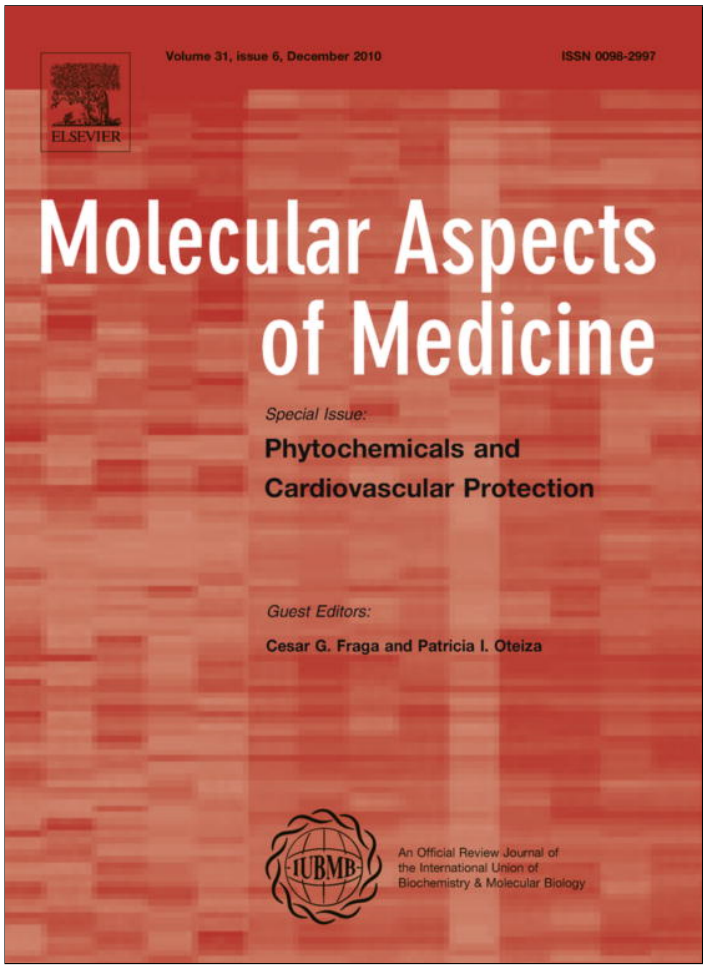

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Review

Basic biochemical mechanisms behind the health benefits of polyphenols

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ABSTRACT

Polyphenols and consequently many flavonoids have several beneficial actions on human health. However, the actual molecular interactions of polyphenols with biological systems remain mostly speculative. This review addresses the potential mechanisms of action that have been so far identified, as well as the feasibility that they could occur in vivo. Those mechanisms include: i) non specific actions, based on chemical features common to most polyphenols, e.g. the presence of a phenol group to scavenge free radicals; and ii) specific mechanisms; based on particular structural and conformational characteristics of select polyphenols and the biological target, e.g. proteins, or defined membrane domains. A better knowledge about the nature and biological consequences of polyphenol interactions with cell components will certainly contribute to develop nutritional and pharmacological strategies oriented to prevent the onset and/or the consequences of human disease.

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Abbreviations: AH, antioxidant; A$^{\cdot}$, antioxidant radical; CVD, cardiovascular disease; EC, (−)−epicatechin; EGCG, (−)−epigallocatechin gallate; LH, lipid; L$^{\cdot}$, lipid radical; LOOH, lipid hydroperoxide; LOO$^{\cdot}$, lipid peroxyl radical; NF-κB, nuclear factor kappa-B; PO$^{\cdot}$, polyphenol radical; POH, polyphenol.

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1. Introduction

A large number of studies has identified cellular targets that could be involved in the health promoting actions of dietary plant polyphenols. However the actual molecular interactions of polyphenols with those cellular targets remain mostly speculative. This paper summarizes the most important mechanisms proposed for the actions of polyphenols in animal settings. Although the particular focus will be on the effects of polyphenols on cardiovascular disease (CVD), the same potential mechanisms could occur in other animal tissues and systems. It is important to mention that this paper was mostly written based on the positive aspects of fruits and vegetables, and hence that polyphenol interactions with animal tissues provide beneficial effects. However, negative effects of polyphenols cannot be totally disregarded. In this regard, caution should be taken when the supplementation of polyphenols is beyond an upper limit that could be set as a generous intake of fruits and vegetables, e.g. 10 servings a day.

2. Polyphenols and cardiovascular disease

Epidemiological evidence demonstrates that diets rich in fruits and vegetables promote health and attenuate, or delay, the onset of CVD (Hertog et al., 1993; Appel et al., 1997; Liu et al., 2000; Joshipura et al., 2001; Hung et al., 2004; Buijsse et al., 2006; Lichtenstein et al., 2006; Iqbal et al., 2008; Mursu et al., 2008; Holt et al., 2009). The beneficial effects of fruits and vegetables have been largely ascribed to polyphenols, since the ingestion of foods rich in polyphenols is associated in humans and experimental animals with diminutions in: i) dyslipidemia and atherosclerosis; ii) endothelial dysfunction and hypertension; iii) platelet activation and thrombosis; iv) the inflammatory process associated with the induction and perpetuation of CVD. This evidence has been revised by different authors (Dohadwala and Vita, 2009; Bertelli and Das, 2009; Corti et al., 2009; Desch et al., 2010; Galleano et al., 2009). A definitive understanding of the mechanisms behind the health effects of polyphenols will allow to identify the fruits and vegetables, and the chemical compounds, responsible for those effects, and finally define the best health promoting diets.

3. Chemical aspects of dietary polyphenols

Plants produce polyphenols as secondary metabolites involved in diverse processes, such as growth, lignification, pigmentation, pollination, and resistance against pathogens, predators, and environmental stresses (Duthie et al., 2003). Chemically, polyphenols are compounds having one or more hydroxyl groups attached to a benzene ring. Edible plants provide to the human diet with more than 8000 different polyphenols that can be categorized as flavonoids and non-flavonoid compounds.

Flavonoids have a common C6–C3–C6 structure consisting of two aromatic rings (A and B) linked through a three carbon chain, usually organized as an oxygenated heterocycle (ring C) (Fig. 1). Flavonoids can be divided into several subfamilies according to the degree of oxidation of the oxygenated heterocycle, being flavanols, flavanones, flavones, flavonols (essentially, flavan-3-ols), isoflavones, and anthocyanidins, the most relevant for human diets (Scalbert and Williamson, 2000). Starting from a basic chemical structure, plant biosynthetic pathways introduce different hydroxyl group patterns, methyl groups, and sugars (Jaganatah and Crozier, 2010). In certain cases, oligomerization and polymerization of the flavonoid units occur. Oligomers and polymers of flavonoids are called tannins and are classified in two groups, condensed tannins and hydrolyzable tannins. Condensed tannins (also known as proanthocyanidins or procyanidins) are oligomers of flavanols, and their chemical structures are defined not only by the kind of monomer, but also according to the kind of link among monomers. There are several oligomerization patterns and some plants present characteristic manners of oligomerization, e.g. in cocoa the monomeric units are linked through 4→8 carbon–carbon bonds forming mostly B-type dimers (Jaganatah and Crozier, 2010). Hydrolyzable tannins are polymers readily hydrolyzed by acids into their components: a central core constituted by a polyol (a sugar, generally D-glucose, or a flavonoid, as catechin) and a phenolic carboxylic acid esterifying partially or totally that core molecule. These tannins are classified according to the phenolic carboxylic acids present that could be gallic acid (gallotannins) or ellagic acid (ellagitannins) (Jaganathan and Mandal, 2009).

Among the non-flavonoid polyphenols, stilbenes have gained attention due to their proposed biological actions in animals. Stilbenes have a common C6–C2–C6 structure, consisting in two aromatic rings linked through a two carbon bridge with a double bond. The parent compound of this family is resveratrol (Fig. 1) that occurs i) in *trans* and *cis* configurations; ii) as free forms (aglycones) and as glucosides; and iii) as monomers, oligomers and polymers (viniferins). As it can be inferred from the above brief description of the chemical structures of plant polyphenols, although the phenol group is the base of their classification, its relevance for chemical reactions in animals is as important as that of other chemical groups that are part of the polyphenol molecule.

