

Proanthocyanidins: Biological Activities Associated with Human Health

Gary R. Beecher

Foods and Nutrition Consultant, Lothian, Maryland, USA

Abstract

Proanthocyanidins, also called condensed tannins, are oligomers and polymers of monomeric flavans linked through specific single (B linkages) and double (A linkages) bonds. These secondary plant metabolites have substantial antioxidant activity. They are prevalent in some foods and dietary supplements including several berries, red grapes and their wines, and seeds, baking chocolate, cinnamon, pycnogenol, and *Ginkgo biloba*. Calculations based on limited food composition data suggest daily intakes of about 54 mg/day per person in the United States. Similar data are unavailable to estimate intakes from dietary supplements. Studies on digestion of proanthocyanidins indicates only monomers and dimers are absorbed; however, preliminary evidence suggests hydroxylated phenolic acids are important products of gastrointestinal microflora activity that also may be absorbed. Several types of investigations support improved vascular health after short- or long-term consumption of proanthocyanidins or foods and supplements that contain them. These effects include vasodilation, presumably as a result of increased NO production, decreased platelet aggregation, reduced sensitivity of low-density lipoproteins (LDL) to oxidation, and modulation of several reactions associated with inflammation. Studies with cranberries and cinnamon, both of which contain uniquely linked proanthocyanidins, support a role for bacterial antiadhesion and improved glucose metabolism in type 2 diabetics, respectively. Results from a variety of experiments indicate proanthocyanidins may modulate several reactions involved in cancer processes. A crucial research need is to identify further biologically active components of proanthocyanidins so that mechanisms of action at the tissue, cellular, and subcellular levels can be elucidated.

Keywords: Atherosclerosis, bacterial antiadhesion, botanicals, cancer, condensed tannins, diabetes, dietary supplements, foods, herbals, immune function.

Introduction

Name and general description

Proanthocyanidins, also named condensed tannins, are oligomers and polymers of monomeric flavonoids. More specifically they are polyflavans: condensed molecules of those flavonoids with a saturated C ring (Fig. 1A). Fifteen subclasses of proanthocyanidins have been identified (Porter, 1994); however, only a few are prominent in foods and supplements of plant origin. The various subclasses are named based on the conversion of the “interior” monomeric units (M) to the corresponding anthocyanidin during acid-catalyzed depolymerization; hence this broad class of polymers is named proanthocyanidins. Examples include conversion of (epi)catechin monomers to cyanidin (procyanidins) and (epi)gallocatechin monomers to delphinidin (prodelphinidins). In these tannins, the monomeric units are primarily linked through single 4→6 or 4→8 carbon-carbon bonds (B linkages) or through 4→8 carbon-carbon and 2→7 ether bonds (A linkages) (Fig. 1). Other linkages also have been identified but have been isolated from nonfood plants or constitute minor compounds of foods such as cocoa (Porter, 1994). Proanthocyanidins range in size from dimers through very large polymers and are found in many plant-based foods and several dietary supplements.

Biochemistry

Proanthocyanidins are secondary metabolites of plants; that is, they are not required for the structural or

Accepted: September 15, 2004

Address correspondence to: Dr. Gary R. Beecher, 1123 Pemberton Lane, Lothian, MD 20711 USA. Tel.: 301-627-6560; Fax: 410-741-9888; E-mail: beecher@earthlink.net

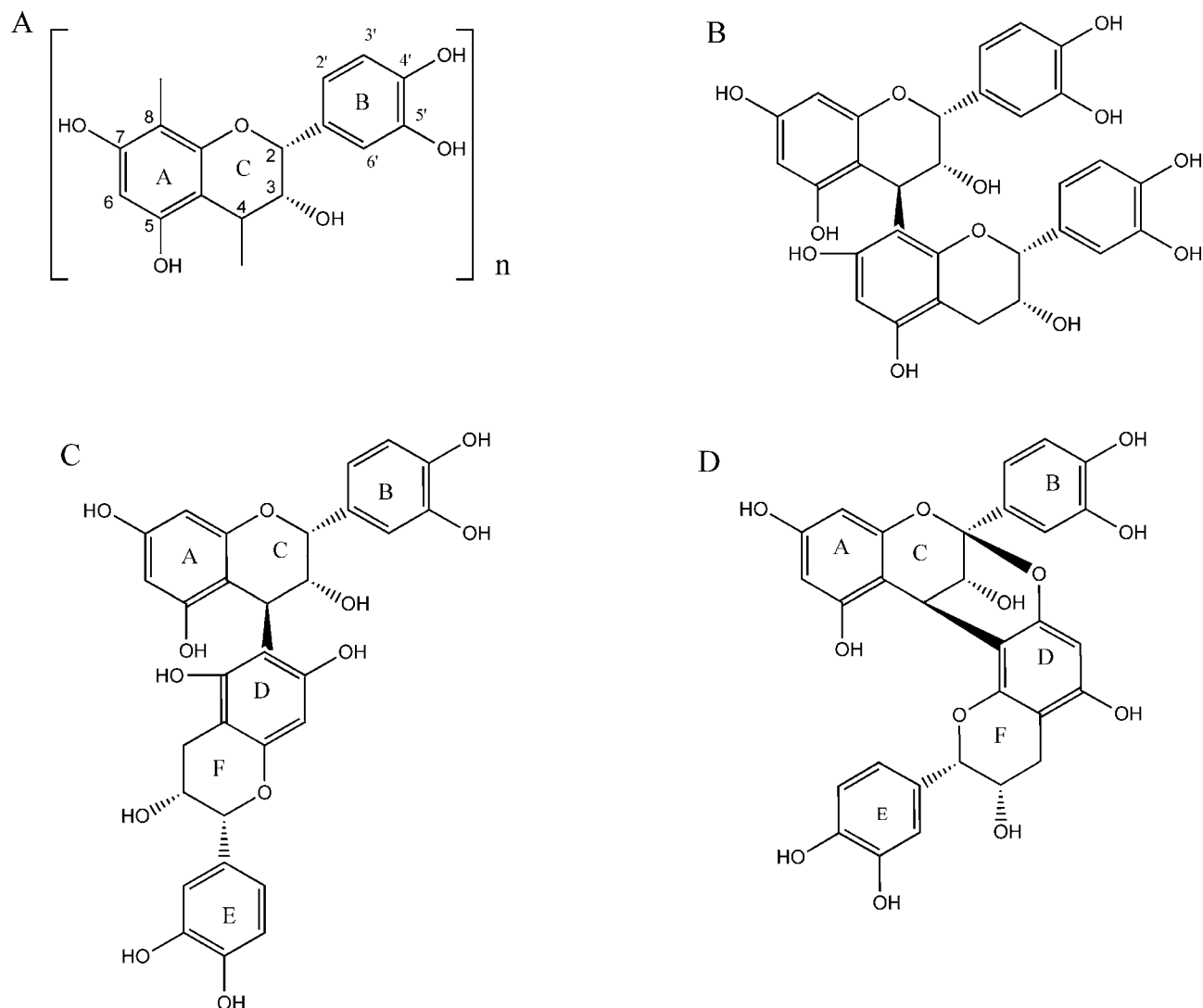


Figure 1. Representative linkages within proanthocyanidin molecules. (A) Monomeric representation with carbon 4 and 8 shown as potential linkages. Structure of (-)-epicatechin shown as example. Letters within rings identify individual phenolic or heterocyclic ring. n may equal 2 (dimer) to ~ 50 . (B) Example of B type (4→8) linkage. Specific compound is procyanidin B2 (dimer), epicatechin-(4β→8)-epicatechin. (C) Example of B type (4→6) linkage. Specific compound is procyanidin B5 (dimer), epicatechin-(4β→6)-epicatechin. (D) Example of A-type (4→8, 2→7) linkage. Specific compound is procyanidin A2 (dimer), epicatechin-(2β→7, 4β→8)-epicatechin. Adapted from Santos-Buelga and Scalbert (2000) and Qin et al. (2002).

metabolic integrity of the organism. However, they have several biological activities that protect plants from harmful intruders such as microbes, fungi, and animals. These biological properties and others currently are being investigated as “natural” sources of drugs (Cowan, 1999).

One of the earliest biochemical properties of proanthocyanidins to be realized was their ability to bind to and denature proteins. Their use in the conversion of animal hides into leather, a process called tanning (protein denaturation), led to the generic name of tannins for these compounds. The interaction between proanthocyanidins and proline/hydroxyproline-rich proteins and other polymers is very strong (Salunkhe et al., 1989),

thus explaining the excellent tanning effect of these natural constituents (collagen, a prominent protein of animal skin and hides, is rich in proline and hydroxyproline).

The unique polyhydroxy phenolic nature of proanthocyanidins and the resulting electronic configuration allows relatively easy release of protons and, as a result, they have substantial antioxidant activity. Employing many antioxidant systems, investigators have shown that proanthocyanidins have high antioxidant and radical scavenging activity (Bagchi et al., 1997; Ho et al., 1999; Santos-Buelga & Scalbert, 2000; Bors et al., 2001; Hatano et al., 2002; Beninger & Hosfield, 2003), usually greater than vitamins C and E, the gold standards. In

addition, these unique chemical structures bind divalent cations, such as iron and copper, which also contributes to their antioxidant activity. Such antioxidant activity is indirect because both iron and copper stimulate oxidative type reactions (Fenton reaction), but by effectively reducing the concentration of these cations (through binding), the extent of oxidative activity is greatly reduced. Conversely, the role that proanthocyanidin-cation binding has on the bioavailability of such minerals as copper, iron, or aluminum is uncertain (Salunkhe et al., 1989).

Physiology

One of the first responses when a food or supplement containing high levels of proanthocyanidins is consumed is an astringent sensation. This is due in part to the binding of these dietary constituents to proline-rich salivary proteins as described above (Salunkhe et al., 1989). As a result, formulation of palatable foods and supplements containing substantial levels of proanthocyanidins has been a challenge for food technologists and supplement formulation experts.

Although binding of proanthocyanidins to digestive enzymes has been a concern in animal nutrition where dietary concentrations of these components may be as high as a few percent, human foods contain much lower levels, and as a result, interference with digestive enzymes is of little concern (Salunkhe et al., 1989). However, the gastrointestinal (GI) metabolism and absorption of proanthocyanidins *per se* have been of interest relative to human health.

