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Cocoa, chocolate and cardiovascular disease

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Abstract

A significant body of evidence demonstrates that diets rich in fruit and vegetables promote health, and attenuate, or delay, the onset of various diseases, including cardiovascular disease (CVD), diabetes, certain cancers, and several other age-related degenerative disorders. The concept that moderate chocolate consumption could be part of a healthy diet has gained acceptance in the last years based on the health benefits ascribed to selected cocoa components. Specifically, cocoa as a plant and chocolate as food contain a series of chemicals that can interact with cell and tissue components providing protection against the development and amelioration of pathological conditions. The most relevant effects of cocoa and chocolate have been related to CVD. The mechanisms behind these effects are still under investigation. However the maintenance or restoration of vascular NO production and bioavailability and the antioxidant effects are the mechanisms most consistently supported by experimental data. This review will summarize the most recent research on the cardiovascular effects of cocoa flavanoles and related compounds.

Keywords

flavonoids; flavanols; hypertension; oxygen radicals; antioxidant; cardiovascular health

CARDIOVASCULAR DISEASE AND DIET

CVD, including stroke, is the leading cause of death and disability in developed countries. Atherosclerosis, vascular dysfunction, platelet aggregation, and other inflammation-associated conditions are central to CVD. Diet is a major factor contributing to the onset and development of CVD, by primarily affecting all the above mentioned conditions. Nevertheless, high intake of calories and certain fats increase the risk for CVD[1], diet also provides micronutrients that appear fundamental in controlling CVD[2]. Defining specific modifications of dietary habits in a population can have a major impact on CVD, especially during the long period in which the disease is silent[3]. To set new goals to improve human diets, it is important to understand how macro and micronutrients can interact with biological systems to enhance health.

CARDIOVASCULAR DISEASE AND COCOA

Robust epidemiological evidence demonstrates that diets rich in fruits and vegetables promote health, and attenuate, or delay the onset of CVD[3–6] (and references therein). The questions that remain open are: i) are all fruits and vegetables equivalent? if not, can we identify those with best health benefits?; and ii) how can we recognize the compounds responsible for such

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effects? A pioneer population study showed an inverse association between flavonoid consumption and the risk of coronary heart disease[7]. Under this paradigm cocoa and chocolate have been intensively studied in the last years mostly driven by the large content of flavanols and related compounds in cocoa beans that are generally conserved in the commercially available chocolate.

High cocoa and chocolate consumption have been associated with a decreased risk for CVD in a few population studies. A sub-study of a population from the Zutphen Elderly Study showed that cocoa consumption was associated with a decrease in blood pressure and overall cardiovascular mortality[8]. A case-control study done in Italy showed that the risk for myocardial infarction was inversely associated to chocolate consumption, reaching a 77% decrease in risk when comparing the population that ate more than three portions of chocolate per day with the population that consumed less than one[9]. Several dietary intervention studies in humans and animals demonstrated that cocoa[10–18] and other flavanol-rich foods/beverages[19–21] may exert protective vascular effects.

Although data for the above mentioned population and clinical studies show a similar trend suggesting flavanols as cardioprotective agents, the biochemical mechanisms behind that cardioprotection are not conclusively identified. Based on a number of in vitro studies, it has been speculated that biochemical mechanisms contributing to the health effects of flavanols include: antioxidant effects[22]; modulation of cell signaling and gene expression[23–24]; and alterations of certain cell membrane properties and receptor functions[25–26]. In addition to the above mentioned mechanisms, flavanols can inhibit several enzyme activities[27–30]. These mechanisms are not necessary independent, and then could occur concurrently or synergistically, as will be discussed in the following sections.

CHEMICAL STRUCTURE OF COCOA FLAVANOLS AND PROCYANIDINS

Polyphenols occur as plant secondary metabolites. Their ubiquitous occurrence in plants and plant foods, favors animal consumption and indeed human and animal tissue presence. Flavonoids are a chemically defined family of polyphenols that includes several thousand compounds. The flavonoids have a basic structure (Fig. 1), and several subclasses of flavonoids are characterized by a substitution pattern in the B- and C-rings. The main subclasses include flavanols, flavanones, flavones, isoflavones, flavonols, and anthocyanidins[31]. Flavanols are compounds present in high concentrations not only in cocoa, but also in grapes, apples, pomegranates, tea among other widely consumed fruits and vegetables. In cocoa and cocoa products, flavanols are present as: i) monomers, i. e. (−)-epicatechin (EC) and (+)-catechin (CT); and ii) oligomers of EC (procyanidins)[32]. EC (and CT) oligomers are denominated procyanidins, condensed tannins, or proanthocyanidins. Different plants present a particular pattern of monomers and oligomerization derivatives, e.g. cocoa procyanidins are mostly of the denominated B-type, e.g. B2-dimer (Fig. 1); while in tea (*Camellia sinensis*) predominate the gallolyated catechins.