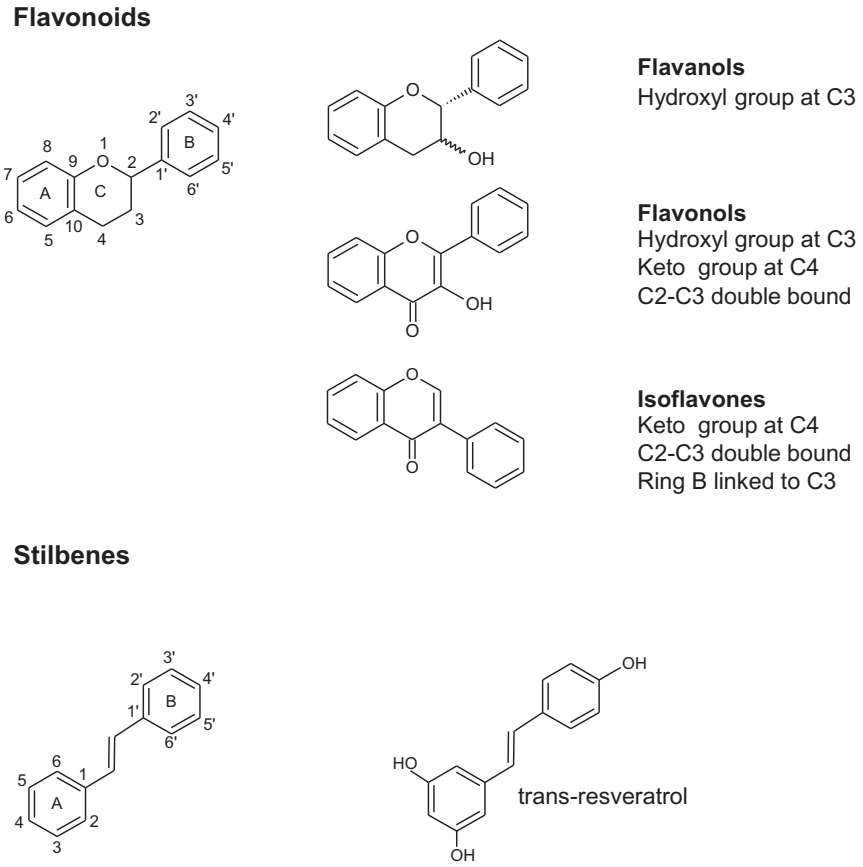

Fig. 1. Chemical structure of selected polyphenols.

4. Mechanisms involved in the health benefits of polyphenols

Several mechanisms have been proposed to explain the biological actions of plant polyphenols in animals. We will analyze the available data, classifying the biological mechanisms in two groups: i) general or nonspecific mechanisms, essentially related to the presence of phenolic groups, and ii) specific mechanisms, dependent on the particular chemical and structural characteristics of the active polyphenol.

4.1. Nonspecific mechanisms

4.1.1. Polyphenols as antioxidants: free radical scavenging and metal sequestration

Thermodynamics. A major event in the oxidation of biological systems is lipid oxidation, which is a free radical chain reaction (reactions 1–6).

$$LH \rightarrow L^{\bullet} \quad \text{(reaction 1)}$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad \text{(reaction 2)}$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \quad \text{(reaction 3)}$$

$$L^{\bullet} + L^{\bullet} \rightarrow \text{non radical products} \quad \text{(reaction 4)}$$

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow \text{non radical products} \quad \text{(reaction 5)}$$

$$L^{\bullet} + LOO^{\bullet} \rightarrow \text{non radical products} \quad \text{(reaction 6)}$$

"Breaking" these reactions, by inhibiting or retarding lipid oxidation (interfering with initiation (reaction 1), or with propagation (reactions 2 and 3)), that is scavenging free radicals, may be considered as one of the most important antioxidant strategy. Generally, free radical scavengers are chain-breaking antioxidants (AH) able to react with free radicals ($LOO^{\cdot} + AH \rightarrow LOOH + A^{\cdot}$). Phenols, and in consequence polyphenols, are highly efficient in breaking free radical chain reactions. Two chemical features shared by the majority of polyphenols are responsible for the efficiency of the antioxidant reaction ($LOO^{\cdot} + POH \rightarrow LOOH + PO^{\cdot}$): i) the phenolic OH groups that are able to reduce free radicals through a one-electron donation; and ii) the aromatic structures that allow the stabilization by resonance of the resultant aroxyl radicals ($PO^{\cdot}$) (Bors et al., 1990).

Table 1
Thermodynamic and kinetic aspects of polyphenols, α-tocopherol, and ascorbate as free radical scavengers.

Antioxidant reactions[a]	$-E^{\circ\prime}_{(AH/A^{\bullet})}$[b] (mV)	Rate law	k ($\mu M^{-1}\ s^{-1}$)	[AH][c] (μM)	Relative rate[d] (s^{-1})
$LOO^{\bullet} + Asc \rightarrow LOOH + Asc^{\bullet}$	282[e]	$v_{ASC} = k_{ASC}\ [LOO^{\bullet}]\ [Asc]$	1.0[e]	50[e]	50
$LOO^{\bullet} + TP \rightarrow LOOH + TP^{\bullet}$	500[e]	$v_{TP} = k_{TP}\ [LOO^{\bullet}]\ [TP]$	1.0[e]	28[e]	28
$LOO^{\bullet} + EC \rightarrow LOOH + EC^{\bullet}$	570[e]	$v_{EC} = k_{EC}\ [LOO^{\bullet}]\ [EC]$	7.3[e]	0.3[e]	2.0
$LOO^{\bullet} + QC \rightarrow LOOH + QC^{\bullet}$	330[e]	$v_{QC} = k_{QC}\ [LOO^{\bullet}]\ [QC]$	15.0[g]	0.1[i]	1.5
$LOO^{\bullet} + RV \rightarrow LOOH + RV^{\bullet}$	650[f]	$v_{RV} = k_{RV}\ [LOO^{\bullet}]\ [RV]$	0.03[h]	0.4[j]	0.01

[a] Reaction of antioxidants (AH) with peroxyl radicals ($LOO^{\bullet}$). Asc = ascorbic acid; TP = α-tocopherol; EC = (−)–epicatechin; QC = quercetin; and RV = resveratrol.
[b] Reduction potentials are defined for $A^{\bullet} \rightarrow AH$; the minus sign makes $E^{\circ\prime}$ consistent with the proposed antioxidant reactions ($AH \rightarrow A^{\bullet}$).
[c] Estimated plasma concentration of antioxidant compounds.
[d] Relative rate is defined as rate divided by $[LOO^{\bullet}]$.
[e] Fraga et al., 2010.
[f] Piljac et al., 2004.
[g] Erben-Russ et al.,1987.
[h] Rhayem et al., 2008.
[i] Conquer et al., 1998.
[j] Ortuño et al., 2010.

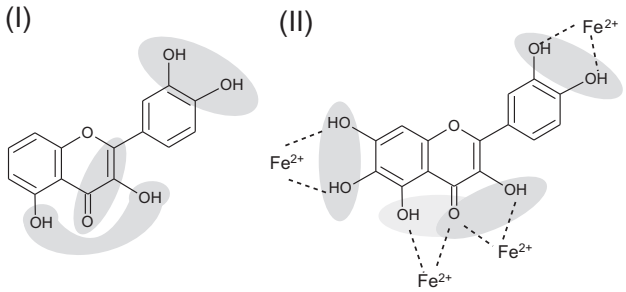

Fig. 2. Structural features defining antioxidant actions of flavonols. Structural requirements associated with antioxidant possibilities of flavonols as chain breaking antioxidant (I) and metal (iron) chelators (II). In I), the highlighted areas define the three criteria for maximal antioxidant activity of flavonoids (Bors et al., 1990): i) a catechol group (3'- and 4'-hydroxyl groups) in ring B; ii) 2–3 double bond in conjugation with a 4-keto function in ring C; and iii) presence of 3- and 5-hydroxyl groups in rings C and A. In II), highlighted areas and dotted lines define iron chelation sites.