Early studies employing extracts of whole grapes suggested proanthocyanidins were absorbed and metabolized in rats and mice (reviewed by Santos-Buelga & Scalbert, 2000). Later experiments using purified proanthocyanidin fractions or grape seed extracts failed to demonstrate absorption of these food components in rats (Donovan et al., 2002; Nakamura & Tonogai, 2003), chickens, or sheep (Santos-Buelga & Scalbert, 2000). A few studies have demonstrated limited absorption of purified dimers in rats (Baba et al., 2002; Tanaka et al., 2003), which may be a result of strain specificity or dietary component interactions (Schramm et al., 2003). However, when absorption studies were conducted with Caco-2 cells, dimers and trimers of proanthocyanidins were readily transported across the cell monolayers, whereas higher polymers [degree of polymerization (Dp6), MW 1740 Da] were adsorbed onto the epithelial cells and permeability was greatly reduced (Deprez et al., 2001). Studies with human subjects have corroborated these results, showing that dimeric proanthocyanidins, but not higher polymers, were identified in plasma after consumption of flavonol-rich cocoa (containing primarily large polymers) or proanthocyanidin-rich grape seed extract (Holt et al., 2002; Sano et al., 2003).

It is difficult to ascribe the positive effects on health biomarkers to the relatively low (nanomolar concentrations) and transient plasma levels of only proanthocyanidin dimers, when foods or supplements containing high levels of proanthocyanidins are consumed. Metabolism of monomeric polyphenols by microflora of the lower GI tract has been recognized for many years (De Eds, 1959). The same concept recently has been applied to the metabolism of proanthocyanidins. *In vitro* experiments employing human colonic microflora demonstrated that semipurified proanthocyanidins (primarily hexamers and heptamers, but free of monomers, dimers, and trimers) were nearly totally degraded after 48 h of incubation (Deprez et al., 2000). The primary products of these incubations were monohydroxylated derivatives (*meta* and *para* isomers) of phenylacetic, phenylpropionic, and phenylvaleric acids (Deprez et al., 2000), which are similar to those resulting from the metabolism of monomeric flavonoids (De Eds, 1959). Studies with human subjects showed that cocoa proanthocyanidins were stable during transit through the high-acid environment of the stomach (Rios et al., 2002), but subsequently increased the urinary excretion of several phenolic acids (many the same as above) suggesting extensive metabolism in the lower GI tract (Rios et al., 2003). Additional studies with human beings identified the circulating forms of these phenolic acids as glucuronides, which were in much higher concentrations than the intact monomeric polyphenols that were fed (Rechner et al., 2002). Presumably, the glucuronides, along with sulfates and *O*-methyl derivatives of the phenolic acids, are the primary urinary products of proanthocyanidins (Duweler & Rohdewald, 2000; Scalbert et al., 2002), although a dearth of clinical observations limits validation of these assumptions. Radiolabeled proanthocyanidin trimers and higher oligomers have been identified in organs of mice after oral administration (Ammon & Kaul, 1994); however, there is a lack of similar scientific evidence for human beings. In addition, tissue distribution of hydroxylated phenolic acids and similar colonic metabolic products have not been investigated.

One of the shortcomings of many of the investigations on proanthocyanidins has been the commercial availability of purified individual oligomers and polymers. As a result, foods, supplements, and medicinal plant preparations, which contain proanthocyanidins, have been tested for effectiveness. Unfortunately, these materials often contain a host of other compounds that also may alter various biological responses; that is, terpene trilactones of *Ginkgo biloba* with neuromodulatory properties (Stromgaard & Nakanishi, 2004). Nonetheless, results employing these dietary materials give an indication of the potential proanthocyanidins may have in the beneficial alteration of disease markers. Typical natural product preparations that have been employed in such

experiments include *Eriobotrya japonica* (loquat) leaf and seed extracts, grape seed extract, *Ginkgo biloba*, pycnogenol (extract of French maritime pine bark), and red alder bark extract and witch hazel (extract of *Hamamelis virginiana* L. bark). Foods and spices that often have been examined for health-promoting activity and that contain proanthocyanidins include cinnamon, cranberries, cocoa powder and dark chocolate, and red wines.

Alteration of biological markers associated with chronic and other diseases

Free radicals, human diseases, and proanthocyanidins as antioxidants

Many life processes generate free radicals. The resulting reactive oxygen and nitrogen species (ROS, RNS), if left unchecked, have the potential to cause oxidative damage to DNA, lipids, and proteins, resulting in a cascade of degradative effects that contributes to human disease pathophysiology (Bagchi et al., 2000). Free-radical scavengers and/or antioxidants provide protection of cells against oxidative damage (Halliwell et al., 1992). Proanthocyanidins and their purported digestion products, hydroxylated phenolic acids, have high antioxidant activity (Rice-Evans et al., 1996; Bors & Michel, 2002). In the case of proanthocyanidins, major contributions to this activity are the presence of a catechol group (adjacent hydroxyls) on the B ring and the stability of the reduction products, semiquinones and quinones (Rice-Evans et al., 1996).

A wide variety of *in vitro* measurement systems have been employed to assess antioxidant activity. Depending on chemical structure(s), a specific molecule or natural product may have quite different affinities toward the various radicals employed in each system (Prior & Cao, 2000). The data in Table 1 are a compilation of relative antioxidative activities generated by such systems for several purified proanthocyanidins as well as selected foods and supplements that are known to contain them. In general, these data show that proanthocyanidins and proanthocyanidin-rich products have substantial ROS/RNS scavenging activity, often orders of activity greater than vitamins C and E. Although the observations in Table 1 suggest foods and spices may have greater relative antioxidant activity than pure compounds or supplements (those listed), similar measurement systems have not been applied across all materials, thus precise interpretation of these data is difficult (Prior & Cao, 2000). The general agreement of high relative antioxidant activity (Table 1) strongly suggests proanthocyanidins may play a role in the amelioration of oxidative processes that have the potential to accelerate several diseases.

Cancer

Many *in vitro* and *in vivo* systems have been employed to investigate the role of proanthocyanidins on markers associated with cancer processes. Studies with DNA indicated cocoa-related flavonols and proanthocyanidins were effective as ROS scavengers at physiological concentrations in the inhibition of oxo⁸dG formation induced by UV-C light energy (Ottaviani et al., 2002). Relative to protection against oxidative stress, pretreatment of cultured J774A.1 macrophage and neuroactive PC-12 cells with grape seed proanthocyanidin-rich extract (GSPE) reduced hydrogen peroxide-induced stress substantially (36–70%) and in a dose-dependent manner (Bagchi et al., 2002). Procyanidin B2 (dimer), isolated from loquat leaves, also was found to inhibit the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced activation of Epstein-Barr virus early antigen in Raji cells (Ito et al., 2002). *In vivo* studies with mice indicated that pretreatment with dietary GSPE greatly reduced TPA-induced lipid peroxidation and DNA fragmentation in brain and hepatic tissues (Bagchi et al., 1998). In addition, production of ROS by peritoneal macrophages was significantly decreased in GSPE-pre-fed and TPA-treated mice as evidenced by decreased cytochrome c reduction and chemiluminescence response. The responses of all of the biological markers were dose dependent with respect to dietary GSPE levels and were also greater than the response when vitamin C or E or beta-carotene was fed at similar concentrations.

Cytotoxicity

The 3,3'-di-*O*-gallate derivative of procyanidin B2 was active against human leukemic cells (HL-60) and a melanoma cell line but was inactive toward several other tumor cell lines (Santos-Buelga & Scalbert, 2000). Several other galloylated procyanidin dimers were also shown to inhibit the growth of human lung and colon carcinoma cell lines. However, several non-galloylated dimers, dimers and trimers containing A-type linkages, and a galloylated pentamer were less active (Santos-Buelga & Scalbert, 2000). A procyanidin oligomer mixture isolated from loquat leaves, composed primarily of undecameric procyanidin (Dp11), showed cytotoxic activity against human squamous cell carcinoma and a human salivary gland tumor line (Ito et al., 2000). Two fractions of proanthocyanidins, isolated from the bark of witch hazel, were tested in a human derived, metabolically competent hepatoma cell line (Hep G2) for protection against benzo(a)pyrene [B(a)P]-induced DNA damage (Dauer et al., 2003a). The low-molecular-weight fraction and catechin provided only limited protection of DNA, whereas the higher molecular weight fraction and hamamelitannin (a hydrolyzable tannin), isolated from

Table 1. Reactive oxygen and nitrogen species (ROS/RNS) scavenging activities of several proanthocyanidins, foods, and supplements.

Proanthocyanidin, food, supplement	Relative ROS scavenging activity ^a	<i>In vitro</i> measurement ^b	Ref. ^c
Flavonoids/proanthocyanidins			
Catechin	+	TEAC	1
(-)-Epicatechin	+	TEAC, OH ⁻ scavenger	1, 2
Quercetin	+	TEAC, OH ⁻ scavenger	1, 2
Procyanidin B ₂ (dimer)	+	OH ⁻ scavenger	2
Proanthocyanidin C ₁ , trimer	+	Peroxy nitrite and OH ⁻ scavenger	2, 3
Proanthocyanidin tetramer	+	OH ⁻ scavenger	2
Foods/spices			
Apple, including peel	++	Total ORAC	4
Blueberries	++	Total ORAC	4
Cinnamon	+++	Total ORAC	4
Chocolate, baking	+++	Total ORAC	4
Tea, green and black	+	TEAC, Total ORAC	1, 5
Wine, red	++	TEAC, Total ORAC, peroxy nitrite scavenger	1, 6, 7
Supplements			
Ginko biloba	+	Peroxy nitrite scavenger	6
Grape seed extract	+	Superanion and peroxy nitrite scavenger	6, 8
Pine bark extract	++	ORAC	9

^aBecause of the wide variety of measurement systems employed, relative values are only approximations. Relative values are based on mass (volume) of compound/food/supplement. Reference compounds for many entries were vitamins C or E, or Trolox (water-soluble form of vitamin E), all of which exhibited ROS scavenger activities less than any entry in the table.

^bAbbreviations of *in vitro* systems: ORAC, oxygen radical absorbance capacity (hydrophilic fraction only); TEAC, Trolox equivalent antioxidant capacity; Total ORAC, summation of lipophilic and hydrophilic oxygen radical absorbance capacity.

