The vasculoprotective effects of flavonoid-rich cocoa and chocolate

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Abstract

The current evidence on the vasculoprotective effects of flavonoid-rich cocoa and chocolate is reviewed. Cocoa flavonoids are derived from the cacao bean, found in the fruit pod of the cacao tree, Theobroma cacao. There is growing evidence that dietary supplementation with flavonoid-rich cocoa and chocolate may be cardioprotective because of their interference in many pathophysiological mechanisms associated with atherosclerosis. Possible beneficial effects by cocoa flavonoids include: antioxidant properties, improvement in endothelial function, blood pressure lowering, decreased platelet activation and function, and modulation of immune function and inflammation. Larger, long-term clinical trials investigating the potential beneficial effects of purified flavonoids and food sources rich in flavonoids, including cocoa and chocolate, are certainly warranted. © 2004 Elsevier Inc. All rights reserved.

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1. Introduction

Flavonoids represent a ubiquitous and abundant group of polyphenols consumed in the diet, primarily from fruits and vegetables. These compounds are derived from plants and act as antioxidants due to their free radical scavenging properties, their ability to reduce the formation of free radicals [1], and their ability to stabilize membranes by decreasing membrane fluidity [2]. This effect may inhibit lipid peroxidation in vascular endothelial cell
membranes, reducing mobility of free radicals in the lipid bilayer. The antioxidant properties of flavonoids may contribute to mounting evidence that a diet rich in fruits and vegetables reduces the risk of cardiovascular disease [3]. The American Heart Association dietary guidelines currently recommend five or more servings of fruit and vegetables per day [4]. It is estimated that the daily intake of flavonoids ranges from 50 to 800 mg depending on the amount consumed in the diet [1].

The common classes and food sources of flavonoids include: flavanol (quercetin, kaempferol, myricetin [in onions, apples, tea, and red wine]), isoflavones (daidzein, genistein [in soy]), flavan-3-ols or flavanols (catechin, epicatechin [in tea, chocolate, red wine]), flavanones (naringenin, hesperitin [in citrus fruits]), flavones (apigenin [in celery], luteolin [in red pepper]), and anthocyanins (in pigments of red fruits such as berries and red grapes). These different classes of flavonoids are based on the level of oxidation in the basic flavonoid structure (C6-C3-C6), a 15-carbon atom structure arranged in three rings (two aromatic rings on the ends with an oxygenated heterocycle in the middle). Flavonoids have been reported to have a beneficial influence on oxidative stress, vascular function, platelet function, and immune responses that may be involved in the process of atherogenesis.

Recent improvements in analytical methodology, such as high-performance liquid chromatography (HPLC) coupled with electrochemical detectors, have advanced the field of flavonoid research from analysis of plant composition to bioavailability and bioactivity studies in which the detection limits of flavonoids are 100-fold lower [5]. Identification and quantification of flavonoids in chocolate have only been developed in the past few years. Chocolate has been found to be a rich food source of flavonoids in comparison to many common food and beverages [6]. Cocoa flavonoids are characterized as flavan-3-ols or flavanols and include the monomeric forms, (−)-epicatechin and (+)-catechin, and the oligomeric form of the monomeric units, the procyanidins (Fig. 1). These flavonoids are found in the cacao bean, the fruit seed of the cacao tree (*Theobroma cacao*), which is used
in the manufacture of cocoa products and chocolate. Interestingly, there is a linear relationship between the amount of cocoa flavonoids in samples of cocoa and chocolate to antioxidant capacity, which is measured using the oxygen radical absorbance capacity (ORAC) assay [7]. The processing of the cacao beans in the manufacture of chocolate can affect the amount of flavonoids retained and therefore antioxidant capacity. Flavonoid levels in chocolate are preserved approximately 70–95% when heat and alkalization are reduced during processing. Traditional processing conserves only 50–75% of the total cocoa flavonoids [8,9].

It is also important to note that most dark chocolate contains a higher amount of antioxidant cocoa flavanols than does milk chocolate. For example, a 40-g serving of milk chocolate provides 394 mg of cocoa flavonoids, whereas dark chocolate contains 951 mg [10]. Hot cocoa mix, in contrast, contains 45 mg of cocoa flavonoids in a 240-mL serving. These numbers represent typical cocoa flavonoid concentrations and are dependent on the chocolate processing methods, which may reduce or retain the amount of flavonoids derived from the cacao bean.