The standard reduction potentials (E°) represent the tendency of the oxidized form of a molecule or atom (the free radical in this case) to be reduced by receiving one electron. The E° calculated for biological conditions ($E^{\circ\prime}$) for each of the compounds involved in a free radical breaking reaction can be used for predicting the feasibility that such antioxidant reaction can occur (Table 1). Comparing the $E^{\circ\prime}$ of two compounds, the one with the highest $E^{\circ\prime}$ will receive the electron from the one with the lowest $E^{\circ\prime}$. Biologically relevant free radicals, such as $LOO^{\bullet}$, have high $E^{\circ\prime}$ values $E^{\circ\prime}_{LOO^{\bullet}/LOOH}$, while the $E^{\circ\prime}$ for polyphenols ranges within 330 to 840 mV (Fraga et al., 2010) (Table 1). These relatively low $E^{\circ\prime}$ values are the forces driving the electron donation by polyphenols. Considering that ascorbic acid and α-tocopherol have similar $E^{\circ\prime}$ values, polyphenols will be able to act as free radical chain breakers with activities comparable with that of those two well-known antioxidants (Table 1). However kinetic aspects should also be considered and this will be discussed below.

The stability of the formed $PO^{\bullet}$ will determine the potency of the parent polyphenol in the breaking of the chain reaction. A relatively non reactive $PO^{\bullet}$ will inhibit or decrease the rate of lipid oxidation, meanwhile a highly reactive $PO^{\bullet}$ would propagate rather than interrupt the reaction chain. Based on this rationale, it has been established some structural requirements in the flavonoid molecule to obtain a stable $PO^{\bullet}$ that can be assimilated to the requirements for chain breaking antioxidant (Fig. 2).

In biological systems redox active metals (e.g. iron and copper) catalyze free radical-producing reactions that are slow in their absence. Thus, sequestration of iron and/or copper to prevent metal-catalyzed free radical formation is another antioxidant strategy (Guo et al., 1996; Brown et al., 1998; Morel et al., 1998). Catechol moieties and combinations of hydroxyl and carbonyl groups present in polyphenols are centers of high affinity for metal ions; however, large differences have been observed in the metal-chelating capacity of different polyphenols (Thompson et al., 1976; van Acker et al., 1996; Arora et al., 1998; Perron and Brumaghim, 2009). Iron and flavonoids are the most studied transition metal and polyphenol family, respectively. From those studies, there is a consensus on the presence of "iron chelation sites" or "iron binding motifs" in an important number of flavonoids (Fig. 2): flavonoid-metal binding occurs preferentially at the 3-hydroxyl-4-carbonyl group, followed by 4-carbonyl-5-hydroxyl group, and the 3′-4′ hydroxyl (if present) (Guo et al., 1996; Ren et al., 2008). Recently, the 6–7 hydroxyl groups have been indicated as the iron chelation site for baicalein (Perez et al., 2009).

It is important to consider that chelation of transition metals not always will result in an antioxidant action. As the redox properties of a metal change by chelation, it will be the $E^{\circ\prime}$ of each polyphenol-metal complex the determinant of the subsequent behavior (Fraga et al., 2010). In the intracellular milieu, Fe^{2+} is complexed with a variety of compounds, e. g. phosphates, citrate, etc., with different abilities to promote radical formation (Rush et al., 1990; Yamazaki and Piette, 1990). To operate as an antioxidant, the polyphenol-metal complex has to be less efficient in promote radical formation than the physiological metal-complexes. Finally it is important to indicate that polyphenol-metal complexes have also shown other antioxidant actions, such as mimicking the effects of superoxide dismutase as it was reported for the quercetin–Fe^{2+} complex (de Souza and De Giovani, 2004).

Kinetics. A major limitation for the 'antioxidant action' of polyphenols associated to free radical chain reactions breaking and metals sequestration, is the relatively low polyphenol bioavailability observed even after the consumption of foods rich in these compounds (Fraga 2007). The highest concentrations that can be reached in humans after a realistic polyphenol consumption are in the nanomolar range, showing a peak at 2–4 h post-ingestion, and being rapidly removed from plasma (Rein et al., 2000; Holt et al., 2002; Schroeter et al., 2006). This low bioavailability leads to a kinetically unfavorable condition with respect to other compounds with similar free radical scavenger capabilities, which are present in blood or tissues in significantly higher concentrations (28 and 50 μM for α-tocopherol and ascorbate, respectively). For example, in Table 1, it can be observed that ascorbate and quercetin have similar $E^{\circ\prime}$ values, but their rate constant (k) and plasma concentration define a rate of reaction (antioxidant effect) that is more than 30-times higher for ascorbate. Thus, a potential action of polyphenols as free radical scavengers is unlikely to be physiologically relevant in most organs, except for those exposed to a high polyphenol concentration such as the gastrointestinal tract and perhaps, the blood (Fraga, 2007; Galleano et al., 2010b).

From a similar analysis, it can be inferred that the concentration of polyphenols in human and animal tissues would not be enough to displace physiological metal chelators. This situation would limit the role of polyphenols in transition metal sequestration to those conditions characterized by excessive amounts of redox active metals, and/or to compartments with high polyphenol concentration, e.g. the gastrointestinal tract.

4.1.2. Interactions of polyphenols with membranes

The interactions with membrane lipids and proteins could mediate certain biological effects of polyphenols, as it is suggested by consistent observations showing that many of these compounds generate a cell response despite the fact that they are not internalized (Erlejman et al., 2006; Erlejman et al., 2008; Verstraeten et al., 2008). The presence of both hydrophobic and hydrophilic domains in most polyphenol molecules, allow them to localize at different levels in the membrane: i) at the surface of the bilayer adsorbed on the polar head of lipids; and/or ii) inserting into the bilayer and interacting with the hydrophobic chains of lipids (Hendrich et al., 2002; Yoshioka et al., 2006; Sirk et al., 2009). Experiments in liposomes with different polar groups and a series of flavonoids, suggest the formation of hydrogen-bonds between the hydroxyl groups of the flavonoid and the phospholipid polar headgroups favoring the interaction with the membrane (Verstraeten et al., 2003; Oteiza et al., 2005).

A set of structural characteristics determine the adsorption or penetration of the polyphenol into the lipid bilayer. The interaction with lipid headgroups positively correlates with the number of polyphenol hydroxyl groups, and inversely with the hydrophobicity of the molecules (Ollila et al., 2002). However, large amounts of hydroxyl moieties in small flavonoids can prevent their membrane adsorption by causing a large increase in their hydrophylicity (Ratty et al., 1988). The tridimensional structure of flavonoids will also determine the extent of their interaction with lipid bilayers. For instance, the planar structure adopted by the flavanols morin and quercetin makes these compounds more avid for membranes than flavones, as naringenin, eriodictyol, and hesperetin, which adopt a tilted configuration (van Dijk et al., 2000). In addition, methylation of hydroxyl groups of flavonoids could prevent their interaction with membranes, as observed for epigallocatechingallate (EGCG). Mono and dimethylation of hydroxyl groups present in ring B and in the gallate group markedly decreased EGCG affinity for lipid bilayers, which was completely abolished by the simultaneous methylation at 4' and 4'' positions. These findings suggest that those hydroxyl groups are central for the cell surface binding activity of EGCG (Yano et al., 2007).

Then, polyphenols could affect cell function by modifying plasma membrane structure and physical characteristics such as fluidity and electrical properties. These effects can be observed both, when polyphenols are adsorbed on the membrane or when they are inserted into the bilayer. These modifications can result in functional changes of several membrane-associated events including the activity of membrane-associated enzymes, ligand-receptor interactions, ion and/or metabolite fluxes, and the modulation of signal transduction. Taking in consideration the antioxidant effects, when adsorbed on the membrane surface, polyphenols could provide a physical barrier for hydrosoluble radicals (Verstraeten et al., 2003). Inserted into the lipid bilayer, polyphenols would be in close proximity to scavenge $L^{\cdot}$, $LOO^{\cdot}$, and other lipid soluble radicals (Verstraeten et al., 2003; Verstraeten et al., 2005). Thus, flavonoids can protect membranes and membrane components from oxidation by providing an antioxidant protection through mechanisms not completely related to free radical scavenging or metal chelating actions.