^cReferences code as follows: 1, Rice-Evans et al. (1996); 2, Shahat et al. (2002); 3, Stevens et al. (2002); 4, Wu et al. (2004); 5, Prior and Cao (1999b); 6, Valdez et al. (2002); 7, Sanchez-Moreno et al. (2003); 8, Bagchi et al. (2002); 9, Prior and Cao (1999a).

the same source, showed a two-fold enhancement of the DNA-protection marker (Olive tail moment of single-cell gel electrophoresis). Exposure of the cells to the isolated preparations prior to, but not after, B(a)P treatment led to a significant reduction of induced DNA damage, which was dose dependent, for both proanthocyanidin fractions. Results of studies to elucidate the mechanism of protective action suggested the proanthocyanidin fractions scavenged an active mutagenic form of B(a)P, (\pm)-anti-benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide, and that DNA repair mechanisms were unaffected. Additional research is needed to determine if the large proanthocyanidin molecules, *per se*, were the active scavengers or if a metabolite was effective in neutralizing B(a)P or its mutagenic form(s).

Grape seed extract, a nutritional supplement, is currently being used by many individuals. A standardized, water-ethanol extract from California red grape seeds (IH636) (InterHealth Nutraceuticals, Benicia, CA, USA) contains about 75–80% oligomeric proanthocyanidins and 3–5% monomeric flavonoids (Bagchi et al., 2003). The cytotoxicity of this novel extract (IH636) has been evaluated with several cancer cell lines. Concentration- and time-dependent cytotoxic effects of IH636 were observed on MCF-7 breast cancer, A-427 lung cancer, and gastric adenocarcinoma cells, but not against

neoplastic K562 myelogenous leukemic cells (Ye et al., 1999). However, IH636 enhanced growth and viability of normal human gastric mucosal cells and J774A.1 murine macrophage cells. Treatment with GSPE ameliorated the chemotherapy-induced toxic effects of idarubicin and 4-hydroxyperoxycyclophosphamide of normal human Chang liver cells (Joshi et al., 2001). The GSPE-treated cells in these studies had reduced apoptosis, increased antiapoptotic gene *Bcl-2*, and decreased *c-myc* and p53 (the product of TP53 genes).

The influence of proanthocyanidins has been investigated on biological markers for cancer in several animal models. Proanthocyanidins fed as a condensed tannin extract of red alder bark or as GSPE significantly inhibited the multiplicity, size, and distribution of chemically induced colonic aberrant crypt foci in mice and rats (Gali-Muhtasib et al., 2001; Singletary & Meline, 2001). Interestingly, one of the most effective forms of administration of red alder bark proanthocyanidins was via drinking water. Experiments with proanthocyanidins isolated from cacao liquor and fed to Sprague-Dawley rats substantially inhibited the initiation of 2-aminomethyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced pancreatic carcinogenesis (Yamagishi et al., 2002). *In vitro* studies suggested proanthocyanidins also directly inhibited the mutagenic

activity of PhIP, perhaps through nonspecific binding. Feeding proanthocyanidins extracted from grape seeds to SKH-1 hairless mice also decreased both UVB-induced skin carcinogenesis and malignant transformation in terms of incidence, multiplicity, and size (Mittal et al., 2003). A suggested mechanism of the inhibition of carcinogenesis is the antioxidant activity conferred by the dietary proanthocyanidins. Grape seed proanthocyanidins fed to mice or rats, however, were not effective in curtailing chemically induced mammary tumorigenesis (Singletary & Meline, 2001; Yamagishi et al., 2002). Several foods also contain proanthocyanidins; however, there is a paucity of observations on their effect on carcinogenic processes. Although black and green teas have extensively been investigated for their anticancer activity, green teas contain only limited proanthocyanidins (Lakenbrink et al., 1999), and black teas have substantial concentrations of derived tannins (theaflavins, thearubigins, and others), which are a heterogeneous mixture of oxidation products of monomeric flavonoids and structurally different from proanthocyanidins (Beecher, 2002).

Mechanisms of action

Investigators have suggested several mechanisms of action for proanthocyanidins toward inhibition of carcinogenic processes. Foremost among these is their antioxidant or ROS/RNS scavenging activities (Bagchi et al., 2002). However, both caffeic acid (a phenolic acid) and pycnogenol inhibited purified protein kinases A and C, as well as phosphorylase kinase (Nardini et al., 2002). In addition, caffeic acid supplementation, but not antioxidant addition, to U937 monocytic cells significantly inhibited ceramide-induced apoptosis and protein tyrosine kinase activity. A procyanidin-rich fraction isolated from cranberries was effective in the inhibition of TPA-induced ornithine carboxylase activity, a marker of chemopreventive activity (Kandil et al., 2002).

Studies with GSPE have linked its protective abilities with DNA repair, lipid peroxidation, and intracellular calcium homeostasis (Bagchi et al., 2002). In addition, GSPE provided protection against chemically induced hepato- and nephrotoxicity, increased *bcl-X_L* expression in liver tissue, and antagonized both necrotic and apoptotic liver cell death. Further attempts to elucidate the antiapoptotic and antinecrotic mechanism suggested that liver cytochrome P450 2E1 activity, responsible for metabolism of drugs and chemicals, was substantially decreased by feeding GSPE to mice (Bagchi et al., 2002) but not in the same organ of rats (Singletary & Meline, 2001). Also, cytochrome P450 1A and glutathione *S*-transferase activities (liver carcinogen metabolism) were unaltered by a similar feeding regimen of rats (Singletary & Meline, 2001).

Atherosclerosis

Atherosclerosis is an inflammatory disease process (Hannum et al., 2002; Kris-Etherton et al., 2004). Endothelial injury is one of the first events in this process, which is followed by a large number of reactions and molecular responses. All of these events may lead to the formation of atherosclerotic plaques, which results in constriction of blood vessels that also may have reduced capacity to dilate. Proanthocyanidins and/or their metabolic products appear to ameliorate several of the steps in this complex process.

Inhibition of liposome and LDL oxidation

Experiments with synthetic liposomes and proanthocyanidins isolated from cocoa revealed that oxidation originating in the aqueous phase was inhibited most effectively by flavan-3-ol monomers and proanthocyanidin dimers and trimers (Lotito et al., 2000). Conversely, protection was greatest with higher polymers (Dp3–6) when oxidation was initiated in the lipid phase. Elucidation of the antioxidant mechanism suggested proanthocyanidins interacted with the phospholipid “head” groups of the liposome, which restricted both access of oxidants to the membrane surface and movement of oxidants through the internal hydrophobic region of the membrane (Verstraeten et al., 2003).

Relative to oxidation of low-density lipoproteins, *in vitro* studies with isolated LDL particles showed that individual isolated procyanidins (monomer through hexamer) or several natural products rich in proanthocyanidins (cranberry extract, grape seed extract) inhibited chemically induced oxidation of LDL (Lotito et al., 2000; Pearson et al., 2001; Porter et al., 2001; Reed, 2002; Steinberg et al., 2002). In a copper-catalyzed LDL system, equimolar concentrations of individual procyanidins indicated antioxidation activity proportional to degree of polymerization (Pearson et al., 2001). Employing a similar system, isolated fractions from cranberries rich in proanthocyanidin oligomers (Dp3–9) and containing 1–3 A-type linkages were also effective in delaying LDL oxidation (Porter et al., 2001). When results from an 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH)-induced LDL conjugated diene formation system were expressed on a monomer equivalent basis, inhibitory activity of the various polymers was similar, suggesting antioxidant capacity was a function of the number of available catechol groups (Steinberg et al., 2002). Studies with fractions containing mixed oligomers gave similar results in terms of antioxidant capacity, but higher polymers (Dp5–9) appeared to have greater affinity for LDL particles than oligomers with a lower degree of polymerization (Reed, 2002). Similar studies in a cellular system (endothelial cell-mediated LDL oxidation) changed preference of antioxidant to monomeric catechin and dimers rather than higher polymers of

procyanidin (Pearson et al., 2001). It is difficult to interpret results with polymeric proanthocyanidins and chemically induced LDL oxidation in terms of biological activity, because studies to date suggest oligomers greater than Dp2 are only minimally absorbed and circulated in the bloodstream. Results from endothelial cell-mediated oxidation of LDL appear to be most comparable to *in vivo* systems in that monomers and dimers, those fractions quantified in blood, were most effective as antioxidants.

A highly controlled, double-blind randomized crossover human study demonstrated that proanthocyanidin-containing cocoa powder and chocolate were effective in decreasing LDL oxidation susceptibility and slightly increasing serum total antioxidant capacity as well as high-density lipoprotein (HDL) cholesterol levels (Wan et al., 2001). The diets of this study were based on an average American diet and identical in content except for daily addition of 16 g dark chocolate and 22 g of cocoa powder to the test diet. Similar results of cocoa ingestion on LDL oxidation susceptibility were observed in additional studies for which dietary control was less rigorous (Steinberg et al., 2003 and references therein). Pycnogenol (150 mg/day) fed to healthy individuals for 6 weeks did not alter LDL oxidizability but reduced LDL-cholesterol and increased HDL-cholesterol levels in plasma of two-thirds of the subjects (Devaraj et al., 2002). However, the same extract (360 mg/day) given to patients with chronic venous insufficiency decreased blood total cholesterol and LDL-cholesterol values but did not alter HDL-cholesterol levels (Koch, 2002). Addition of GSPE to the diet of hypercholesterolemic subjects for 8 weeks substantially reduced the level of antibodies to oxidized LDL (measure of oxidized LDL) compared to results of the placebo control group (Bagchi et al., 2003). Results from *in vitro* studies suggested isolated cocoa-proanthocyanidins were inhibitors of mammalian 15-lipoxygenase-1, an enzyme that oxygenates LDL to an atherogenic form (Schewe et al., 2001b). Studies with red wine or red wine polyphenol-containing diets (rich in proanthocyanidins) gave mixed results in terms of plasma antioxidant capacity and resistance to *ex vivo* LDL oxidation (Santos-Buelga & Scalbert, 2000; Steinberg et al., 2003).