2. Antioxidant properties

Oxidative stress or the imbalance between increased generation and decreased elimination of free radicals is well described as a causal process in the oxidation of low-density lipoprotein cholesterol (LDL-C) and the subsequent development of atherosclerosis. Free radicals are cytotoxic to vascular endothelial cells and decrease the bioavailability of nitric oxide (NO). The physiological effects of endothelial- and platelet-derived NO are shown in Fig. 2. Oxidative stress causes damage to molecules such as DNA, lipids, and proteins and contributes to impaired endothelial function or endothelial dysfunction. NO bioavailability is reduced as a result and a host of pathophysiological consequences such as vasoconstriction, thrombosis, and inflammation can occur (Fig. 3). Vascular lesion progression may then evolve to plaque rupture, thrombosis, and vasospasm with eventual coronary events. Many antiatherosclerotic properties have been attributed to NO including inhibition of the following: oxidation of LDL-C, smooth muscle proliferation, expression of adhesion molecules, endothelin production, platelet aggregation, smooth muscle contraction, and monocyte and platelet adhesion [11].

Endothelial dysfunction due to cardiovascular risk factors such as high cholesterol, smoking, hypertension, or diabetes promotes LDL-C penetration and oxidation in the subendothelial space of the arterial wall. Oxidized LDL stimulates an inflammatory response, including release of chemotactic and cell adhesion molecules, i.e., vascular adhesion molecules (V-CAM), intracellular adhesion molecules (I-CAM), and E-selectin, which recruit and promote penetration of monocytes into the subendothelial layer. These monocytes are transformed into macrophages and engulf oxidized LDL, with eventual formation of foam cells and fatty streaks, leading to advanced atherosclerotic lesions within the arterial wall.

It is clear that oxidized LDL may play a significant role in the initial endothelial damage that leads to atherogenesis. The hypothesis that inhibition of the production of oxidized LDL will prevent atherosclerosis and cardiovascular disease has stimulated epidemiological,
experimental, and clinical trials with flavonoids and food or beverage sources rich in flavonoids. Polyphenol constituents of cacao liquor, the key ingredient of chocolate, have been shown to have antioxidant effects [12,13]. Consumption of cocoa powder (37.5 g) rich in flavonoids (catechin, epicatechin, and oligomeric procyanidins) in six healthy adults decreases LDL susceptibility to metal ion–dependent and metal ion–independent oxidation and spared α-tocopherol in vitro [14].

Cocoa supplementation (36.9 g of dark chocolate bar and 30.9 g of cocoa powder drink) daily for 6 weeks in healthy adults increases LDL oxidation lag time but has no effect on urinary F2 isoprostanes [15]. The amount of cocoa flavonoids was equivalent to 651 mg/day. In another study with cocoa supplementation for 4 weeks (22 g cocoa powder and 16 g dark chocolate) serum total antioxidant capacity was increased and LDL oxidation susceptibility was reduced in 23 healthy subjects [16]. Even an acute study with an 80 g bolus of procyanidin-rich semi-sweet chocolate was shown to induce a significant increase (31%) in plasma total antioxidant capacity and a decrease (40%) in plasma 2-thiobarbituric acid reactive substances [17]. Importantly, an increase in plasma epicatechin, the major cocoa flavanol, was associated with the antioxidant effects [14,16,17].

In our short-term study with 21 healthy adults, 46 g of flavonoid-rich dark chocolate (259 mg of cocoa flavonoids) was consumed daily for 2 weeks. A significant increase in plasma epicatechin concentrations was observed after consumption (Fig. 4), but no change was found in plasma oxidation measurements, i.e., LDL oxidation, 8-isoprostanes, or oxygen radical absorbance capacity (ORAC) [18,19]. Consumption of one dose of procyanidin-rich chocolate (27, 53, and 80 g) produced a weak trend for dose-response increases in plasma antioxidant capacity as well as decreases in plasma lipid oxidation products, but no change was seen in plasma 8-isoprostanes [20]. Although most of the current studies have demon-
strated antioxidant effects with cocoa and/or chocolate consumption, the variable results may be related to differences in baseline epicatechin concentrations and the magnitude of increase seen after consumption. These may result from differences in baseline diets or in the sensitivity for detecting low concentrations of epicatechin using different HPLC coupled with electrochemical (coulometric detection) methodologies.

Interestingly, Serafini et al. recently demonstrated an increase in total antioxidant capacity and plasma epicatechin concentrations after consumption of dark chocolate (100 g) in 12 healthy volunteers; however, these effects were reduced by the presence of milk [21]. This amount (100 g) of dark chocolate represents approximately 2.5–3 times the size of a standard size chocolate bar. Communications by Halliwell [22] and Schroeter et al. [23] in response to this study raise important concerns regarding the methods used and results. As Halliwell points out, the unusual high total plasma antioxidant capacity of 18% after chocolate consumption reported by Serafini et al. [21] may be due to an increase in plasma urate levels rather than due to the antioxidant properties of epicatechin or its metabolites. Hyperuricemia may lead to deleterious health effects.