Although the interactions of flavonoids with membranes can be considered as a nonspecific mechanism of action, recent evidence suggests a selectivity of certain polyphenols for specialized areas of the membrane, namely the lipid rafts (Fujimura et al., 2004a,b; Adachi et al., 2007; Xia et al., 2007; Maldonado-Celis et al., 2009; Annaba et al., 2010). Lipid rafts are known to have a particular lipid composition being enriched in cholesterol and sphingolipids, and containing proteins involved in membrane signaling and trafficking (Lingwood and Simons, 2010). For example, the flavanol EGCG interacts with lipid rafts affecting the activity of the 67 kDa laminin receptor and of the epidermal growth factor receptors. As a consequence, EGCG

affects the downstream activation of signals involved in the allergic response (Fujimura et al., 2007) and in cell proliferation (Shimizu et al., 2010). Thus, polyphenols interactions with lipid raft proteins and/or lipids constitute a major potential site in the regulation of cellular events by these compounds.

4.2. Specific mechanisms

The interaction with proteins has emerged as a relevant mechanism to explain several of polyphenol biological effects. For the nonspecific type of mechanisms, the chemical features shared by most of the polyphenols, i.e. the phenol group, are key to their biological actions. On the other hand, a select chemical structure of a particular polyphenol becomes relevant for a specific mechanism of action. Polyphenols interactions with proteins are examples of such specific actions, and will result in biological effects depending on the function of the protein involved, including the modification of enzymatic activities, receptors-ligand binding, and transcription factors binding to their specific sites in DNA, among others.

It is worth mentioning that under certain circumstances the interaction between polyphenols and proteins can also be considered as nonspecific. Polyphenols interact with proline-rich proteins in a process that starts with a primary hydrophobic association between proline residues and aromatic phenolic rings, followed by the formation of small size aggregates, and ending with protein precipitation (Poncet-Legrand et al., 2007). This mechanism is responsible, for example, for the astringent sensation felt in the oral cavity during red wine drinking, due to the precipitation of the salivary proline-rich proteins (Baxter et al., 1997).

4.2.1. Interaction of polyphenols with enzymes

Different polyphenols are efficient inhibitors of the activity of a broad number of enzymes. Analyzing the data reviewed by Middleton et al. (2000) it is possible to infer that an important proportion of the enzymes included as targets of polyphenols are enzymes with: i) purines (e.g. ATP) as substrates (kinases, ATPases, cyclic nucleotide phosphodiesterase, adenylate cyclase, reverse transcriptase, xanthine oxidase, RNA and DNA polymerases, ribonuclease, human DNA ligase); and ii) enzymes with NADPH as a cofactor (aldose reductase, malate dehydrogenase, lactic dehydrogenase, nitric oxide synthase, glutathione reductase, 11-β-hydroxysteroid dehydrogenase). It can be hypothesized that certain polyphenols act inhibiting ATP-dependent enzymes, through a competitive binding to the enzyme ATP-binding site. This competition seems to rely on the presence of two hydroxyl substitutions in 5,7 position in the flavonoid A ring, and a 2,3 unsaturation together with a 4-keto group in the C ring (Lotito and Frei, 2006). Given the similarity between ATP and NADPH structures, NADPH-dependent enzymes would be also affected by polyphenols.

A specific interaction between a protein and a particular flavonoid has been proposed for the enzyme NADPH-oxidase (NOX) and the flavan-3-ol (−)−epicatechin (EC). NOX inhibition by the *O*-methylated metabolite of EC results in a decrease in superoxide anion production. Interestingly, this metabolite has structural similarities with apocynin, considered a typical NOX inhibitor (Fig. 3) (Steffen et al., 2007a,b). This mechanism of action is important for the regulation of vascular function and, consequently, of blood pressure because of the limitation imposed by superoxide anion on nitric oxide availability (Galleano et al., 2010a; Steffen et al., 2007b).

4.2.2. Interactions of polyphenols with transcription factors

Among polyphenols, select flavanols (such as EC, (+)−catechin, and certain procyanidins) can modulate the expression of numerous NF-κB-regulated genes involved in inflammation and carcinogenesis (Park et al., 2000; Mackenzie et al., 2004; Mackenzie and Oteiza, 2006; Erlejman et al., 2008; Mackenzie et al., 2008). EC and dimeric procyanidins B2 inhibited NF-κB activation at multiple levels in the NF-κB pathway in Jurkat T (Mackenzie et al., 2004) and in Hodgkin's lymphoma (Mackenzie et al., 2008) cells. Functional evidence supported by a putative molecular model suggests that B2 could interact with NF-κB proteins and prevent the binding of NF-κB to the DNA κB sites (Mackenzie et al., 2009). Supporting that interaction, in vitro experiments showed that B1 and B2 dimers inhibit NF-κB-DNA binding both in isolated nuclear fractions and in purified p50 and Rel A proteins (constituents of NF-κB) (Mackenzie et al., 2009). Under similar experimental conditions, dimeric procyanidins A1 and A2 did not affect the binding of NF-κB to DNA, indicating a selective effect of B1 and B2 (Mackenzie et al., 2009). The relevance of phenolic conformation is stress by the finding that rotationally constrained

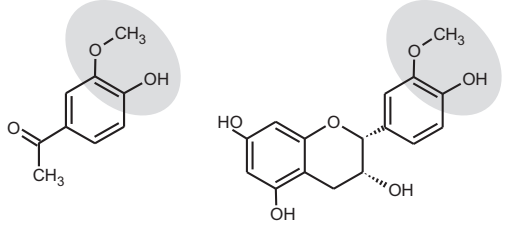

Fig. 3. Structural features of apocynin and 3'-*O*-methyl-(−)−epicatechin as NADPH oxidase (NOX) inhibitors. Highlighted areas indicate the structural similarities between 3'-*O*-methyl-(−)−epicatechin and apocynin associated to the inhibition of NOX activity. Summarized from Steffen et al. (2007a,b).

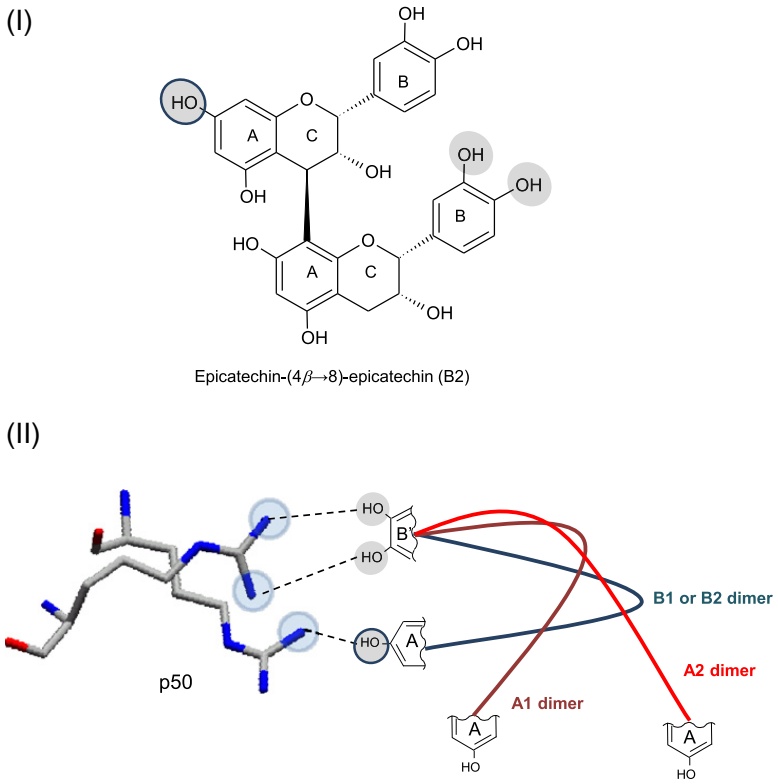

Fig. 4. Interaction of (−)–epicatechin dimers with NF-κB. I) Chemical structure of dimeric procyanidin B2. Highlighted hydroxyl groups are central to the interaction of B2 with NF-κB proteins RelA and p50. II) Potential hydrogen bonding interactions (indicated as dashed lines) between p50 arginine residues of RelA and p50 and the different dimers. The global minimum energy conformer of A1, A2, B1 or B2 is represented as a line, and interactions were arbitrarily forced for all dimers to those established by hydroxyl groups in ring B'.