Inhibition of inflammatory response

Studies with isolated or purified cyclooxygenase-1, cyclooxygenase-2, and 5-lipoxygenase demonstrated they were inhibited by flavonols and oligomeric proanthocyanidins from cocoa at concentrations similar to drugs used for the same purpose; for example, indomethacin (Schmitz & Romanczyk, 2000; Schewe et al., 2001a). Short-term (6 h) *in vivo* experiments with human subjects fed high proanthocyanidin-containing chocolate resulted in increased plasma levels of prostacyclin, decreased concentrations of leukotrienes, and a decreased leukotriene/

prostacyclin ratio, a measure of the proinflammatory/anti-inflammatory eicosanoid balance (Schramm et al., 2001). Similar results were observed with *in vitro*-treated aortic endothelial cells. Longer-term studies (4 and 6 weeks) with subjects consuming a combination of cocoa powder and dark chocolate daily, in addition to an average American diet or a low-flavonoid diet, failed to alter the urinary excretion of F₂ isoprostane, thromboxane B₂, 6-keto-prostaglandin F_{1 α} , or their ratio (Wan et al., 2001; Mathur et al., 2002). Consumption of purple grape juices (proanthocyanidin-containing juice), but not several other juice or coffee devoid of proanthocyanidins, significantly increased 6-keto-prostaglandin F_{1 α} at 2 h postconsumption (Polagruto et al., 2003). Pycnogenol (200 mg/day) consumption reduced thromboxane B₂ levels in smokers compared to those of nonsmokers but did not alter levels of nonsmokers (Araghi-Niknam et al., 2000). Intake of *Ginkgo biloba* extract (120 mg/day, 3 months) by healthy subjects reduced excretion of both 11-dehydro thromboxane B₂ and prostacyclin but had no effect in type 2 diabetics, which suggested a differential modulation of cyclooxygenases depending on their cellular location (platelets or endothelial cells) (Kudolo et al., 2002). Results from these experiments suggest proanthocyanidins and proanthocyanidin-containing foods and supplements may alter eicosanoid metabolism in favor of an anti-inflammatory environment. However, environmental interactions as well as time-course and magnitude of this response require further investigation.

Endothelial injury causes increased expression of cellular adhesion molecules [CAMs; i.e., ICAM-1 (intra-cellular CAM), VCAM-1 (vascular CAM), E-selectin] that mediate recruitment of monocytes and their subsequent differentiation into phagocytic macrophages (Reed, 2002). Employing HaCaT cells (human keratinocyte), pycnogenol pretreatment inhibited IFN- γ -induced adherence of these cells to Jurkat T cells and expression of ICAM-1 (Bito et al., 2000). Pycnogenol also inhibited NF- κ B activation and VCAM-1 and ICAM-1 expression in TNF- α -treated human umbilical vein endothelial cells (HUVECs) (Peng et al., 2000). A gene that codes for an oxidized LDL receptor directly linked to foam cells and atherosclerosis, CD 36, was found to be downregulated by GSPE in TNF- α -induced HUVECs (Bagchi et al., 2003). *In vitro* studies with peripheral blood mononuclear cells (PBMCs), isolated from human subjects that had low production of transforming growth factor (TGF) beta1, showed that TGF- β 1 production was greatly stimulated by dimer and tetramer proanthocyanidins isolated from cocoa compared to higher polymers (Dp > 5) (Mao et al., 2003). In contrast, TGF- β 1 secretion from high-producing PBMCs at baseline was inhibited by all cocoa proanthocyanidin fractions tested (Dp2–10). A study with cultured vascular smooth muscle cells demonstrated that exposure to red wine polyphenolic compounds inhibited both mRNA expression for and

vascular endothelial growth factor in response to platelet-derived growth factor AB, TGF- β -1, or thrombin (Oak et al., 2003). Elucidation of the mechanism suggested the redox-sensitive activation of the p38 mitogen-activated protein kinase (MAPK) had been inhibited. Notwithstanding these observations from *in vitro* experiments, a 6-week-long study with human subjects who received a combination of dark chocolate and cocoa powder (~650 mg procyanidins per day) in addition to a low flavonoid diet failed to alter the response of several markers of inflammation, including interleukin-1 β , interleukin-6, TNF- α 1, C-reactive protein, and P-selectin (Mathur et al., 2002).

Decreased platelet aggregation

In vitro experiments with whole blood showed that cocoa procyanidin trimers and pentamers as well as dealcoholized red wine (DRW) increased expression of platelet activation markers (fibrinogen binding conformation of GPIIb-IIIa and P-selectin) in unstimulated platelets but suppressed platelet activation response to epinephrine (Rein et al., 2000a). Both short-term (2–6 h) studies and a long-term (28 day) study with human subjects demonstrated that consumption of proanthocyanidin-rich cocoa beverage lowered P-selectin expression and platelet aggregation (ADP-, collagen-, epinephrine-induced) in *ex vivo* experiments (Rein et al., 2000a, 2000b; Pearson et al., 2002; Murphy et al., 2003). The effects observed were qualitatively similar to aspirin but less profound (Pearson et al., 2002). Other food sources of proanthocyanidins (and minor constituents), such as purple grape juice, and combined extracts of grape seeds and grape skins, but not citrus juices, also were active in the reduction of platelet aggregation when administered to dogs, monkeys, or human beings (Reed, 2002; Shanmuganayagam et al., 2002). Extract of *Ginkgo biloba* (120 mg/day for 3 months) fed to healthy volunteers modulated collagen- but not PAF-mediated platelet aggregation (Kudolo et al., 2002). However, giving the same extract to subjects with type 2 diabetes mellitus decreased platelet aggregation stimulated by both systems.

An *in vivo* model based on cyclic flow reductions caused by platelet aggregation in the partially occluded circumflex coronary artery of anesthetized dogs has been employed to test platelet activity and platelet-vessel wall interactions (Reed, 2002 and references therein). Several of the same dietary sources of proanthocyanidins (red wine, purple grape juice) that were active *in vitro*, were also active in preventing thrombus formation in this model. A similar model, based on experimental venous thrombosis in spontaneously normolipidemic rats fed a cholesterol-rich diet, demonstrated that DRW added to their diet reversed the prothrombotic effect of the hyperlipidemic factors (De Gaetano et al., 2002).

Animal models

Two animal models have been developed to study dietary and other effects on progression of atherosclerosis. Golden Syrian hamsters, when fed diets of high cholesterol and coconut oil for 10 weeks, have a lipid profile similar to hypercholesterolemic human beings. This treatment also results in the formation of foam cells on aorta walls, the extent of which has been used as a biomarker of the early stages of atherosclerosis (atherosclerotic index) (Bagchi et al., 2003). Addition of GSPE to hypercholesterolemic hamster diets (50 or 100 mg/kg body weight) resulted in a substantial and significant reduction of the atherosclerotic index. In addition, total plasma cholesterol and triglyceride levels also were significantly reduced in the GSPE-fed animals.

New Zealand White rabbits fed hypercholesterolemic diets respond with high plasma total cholesterol levels (400+ mg/dl) and the formation of Sudan-positive stained lesions (fatty streaks) on the walls of their aorta (biomarker of atherosclerosis potential) (Ursini & Sevanian, 2002). Addition of GSPE (Leucoselect-Phytosome) to hypercholesterolemic diets of a group of rabbits reduced aortic arch lesions to nearly control levels (3%), whereas atherosclerotic diets alone resulted in lesions that covered 18% of the vessel wall.

Studies with human beings who consumed a combination of cocoa powder and dark chocolate for relatively long periods (4 and 6 weeks) only slightly but significantly increased HDL levels in one experiment, but did not significantly alter plasma cholesterol, triglyceride, or other lipoprotein concentrations (Wan et al., 2001; Mathur et al., 2002). In contrast, cinnamon, which contains a series of unique trimeric and tetrameric procyanidins with A-type linkages (Anderson et al., 2004), significantly decreased plasma levels of triglycerides as well as total and LDL cholesterol when administered (1–6 g/day) for only 20 days (Khan et al., 2003). Grape seed proanthocyanidins fed to rats, along with high-cholesterol diets, also reduced serum cholesterol levels compared to non-proanthocyanidin fed controls (Santos-Buelga & Scalbert, 2000). Studies with proanthocyanidin-rich cranberry juice powder fed to familial hypercholesterolemic pigs significantly lowered plasma total cholesterol and LDL and slightly raised HDL (Reed, 2002). However, the same powder fed to normocholesterolemic pigs did not alter levels of circulating cholesterol fractions.

Nitric oxide-dependent vasodilation

The enzyme, nitric oxide synthase (NOS), uses L-arginine and oxygen as substrates to produce NO, which interacts with smooth muscle cells to cause vasorelaxation. A common inhibitor of NOS, N^G-nitro-L-arginine methyl

ester (L-NAME), when infused, nullified vasodilation observed with treatments that stimulate NO production (De Gaetano et al., 2002; Fisher et al., 2003), thereby validating the action of NOS and role of NO in vasodilation. Three distinct NOS isozymes have been identified: endothelial (eNOS), the crucial isozyme relative to maintenance of vascular function, neuronal (nNOS), and an inducible form (iNOS) found in a number of cell types, including macrophages and vascular smooth muscle cells (Parks & Booyse, 2002).

In vitro studies demonstrated that red wine and pycnogenol, but not white wine, improved vasodilation and simultaneously increased endothelial NO production (Reed, 2002). Further characterization of proanthocyanidin fractions isolated from red wine showed that vasodilation activity was greatest in the presence of low-molecular-weight oligomers (Dp2–3), whereas higher polymers were inactive. Examination of the mechanism of increased NO production with rat aorta ring strips and *Gingko biloba* extract suggested inhibition of Ca^{2+} -influx through Ca^{2+} -channels thereby activating NO release (Nishida & Satoh, 2003). Contrary to the above findings, proanthocyanidins isolated from female inflorescences of hops (*Humulus lupulus*), a common ingredient of beer, were strong inhibitors of nNOS activity, with procyanidin dimer B2 having the highest inhibitory activity (Stevens et al., 2002). Procyanidin dimer B3, an isomer of B2, was noninhibitory in this system. An explanation for the differential action of these isomers on two isoforms of NOS is not apparent at this time.

Two noninvasive *in situ* systems have been developed to test the efficacy of various dietary components, drugs, and environmental conditions on vasodilation. A study in patients with coronary artery disease had improved flow-mediated vasodilation of the brachial artery (FMD) when purple grape juice was consumed compared to beverages that did not contain proanthocyanidins (Reed, 2002). A similar experiment with subjects that had at least one cardiovascular risk factor and who consumed a high-proanthocyanidin cocoa beverage had a significant increase in FMD response (4 h postconsumption) relative to response, at baseline and after consumption of a low-proanthocyanidin cocoa drink (Heiss et al., 2003). In addition, plasma NO concentrations paralleled FMD response, supporting the concept that improved endothelial function, in part, may be due to a favorable modulation of NO bioavailability. Employing pulsatile artery dilation in a fingertip, healthy subjects who consumed a proanthocyanidin-rich cocoa for 4 days had consistent and striking peripheral vasodilation compared to baseline (Fisher et al., 2003). Consumption of test cocoa on day 5 gave an additional significant acute vasodilation response, whereas infusion of L-NAME completely reversed the cocoa-induced vasodilation.

