importantly, a second equal dose given to female mice 6 h after the first dose resulted in four- to six-fold increases in tissue levels of radioactivity in blood, brain, liver, pancreas and bone, compared to levels from a single dose.

Adverse events associated with high flavanol intake are rare, which may be explained by their relatively low absorption from botanical sources, and their rapid metabolism and quick elimination. A 4-week trial in healthy subjects provided a decaffeinated green tea extract containing 800 mg/day of EGCG reported a small number of complaints of stomach upset, mild nausea, and dizziness (Chow et al. 2003). Cancer patients consuming 6 g/day of a caffeinated green tea extract in three to six equal doses reported mild to moderate nausea, abdominal pain and diarrhea, along with agitation, insomnia, dizziness and tremors, which was likely due to the caffeine content of the extract (Jatoi et al. 2003; Pisters et al. 2001). The above would suggest that relatively large amounts of flavanols are well tolerated. Given the increasing interest that is occurring regarding the potential health benefits of this class of nutrients, the identification of a suitable "safe" intake of flavanols will be a subject of importance. At present, it would seem that a safe upper limit (UL; an intake that can occur on a chronic basis with no evidence of harm) is on the order of one gram or more per day.

Flavanols as antioxidants

Flavanols can scavenge free radicals in vitro and in vivo. Hydroxylation of the flavan ring, particularly the 3′,4′-dihydroxylation of the B-ring, as well as the stereochemical features and degree of oligomerization, are likely reasons for the antioxidant properties (Lotito et al. 2000; Sekher Pannala et al. 2001). In vitro, the flavanol content of a variety of teas and green tea extract, ranging from 5 to 100 mg/g, was significantly correlated with a modified oxygen radical absorbance capacity assay (ORAC) (Henning et al. 2003). Oolong tea has also been shown to have strong antioxidant properties in vitro as well as in vivo (Zhu et al. 2002a). Cocoa and purified cocoa extracts produce numerous antioxidant-related effects, including reduction of the production of reactive oxygen species by activated leukocytes (Sanbongi et al. 1997), reduction of endothelial cell-mediated and copper-mediated oxidation of low-density lipoprotein (Pearson et al. 2001), reduction of plasma F(2)-isoprostanes (indicators of in vivo lipid peroxidation) from exercise-induced oxidative stress (Wiswedel et al. 2004), inhibition of ultraviolet C-induced DNA oxidation (Ottaviani et al. 2002), and protection against oxidant-induced erythrocyte hemolysis (Zhu et al. 2002b; Zhu et al. 2005). A standardized grape seed extract (Leucoselect® Phytosome) containing 15% (+)-catechin, (−)-epicatechin and gallic acid, plus 80% dimers, trimers, tetramers and their esters with gallic acid produced significant reductions in thiobarbituric acid reactive substances (TBARS) and prolonged lag phase time to oxidation of low-density lipoproteins when fed to a group of 24 male smokers for 4 weeks in a double-blind, placebo-controlled crossover study (Vigna et al. 2003). Interestingly, using a variety of in vitro tests, cocoa was assessed to exhibit greater antioxidant capacity than red wine, green tea and black tea (Lee et al. 2003). However, caution must be used in interpreting these data, since in vitro testing does not necessarily reflect the profile and activity of ingested flavanols once they have been absorbed and biotransformed in the intestine and liver.

The in vitro antioxidant effects correspond, in part, to results in vivo. In rats, the chronic intake of a diet containing 2% of a cocoa powder that contained 1.57 mg/g diet of flavanols and procyanidins was associated with a reduced concentration of 8-oxo-DNA (Orozco et al. 2003). Plasma antioxidant capacity (Schramm et al. 2003a) in rats rose approximately 15% ($P < 0.01$) 2 h following administration of the flavanol-rich extract Oligonol® compared to baseline levels, and this capacity remained significantly elevated at 4 h before returning to baseline at 6 h (Fig. 5). In humans consuming a supplement of grape seed extract or 250 or 500 mg Oligonol® (Table 2), plasma antioxidant capacity rose significantly when the monomer/dimer-rich Oligonol® was consumed, in contrast to no changes seen in those taking GSE (Fig. 6). Oligonol® has also been