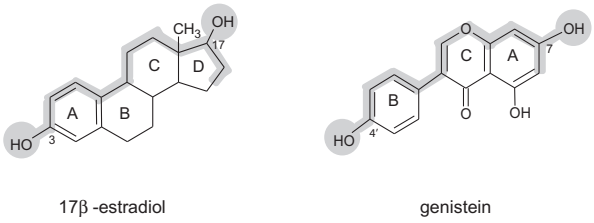

Fig. 5. Structural features of 17β-estradiol and genistein as ligands of estrogen receptors. The highlighted areas define the part of the molecules and the numbers of the most important positions to define an effective binding to estrogen receptors.

variants of caffeic acid phenethyl ester have less ability to inhibit NF-κB-DNA binding than the parent molecule (Natarajan et al., 1996). To better define the stereo-chemical possibilities for the interaction between dimeric procyanidins and NF-κB proteins these interactions were analyzed by molecular modeling (Fig. 4). The minimum energy conformers for B1 and B2 showed a folded structure where ring B' stacks onto ring A orienting the hydroxyl groups toward the same edge of the molecule. However, the above was not observed for A1 and A2 dimers, where rings B and A cannot stack due to the extra covalent bridge present between the two monomers. This extra covalent bridge (2β→O7 ether bond) generates a more rigid molecule that impairs the formation of a stacked molecule. Stacked rings B and A of dimers B1 and B2 lie very close to the positions occupied by the two guanine rings in the NF-κB DNA consensus sequence. Also, the polar atoms of B1 and B2 are favorably placed to give rise to a similar hydrogen bonding pattern to that observed in the complex. The differences between the spatial disposition of A and B dimers series can determine the differential inhibitory effects of these dimeric procyanidins on NF-κB activation to its consensus sequence. This is just one example on how specific polyphenol–protein interactions, can be driven not only by the chemical composition, but also by the structural and conformational characteristics of polyphenols.

4.2.3. Interaction of polyphenols with receptors

Animal estrogens are steroidal compounds which physiological responses are mediated by their interaction with two estrogen receptors (ERs): ERα and ERβ. Isoflavones possess estrogenic activity in animals at concentrations lower than 0.1 μM based on the direct interactions between isoflavones and ERs (Kuiper et al., 1998). The similarity of estrogens and

isoflavones structures (Fig. 5) provides these polyphenols the ability to act as estrogen agonists or antagonists (Messina, 2010).

From structure–activity relationships five molecular characteristics were postulated as essential for ER binding and these characteristics fit well with isoflavone structures (Fang et al., 2001): i) the presence of the phenolic ring, mimicking the 17β-estradiol 3-OH (for genistein it has been suggested that ring B corresponds to 17β-estradiol ring A, and hence 4'-OH would correspond with 17β-estradiol 3-OH (Pike et al., 1999); ii) a second OH group resembling both, 17β-estradiol 17-OH, and an optimal distance between 2 two OH (11.0 Å) similar to 3-OH and 17-OH in 17β-estradiol (Vaya and Tamir, 2004); iii) precise steric hydrophobic centers mimicking steric 7α- and 11β-substituents; iv) a certain degree of hydrophobicity, given by the benzenic rings; and v) a ring structure, usually aromatic, which provides the rigidity and the hydrophobic flat structure. These features generate a large zone adequate to interact with the receptor as schematized in Fig. 5.

Genistein, for example, has a binding affinity for ERβ similar to 17β-estradiol while its binding affinity for ERα is 20-fold lower (Kuiper et al., 1998). Elimination of one or two hydroxyls groups from genistein, rendering daidzein or formononetin, respectively, causes a significant loss in their binding affinity (Kuiper et al., 1998). Not only isoflavones can interact with the ERs, but also other flavonoids such as the anthocyanin delphinidin could bind to the ERα (Chalopin et al., 2010). A docking study predicted that the manner that delphinidin binds at the ligand binding domain on ERα is similar to that observed in the X-ray structure of the ERα-17β-estradiol complex. Finally, it is important to stress that resveratrol and related stilbenes present some of the mentioned characteristics and also interact efficiently with ERs (Lappano et al., 2009).

4.3. Other mechanisms

Polyphenols have been proposed to participate in many other biological processes through mechanisms that cannot be included in the preceding classifications. A few examples will subsequently provide evidence on the complexity of the subject.

Gastrointestinal lipid digestion and absorption requires the enzymatic hydrolysis of triglycerides, thus, the inhibition of pancreatic lipase is a possible strategy to prevent hyperlipidemia by decreasing lipid absorption. Polyphenols have been reported to decrease pancreatic lipase activity (Kawaguchi et al., 1997; Sbarra et al., 2005); however the direct interaction with the pancreatic lipase active site is not the only mechanism of inhibition. Triglycerides must emulsify into fat droplets to generate a suitable substrate for pancreatic lipase activity (Armand, 2007). The size of the droplet will define enzyme activity being the smaller droplets (larger surface) the ones that allow a higher catalytic activity. Flavanols present in tea, particularly EGCG, change the properties of lipid emulsions by increasing the droplet size and reducing the surface area. Then the overall effect of EGCG is an inhibition of enzyme activity. It has been proposed that the hydroxyl groups of EGCG interact with the hydrophilic head group of the phosphatidylcholines oriented to the exterior of fat droplets through hydrogen bonding (Shishikura et al., 2006). Such interactions can lead to the formation of cross links between droplets resulting in an increase of fat droplet size, and to a decreased capacity of pancreatic lipase to hydrolyze triglycerides (Babu and Liu, 2008).

Other example is the postprandial increased levels of hydroperoxides and aldehydes (malondialdehyde) in plasma following consumption of certain foods. The presence of oxidized compounds in foods might trigger further oxidant production, resulting in higher levels of hydroperoxide and malondialdehyde in the stomach that can pass to the intestine and be absorbed. The absorption of those toxic compounds is significantly decreased if dietary polyphenols are present in the meal (Gorelik et al., 2008). It has been proposed that the occurrence of more than one mechanism can explain the polyphenol effect: i) the antioxidant (reducing) action of polyphenols in the stomach, kinetically favored by a high polyphenol concentration (≈50 μM) (Kwon et al., 2007); ii) the formation of polyphenol–aldehyde complexes (Lo et al., 2006; Totlani and Peterson, 2006) preventing the absorption in the gut; and iii) the decrease of MDA release from protein-bound MDA in the gut due to the inhibition by polyphenols of proteolytic enzymes (Kandra et al., 2004; McDougall and Stewart, 2005).

Finally, as a third example we can consider the formation of nitric oxide in the stomach. It has been demonstrated that nitric oxide can be generated from nitrite through a non enzymatic pathway in an acidic/reducing environment as it happens in the stomach (Weitzberg and Lundberg, 1998). Experiments in simulated gastric juice (pH 2.0) show that the interaction of dietary polyphenols with nitrites produces nitric oxide (Rocha et al., 2009). In healthy volunteers, the consumption of polyphenol- and nitrite-rich foods mediates an increase of nitric oxide in the stomach (Rocha et al., 2009). The physiological relevance of this process should be related to the diffusion of the locally produced nitric oxide through the stomach inducing smooth muscle relaxation (Rocha et al., 2010).