A surgical *in situ* system was employed to assess the response of cerebral microcirculation of rats to intake of an extract of *Gingko biloba* leaves (EGb 761) (Zhang et al., 2000). Normotensive and spontaneously hypertensive (SHR) rats were fed EGb 761 for 9 days, after which several parameters of cerebral circulation were compared with control animals. The most significant and dramatic changes observed in EGb 761-fed SHR rats were a blood pressure decrease of nearly 30%, increased numbers of open capillaries, and increased numbers of circulating endothelial cells compared to SHR controls. Only minor changes were observed (increased cerebral blood flow velocity) in EGb 761-treated normotensive animals. Although NOS activity and NO production were not measured, these observations suggest EGb 761 may have improved cerebral vascular parameters through increased production of NO and other factors.

Vasoconstriction

Angiotensin II is a vasoconstrictor that is produced in the pulmonary capillaries by angiotensin, converting enzyme (ACE) and can be involved in the development of hypertension and atherosclerosis (Reed, 2002). Several proanthocyanidins and preparations containing them inhibited ACE activity in both *in vitro* and *in vivo* experiments. These included pycnogenol, proanthocyanidins isolated from red grapes, and extracts of *Erythroxylum laurifolium* (endemic species on Reunion Island in the Indian Ocean) and of fruits of *Cupressus sempervirens* L. (Italian cypress).

Reperfusion

Induced ischemia-reperfusion studies in hearts isolated from laboratory animals simulates myocardial infarct and recovery in human beings. This model permits investigation of various dietary interventions and other environmental and circulatory alterations on recovery of hearts postischemia. Hearts from GSPE-, red wine-, or red wine proanthocyanidin-fed rats were more resistant to ischemia-reperfusion injury than hearts from control animals (Sato et al., 2002; Bagchi et al., 2003). Blood flow parameters were improved, whereas infarct size, formation of hydroxyl radicals, and malondialdehyde levels of heart perfusate were all modulated as a result of feeding animals proanthocyanidins or proanthocyanidin-containing ingredients. These same dietary treatments also reduced the levels of proapoptotic factors JNK and c-Jun, as well as the proportion of apoptotic cardiomyocytes. Similar studies with a short-term recovery (12 min) showed opposite effects of EGb 761 pretreatment in terms of decreased iNOS mRNA expression and NO production (Varga, 2002).

Other metabolic alterations

Bacterial anti-adhesion

Anecdotal observations and recent critical evaluation of scientific literature indicates consumption of cranberries or its products is effective in the prevention of urinary tract infections (Leahy et al., 2001; Jepson et al., 2004). Although the therapeutic effect was long-thought to be increased urinary acidity due to hippuric acid excretion (Howell, 2002), it is now attributed to a family of unique proanthocyanidins and/or their metabolites (Winston et al., 2002 and references therein), which have been characterized as containing a high proportion of A-type linkages (Foo et al., 2000a 2000b; Porter et al., 2001). In the case of urinary tract infection, the primary effect is inhibition of cellular adherence of P-type (mannose-resistant) uropathogenic strains of *Escherichia coli* (Howell, 2002; Sharon & Ofek, 2002). In addition, evidence has been presented for similar responses with *Helicobacter pylori* to gastric epithelial cells (Burger et al., 2002) and a host of organisms commonly found in the oral cavity (Weiss et al., 2002).

Diabetes, glucose, and insulin metabolism

Impaired glucose uptake and insulin resistance are subtle but common metabolic alterations that may be general etiologies to several age-related disorders and chronic diseases (Preuss et al., 2002). Thus, identification of dietary components and natural products that have the potential to maintain these metabolisms throughout life has a highly favorable risk/benefit ratio. Several foods, biological materials, and synthetic preparations, such as tea, several spices, grape seed proanthocyanidin extract, and niacin-bound chromium, have been found to be effective (Broadhurst et al., 2000; Anderson & Polansky, 2002; Preuss et al., 2002). However, the chromium content of natural materials (long associated with insulin potentiating activity) was not associated with improved insulin action or glucose metabolism (Khan et al., 1990), which suggested other biologically active components were responsible. Relative to proanthocyanidins, an extract of cinnamon, which contained a series of two trimers and a tetramer of flavan-3-ols, each with an A-type linkage (Anderson et al., 2004), was effective in significantly reducing fasting blood glucose in a group of type 2 diabetics (Khan et al., 2003). Elucidation of the mechanism of action suggested that the cinnamon extracts stimulated autophosphorylation and inhibited a tyrosine phosphatase associated with insulin receptors of rat epididymal adipocytes (Imparl-Radosevich et al., 1998; Jarvill-Taylor et al., 2001). In addition, these extracts also stimulated glycogen synthesis by activating glycogen synthase and inhibiting glycogen synthase kinase-3 β activities, which are known effects of insulin treatment in the adipocyte model (Jarvill-Taylor et al., 2001).

Cataract formation and occurrence of retinopathy are two common complications of type 2 diabetes. Cocoa-derived proanthocyanidins fed to diabetes-induced (streptozotocin treated) rats nearly completely inhibited cataract formation (Osakabe et al., 2002). In control animals, lens opacity was first detected at 5 weeks after start of diabetes induction and was prevalent in a majority of rat lenses at 10 weeks of treatment. Although lenses of proanthocyanidin-fed animals revealed some focal hyperplasia of the epithelium and liquefaction of cortical fibers after 10 weeks of dietary treatment, opacity of lens was undetectable. In addition, the lack of hydroxynonenal (marker of oxidative stress) in lenses of these rats suggested very active antioxidative activity.

A review of 5 clinical trials involving nearly 1300 patients in which pycnogenol was tested for treatment and prevention of retinopathy unequivocally showed diminished progression of the disease and partial recovery of visual acuity in subjects ingesting the supplement (Schonlau & Rohdewald, 2001). Pycnogenol treatment effectively improved capillary resistance, reduced blood leakages into the retina, and was as efficacious as the drug, calcium dobesilate.

Immune function

Response of the immune system is one of the first lines of defense of the body to a host of environmental challenges. Many dietary components and drugs stimulate this system to an elevated level of preparedness. Besides those components of the immune system associated with atherosclerosis, the effect of proanthocyanidins also has been tested, *in vitro*, in PBMCS. In a series of experiments investigating the effects of isolated individual proanthocyanidins from cocoa on resting PBMCS, higher molecular weight fractions (Dp5–10) stimulated IL-1 β (proportional to Dp), IL-4 production, and IL-1 β gene expression (Mao et al., 2000a, 2000b), whereas intermediate-sized polymers (Dp4–8) were most active in the stimulation of TNF- α release (Mao et al., 2002a). Employing a similar system, IL-2 and IL-5 secretion was unresponsive to isolated proanthocyanidin treatment (Mao et al., 2000b, 2002b). When phytohemagglutinin (PHA)-stimulated PBMCS were tested, larger polymers (Dp5–10) stimulated gene expression and protein production of IL-1 and IL-1 β and TNF- α release (Mao et al., 2000a, 2000b, 2002a) but greatly inhibited synthesis of IL-5 (Mao et al., 2002b). Intermediate polymers (Dp–57) depressed IL-2 gene expression in a similar system (Mao et al., 2000b).

The influence of pycnogenol has been studied on some of the components of the immune system in cell culture. In RAW 264.7 macrophages, pycnogenol treatment of lipopolysaccharide (LPS)-stimulated cells reduced production of IL-1 β and its mRNA levels in a dose-dependent manner (Cho et al., 2001). In the same cell line, pycnogenol blocked the activation of NF- κ B and activator protein-1

(AP-1), two transcription factors involved in IL-1 β gene expression, and abolished LPS-induced I κ B degradation. Collectively, these results suggest pycnogenol treatment of this cell line can inhibit expression of proinflammatory cytokine IL-1 through the regulation of redox-sensitive transcription factors. When individual proanthocyanidins were investigated in the same cells induced by IFN- γ , monomers and dimers repressed NO production, TNF- α secretion, and NF- κ B-dependent gene expression, whereas procyanidin C2 (trimer) and pycnogenol enhanced these parameters (Park et al., 2000). These latter two treatments also increased TNF- α secretion in unstimulated RAW 264.7 macrophages. Studies with stimulated Jurkat E6.1 cells indicated that pycnogenol depressed IL-2 mRNA expression but that the mechanism of transcriptional regulation was different from regulation of IL-1 β (Cho et al., 2001).

Using isolated proanthocyanidin fractions from *Ecdysanthera utilis* (Chinese plant) and a PHA-stimulated PBMC system, procyanidin A1 (dimer with A-type linkage) inhibited IL-2 and IFN- γ production, which may have caused suppression of PBMC proliferation (Lin et al., 2002). Two newly identified trimers, each with an A-type linkage, failed to alter the response of cytokines or factors from PBMCs. A polyphenol-rich fraction isolated from cocoa liquor inhibited mitogen-stimulated proliferation of T cells and polyclonal Ig production by B cells (Sanbongi et al., 1997). In addition, this cocoa-liquor fraction also inhibited IL-2 mRNA expression and IL-2 secretion by T cells. Following on bacterial antiadhesion factors in cranberries, a high-molecular-weight-containing fraction inhibited hemagglutination of A (H1N1) and B (H3N2) virus strains as well as decreased viral infectivity (Schlesinger et al., 2003).

Dietary sources and intake

Dietary sources

Foods

A wide variety of analytical procedures have been employed for the bulk measurement of proanthocyanidins (Cunningham et al., 2002). Although individual dimers and trimers traditionally have been quantified with reversed-phase high-performance liquid chromatography systems (HPLC) (Lamuela-Raventos & Waterhouse, 1994), only recently have normal-phase HPLC techniques been coupled with sophisticated detection instrumentation for quantification of individual proanthocyanidin oligomers (Dp \leq 12) (Hammerstone et al., 1999; Gu et al., 2002, 2003). In addition, higher molecular weight proanthocyanidins (Dp > 12) can now be quantified as a single chromatographic peak (Gu et al., 2003).