BIOAVAILABILITY AND METABOLISM OF COCOA FLAVANOLS AND PROCYANIDINS

When discussing the biological activity of flavonoids in general, and flavanols in particular, four are the major factors to be considered: i) bioavailability from foods; ii) absorption and metabolism at the gastrointestinal tract; iii) tissue and cellular distribution after absorption; and iv) which are the chemical form(s) biologically available to the cell/tissue and their potential metabolism at cellular level. As indicated above, flavanols are present as monomers or forming procyanidins. Although it was initially thought that the procyanidins could not pass the acidic conditions of the stomach[33], data from human subjects show that flavanols and procyanidins

are stable during gastric transit[34–35]. Once in the mesenteric circulation, flavanols predominately exist in a conjugated form, both as methylated and glucuronidated flavanols[36–38]. In the liver, further glucuronidation and methylation can take place, as well as sulfation[36–38]. Metabolic studies have confirmed the presence of these conjugates in the plasma and urine of rodents and humans[38–41], as well as in the bile[38] and brain of rats[42]. It has been reported that colonic microflora can break flavonoids flavan structure to form simple phenolics and ring-fission metabolites that may be physiologically relevant[43–44]. In summary, non-metabolized flavanols or metabolites of flavanols can exert biological effects depending essentially on flavanol metabolism and presence in the target tissue[28–30].

In humans, plasma concentrations of EC plus EC-metabolites can be found in the micromolar range as soon as 1 h after cocoa consumption[12, 39, 45], with the major metabolite being 4'-o-methyl-epicatechin-7-β-D-glucuronide[12]. There is some evidence that certain flavanols are better absorbed than others. After human subjects consumed a cocoa beverage containing EC and CT in a 1:1 ratio, peak plasma CT concentrations were typically less than 10% of EC (0.16 vs. 5.92 μM)[45]. Part of these differences in plasma flavanol concentrations, could be due to procyanidin degradation; for example, dimers has been shown to form EC and methylated EC under certain conditions[41, 46–47], although the physiological relevance of such degradation remains to be confirmed.

While several research groups have examined the bioavailability of the monomeric flavanols, there is limited information on the bioavailability and metabolism of procyanidins. B2-dimer has been detected in the plasma of humans and rats[41, 45, 49]; B5-dimer (EC-(4β-6)-EC) has been detected in very minute quantities in simulated gastric and intestinal juice[47]; and no other type of dimer has been detected in rats[50]. It is important to note that the dimers that have been detected in the plasma are those made up of EC, not of CT subunits. A chemical explanation for these discrepancies is the different hydroxyl group orientation in the C3 position of the flavonoid B ring, which affects the interaction between the 3-OH on the C ring and the B ring resulting in dissimilar biological actions[51–52].

COCOA FLAVANOLS AS ANTIOXIDANTS: FREE RADICAL SCAVENGERS AND METAL CHELATORS

Plant polyphenols has been considered for long as physiologically relevant antioxidants based on the facts that: i) polyphenols have chemical structures favoring antioxidant actions, i.e. free radical scavenging and chelation of redox-active metals; ii) many polyphenols retain key features of their structure after ingestion and metabolism by mammals; and iii) certain polyphenols can provide physiological benefits in pathological situations associated with high free radical production, e. g. hypertension. Flavanols shared these properties, as confirmed by the extensive literature demonstrating that flavanols have free radical scavenging activity in a myriad of biochemical and ex vivo systems[23, 25, 26, 53–61], and also in animal models and in humans[62–64]. In theory, these antioxidant actions can result in a reduction of the steady state concentration of free radicals and other oxidants, diminishing the subsequent oxidation of target molecules such as lipids, proteins and nucleic acids. However, one important limitation for the "antioxidant action" hypothesis resides in the relatively low flavanol and procyanidin plasma concentrations observed even after the consumption of foods rich in these compounds[45, 65]. The actual concentrations that can be reached in plasma of humans subjected to realistic polyphenol consumption are in the nanomolar range and are transient in nature (peaking at 2–4 h)[12, 45]. This low bioavailability leads to a kinetically unfavourable condition with respect to other compounds with similar free radical scavenger capabilities, that are present in blood in significantly higher micromolar concentrations i.e. tocopherols and ascorbate. Thus, a function of flavanols as direct free radical scavengers is unlikely to be relevant, and could be limited to the blood and other tissues directly exposed after consumption,