In a separate study with 12 healthy volunteers, Schroeter at al [23] reported that the presence of milk in cocoa products did not influence the absorption or biological activity of epicatechin from the cocoa product. A milk-independent increase in plasma antioxidant

Fig. 3. Pathogenesis of atherothrombotic disease, and possible inhibition of oxidative stress and endothelial dysfunction by the cocoa flavonoids.
capacity and epicatechin concentrations was observed as well as, a reduction in platelet-mediated hemostasis after consumption of a milk-containing cocoa beverage. The authors highlighted the matrix-dependent and temporal effects on the absorption of dietary flavanols that need to be accounted for when comparisons of different food formulations and processing methods are made in the context of the biological effects of flavanols. It is acknowledged that an understanding of the mechanisms that regulate total plasma antioxidant capacity and the effects of food in plasma redox status and oxidative disease are imperative in future research [24].

Thus, it is apparent in most of the above studies, which examined the antioxidant properties of chocolate or cocoa beverage that the major cocoa flavanol, epicatechin, is readily absorbed and peaks in the plasma concentration at 2 hours. The antioxidant properties of cocoa or chocolate consumption are associated with an increase in plasma epicatechin concentrations.

### 3. Improved endothelial function

In an experimental study using isolated rabbit aortic rings, extracts of cocoa rich in procyanidins induced endothelium-dependent relaxation and activated endothelial NO synthase, the enzyme required for synthesis of NO [25]. The tetramers and higher polymers of epicatechin were associated with these effects. Cultured human aortic endothelial cells exposed to cocoa flavanols produce twice as much 6-keto-prostaglandin F$_{1\alpha}$ (a stable
metabolite of prostacyclin) and 16% less leukotrienes when compared to control cells [26].

This favorable alteration in eicosanoid synthesis (specifically, the decrease in the plasma leukotriene–prostacyclin ratio) would promote more vasodilation and inhibit platelet aggregation as well as provide an anti-inflammatory eicosanoid balance. Interestingly, similar alterations in eicosanoid synthesis were seen in plasma samples obtained from 10 subjects 2 hours after consumption of 37 g of a high-procyanidin chocolate. A concomitant increase in plasma epicatechin was also reported [26].

Our recent randomized, double blind, placebo-controlled study conducted over a 2-week period in 21 healthy subjects demonstrated an improvement in endothelium-dependent flow-mediated dilation (FMD) of the brachial artery [18,19] after daily consumption of high-flavonoid (259 mg) dark chocolate (46 g). Heiss et al. reported a reversal of endothelial dysfunction and an increase in NO bioactivity in human plasma after a single dose of a cocoa beverage (100 mL) high in cocoa flavanols (176 mg) [27]. The study included 26 participants with at least 1 cardiovascular risk factor, including a history of coronary artery disease, hypertension, hyperlipidemia, diabetes, or current tobacco use.

Another recent investigation with 27 healthy subjects reported increased fingertip pulse wave amplitude after 4 days of cocoa beverage consumption (821 mg cocoa flavonoids per day) [28]. The NO synthase inhibitor, N^G^-nitro-L-arginine methyl ester (L-NAME), given intravenously, reversed a further increase in dilation after an additional dose of cocoa was ingested on the last day of the study. No measurements of plasma epicatechin were taken in this study. Notably, this study is novel in its use of peripheral arterial tonometry in the fingertip as a methodology to assess vasodilation mediated by reactive hyperemia. However, the fingertip represents a microcirculatory bed with arterioles, metarterioles, capillaries, and venules where, because of their large cross-sectional area, flow velocity is the least.

Vasodilation in arteries <2.5 mm in diameter are difficult to measure. Reactive hyperemic responses in the fingertip are the lowest as compared to responses in larger arteries, such as the brachial artery. Therefore, endothelium-dependent flow-mediated dilation due to increased flow and shear stress after reactive hyperemia is commonly assessed in the brachial artery, which correlates closely to responses in the coronary arteries [19,29]. Although experimental and clinical data are limited on the effects of cocoa flavanols in improving endothelial function, these studies suggest a beneficial mechanism for improving vascular health in both normal subjects and those with cardiovascular risk factor or evidence of coronary artery disease.