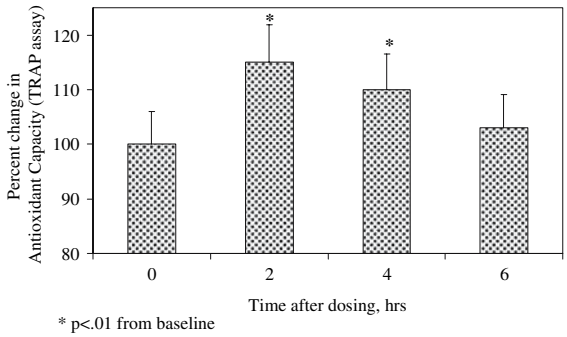


Fig. 5 Influence of Oligonol® on plasma antioxidant capacity in Sprague-Dawley rats receiving 4 mg/kg

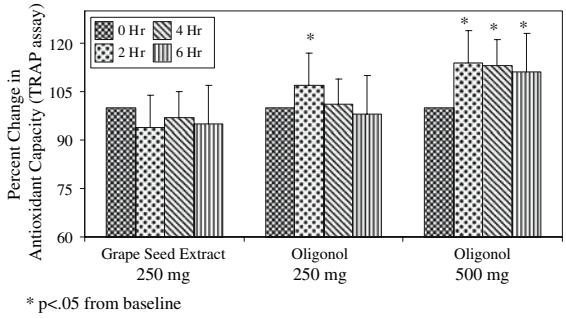


Fig. 6 Influence of grape seed extract and Oligonol on plasma antioxidant capacity in humans

reported to enhance antioxidant protection against β-amyloid-induced oxidative cell death in a neurodegenerative model. Rat pheochromocytoma (PC12) cells treated with β-amyloid and supplemented with Oligonol® showed reduced intracellular reactive oxygen species accumulation and lipid peroxidation, and an increase in the cellular glutathione pool compared to untreated cells (Li et al. 2004). Oligonol®-treated cells also showed less β-amyloid induced cytotoxicity and fewer apoptotic features than untreated cells, and pretreatment with Oligonol® suppressed the transient activation of nuclear factor-κB (NF-κB) induced by β-amyloid.

Genetically hypercholesterolemic rabbits fed cocoa powder (7.8% polyphenol content) for six months showed less LDL oxidation and decreased TBARS compared to animals consuming a control diet (Kurosawa et al. 2005). The area of atherosclerotic lesion was significantly smaller in the supplemented group (31%) compared to the control group (52%). Humans consuming flavanol-rich chocolate showed a dose-dependent increase in plasma antioxidant capacity (Wang et al. 2000). Such increases may manifest as protection against LDL oxidation, as noted acutely (within 2 h) in subjects ingesting a flavanol-rich cocoa (Kondo et al. 1996) and chronically in men ingesting 36 g of cocoa powder containing 2.62 g polyphenols for 2 weeks (Baba et al. 2001). Another indicator of lipid peroxidation, plasma F(2)-isoprostane was significantly reduced in humans engaged in strenuous physical activity when they took a high-flavanol cocoa drink (187 mg flavanols/100 ml) compared to when they took a low-flavanol cocoa drink (14 mg flavanols/100 ml) (Wiswedel et al. 2004).

Vascular effects of flavanols

Numerous risk factors have been identified that promote the pathogenesis of atherothrombotic disease, including smoking, dyslipidemia, hypertension, diabetes, estrogen withdrawal, and elevated homocysteine levels. Collectively, as well as individually, these risk factors can contribute to oxidative stress of the cardiovascular system. Oxidative stress in the vascular endothelium reduces the availability of nitric oxide (NO), resulting in endothelial dysfunction that can manifest as increased platelet aggregation, vasoconstriction and inflammation. Any of these alterations can result in vascular lesion initiation and progression, plaque rupture, vasospasm and thrombosis.