i.e. gastrointestinal tract. It has been suggested that other mechanisms, compatible with the physiological levels reached by flavanols, may explain the observed changes in cell or tissue oxidation levels after flavanol consumption. These mechanisms are beyond the ability of flavanols and other flavonoids to directly prevent free radical-mediated tissue damage[65–66].

Another relevant detrimental effect of oxidants is the reaction of nitric oxide (NO) with superoxide to form peroxynitrite[67]. This reaction, that occur in a near diffusion controlled rate, and is important at the vascular level, leads to two undesirable conditions: i) reduction of NO availability necessary for proper smooth muscle cell function in vessel relaxation; and ii) increased formation of peroxynitrite that will promote oxidative and nitrosative damage (Fig. 2). NO production in mammalian cells is catalyzed by the enzyme nitric oxide synthase, which activity is by four isoforms, i.e. endothelial (eNOS) expressed mostly in endothelial cells; neuronal (nNOS) present mainly in neurons; inducible (iNOS) expressed in response a variety of proinflammatory stimuli; and mitochondrial (mtNOS) present in the inner membrane of the mitochondrion[68–69]. In the vascular environment, superoxide is not only originated by the mitochondria respiratory chain[70–71], xanthine oxidase, and cytochrome P450[72–73], but by other two enzymes: a non phagocytic NADPH oxidase[74] and an uncoupled endothelial NOS[75]. Then, flavanols and procyanidins present in circulation or in the vasculature can improve NO vascular concentration by interfering with the reaction between NO and superoxide by: i) inhibiting NADPH oxidase-dependent superoxide production [30]; ii) optimizing NO generation by NOS[76]; and/or iii) scavenging superoxide, H_2O_2, and other oxidants that mediate damage to cell components with superoxide and other oxidants[77]; and iv) modifying membrane-related events leading to changes in NO and superoxide production.

COCOA FLAVANOLS AS ANTIOXIDANTS: PROTEIN AND LIPID INTERACTIONS

Flavanols and procyanidins have multiple phenolic hydroxyl groups (Fig. 1) that favour their interaction with biological membranes which can occur via the formation of hydrogen bonds. Furthermore, the presence of both, hydrophobic and hydrophilic residues within the flavanol molecule, allows these compounds to interact with phospholipid head groups and be adsorbed onto the surface of membranes. These interactions can result in changes in a number of membrane properties leading to alterations in the regulation of membrane associated molecules and events, including, enzymes, receptors, and other functional proteins receptors[78–80].

Some enzymes affected by cocoa consumptions are directly associated with CVD and oxidant m e tabolism, such as 5-lipoxygenase[81–82], cyclooxygenase-2[83–84], and metalloproteinases[85]. The interactions of flavanols and proteins can also lead to changes in the modulation of gene expression. A direct interaction between nucleic acids and flavanols is thermodynamically feasible[86], but the possibility that these compounds reach the DNA and achieve mechanistically-relevant concentrations is rather low. The modulation of signaling pathways by flavanols has been extensively studied[87–89]. More specifically, the effects of flavanols and procyanidins on the oxidant-regulated NF-kB activation pathway have received special attention. In Jurkat T cells, we demonstrated that EC and CT, and B2-dimer inhibited phorbol mirystate acetate (PMA)-induced IL-2 production, and interfere with several steps of the NF-κB activation cascade[23]. Essentially, since monomers and dimers (and their metabolites) can be transported into the cells they can act by: i) attenuating intracellular oxidants associated with select stimuli, and the subsequent activation of NF-κB (antioxidant effect); and/or ii) interacting with specific proteins, resulting in the inhibition of the phosphorylation and/or degradation of the inhibitory protein IκBα, the transport of active NF-κB from the cytosol into the nucleus, and/or the binding of NF-κB to κB DNA[23, 90]. Large procyanidins (with 3 or more units), that are mostly affecting cells from outside, modulate NF-κB activation by modulating the binding of the ligand (stimuli) to its receptor, as we observed

in Caco-2 cells exposed to tumor necrosis factor alpha[91]. Another example of flavanols interaction with membranes is the finding that EC, B2-dimer and C1-trimer modulate intracellular calcium in Jurkat T cells[92].