Employing these normal-phase HPLC procedures, a large number of food samples, selected on the basis of

market share and demographics within the United States (Pehrsson et al., 2000), were analyzed for proanthocyanidin content (Gu et al., 2004). These data and others have been combined into a database of values for foods available online from the USDA Nutrient Data Laboratory at <http://www.nal.usda.gov/fnic/foodcomp>. Data for the proanthocyanidin content of selected foods containing substantial amounts are tabulated in Table 2. The data for red grapes reported in Table 2 are for seedless "eating" grapes, whereas cultivars of red-wine grapes and their wines have higher proanthocyanidin contents (Monagas et al., 2003; Sanchez-Moreno et al., 2003). This is reflected in the data for several red wines common in Spain, which contained dimers through polymers Dp 13 and represented 77–84% of total flavanols (Monagas et al., 2003). In general, a large number of vegetables, many spices, and some fruits, particularly citrus, had undetectable levels of proanthocyanidins (Gu et al., 2004). Fifty-six different kinds of common Spanish foods have been analyzed for flavanols, including dimers and trimers, but not higher oligomers (de Pascual-Teresa et al., 2000). Results indicated procyanidin B2 was the most abundant dimer or trimer, and flavanols were very low or nondetected in most vegetables.

Supplements

Qualitative and quantitative data on the proanthocyanidin content of dietary supplements, botanicals, and herbals is less precise than for foods because these dietary components have not been subjected to the same rigorous sampling and analysis programs. Relative to *Ginkgo biloba*, a fraction has been isolated that contained monomeric and polymeric flavonoids and accounted for 24% to 36% of the mass, depending on the method of extraction (Christen & Maixent, 2002; Yang et al., 2002). This fraction contained many flavonol glycosides, biflavones, and proanthocyanidins (van Beck, 2002); however, qualitative and quantitative characterization of the polymeric flavonoids awaits application of the techniques that have been applied to foods.

Grape seed proanthocyanidins are unique in that they contain a high level of galloyl derivatives, about one per each monomeric unit (Krueger et al., 2000; Hayasaka et al., 2003). Employing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), masses as high as nonamers (Dp9) and undecamers (Dp11) were observed, depending on the mode of detection (Krueger et al., 2000). These results agree with data from electrospray ionization mass spectrometry (ESI-MS) studies, which showed a mean Dp3 and Dp9, respectively, for two proanthocyanidin-rich fractions isolated from grape seed extract (Hayasaka et al., 2003). However, the largest polymer observed in each fraction was Dp9 and Dp28, respectively. Employing the traditional thiolysis method, an average of Dp8 was calculated for grape seed

Table 2. Proanthocyanidin content of selected foods^a.

Food/spice	mg/100 g of food or mg/l of beverage					Total	Type ^c
	Dimers	Trimers	4–6 mers ^b	7–10 mers ²	>10 mers ^b		
Apples, red delicious, with peel	14	9	30	25	38	116	PC
Beans, red kidney	32	28	126	136	460	782	PC, PP
Blueberries	7	5	20	15	129	176	PC
Chocolate, baking	207	131	333	216	551	1437	PC
Chocolate, milk	26	19	51	35	33	164	PC
Cinnamon, ground	256	1252	2609	1458	2509	8084	A, PC, PP
Cranberries	26	19	70	63	234	412	A, PC
Cranberry juice cocktail	29	17	49	41	89	225	A, PC
Grape seed (dry)	417	290	664	400	1100	2872	PC
Grapes, green	2	2	8	9	59	78	PC, PD
Grapes, red	2	2	6	6	45	61	PC
Hazelnuts	13	14	68	75	322	492	PC, PD
Pecans	42	26	101	84	223	476	PC, PD
Plums, black	16	15	50	35	115	231	A, PC

^aAdapted from Gu et al. (2004).

^b4–6mers indicates values for tetramers through hexamers summed, 7–10mers indicates values for heptamers through decamers summed, >10mers indicates values for polymers Dp>10, which eluted as a single chromatographic peak.

^cA indicates A-type interflavan linkages were identified; PC indicates procyanidins; PD indicates propdelphinidins; and PP indicates propelargonidins were characterized.

extracts with polymerization ranging from Dp5 to Dp17 (Labarbe et al., 1999). Relative to quantification, monomers (+)-catechin and (–)-epicatechin constituted about 15% of the mass; galloylated monomers, dimers, trimers, and tetramers about 80%; and pentamers, hexamers, heptamers, and their galloylates about 5% (Gabetta et al., 2000). Accurate quantitative data for each oligomer/polymer of grape seed proanthocyanidins are unavailable at this time.

Pycnogenol has been characterized as having proanthocyanidins that range from monomers of catechin and taxifolin to oligomers (Dp7) or higher (Rohdewald, 2002). Witch hazel (extract of bark of *Hamamelis virginiana*) contains about 5% proanthocyanidins, characterized by polymers of Dp17–29 as measured by thiolysis and Dp11–20 when gel permeation chromatography was employed (Dauer et al., 2003b). A unique characteristic of these compounds was complete 3-*O*-galloylation of chain extension units. Extracts of loquat (*Eriobotrya japonica*) leaves contained procyanidin B2 as one active ingredient (Ito et al., 2002), whereas *Crataegus sinaica* isolates were characterized as having a series of dimers through pentamers, some of which had A-type interflavan linkages (Shahat et al., 2002). Preparations from *Ecdysanthera utilis* contained two procyanidin trimers that each had an A-type linkage between monomeric unit T (top) and M (interior) as well as several common procyanidin dimers (A1, A2, B2) (Lin et al., 2002).

Dietary intake

Foods

Based on proanthocyanidin content for over 60 United States foods and daily food intake data [USDA Continuing Survey of Food Intakes by Individuals (CSFII) for 1994–1996], consumption by individuals in the United States was calculated for the first time (Gu et al., 2004). The mean intake for all ages (>2 years old) was estimated at 54 mg/day per person for all proanthocyanidins of Dp ≥ 2. Detailed examination of intakes for age/sex groups indicated a bimodal high intake phenomenon for children (2–5 years and 6–11 years) and older males (40–59 years and >60 years) each of whom consumed 59 mg/day or more. Proanthocyanidin consumption among adults ranged from 46 mg/day (20–39 years, female) to 66 mg/day (>60 years, male). As outlined above, these data do not include proanthocyanidins that might be included in the consumption of red wines or other commonly consumed foods that have substantial polymer content but were not analyzed. Nonetheless, these results provide the scientific community with the first estimates of proanthocyanidin consumption.

Supplements

Because of the dearth of analytical data for proanthocyanidin content of supplements, botanicals, and herbals, comprehensive intakes from these dietary sources are

not available. A recent survey of more than 23,000 women in the United States indicated that nearly one-sixth took at least one herbal supplement (including *Gingko biloba*) in the year 2000 (Yu et al., 2004). Based on this study, those women characterized as more likely to consume herbal supplements were non-Hispanic white, more educated, higher income, residence in the South or the West, and who had functional limitations and chronic conditions. Although recent results from two large cohorts indicated that individual vitamins and multivitamins, as well as selected minerals, were the most frequently consumed supplements (Murphy et al., 2002; White et al., 2004), the question must be raised whether appropriate probes were included in the survey instruments of these studies to capture intakes of botanicals and herbals. Nonetheless, plans and approaches have been discussed for inclusion of nutrients and health-related components provided by all supplements into United States National Nutrition Surveys (Dwyer et al., 2003).

Adverse biological effects

Traditionally, condensed tannins (pranthocyanidins) have been considered antinutrients in animal nutrition due to their astringency (reduced feed intake) and ability to bind several macronutrients, thus reducing their digestion and absorption (Salunkhe et al., 1989; Reed, 1995). However, specific digestive advantages (e.g., reduced incidence of bloat in ruminants) have been realized for some of the proanthocyanidin unique chemical traits (protein binding), which are now being pursued to advantage (Reed, 1995; Dixon & Sumner, 2003; Waghorn & McNabb, 2003). Although pycnogenol has been shown to bind selected purified intracellular enzymes (Moini et al., 2000), the precise role of these polymers in the alteration of the metabolic equilibrium in the gastrointestinal tract of human beings is unknown. Toxicological studies on long-term (90 day) oral administration of GSPE to rats established a no-observed-adverse-effect level (NOAEL) of 1.4 g kg BW⁻¹day⁻¹ for males and 1.5 g kg BW⁻¹day⁻¹ for females (Yamakoshi et al., 2002). Similarly, the LD₅₀ of a single oral dose of grape seed extract IH636 was greater than 5 g/kg BW for both male and female rats (Ray et al., 2001). Feeding IH636 at the rate of 100 mg kg⁻¹day⁻¹ to male B6C3F1 mice for a year or 500 mg kg⁻¹day⁻¹ to female mice for 6 months had no detectable adverse effects on the pathologies of vital organs or on serum chemistries (Ray et al., 2001). In terms of dermal irritation, IH636 was rated as moderately irritating and the no-observed-effect level (NOEL) for systemic toxicity was set at 2 g/kg for male and female albino rats (Ray et al., 2001). Observations in both rats and human subjects consuming FastOne, an herbal supplement containing extracts of

kola nut, grape, green tea, and *Gingko biloba*, suggested an increased risk of colorectal cancers as substantiated by induced activity of CYP1A2 (Ryu & Chung, 2003).

In a review of potential drug-dietary supplement interactions, about one-half of patients taking prescription medication and at least one dietary supplement had potential for an “interaction of significance” (Peng et al., 2004). Of these patients, only 6% had the potential of a severe interaction. Spontaneous bleeding in the anterior chamber of the eye (hyphema) was reported for one case, which was resolved when consumption of *Gingko biloba* was stopped (Schneider et al., 2002). Review of several clinical trials that investigated pycnogenol indicated that tolerance of the supplement was very good with only rare side effects, most referring to gastric discomfort (Schonlau & Rohdewald, 2001). Similar observations were reported for enzogenol, a combination of an extract of *Pinus radiata* bark and vitamin C (Shand et al., 2003).