Flavanol-rich foods and beverages can affect endothelial function (West 2001; Duffy and Vita 2003; Vita 2005). Initial studies in humans determined that consumption of flavanol-rich purple grape juice for 14 days was associated with significant increases in blood flow, measured by flow mediated dilation (FMD), and an increased time before the onset of free radical induced oxidation of low density lipoproteins (lag time to LDL oxidation) (Stein et al. 1999). Flavanol-rich dealcoholized red wine (Agewall et al. 2000) and black tea (Hodgson et al. 2002) intake have been shown to improve brachial artery FMD in both healthy human subjects and those with cardiovascular disease following acute and chronic consumption. In other acute studies, flavanol-rich cocoa was shown to increase FMD significantly more

than flavanol-poor cocoa 2 h after intake of a single dose in outpatients with one cardiovascular risk factor (Heiss et al. 2003). A 3-hour study on the effects of an acute feeding of flavonoid-rich chocolate on endothelial function in healthy volunteers reported a significant increase in resting and hyperemic brachial artery diameter over the course of the trial, and in FMD 60 min after the intake of the chocolate (Vlachopoulos et al. 2005).

One week of daily intake of a flavanol-rich cocoa (three daily doses of 306 mg flavanols each) resulted in increases in FMD ranging from 3.7% after one day to 6.6% after 1 week, which were paralleled by increases in circulating nitrite, but not nitrate (Heiss et al. 2007). These improvements disappeared after a 15-day washout period. Chronic studies in a group of healthy subjects consuming flavanol-rich chocolate for 2 weeks also showed a greater FMD compared to those consuming a low-flavanol chocolate (Engler et al. 2004). A 6-week trial in hypercholesterolemic postmenopausal women reported those consuming a high-flavanol cocoa beverage (446 mg flavanols) showed significant improvements in FMD, while those consuming a low-flavanol beverage (43 mg flavanols) did not (Wang-Polagruto et al. 2006). The changes in FMD were correlated with a decrease in vascular cell adhesion molecule-1, which is associated with early monocyte binding to endothelium and endothelial activation. In contrast to the above, a 6-week study among 40 patients with coronary artery disease consuming either a flavanol-rich chocolate bar and cocoa drink (444 mg/day total flavanols) or a matching isocaloric placebo (20 mg/day total flavanols) showed no acute or chronic differences in FMD, systemic arterial compliance, forearm blood flow or soluble cellular adhesion molecules (Faroque et al. 2006).

The changes in FMD may also be associated with improvements in blood pressure. Never-treated male patients with essential hypertension given either white chocolate or a flavanol-rich dark chocolate (88 mg flavanols) daily for 15 days showed significantly reduced ambulatory 24-hour systolic blood pressure in those consuming the high-flavanol chocolate but not in those consuming white chocolate (Grassi et al. 2005). The dark chocolate group also showed significant improvement in FMD and insulin sensitivity, and decreased serum LDL cholesterol compared to the white chocolate group. Both systolic and diastolic blood pressure decreased significantly among a group of healthy men and women, ages 55–64, after 10 days of consuming a dark chocolate bar (500 mg polyphenols) but not after consuming a polyphenol-free chocolate bar (Taubert et al. 2003). In contrast, hypertensive individuals consuming a daily supplement of one gram of grape seed polyphenols plus 500 mg vitamin C showed increased systolic and diastolic blood pressure, and no change in FMD or markers of oxidative stress (Ward et al. 2005). Unfortunately, a more detailed composition of the grape seed extract was not provided.

Flavanol regulation of vascular nitric oxide synthase (NOS) metabolism in endothelial cells (Leikert et al. 2002) may help explain improvements seen in FMD. Flavanol-rich red wine produced relaxation in rat aortic rings via enhanced NO synthesis (Andriambeloson et al. 1997). Exposure of aortic tissue from New Zealand White rabbits to oligomeric cocoa flavanols significantly increased NOS activity (Karim et al. 2000).