5. Conclusions

Dietary polyphenols provide a wide spectrum of biological actions potentially beneficial for cardiovascular health. These biological actions, involve different mechanisms. Nonspecific mechanisms require high polyphenol concentrations to be operative in most tissues, being thus restricted to the gastrointestinal tract and, eventually, to the vascular milieu. On the contrary, a myriad of effects can be mediated by more specific mechanisms based on the interaction between select polyphenols and particular proteins. Taking into account the markedly lower polyphenol concentration required to exert an action through those specific actions, they appear as highly plausible to explain polyphenol actions in vivo, including antioxidant actions. Furthermore, interactions of polyphenols with membranes, largely considered as nonspecific, open a

possibility for specific polyphenol actions based on the interaction with particular zones of the lipid bilayers. A better knowledge on the nature and biological consequences of polyphenol interactions with cell components will certainly contribute to develop nutritional and pharmacological strategies oriented to prevent the onset and/or the consequences of human disease.

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References

Adachi, S., Nagao, T., Ingolfsson, H.I., Maxfield, F.R., Andersen, O.S., Kopelovich, L., Weinstein, I.B., 2007. The inhibitory effect of (−)−epigallocatechin gallate on activation of the epidermal growth factor receptor is associated with altered lipid order in HT29 colon cancer cells. Cancer Res. 67, 6493–6501.

Annaba, F., Kumar, P., Dudeja, A.K., Saksena, S., Gill, R.K., Alrefai, W.A., 2010. Green tea catechin EGCG inhibits ileal apical sodium bile acid transporter ASBT. Am. J. Physiol. Gastrointest. Liver Physiol. 298, G467–G473.

Appel, L.J., Moore, T.J., Obarzanek, E., Vollmer, W.M., Svetkey, L.P., Sacks, F.M., Bray, G.A., Vogt, T.M., Cutler, J.A., Windhauser, M.M., Lin, P.H., Karanja, N., 1997. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N. Engl. J. Med. 336, 1117–1124.

Armand, M., 2007. Lipases and lipolysis in the human digestive tract: where do we stand? Curr. Opin. Clin. Nutr. Metab. Care 10, 156–164.

Arora, A., Nair, M.G., Strasburg, G.M., 1998. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radic. Biol. Med. 24, 1355–1363.

Babu, P.V., Liu, D., 2008. Green tea catechins and cardiovascular health: an update. Curr. Med. Chem. 15, 1840–1850.

Baxter, N.J., Lilley, T.H., Haslam, E., Williamson, M.P., 1997. Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. Biochemistry 36, 5566–5577.

Bertelli, A.A., Das, D.K., 2009. Grapes, wines, resveratrol, and heart health. J. Cardiovasc. Pharmacol. 54, 468–476.

Bors, W., Heller, W., Michel, C., Saran, M., 1990. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Methods Enzymol. 186, 343–355.

Brown, K.E., Kinter, M.T., Oberley, T.D., Freeman, M.L., Frierson, H.F., Ridnour, L.A., Tao, Y., Oberley, L.W., Spitz, D.R., 1998. Enhanced gamma-glutamyl transpeptidase expression and selective loss of CuZn superoxide dismutase in hepatic iron overload. Free Radic. Biol. Med. 24, 545–555.

Buijsse, B., Feskens, E.J., Kok, F.J., Kromhout, D., 2006. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. Arch. Intern. Med. 166, 411–417.

Conquer, J.A., Maiani, G., Azzini, E., Raguzzini, A., Holub, B.J., 1998. Supplementation with quercetin markedly increases plasma quercetin concentration without effect on selected risk factors for heart disease in healthy subjects. J. Nutr. 128, 593–597.

Corti, R., Flammer, A.J., Hollenberg, N.K., Luscher, T.F., 2009. Cocoa and cardiovascular health. Circulation 119, 1433–1441.

Chalopin, M., Tesse, A., Martinez, M.C., Rognan, D., Arnal, J.F., Andriantsitohaina, R., 2010. Estrogen receptor alpha as a key target of red wine polyphenols action on the endothelium. PLoS One 5, e8554.

de Souza, R.F., De Giovani, W.F., 2004. Antioxidant properties of complexes of flavonoids with metal ions. Redox Rep. 9, 97–104.

Desch, S., Schmidt, J., Kobler, D., Sonnabend, M., Eitel, I., Sareban, M., Rahimi, K., Schuler, G., Thiele, H., 2010. Effect of cocoa products on blood pressure: systematic review and meta-analysis. Am. J. Hypertens. 23, 97–103.

Dohadwala, M.M., Vita, J.A., 2009. Grapes and cardiovascular disease. J. Nutr. 139, 1788S–1793S.

Duthie, G.G., Gardner, P.T., Kyle, J.A., 2003. Plant polyphenols: are they the new magic bullet? Proc. Nutr. Soc. 62, 599–603.

Erben-Russ, M., Bors, W., Saran, M., 1987. Reactions of linoleic acid peroxyl radicals with phenolic antioxidants: a pulse radiolysis study. Int. J. Radiat. Biol. 53, 393–412.

Erlejman, A.G., Fraga, C.G., Oteiza, P.I., 2006. Procyanidins protect Caco-2 cells from bile acid- and oxidant-induced damage. Free Radic. Biol. Med. 41, 1247–1256.

Erlejman, A.G., Jaggers, G., Fraga, C.G., Oteiza, P.I., 2008. TNFalpha-induced NF-kappaB activation and cell oxidant production are modulated by hexameric procyanidins in Caco-2 cells. Arch. Biochem. Biophys. 476, 186–195.

Fang, H., Tong, W., Shi, L.M., Blair, R., Perkins, R., Branham, W., Hass, B.S., Xie, Q., Dial, S.L., Moland, C.L., Sheehan, D.M., 2001. Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. Chem. Res. Toxicol. 14, 280–294.

Fraga, C.G., 2007. Plant polyphenols: how to translate their in vitro antioxidant actions to in vivo conditions. IUBMB Life 59, 308–315.

Fraga, C.G., Sadgdicoglu Celep, G., Galleano, M., 2010. Biochemical actions of plant phenolics compounds: thermodynamic and kinetic aspects, in plant phenolics and human health. In: Fraga, C.G. (Ed.). John Wiley & Sons Inc., Hoboken, NJ, pp. 91–106.

Fujimura, Y., Tachibana, H., Kumai, R., Yamada, K., 2004a. A difference between epigallocatechin-3-gallate and epicatechin-3-gallate on anti-allergic effect is dependent on their distinct distribution to lipid rafts. Biofactors 21, 133–135.

Fujimura, Y., Tachibana, H., Yamada, K., 2004b. Lipid raft-associated catechin suppresses the FcepsilonRI expression by inhibiting phosphorylation of the extracellular signal-regulated kinase1/2. FEBS Lett 556, 204–210.

Fujimura, Y., Umeda, D., Yano, S., Maeda-Yamamoto, M., Yamada, K., Tachibana, H., 2007. The 67kDa laminin receptor as a primary determinant of anti-allergic effects of *O*-methylated EGCG. Biochem. Biophys. Res. Commun. 364, 79–85.

Galleano, M., Oteiza, P.I., Fraga, C.G., 2009. Cocoa, chocolate, and cardiovascular disease. J. Cardiovasc. Pharmacol. 54, 483–490.

Galleano, M., Pechanova, O., Fraga, C.G., 2010a. Hypertension, nitric oxide, oxidants, and dietary plant polyphenols. Curr. Pharm. Biotechnol., Sep. 28 [Epub ahead of print].

Galleano, M., Verstraeten, S.V., Oteiza, P.I., Fraga, C.G., 2010. Antioxidant actions of flavonoids: thermodynamic and kinetic analysis. Arch. Biochem. Biophys. 501, 23–30.

Gorelik, S., Ligumsky, M., Kohen, R., Kanner, J., 2008. The stomach as a "bioreactor": when red meat meets red wine. J. Agric. Food Chem. 56, 5002–5007.

Guo, Q., Zhao, B., Li, M., Shen, S., Xin, W., 1996. Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. Biochim. Biophys. Acta 1304, 210–222.

Hendrich, A.B., Malon, R., Pola, A., Shirataki, Y., Motohashi, N., Michalak, K., 2002. Differential interaction of Sophora isoflavonoids with lipid bilayers. Eur. J. Pharm. Sci. 16, 201–208.