Research needed

Although there are many areas of research on proanthocyanidins that can be identified for emphasis, two are crucially important for substantial advancement of the association of these dietary components with human health:

- (1) Identify biologically active compounds that are absorbed and their tissue distribution. To date, studies with human subjects indicates that dimeric (and perhaps monomers) proanthocyanidins, but not higher oligomers, are the primary compounds absorbed after consumption of proanthocyanidin-rich cocoa and grape seed extract (Holt et al., 2002; Sano et al., 2003). However, circulating levels are relatively low and transient in nature. The research of Scalbert's group suggests there is considerable metabolism of proanthocyanidins in the lower GI tract to many different phenolic acids (Deprez et al., 2000; Rios et al., 2003). A phenolic acid, 3,4-dihydroxyphenylacetic acid, whose concentration was raised in plasma after consumption of diets rich in fruits and vegetables, significantly modulated platelet activity at concentrations observed in plasma (Kris-Etherton et al., 2004). Such studies serve as a model for identification of biologically active proanthocyanidin metabolites. Characterization of metabolites and their concentrations in various tissues will be of great advantage in terms of designing *in vitro* studies for the elucidation of mechanisms of action of these dietary constituents.
- (2) Assess intake of proanthocyanidins from dietary supplements. The recent development of robust analytical techniques for the measurement of proanthocyanidins resulted in the analysis of a large

number of foods, the development of a database of values for foods, and estimates of intakes of these components from foods (Hammerstone et al., 1999; Gu et al., 2002, 2003, 2004). Similar efforts must be applied to those botanicals and herbals known to contain proanthocyanidins. In addition, accurate estimates of supplement consumption (especially botanicals and herbals) must be included in United States National Nutrition Surveys so that the contribution of these dietary sources can be calculated (Dwyer et al., 2003).

Acknowledgments

The comments and suggestions of Myron Gross, Marge Leahy, Mark Levine, Ron Prior, and Harold Schmitz during the preparation of this manuscript are greatly appreciated.

References

- Ammon H, Kaul R (1994): Herz-Kreislauf-Wirkungen von *Crataegus* extrakten, flavonoiden and procyanidinen Teil 1: Historisches und wirkstoffe. *Dtsch Apoth Ztg 134*: 2631–2636.
- Anderson RA, Polansky MM (2002): Tea enhances insulin activity. *J Agric Food Chem 50*: 7182–7186.
- Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, Schoene NW, Graves DJ (2004): Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *J Agric Food Chem 52*: 65–70.
- Araghi-Niknam M, Hosseini S, Larson D, Rohdewald P, Watson RR (2000): Pine bark extract reduces platelet aggregation. *Integr Med 2*: 73–77.
- Baba S, Osakabe N, Natsume M, Terao J (2002): Absorption and urinary excretion of procyanidin B2 [epicatechin-(4 β -8)-epicatechin] in rats. *Free Radic Biol Med 33*: 142–148.
- Bagchi D, Garg A, Krohn RL, Bagchi M, Tran MX, Stohs SJ (1997): Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract *in vitro*. *Res Commun Mol Pathol Pharmacol 95*: 179–189.
- Bagchi D, Garg A, Krohn RL, Bagchi M, Bagchi DJ, Balmoori J, Stohs SJ (1998): Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. *Gen Pharmacol 30*: 771–776.
- Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG (2000): Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology 148*: 187–197.
- Bagchi D, Bagchi M, Stohs S, Ray SD, Sen CK, Pruess HG (2002): Cellular protection with proanthocyanidins derived from grape seeds. *Ann NY Acad Sci 957*: 260–270.
- Bagchi D, Sen CK, Ray SD, Das DK, Bagchi M, Pruess HG, Vinson JA (2003): Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. *Mut Res 523–524*: 87–97.
- Beecher GR (2002): Overview of dietary flavonoids: Nomenclature, occurrence and intake. *J Nutr 133*: 3248S–3254S.
- Beninger CW, Hosfield GL (2003): Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* seed coat color genotypes. *J Agric Food Chem 51*: 7879–7883.
- Bito T, Roy S, Sen CK, Packer L (2000): Pine bark extract pycnogenol downregulates IFN-gamma-induced adhesion of T cells to human keratinocytes by inhibiting inducible ICAM-1 expression. *Free Rad Biol Med 28*: 219–227.
- Bors W, Michel C (2002): Chemistry of the antioxidant effect of polyphenols. *Ann NY Acad Sci 957*: 57–69.
- Bors W, Foo LY, Hertkorn N, Michel C, Stettmaier K (2001): Chemical studies of proanthocyanidins and hydrolysable tannins. *Antiox Redox Signal 3*: 995–1008.
- Broadhurst CL, Polansky MM, Anderson RA (2000): Insulin-like biological activity of culinary and medicinal plant aqueous extracts *in vitro*. *J Agric Food Chem 48*: 849–852.
- Burger O, Weiss E, Sharon N, Tabak M, Neeman I, Ofek I (2002): Inhibition of *Helicobacter pylori* adhesion to human gastric mucus by a high-molecular weight constituent of cranberry juice. *Crit Revs Food Sci Nutr 42*(Suppl): 279–284.
- Christen Y, Maixent JM (2002): What is *Ginkgo biloba* extract EGb 761? An overview—from molecular biology to clinical medicine. *Cell Mol Biol 48*: 601–611.
- Cho KJ, Yun CH, Packer L, Chung AS (2001): Inhibition mechanics of bioflavonoids extracted from the bark of *Pinus maritima* on the expression of proinflammatory cytokines. *Ann NY Acad Sci 928*: 141–156.
- Cowan MM (1999): Plant products as antimicrobial agents. *Clin Microbiol Rev 12*: 564–582.
- Cunningham DG, Vannozzi S, O'Shea E, Turk R (2002): Analysis and standardization of cranberry products. In: Ho C-T, Zheng QY, eds., *Quality Management of Nutraceuticals*, ACS Symposium Series 803. Washington, DC, American Chemical Society, pp. 151–166.
- Dauer A, Hensel A, Lhoste E, Knasmüller S, Mersch-Sundermann V (2003a): Genotoxic and antigenotoxic effect of catechin and tannins from the bark of *Hamamelis virginiana* L. in metabolically competent, human hepatoma cells (Hep G2) using single cell gel electrophoresis. *Phytochemistry 63*: 199–207.
- Dauer A, Rimpler H, Hensel A (2003b): Polymeric proanthocyanidins from the bark of *Hamamelis virginiana*. *Planta Med 69*: 89–91.

- De Eds F (1959): Physiological effects and metabolic fate of flavonoids. In: Fairbairn JW, ed., *The Pharmacology of Plant Phenolics*. London, Academic Press, pp. 91–102.
- De Gaetano G, De Curtis A, Di Castelnuovo A, Donati MB, Iacoviello L, Rotondo S (2002): Antithrombotic effect of polyphenols in experimental models: a mechanism of reduced vascular risk by moderate wine consumption. *Ann NY Acad Sci* 957: 174–188.
- de Pascual-Teresa S, Santos-Buelga C, Rivas-Gonzalo JC (2000): Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. *J Agric Food Chem* 48: 5331–5337.
- Deprez S, Brezillon C, Rabot S, Philippe C, Mila I, Lapiere C, Scalbert A (2000): Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J Nutr* 130: 2733–2738.
- Deprez S, Mila I, Huneau JF, Tome D, Scalbert A (2001): Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antiox Redox Signal* 3: 957–967.
- Devaraj S, Vega-Lopez S, Kaul N, Schonlau F, Rohdewald P, Jialal I (2002): Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. *Lipids* 37: 931–934.
- Dixon RA, Sumner LW (2003): Legume natural products: understanding and manipulating complex pathways for human and animal health. *Plant Physiol* 131: 878–885.
- Donovan JL, Manach C, Rios L, Morand C, Scalbert A, Révész C (2002): Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B₃. *Br J Nutr* 87: 299–306.
- Duweler KG, Rohdewald P (2000): Urinary metabolites of French maritime pine bark extract in humans. *Pharmazie* 55: 364–368.
- Dwyer J, Picciano MF, Raiten DJ, Members of Steering Committee (2003): Food and dietary supplement databases for What We Eat in America-NHANES. *J Nutr* 133: 624S–634S.
- Fisher NDL, Hughes M, Gerhard-Herman M, Hollenberg NK (2003): Flavonol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens* 21: 2281–2286.
- Foo LY, Howell AB, Vorsa N (2000a): A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. *J Nat Prod* 63: 1225–1228.
- Foo LY, Howell AB, Vorsa N (2000b): The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli* in vitro. *Phytochemistry* 54: 173–181.
- Gabetta B, Fuzzati N, Griffini A, Lolla E, Pace R, Ruffilli T, Peterlongo F (2000): Characterization of proanthocyanidins from grape seed. *Fitoterapia* 71: 162–175.
- Gali-Muhtasib HU, Younes IH, Karchesy JJ, el-Sabban ME (2001): Plant tannins inhibit the induction of aberrant crypt foci and colonic tumors by 1,2-dimethylhydrazine in mice. *Nutr Cancer* 39: 108–116.
- Gu L, Kelm M, Hammerstone JF, Beecher GR, Cunningham DG, Vannozzi S, Prior RL (2002): Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J Agric Food Chem* 50: 4852–4860.
- Gu L, Kelm MA, Hammerstone JF, Beecher GR, Holden J, Haytowitz D, Prior RL (2003): Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J Agric Food Chem* 51: 7513–7521.
- Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL (2004): Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J Nutr* 134: 613–617.
- Halliwell B, Gutteridge JMC, Cross CE (1992): Free radicals, antioxidants, and human disease: Where are we now? *J Lab Clin Med* 119: 598–620.
- Hammerstone JF, Lazarus SA, Mitchell AE, Rucker R, Schmitz HH (1999): Identification of procyanidins in cocoa (*Theobroma cacao*) using high performance liquid chromatography (HPLC)/mass spectrometry (MS). *J Agric Food Chem* 47: 490–496.
- Hannum SM, Schmitz HH, Keen CL (2002): Chocolate: A heart-healthy food? Show me the science. *Nutr Today* 37: 103–109.
- Hatano T, Miyatake H, Natsume M, Osakabe N, Takizawa T, Ito H, Yoshida T (2002): Proanthocyanidin glycosides and related polyphenols from cocoa liquor and their antioxidant effects. *Phytochemistry* 59: 49–58.
- Hayasaka Y, Waters EJ, Cheynier V, Herderich MJ, Vidal S (2003): Characterization of proanthocyanidins in grape seeds using electrospray mass spectrometry. *Rapid Commun Mass Spectrom* 17: 9–16.
- Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M (2003): Vascular effects of cocoa rich in flavan-3-ols. *JAMA* 290: 1030–1031.
- Ho KY, Huang JS, Tsai CC, Lin TC, Hsu YF, Lin CC (1999): Antioxidant activity of tannin components from *Vaccinium vitis-idaea* L. *J Pharm Pharmacol* 51: 1075–1078.
- Holt RR, Lazarus SA, Sullards MC, Zhu QY, Schramm DD, Hammerstone JF, Fraga CG, Schmitz HH, Keen CL (2002): Procyanidin dimer B2 [epicatechin-(4β-8)-epicatechin] in human plasma after the consumption of flavanol-rich cocoa. *Am J Clin Nutr* 76: 798–804.
- Howell AB (2002): Cranberry proanthocyanidins and the maintenance of urinary health. *Crit Revs Food Sci Nutr* 42(Suppl): 273–278.
- Imparil-Radosevich J, Deas S, Polansky MM, Baedke DA, Ingebritsen TS, Anderson RA, Graves DJ (1998): Regulation of PTP-1 and insulin receptor kinase by