Human studies are consistent with the findings in animals. Twenty subjects at risk for cardiovascular disease who consumed a high-flavanol cocoa beverage showed significant increases in plasma nitroso compounds and FMD compared to when they consumed a low flavanol cocoa beverage (Sies et al. 2005). Male smokers consuming a high (176–185 mg) flavanol cocoa drink showed significant increases in FMD and circulating NO species in plasma 2 h after ingestion, while those consuming a low-flavanol (<11 mg) cocoa drink showed no change in either outcome (Heiss et al. 2005). In another study, four days of supplementation with high-flavanol cocoa (821 mg flavanols/day) among 27 healthy adults resulted in strikingly consistent improvements in peripheral vasodilation, as measured by volume-sensitive plethysmography (Fisher et al. 2003). After intake on day 5, an additional acute response was noted, which was completely reversed with infusion of a NOS inhibitor. Flavanol-rich cocoa also amplified the system pressor effects of the NOS inhibitor. These observations were extended and related to age. Flavanol-rich cocoa (four daily doses of 230 mg flavanols each) had a greater effect on blood pressure and peripheral arterial response in 19 participants over 50 years of age compared to 15 subjects less than 50 years old (Fisher and Hollenberg 2006). FMD measured by finger tonometry was

enhanced in both groups, but significantly more in the older group. Changes in basal pulse wave amplitude followed a similar pattern, which was reversed following administration of an NOS inhibitor, suggesting that NO-related vascular effects may be greater among older people, who are more likely to have compromised endothelial function.

(−)-Epicatechin and its metabolite, epicatechin-7-*O*-glucuronide, (Figs. 1, 2) have been identified as independent predictors of the vascular effects after flavanol-rich cocoa intake (Schroeter et al. 2006). Intake of pure (−)-epicatechin closely mimicked the acute vascular effects of flavanol-rich cocoa and further suggested that this flavanol can be causally linked to reported vascular benefits. The authors propose the concept of epicatechin equivalents, defined as the ability of a food, beverage or supplement, on a caloric or per serving basis, to improve surrogate markers for cardiovascular disease, as a potentially useful index for evaluating flavanol- and procyanidin-containing foods. (−)-Epicatechin has also been shown to reduce human LDL oxidation, and protect vascular endothelial cells from damage by oxidized human LDL in vitro (Steffan et al. 2006). Similar protection was not measured with other vasoprotective agents, specifically vitamin C, vitamin E or aspirin.

Flavanols have been shown to influence platelet aggregation. While platelet aggregation is one of the initial steps in blood clotting, which is essential for survival, an overly reactive platelet response can increase risk for blood clots that can occlude coronary or cerebral arteries, resulting in myocardial infarction, stroke or venous thromboembolism. Whole human blood incubated with GSE composed of a large amount of gallated procyanidins showed significant reduction in collagen-induced platelet aggregation (Shanmuganayagam et al. 2002). When fed to dogs, the GSE alone failed to influence platelet aggregation, but did produce a significant decline when combined with grape skin extract (also rich in procyanidins). Isolated human platelets showed decreased aggregation when incubated with either GSE or grape skin extract, along with a marked decrease in superoxide release and an increase in radical-scavenging activity (Vitseva et al. 2005). Intake of flavanol-rich cocoa has been shown to reduce ADP/collagen- and epinephrine/collagen-stimulated platelet aggregation within hours after consumption (Rein et al. 2000; Holt et al. 2002b; Pearson et al. 2002). These antiaggregatory effects were associated with a reduction in the ADP- and epinephrine-induced expression of platelet GPIIa/IIIb surface protein to an extent only slightly less than that achieved by low-dose (81 mg) aspirin (Pearson et al. 2002; Pearson et al. 2005).

The platelet modulating effects of flavanols may be mediated in part through both antioxidant and NO pathways. Purple grape juice intake for 14 days in humans reduced platelet superoxide release and platelet aggregation, with a concomitant increase in platelet NO production (Freedman et al. 2001). Platelet incubation with extracts of purple grape skins or seeds showed dose-dependent decreases in superoxide release and production of reactive oxygen species, inhibition of platelet aggregation, enhanced NO release and immediate reduction of soluble CD40 ligand, an inflammatory mediator (Vitseva et al. 2005).