COCOA, LIPID METABOLISM, AND ATHEROSCLEROSIS

Alterations in plasma cholesterol concentration, especially increased levels of LDL-cholesterol, and decreased levels of HDL-cholesterol are associated to the development of atherosclerosis[93–94] and CVD[1]. Reductions in LDL-cholesterol plasma levels have been reported after treatments with polyphenols from different sources[95–98]. Regarding the action of cocoa, mild hypercholesterolemic subjects lowered significantly (~5%) their LDL cholesterol level after 4 weeks of a dietary intervention with cocoa powder (81–163 mg/day of EC+CT)[99]. Even in normocholesterolemic young subjects a 15% reduction was observed in LDL-cholesterol level after 14 days of a daily consumption of 105 g of flavanol-containing milk chocolate (168 mg of flavanols)[100]. Patients with essential hypertension showed 11% of decrease in LDL cholesterol after 15 days receiving 100 g/day dark chocolate (88 mg of flavonols)[10]. Under an equivalent protocol a similar result was observed in glucose-intolerant hypertensive patients (−7.5%)[101]. Although the above studies did not investigate potential mechanisms, other works have proposed that LDL-cholesterol decrease associated to flavanoids consumption from different sources include: i) inhibition of cholesterol absorption in the digestive tract[96]; ii) inhibition of LDL biosynthesis in the liver[98]; iii) suppression of the hepatic secretion of apolipoprotein B100[95]; and/or iv) increased expression of LDL receptors in the liver[97]. All these mechanisms should be the result of interactions between flavanols and membranes, as whole structures, or with particular lipids or proteins. An increase in HDL-cholesterol has been demonstrated in normo and mildly hypercholesterolemic subjects after dark chocolate or cocoa powder supplementation[99, 102–103]. The mechanisms responsible for these effects on HDL concentrations remain unclear.

It is accepted that oxidized LDL has a role in the development of atherosclerosis[104]. Numerous studies in animals and humans showed that isolated LDLs are more resistant to in vitro oxidation after the consumption of cocoa products[57, 102, 105–109]. One study showed a decrease in plasma levels of oxidized LDL plasma levels after dietary cocoa powder supplementation[99]. All these studies suggest a role for cocoa components in the in vivo protection of LDL. These effects have been mostly ascribed to the scavenging of oxidants formed in the surface of the LDL, and to the chelation of metals catalyst of free radical formation; but they could also be the result of changes in the LDL surface rendering LDL less susceptible to oxidation.

COCOA, ENDOTHELIAL FUNCTION AND HYPERTENSION

The regulation of the vascular tone is the result of a complex network of molecules that includes catecholamines, vasoactive peptides (angiotensin-II or vasopressin), prostaglandins, and importantly, NO. Consumption of a high flavanol cocoa drink (providing 176–185 mg) by patients with cardiovascular risk factors, increased the bioavailability of NO, and an augmented flow-mediated vasodilation, effects that were reversed by the infusion of a NO synthesis inhibitor[13–15]. Studies with isolated flavonoids showed that EC was able to reproduce the vascular effects observed with cocoa products, suggesting that this flavanol should be responsible for the vascular effects[12].

Observational and epidemiological studies indicate that diets rich in polyphenols decrease blood pressure and prevent the increase in blood pressure associated to several pathologies. For years, red wine was considered to have beneficial effects on cardiovascular health, a relationship supported by the French Paradox[110]. Cocoa and cocoa derived products have gained attention because their potential antihypertensive effects. A sub-study of the Zutphen

population showed that cocoa consumption was associated with a decrease in blood pressure[8]. In addition, an association between the intake of cocoa and a low incidence of hypertension was observed in an indigenous population living on the Kuna Islands[111]. The Kuna Indians of Panama have a very low incidence of hypertension and cardiovascular disease, but when members of this tribe moved to urban places, their blood pressure was increased. The relocation led to cultural changes and to a decrease in the consumption of cocoa, suggesting that this dietary change was responsible of the observed changes in blood pressure. These results were complemented by other studies that showed an enhancement of endothelial function including acute positive effects on flow mediated dilation by cocoa consumption in humans[12–13, 15–17].