Hertog, M.G., Feskens, E.J., Hollman, P.C., Katan, M.B., Kromhout, D., 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342, 1007–1011.

Holt, E.M., Steffen, L.M., Moran, A., Basu, S., Steinberger, J., Ross, J.A., Hong, C.P., Sinaiko, A.R., 2009. Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. J. Am. Diet Assoc. 109, 414–421.

Holt, R.R., Lazarus, S.A., Sullards, M.C., Zhu, Q.Y., Schramm, D.D., Hammerstone, J.F., Fraga, C.G., Schmitz, H.H., Keen, C.L., 2002. Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. Am. J. Clin. Nutr. 76, 798–804.

Hung, H.C., Joshipura, K.J., Jiang, R., Hu, F.B., Hunter, D., Smith-Warner, S.A., Colditz, G.A., Rosner, B., Spiegelman, D., Willett, W.C., 2004. Fruit and vegetable intake and risk of major chronic disease. J. Natl. Cancer Inst. 96, 1577–1584.

Iqbal, R., Anand, S., Ounpuu, S., Islam, S., Zhang, X., Rangarajan, S., Chifamba, J., Al-Hinai, A., Keltai, M., Yusuf, S., 2008. Dietary patterns and the risk of acute myocardial infarction in 52 countries: results of the INTERHEART study. Circulation 118, 1929–1937.

Jaganatah, I.B., Crozier, A., 2010. Dietary flavonoids and phenolic compounds in: Plant Phenolics and Human Health, Fraga, C.G. (Ed.). In: Fraga, C.G. (Ed.). John Wiley & Sons Inc., Hoboken, NJ. pp. 1–50.

Jaganathan, S.K., Mandal, M., 2009. Antiproliferative effects of honey and of its polyphenols: a review. J. Biomed. Biotechnol. 2009, 830616.

Joshipura, K.J., Hu, F.B., Manson, J.E., Stampfer, M.J., Rimm, E.B., Speizer, F.E., Colditz, G., Ascherio, A., Rosner, B., Spiegelman, D., Willett, W.C., 2001. The effect of fruit and vegetable intake on risk for coronary heart disease. Ann. Intern. Med. 134, 1106–1114.

Kandra, L., Gyemant, G., Zajacz, A., Batta, G., 2004. Inhibitory effects of tannin on human salivary alpha-amylase. Biochem. Biophys. Res. Commun. 319, 1265–1271.

Kawaguchi, K., Mizuno, T., Aida, K., Uchino, K., 1997. Hesperidin as an inhibitor of lipases from porcine pancreas and Pseudomonas. Biosci. Biotechnol. Biochem. 61, 102–104.

Kuiper, G.G., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., van der Burg, B., Gustafsson, J.A., 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139, 4252–4263.

Kwon, O., Eck, P., Chen, S., Corpe, C.P., Lee, J.H., Kruhlak, M., Levine, M., 2007. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J. 21, 366–377.

Lappano, R., Rosano, C., Madeo, A., Albanito, L., Plastina, P., Gabriele, B., Forti, L., Stivala, L.A., Iacopetta, D., Dolce, V., Ando, S., Pezzi, V., Maggiolini, M., 2009. Structure-activity relationships of resveratrol and derivatives in breast cancer cells. Mol. Nutr. Food Res. 53, 845–858.

Lichtenstein, A.H., Appel, L.J., Brands, M., Carnethon, M., Daniels, S., Franch, H.A., Franklin, B., Kris-Etherton, P., Harris, W.S., Howard, B., Karanja, N., Lefevre, M., Rudel, L., Sacks, F., Van Horn, L., Winston, M., Wylie-Rosett, J., 2006. Summary of American Heart Association Diet and Lifestyle Recommendations revision 2006. Arterioscler. Thromb. Vasc. Biol. 26, 2186–2191.

Lingwood, D., Simons, K., 2010. Lipid rafts as a membrane-organizing principle. Science 327, 46–50.

Liu, S., Manson, J.E., Lee, I.M., Cole, S.R., Hennekens, C.H., Willett, W.C., Buring, J.E., 2000. Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. Am. J. Clin. Nutr. 72, 922–928.

Lo, C.Y., Li, S., Tan, D., Pan, M.H., Sang, S., Ho, C.T., 2006. Trapping reactions of reactive carbonyl species with tea polyphenols in simulated physiological conditions. Mol. Nutr. Food Res. 50, 1118–1128.

Lotito, S.B., Frei, B., 2006. Dietary flavonoids attenuate tumor necrosis factor alpha-induced adhesion molecule expression in human aortic endothelial cells. Structure–function relationships and activity after first pass metabolism. J. Biol. Chem. 281, 37102–37110.

Mackenzie, G.G., Adamo, A.M., Decker, N.P., Oteiza, P.I., 2008. Dimeric procyanidin B2 inhibits constitutively active NF-kappaB in Hodgkin's lymphoma cells independently of the presence of IkappaB mutations. Biochem. Pharmacol. 75, 1461–1471.

Mackenzie, G.G., Carrasquedo, F., Delfino, J.M., Keen, C.L., Fraga, C.G., Oteiza, P.I., 2004. Epicatechin, catechin, and dimeric procyanidins inhibit PMA-induced NF-kappaB activation at multiple steps in Jurkat T cells. FASEB J. 18, 167–169.

Mackenzie, G.G., Delfino, J.M., Keen, C.L., Fraga, C.G., Oteiza, P.I., 2009. Dimeric procyanidins are inhibitors of NF-kappaB-DNA binding. Biochem. Pharmacol. 78, 1252–1262.

Mackenzie, G.G., Oteiza, P.I., 2006. Modulation of transcription factor NF-kappaB in Hodgkin's lymphoma cell lines: effect of (−)−epicatechin. Free Radic. Res. 40, 1086–1094.

Maldonado-Celis, M.E., Bousserouel, S., Gosse, F., Lobstein, A., Raul, F., 2009. Apple procyanidins activate apoptotic signaling pathway in human colon adenocarcinoma cells by a lipid-raft independent mechanism. Biochem. Biophys. Res. Commun. 388, 372–376.

McDougall, G.J., Stewart, D., 2005. The inhibitory effects of berry polyphenols on digestive enzymes. Biofactors 23, 189–195.

Messina, M., 2010. A brief historical overview of the past two decades of soy and isoflavone research. J. Nutr. 140, 1350S–1354S.

Middleton Jr., E., Kandaswami, C., Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52, 673–751.

Morel, I., Abalea, V., Sergent, O., Cillard, P., Cillard, J., 1998. Involvement of phenoxyl radical intermediates in lipid antioxidant action of myricetin in iron-treated rat hepatocyte culture. Biochem. Pharmacol. 55, 1399–1404.

Mursu, J., Voutilainen, S., Nurmi, T., Tuomainen, T.P., Kurl, S., Salonen, J.T., 2008. Flavonoid intake and the risk of ischaemic stroke and CVD mortality in middle-aged Finnish men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Br. J. Nutr. 100, 890–895.

Natarajan, K., Singh, S., Burke Jr., T.R., Grunberger, D., Aggarwal, B.B., 1996. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. Proc. Natl. Acad. Sci. USA 93, 9090–9095.

Ollila, F., Halling, K., Vuorela, P., Vuorela, H., Slotte, J.P., 2002. Characterization of flavonoid–biomembrane interactions. Arch. Biochem. Biophys. 399, 103–108.

Ortuño, J., Covas, M.I., Farre, M., Pujadas, M., Fito, M., Khymenets, O., Andres-Lacueva, C., Roset, P., Joglare, J., Lamuela-Raventós, R.M., de la Torre, R., 2010. Matrix effects on the bioavailability of resveratrol in humans. Food Chem. 120, 1123–1130.

Oteiza, P.I., Erlejman, A.G., Verstraeten, S.V., Keen, C.L., Fraga, C.G., 2005. Flavonoid-membrane interactions: a protective role of flavonoids at the membrane surface? Clin. Dev. Immunol. 12, 19–25.