Modulation of the inflammatory response may be another mechanism through which flavanols influence vascular function. High flavanol cocoa extract has been shown to stimulate NO production and reduce the activity of the proinflammatory enzymes xanthine oxidase and myeloperoxidase after ethanol-induced oxidative stress (Osakabe et al. 1998). Flavanol monomers and procyanidins added to peripheral blood mononuclear cells were shown to suppress production of the proinflammatory cytokines interleukin (IL)-1β and IL-2 (Mao et al. 2000) and 15-lipoxygenase activity (Sadik et al. 2003; Schewe et al. 2001), and favorably modify transforming growth factor-β_1 and tumor necrosis factor alpha (Mao et al. 2002). NF-κB regulates gene transcription of cytokines and adhesion molecules involved in the onset and progression of vascular disease. Epicatechin, catechin and procyanidin dimers B2 and B5 have been determined to regulate NF-κB and inhibit increases in cell oxidant concentrations (Mackenzie et al. 2004). Dealcoholized wine from pomegranates has also been shown to act as a potent inhibitor of NF-κB activation in vascular endothelial cells grown in vitro (Schubert et al. 2002). 5-Lipoxygenase, the key enzyme in the synthesis of proinflammatory leukotrienes in humans, was inhibited in the presence of monomers or oligomers of two to five epicatechin subunits in vitro (Sies et al. 2005). Larger oligomers produced no

effect. The tea flavanol, EGCG, has been shown to be an even more potent inhibitor of 5-lipoxygenase than cocoa monomers (Schewe et al. 2002).

Meta-analysis and epidemiology

A systemic review of 136 publications related to chocolate and cardiovascular disease concluded that intake of flavonoids from cocoa may lower risk of cardiovascular (CV) mortality (Ding et al. 2006). An updated meta-analysis from this review found a significantly lower risk of CV mortality when comparing the highest and lowest tertiles of flavonoid intake (Relative Risk = 0.81 (95% CI: 0.71–0.92). The authors urge larger randomized trials to more definitively assess the impact of chocolate specifically, and flavanols in general, on long-term CV measures.

Results from the Zutphen Elderly Study have shown an inverse relationship between cocoa intake and 15-year cardiovascular and all-cause mortality, as well as blood pressure (Buijsse et al. 2006). A total of 470 healthy men aged 65–84 years were followed over a 15 year period. Blood pressure was measured at baseline and five years later, and food consumption was calculated at baseline and at five and ten years. Cocoa intake was estimated from food consumption data. The median cocoa intake among users was 2.11 g/day, though the flavanol intake is not reported. After 15 years, 314 men died, of which 152 died from CV diseases. Compared with men in the lowest tertile, men in the highest tertile of cocoa intake had an adjusted relative risk for cardiovascular mortality of 0.50 (95% CI, 0.32–0.78, $P = 0.004$) and for all-cause mortality of 0.53 (95% CI, 0.39–0.72, $P < 0.001$). After adjustment, the mean systolic blood pressure was 3.7 mm Hg lower, and the mean diastolic blood pressure 2.1 mm Hg lower in the highest tertile of cocoa consumers compared to the lowest tertile ($P = 0.03$ for each trend).

Conclusion

Understanding of flavanol chemistry has advanced greatly in the past decade, but many key questions remain, including clarification of which metabolites produce the most biological activity and mechanisms of action. Caution must be used in translating in vitro studies and methods of flavanol delivery that bypass phase I/II metabolism in the gut and liver. Additionally, future studies must clearly define the composition of extracts. Studies must move beyond testing a "soup" to identification and testing of purified compounds.

While it is tempting to suggest that flavanol-induced changes in NO-dependent pathways provide a central model for effects on the vascular system, other factors such as platelet aggregation and inflammatory mediators are also affected by flavanols and may act independently from NO. Individual genetic variations may also influence the effect of flavanols.

Targeted dietary components and nutrition supplements that can influence the redox-sensitive components of the vascular system will be of great value in the prevention and treatment of cardiovascular disease, stroke, hypertension, pulmonary disease and possibly neurodegeneration. While measurements of oxidative protection and changes to the antioxidant parameters will aid in the determination of mechanisms of flavanol activity, changes to clinically relevant indices that consumers can feel (e.g., more energy, improved cognition) may ultimately drive consumer demand for flavanol-rich products. Enhancement of bioavailable flavanols through improvements in harvesting and food processing will likely lead to new flavanol-rich foods, beverages and supplements.

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