Consistent with the association between cocoa consumption and low incidence of hypertension are the results from several short term clinical studies showing that the intake of certain chocolates can decrease blood pressure in humans[10–11, 100, 112–113]. Grassi et al. studied 15 healthy young adults with typical Italian diets isocalorically supplemented daily with daily 100 g dark chocolate or 90 g white chocolate (assuming 500 and 0 mg of polyphenols, respectively). They observed that the dark chocolate supplement was associated with decreased systolic blood pressure, whereas the white chocolate had no effect[112]. Results were extended to essential hypertensive patients[10] and more recently to glucose-intolerant hypertensive patients[101]. We studied the effects of the regular consumption of a flavanol-containing milk chocolate on blood pressure and on oxidative stress parameters in healthy young soccer-players[100]. The consumption of the flavanol-containing milk chocolate was significantly associated with a decrease in blood pressure, and an amelioration of oxidative stress. Taubert et al. studied the effects of low doses of polyphenol-rich dark chocolate in humans during 18 weeks[11]. Dark chocolate intake reduced mean, systolic, and diastolic blood pressure and oxidative stress. Blood pressure decrease was accompanied by a sustained increase of S-nitrosoglutathione, suggesting an improve formation of NO. The above studies provide support for the involvement of oxidative stress in the vascular tone regulation by NO availability. A meta-analysis of 5 studies relating cocoa consumption with decreases in blood pressure confirmed the individual results[114]. Significantly, the reductions in systolic (4.7 mm Hg) and diastolic (2.8 mm Hg) blood pressure associated to cocoa and chocolate consumption were similar to those obtained with antihypertensive drugs[114]. In another study, a dark chocolate and a sugar-free cocoa drink were effective in improving endothelial function and decreasing blood pressure in overweight adults[115]. In accordance with all these results, several studies have shown significant decreases in blood pressure following the consumption of other flavanol-containing beverages such as wine[19, 110, 116] and tea[117–120]. On the other hand, one study showed that the administration of 900 mg of flavanoles per day during two weeks enhanced insulin-mediated vasodilation but did not modify blood pressure in patients with essential hypertension. These authors concluded that the treatment was not long enough[121].

COCOA, PLATELET ACTIVATION AND THROMBOSIS

Platelet activation is a central event in coagulation, but it is also related to the acute development of thrombosis and to the long term CVD pathogenesis. A thrombi release from an unstable atherosclerotic plaque is very often the first clinical manifestation of a myocardial ischemia or infarction or stroke[122–123]. Regarding the effects of cocoa on platelet function, Rein et al. administered a cocoa beverage (~897 mg of total EC and oligomeric procyanidins) or placebo to healthy subjects. Blood obtained 2 h after was stimulated with epinephrine and ADP. Platelets present in those samples were studied by the detection of activated conformation of the fibrinogen-binding receptor GPIIb-IIIa and the expression of CD62P (associated with platelet activation). Both parameters were significantly reduced in cocoa-treated individuals. Cocoa also inhibited coagulation, by reducing the formation of hemostatically active platelet microparticles, and increasing platelet-related hemostasis time[124]. In another study, platelet

function was evaluated in smokers 2 h after receiving 40 g of dark chocolate (~47 mg of EC +CT)[125]. Platelet adherence as a result of a shear stress that mimics severely stenotic or disrupted plaques was significantly reduced (−5%) in association with dark chocolate supplementation. Using a similar assay in a protocol including heart transplant recipients, it was also found a reduction in platelet adherence[126]. Both studies propose that the antioxidant properties of cocoa flavanols can lead to an increase in NO bioavailability, which would be associated to the decrease in platelet reactivity, given the potent action of NO as platelet inhibitor[127]. In isolated platelets it was shown that a mixture of CT and quercetin can cause a similar effect increasing NO levels through the inhibition of protein kinase C-dependent NADPH oxidase[128]. Other mechanisms for the effects of flavonoids on platelet reactivity that may be associated with a NO availability because the inhibition of oxidant production include: i) a the reduction in phospholipase C activity associated to hydrogen peroxide production[129]; ii) the modulation of eicosanoide metabolism[130]; iii) the blockade of platelets TxA2 receptors[131]; and iv) the inhibition of platelet lipooxygenase[132–133].