4. Effects on blood pressure

Data published from a randomized crossover trial in 13 healthy individuals (aged 55–64 years) with recently diagnosed and untreated stage 1 mild isolated systolic hypertension demonstrate significantly lower systolic and diastolic blood pressure within 10 days of a 14-day intervention of high-flavonoid (500 mg) dark chocolate (100 g). The mean systolic blood pressure declined by 5.1 mm Hg and the mean diastolic blood pressure decreased by 1.8 mm Hg compared to controls [30]. A recent study by Actis-Goretta et al., which measured angiotensin I converting enzyme (ACE) activity, showed cocoa flavonoids com-
pete for enzyme-active sites with synthetic ACE substrates [31]. The authors speculated that the cocoa flavanols may lower blood pressure by acting as ACE inhibitors, which are known to modulate antioxidant defenses and to regulate mitochondrial NO production.

In a report by Engler et al., no significant changes in systolic or diastolic blood pressure measurements were seen after 2 weeks of daily flavonoid-rich dark chocolate supplementation in healthy adults (aged 21–55 years) with normal baseline blood pressure [18,19]. Mean arterial pressure in healthy subjects (aged 26–49 years) was also not changed 2 or 6 hours after a single-dose ingestion of 80 g of flavonoid-rich (557 mg) chocolate baking bits [17]. Another acute study in 13 healthy subjects (aged 23–32 years) found no change in systolic or diastolic blood pressure at 2, 4, or 6 hours after ingestion of chocolate (1.5 g/kg of body weight) [32]. The type of chocolate was not specified in this study. Fisher et al. [28] similarly found no effect with 4 days of cocoa beverage supplementation (4 daily 230-mL doses with 821 mg cocoa flavanols) on blood pressure.

5. Effects on platelets activation and function

Platelet and coagulation abnormalities lead to increased platelet activation and a pro-thrombotic milieu within the arterial lumen. Basically, normal platelet function represents four phases including activation, adhesion, aggregation, and secretion. Numerous stimuli from various substances, such as thromboxane A$_2$, thrombin, adenosine diphosphate, and epinephrine can activate the platelet initiating a spherical change in shape with extensions. Adhesion to the endothelium and other cells is facilitated and mediated by platelet membrane glycoproteins, mainly GPIIb/IIIa. GPIIb/IIIa binds to fibrinogen and promotes platelet aggregation, which can stimulate the coagulation cascade. Secretion of $\alpha$-granules and dense granules induces a number of effects, ranging from recruitment of other platelets and white blood cells, to vasodilation and vasoconstriction [33]. Since platelets play a significant role in the pathophysiology of cardiovascular disease, antiplatelet regimens with platelet GIIb/IIIa antagonists and aspirin have been used in the secondary prevention of acute coronary syndromes.

A recent study by Murphy et al. [34] in a blinded parallel design showed lower P-selectin expression indicating diminished platelet activation as well as lower ADP- and collagen-induced aggregation in healthy subjects after 28 days of cocoa tablets (234 mg of cocoa flavonoids per day) supplementation. Plasma epicatechin and catechin concentrations were also increased; however, no change was noted in plasma oxidation markers or antioxidant status.

In an interesting study with 16 healthy adults, the effects of one dose of flavanol-rich cocoa beverage (300 mL, 897 mg cocoa flavanols) and aspirin (81 mg) on ex vivo platelet function were examined. Both cocoa and aspirin (consumed separately or together) suppressed collagen–epinephrine–induced platelet plug formation or closure time under simulated small vessel shear conditions [35]. Cocoa treatment also prolonged collagen-ADP–induced platelet plug formation. Measurements that indicate platelet activation, i.e., epinephrine-stimulated expression of the activated conformation of the fibrinogen binding receptor GPIIb/IIIa, were reduced at 2 hours after consumption of the cocoa beverage, but
only 6 hours after the aspirin dose [35]. Both aspirin and cocoa combined reduced platelet activation at both 2 and 6 hours after consumption, suggesting a synergistic effect. Plasma epicatechin was also significantly increased at 2 hours in the cocoa and aspirin-plus-cocoa group, which suggests that the inhibition of platelet function was due to the flavanol content of the cocoa.

Consumption of flavonoid-rich semi-sweet chocolate bits (25 g), providing approximately 220 mg cocoa flavonoids as one dose, decreased platelet-related primary hemostasis in healthy adults [36]. This was measured by the time to occlude an aperture in a collagen membrane. Two earlier studies with higher single doses of cocoa flavonoids (897 mg) in healthy subjects also showed a suppressive effect on platelet reactivity, i.e., ADP- or epinephrine-stimulated platelet activation and platelet microparticle formation 2 and 6 hours after ingestion of the cocoa beverage [37,38].