Park, Y.C., Rimbach, G., Saliou, C., Valacchi, G., Packer, L., 2000. Activity of monomeric, dimeric, and trimeric flavonoids on NO production, TNF-alpha secretion, and NF-kappaB-dependent gene expression in RAW 264.7 macrophages. FEBS Lett. 465, 93–97.

Perez, C.A., Wei, Y., Guo, M., 2009. Iron-binding and anti-Fenton properties of baicalein and baicalin. J. Inorg. Biochem 103, 326–332.

Perron, N.R., Brumaghim, J.L., 2009. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem. Biophys. 53, 75–100.

Pike, A.C., Brzozowski, A.M., Hubbard, R.E., Bonn, T., Thorsell, A.G., Engstrom, O., Ljunggren, J., Gustafsson, J.A., Carlquist, M., 1999. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. EMBO J. 18, 4608–4618.

Piljac, J., Martinez, S., Stipèeviæ, T., Petroviæ, S., Metiko-Hukoviæ, M., 2004. Cyclic voltammetry investigation of the phenolic content of Croatian wines. Am. J. Enol. Vitic. 55, 417–422.

Poncet-Legrand, C., Gautier, C., Cheynier, V., Imberty, A., 2007. Interactions between flavan-3-ols and poly(L-proline) studied by isothermal titration calorimetry: effect of the tannin structure. J. Agric. Food Chem. 55, 9235–9240.

Ratty, A.K., Sunamoto, J., Das, N.P., 1988. Interaction of flavonoids with 1, 1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase-1. Biochem. Pharmacol. 37, 989–995.

Rein, D., Lotito, S., Holt, R.R., Keen, C.L., Schmitz, H.H., Fraga, C.G., 2000. Epicatechin in human plasma: in vivo determination and effect of chocolate consumption on plasma oxidation status. J. Nutr. 130, 2109S–2114S.

Ren, J., Meng, S., Lekka, Ch. E., Kaxiras, E., 2008. Complexation of flavonoids with iron: structure and optical signatures. J. Phys. Chem. B 112, 1845–1850.

Rhayem, Y., Therond, P., Camont, L., Couturier, M., Beaudeux, J.L., Legrand, A., Jore, D., Gardes-Albert, M., Bonnefont-Rousselot, D., 2008. Chain-breaking activity of resveratrol and piceatannol in a linoleate micellar model. Chem. Phys. Lipids 155, 48–56.

Rocha, B.S., Gago, B., Barbosa, R.M., Laranjinha, J., 2009. Dietary polyphenols generate nitric oxide from nitrite in the stomach and induce smooth muscle relaxation. Toxicology 265, 41–48.

Rocha, B.S., Gago, B., Barbosa, R.M., Laranjinha, J., 2010. Diffusion of nitric oxide through the gastric wall upon reduction of nitrite by red wine: physiological impact. Nitric oxide 22, 235–241.

Rush, J.D., Maskos, Z., Koppenol, W.H., 1990. Reactions of iron(II) nucleotide complexes with hydrogen peroxide. FEBS Lett. 261, 121–123.

Sbarra, V., Ristorcelli, E., Petit-Thevenin, J.L., Teissedre, P.L., Lombardo, D., Verine, A., 2005. In vitro polyphenol effects on activity, expression and secretion of pancreatic bile salt-dependent lipase. Biochim. Biophys. Acta 1736, 67–76.

Scalbert, A., Williamson, G., 2000. Dietary intake and bioavailability of polyphenols. J. Nutr. 130, 2073S–2085S.

Schroeter, H., Heiss, C., Balzer, J., Kleinbongard, P., Keen, C.L., Hollenberg, N.K., Sies, H., Kwik-Uribe, C., Schmitz, H.H., Kelm, M., 2006. (−)–Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc. Natl. Acad. Sci. USA 103, 1024–1029.

Shimizu, M., Shirakami, Y., Sakai, H., Yasuda, Y., Kubota, M., Adachi, S., Tsurumi, H., Hara, Y., Moriwaki, H., 2010. (−)–Epigallocatechin gallate inhibits growth and activation of the VEGF/VEGFR axis in human colorectal cancer cells. Chem. Biol. Interact. 185, 247–252.

Shishikura, Y., Khokhar, S., Murray, B.S., 2006. Effects of tea polyphenols on emulsification of olive oil in a small intestine model system. J. Agric. Food Chem. 54, 1906–1913.

Sirk, T.W., Brown, E.F., Friedman, M., Sum, A.K., 2009. Molecular binding of catechins to biomembranes: relationship to biological activity. J. Agric. Food Chem. 57, 6720–6728.

Steffen, Y., Jung, T., Klotz, L.O., Schewe, T., Grune, T., Sies, H., 2007a. Protein modification elicited by oxidized low-density lipoprotein (LDL) in endothelial cells: protection by (−)–epicatechin. Free Radic. Biol. Med. 42, 955–970.

Steffen, Y., Schewe, T., Sies, H., 2007b. (−)–Epicatechin elevates nitric oxide in endothelial cells via inhibition of NADPH oxidase. Biochem. Biophys. Res. Commun. 359, 828–833.

Thompson, M., Williams, C.R., Elliot, G.E., 1976. Stability of flavonoid complexes of copper(II) and flavonoid antioxidant activity. Anal. Chim. Acta 85, 375–381.

Totlani, V.M., Peterson, D.G., 2006. Epicatechin carbonyl-trapping reactions in aqueous maillard systems: Identification and structural elucidation. J. Agric. Food Chem. 54, 7311–7318.

van Acker, S.A., van den Berg, D.J., Tromp, M.N., Griffioen, D.H., van Bennekom, W.P., van der Vijgh, W.J., Bast, A., 1996. Structural aspects of antioxidant activity of flavonoids. Free Radic. Biol. Med. 20, 331–342.

van Dijk, C., Driessen, A.J., Recourt, K., 2000. The uncoupling efficiency and affinity of flavonoids for vesicles. Biochem. Pharmacol. 60, 1593–1600.

Vaya, J., Tamir, S., 2004. The relation between the chemical structure of flavonoids and their estrogen-like activities. Curr. Med. Chem. 11, 1333–1343.

Verstraeten, S.V., Hammerstone, J.F., Keen, C.L., Fraga, C.G., Oteiza, P.I., 2005. Antioxidant and membrane effects of procyanidin dimers and trimers isolated from peanut and cocoa. J. Agric. Food Chem. 53, 5041–5048.

Verstraeten, S.V., Keen, C.L., Schmitz, H.H., Fraga, C.G., Oteiza, P.I., 2003. Flavan-3-ols and procyanidins protect liposomes against lipid oxidation and disruption of the bilayer structure. Free Radic. Biol. Med. 34, 84–92.

Verstraeten, S.V., Mackenzie, G.G., Oteiza, P.I., Fraga, C.G., 2008. (−)–Epicatechin and related procyanidins modulate intracellular calcium and prevent oxidation in Jurkat T cells. Free Radic. Res. 42, 864–872.

Weitzberg, E., Lundberg, J.O., 1998. Nonenzymatic nitric oxide production in humans. Nitric oxide 2, 1–7.

Xia, M., Ling, W., Zhu, H., Wang, Q., Ma, J., Hou, M., Tang, Z., Li, L., Ye, Q., 2007. Anthocyanin prevents CD40-activated proinflammatory signaling in endothelial cells by regulating cholesterol distribution. Arterioscler. Thromb. Vasc. Biol. 27, 519–524.

Yamazaki, I., Piette, L.H., 1990. ESR spin-trapping studies on the reaction of Fe^{2+} ions with H_2O_2-reactive species in oxygen toxicity in biology. J. Biol. Chem. 265, 13589–13594.

Yano, S., Fujimura, Y., Umeda, D., Miyase, T., Yamada, K., Tachibana, H., 2007. Relationship between the biological activities of methylated derivatives of (−)–epigallocatechin-3-O-gallate (EGCG) and their cell surface binding activities. J. Agric. Food Chem. 55, 7144–7148.

Yoshioka, H., Haga, H., Kubota, M., Sakai, Y., 2006. Interaction of (+)-catechin with a lipid bilayer studied by the spin probe method. Biosci. Biotechnol. Biochem. 70, 395–400.