COCOA AND INFLAMMATION

There is increasing evidence that inflammation is pivotal in the induction and perpetuation of CVD. A participation of dietary flavonoids in the modulation of inflammation would contribute to reduce cardiovascular risk. An inverse association was observed between dietary flavonoid intake and serum C-reactive protein (CRP), that is both a biomarker for chronic inflammation and a sensitive risk factor for CVD[134]. Moreover, data from the NHANES 1999–2002 has shown an inverse association between particular flavonols, i.e. quercetin and kaempferol and C-reactive protein. A significant association between inflammation and moderate consumption of cocoa products was found in a study comparing subjects that ate chocolate regularly in the form of dark chocolate (n = 824) with subjects that did not eat chocolate for at least one year (n = 1,317). Serum C-reactive protein concentration in the subgroup having up to one serving (20 g of cocoa) every 3 days was significantly lower than in both, non consumers and subjects having higher consumption[135]. The possible mechanisms involved in the anti-inflammatory effects of cocoa products have been lately revised[136–137]. Of interest, are cell experiments showing the inhibition of MAPK kinase activities by cocoa procyanidins[138–139], and cocoa extracts[140].

CONCLUDING REMARKS

A full range of health benefits can today be associated to the actions of flavanols and procyanidins on vascular function. These benefits are mainly ascribed to diets rich in flavanols and procyanidins, and chocolate and cocoa derivatives are among the most valuable components of such a diet. Considering the fact that CVD is associated with a series of conditions that can trigger oxidant production and oxidant-regulated cell signaling, it would be logical to relate the free radical scavenging and metal chelating properties of cocoa flavanols to CVD protective effects. However, other biochemical mechanisms related to specific flavanol-lipid and flavanol-protein interactions can partially explain the observed in vitro and in vivo antioxidant effects. These mechanisms are more consistent with the in vivo flavanol and procyanidins levels observed in most human and animal tissues.

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Abbreviations

CT	(+)-catechin
EC	(−)-epicatechin
CVD	cardiovascular disease

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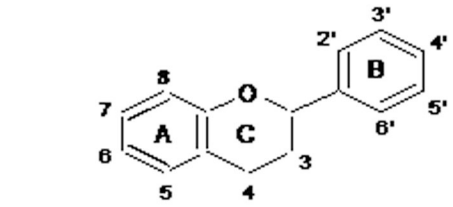
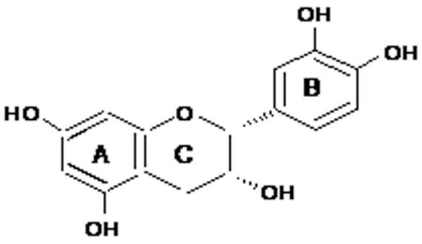
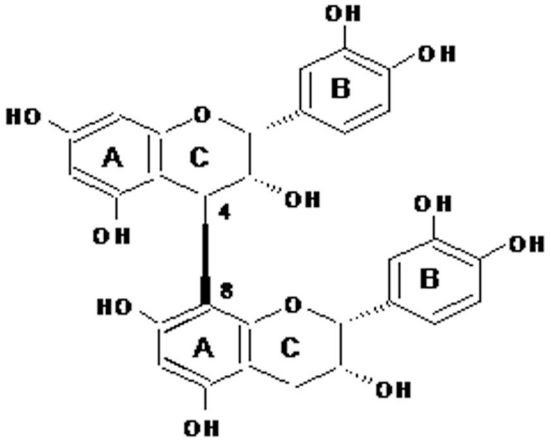


Flavonoid basic structure



(-)-epicatechin



B2 dimer

Fig. 1.
Chemical structure of flavanols and procyanidins. B2-dimer is a characteristic cocoa procyanidin formed by two (−)-epicatechin units linked by 4→8 bonds.