6. Modulation of immune function and inflammation

Inflammation as well as increased oxidative stress in the vessel wall are considered major mechanisms that promote endothelial dysfunction and atherosclerosis. Decreased bioavailability of NO associated with endothelial dysfunction results in loss of inhibition of nuclear transcription factor (NFκB) by NO. NFκB binds to the promoter regions of genes that code for proinflammatory proteins, including adhesion molecules, cytokines, and other molecules that participate in atherogenesis [39]. NO inhibits synthesis and expression of cytokines and cell adhesion molecules that attract inflammatory cells to the endothelium and promote their migration into the vessel wall.

Flavonoids inhibit cytokine transcription possibly by reducing intracellular reactive oxygen species, which activate NFκB [40]. Purified cocoa flavonoid fractions (monomer–tetramer) consistently reduced the proinflammatory cytokine, interleukin-1β (IL-1β) expression in phytohemagglutinin-stimulated peripheral blood mononuclear cells by 1-15%, whereas the larger oligomers (pentamer-decamer) increased expression by 4–50% [40]. Secretion of tumor necrosis factor–α (TNF-α), another proinflammatory cytokine, is increased after exposure of cocoa flavonoid fractions for 72 hours in human peripheral blood mononuclear cells [41]. The authors suggest stimulation of TNF-α secretion may be beneficial against microbial infection and tumorigenesis. Another recent experimental study [42] found flavan-3-ol monomers, (-)-epicatechin and (+)-catechin, or procyanidin oligomers of these monomers, are incorporated in Jurkat T cells after pretreatment. They also inhibit PMA-induced NFκB activation at different levels of the activation cascade, including the PMA-induced increase in cell oxidants.

The immunomodulatory effects of cacao liquor purified flavonoids on lymphocyte and granulocyte functions were studied by Sanbongi et al. in 1997 [43]. Cacao liquor flavonoids were found to inhibit the production of both reactive oxygen species, hydrogen peroxide and superoxide anion, in activated granulocytes and in normal human peripheral blood lymphocytes. Moreover, the cocoa treatment inhibited mitogen-induced proliferation of T cells and Ig production by B cells in a dose-dependent manner. IL-2 mRNA expression of and IL-2 secretion by T cells were also inhibited [43].
Six weeks of chocolate/cocoa supplementation (651 mg of flavonoids per day) as described earlier in the report by Mathur et al. [15] in 2002, demonstrated no effect on whole-blood cytokines, IL-1β, IL-6, TNF-α, high sensitivity C-reactive protein, and P-selectin. Plasma epicatechin concentrations were not detected and may have been a factor with the negative findings reported. The outcome measurements were not taken 2 hours after chocolate/cocoa consumption, when plasma epicatechin has been shown to be at its highest level. Collectively, these studies suggest an important role of the cocoa flavonoids in the modulation of immune function and inflammation.

7. Conclusions

The cocoa flavonoids are important nutritional compounds in human health, as evidenced by their influence on a number of biochemical and physiological functions. They exhibit potent antioxidant effects under in vitro conditions and in vivo after consumption of flavonoid-rich chocolate and/or cocoa products. The antioxidant properties appear to be associated with an increase in plasma epicatechin concentrations. Endothelial-dependent vascular relaxation is promoted by cocoa flavonoids in part due to increased bioavailability of NO and prostacyclin. The antiatherosclerotic properties of NO combined with a favorable shift toward vasodilation may confer a vasculoprotective effect.

Blood pressure lowering is also noted after short-term dark chocolate intervention in the presence of mild isolated systolic hypertension. The antithrombotic properties of the cocoa flavonoids are significant in many studies, as exhibited by a suppressive effect on platelet reactivity and platelet-related primary hemostasis even after a single chocolate dose. Modulation of immune function and inflammation are also emerging as potential cardioprotective effects of the cocoa flavonoids, specifically, a reduction of proinflammatory cytokines.

It is clear that the cocoa flavonoids represent an exciting new area of nutritional research with significant implications for cardiovascular protection. Other food and beverage sources of the flavonoids found in cocoa products and chocolate include green and black tea (especially Ceylon), red wine, cherries (sweet), apples, purple grapes, blackberries, raspberries, and broad-beans [44]. Further experimental studies with cocoa flavonoids are needed to define the specific mechanisms of action. Long-term studies with large sample sizes are also warranted to determine optimal doses and long-term effects of purified flavonoids and food sources rich in flavonoids, including cocoa and chocolate.

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References