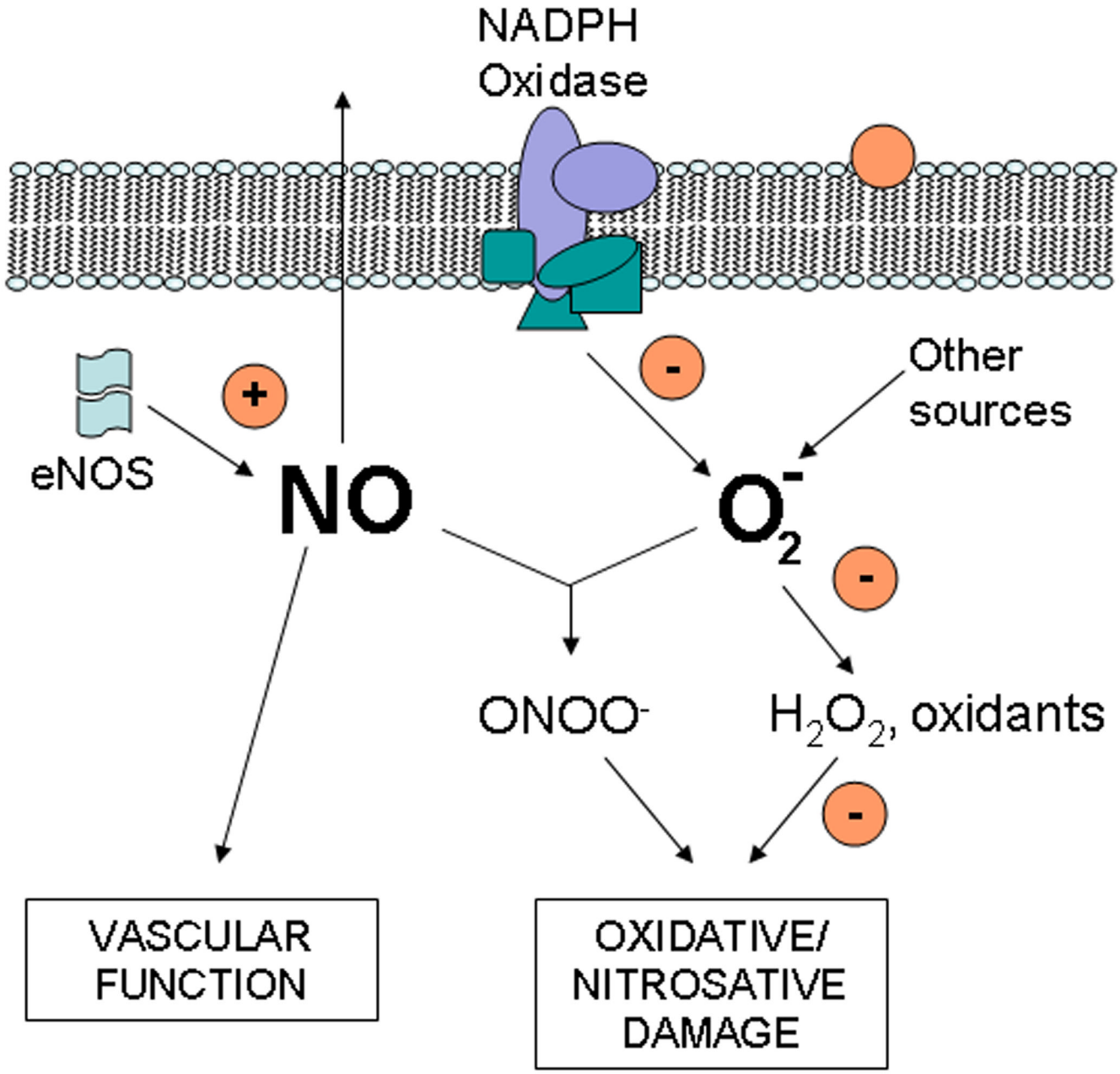


Fig. 2.

Scheme relating flavanols (circles) with NO and superoxide (O_2^-) metabolism in endothelial cells. Flavanols could act by: i) inhibiting NADPH oxidase-dependent superoxide production, ii) activating eNOS; iii) scavenging superoxide, H_2O_2, and other oxidants that mediate damage to cell components; and iv) modifying membrane-related events leading to changes in NO and superoxide production. NO generated in endothelial cells will diffuse outside the cell and reach smooth muscle cells where it will induce vascular relaxation.