- fractions from cinnamon: Implications for cinnamon regulation of insulin signaling. *Horm Res* 50: 177–182.
- Ito H, Kobayashi E, Takamatsu Y, Li SH, Hatano T, Sakangami H, Kusama K, Satoh K, Sugita D, Shimura S, Itoh Y, Yoshida T (2000): Polyphenols from *Eriobotrya japonica* and their cytotoxicity against human oral tumor cell lines. *Chem Pharm Bull* (Tokyo) 48: 687–693.
- Ito H, Kobayashi E, Li SH, Hatano T, Sugita D, Kubo N, Shimura S, Itoh Y, Tokuda H, Nishino H, Yoshida T (2002): Antitumor activity of compounds isolated from leaves *Eriobotrya japonica*. *J Agric Food Chem* 50: 2400–2403.
- Jarvill-Taylor KJ, Anderson RA, Graves DJ (2001): A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. *J Am Coll Nutr* 20: 327–336.
- Jepson R, Mihaljevic L, Craig J (2004): Cranberries for preventing urinary tract infections. *Cochrane Database Syst Rev* 1: CD001321.
- Joshi SS, Kuszynski CA, Bagchi D (2001): The cellular and molecular basis of health benefits for grape seed proanthocyanidin extract. *Curr Pharm Biotechnol* 2: 187–200.
- Kandil FE, Smith MA, Rogers RB, Pepin M-F, Song LL, Pezzuto JM, Seigler DS (2002): Composition of a chemopreventive proanthocyanidin-rich fraction from cranberry fruits responsible for the inhibition of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-induced ornithine decarboxylase (ODC) activity. *J Agric Food Chem* 50: 1063–1069.
- Khan A, Bryden NA, Polansky MM, Anderson RA (1990): Insulin potentiating factor and chromium content of selected foods and spices. *Biol Trace Elem Res* 24: 183–188.
- Khan A, Safdar M, Khan A, Khattak KN, Anderson RA (2003): Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes Care* 26: 3215–3218.
- Koch R (2002): Comparative study of venostasin and pycnogenol in chronic venous insufficiency. *Phytother Res* 16 (Suppl 1): S1–S5.
- Kris-Etherton PM, Lefevre M, Beecher GR, Gross MD, Keen CL, Etherton TD (2004): Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Ann Rev Nutr* 24: 511–538.
- Krueger CC, Dopke NC, Treidchel PM, Folts J, Reed JD (2000): Matrix-assisted LASER desorption/ionization time-of-flight mass spectrometry of polygalloyl polyflavan-3-ols in grape seed extract. *J Agric Food Chem* 48: 1663–1667.
- Kudolo GB, Dorsey S, Blodgett J (2002): Effect of ingestion of *Ginkgo biloba* extract on platelet aggregation and urinary prostanoid excretion in healthy and Type 2 diabetic subjects. *Thromb Res* 108: 151–160.
- Labarbe B, Cheynier V, Brossaud F, Souquet JM, Moutounet M (1999): Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J Agric Food Chem* 47: 2719–2723.
- Lakenbrink C, Engelhardt UH, Wray V (1999): Identification of two novel proanthocyanidins in green tea. *J Agric Food Chem* 47: 4621–4624.
- Lamuella-Raventos RM, Waterhouse AL (1994): A direct HPLC separation of wine phenolics. *Am J Enol Vitic* 45: 1–5.
- Leahy M, Roderick R, Brilliant K (2001): The cranberry—Promising health benefits, old and new. *Nutr Today* 36: 254–265.
- Lin LC, Kuo YC, Chou CJ (2002): Immunomodulatory proanthocyanidins from *Ecdysanthera utilis*. *J Nat Prod* 65: 505–508.
- Lotito SB, Actis-Goretta L, Renart ML, Caligiuri M, Rein D, Schmitz HH, Steinberg FM, Keen CL, Fraga CG (2000): Influence of oligomer chain length on the antioxidant activity of procyanidins. *Biochem Biophys Res Commun* 276: 945–951.
- Mao T, Powell J, Van de Water J, Keen CL, Schmitz HH, Hammerstone JF, Gershwin ME (2000a): The effect of cocoa procyanidins on the transcription and secretion of interleukin 1 beta in peripheral blood mononuclear cells. *Life Sci* 66: 1377–1386.
- Mao T, Van de Water J, Keen CL, Schmitz HH, Gershwin ME (2000b): Cocoa procyanidins and human cytokine transcription and secretion. *J Nutr* 130 (Suppl): 2093S–2099S.
- Mao T, Van de Water J, Keen CL, Schmitz HH, Gershwin ME (2002a): Modulation of TNF- α secretion in peripheral blood mononuclear cells by cocoa flavanols and procyanidins. *Dev Immunol* 9: 135–141.
- Mao T, Van de Water J, Keen CL, Schmitz HH, Gershwin ME (2002b): Effect of cocoa flavanols and their related oligomers on the secretion of interleukin-5 in peripheral blood mononuclear cells. *J Med Food* 5: 17–22.
- Mao T, Van de Water J, Keen CL, Schmitz HH, Gershwin ME (2003): Cocoa flavanols and procyanidins promote transforming growth factor-beta1 homeostasis in peripheral blood mononuclear cells. *Exp Biol Med* (Maywood) 228: 93–99.
- Mathur S, Devaraj S, Grundy SM, Jialal I (2002): Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. *J Nutr* 132: 2663–2667.
- Mittal A, Elmets CA, Katiyar SK (2003): Dietary feeding of proanthocyanidins from grape seed prevents photocarcinogenesis in SKH-1 hairless mice: relationship to decreased fat and lipid peroxidation. *Carcinogenesis* 24: 1379–1388.
- Moini H, Guo Q, Packer L (2000): Enzyme inhibition and protein-binding action of the procyanidin-rich French maritime pine bark extract, pycnogenol: Effect on xanthine oxidase. *J Agric Food Chem* 48: 5630–5639.

- Monagas M, Gomez-Cordoves C, Bartolome B, Laureano O, da Silva JMR (2003): Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Craciano, Tempranillo, and Cabernet Sauvignon. *J Agric Food Chem* 51: 6475–6481.
- Murphy SP, Wilkens LR, Hankin JH, Foote JA, Monroe KR, Henderson BE, Kolonel LN (2002): Comparison of two instruments for quantifying intake of vitamin and mineral supplements: A brief questionnaire versus three 24-hour recalls. *Am J Epidemiol* 156: 669–675.
- Murphy KJ, Chronopoulos AK, Singh I, Francis MA, Moriarty H, Pike MJ, Turner AH, Mann NJ, Sinclair AJ (2003): Dietary flavonols and procyanidin oligomers from cocoa (theobroma cacao) inhibit platelet function. *Am J Clin Nutr* 77: 1466–1473.
- Nakamura Y, Tonogai Y (2003): Metabolism of grape seed polyphenol in the rat. *J Agric Food Chem* 51: 7215–7225.
- Nardini M, Scaccini C, Virgili F (2002): Modulation of kinase activity by polyphenols. *Ann NY Acad Sci* 973: 368–370.
- Nishida S, Satoh H (2003): Mechanisms for the vasodilation induced by *Ginkgo biloba* extract and its main constituent, bilobide in rat aorta. *Life Sci* 72: 2659–2667.
- Oak MH, Chataigneau M, Keravis T, Chataigneau T, Beretz A, Andriantsitohaina R, Stoclet JC, Chang SJ, Schini-Kerth VB (2003): Red wine polyphenolic compounds inhibit vascular endothelial growth factor expression in vascular smooth muscle cells by preventing the activation of the p38 mitogen-activated protein kinase pathway. *Arterioscler Thromb Vasc Biol* 23: 1001–1007.
- Osakabe N, Yamagishi M, Natsume M, Yasuda A, Osawa T (2002) Ingestion of proanthocyanidin derived from cacao inhibits diabetes-induced cataract formation in rats. *Exp Biol Med* (Maywood) 229: 33–39.
- Ottaviani JI, Carrasquedo F, Lotito SB, Keen CL, Schmitz HH, Fraga CG (2002): Influence of cocoa flavanols on UV-C mediated DNA oxidation. *Arch Biochem Biophys* 406: 203–208.
- Park YC, Rimbach G, Saliou C, Valacchi G, Packer L (2000): Activity of monomeric, dimeric, and trimeric flavonoids on NO production, TNF-alpha secretion, and NF-kappa B-dependent gene expression in RAW 264.7 macrophages. *FEBS Lett* 465: 93–97.
- Parks DA, Booyse FM (2002): Cardioprotection by alcohol and polyphenols: role of nitric oxide. *Ann NY Acad Sci* 957: 115–121.
- Pearson DA, Schmitz HH, Lazarus SA, Keen CL (2001): Inhibition of *in vitro* low-density lipoprotein oxidation by oligomeric procyanidins present in chocolate and cocoa. *Meth Enzymol* 335: 350–360.
- Pearson DA, Paglieroni TG, Rein D, Wun T, Schramm DD, Wang JF, Holt RR, Gosselin R, Schmitz HH, Keen CL (2002): The effects of flavonol-rich cocoa and aspirin on *ex vivo* platelet function. *Thromb Res* 106: 191–197.
- Pehrsson PR, Haytowitz D, Holden JM, Perry CR, Beckler DG (2000): USDA's National Food and Nutrition Analysis Program. Food sampling. *J Food Comp Anal* 13: 379–389.
- Peng Q, Wei Z, Lau BH (2000): Pycnogenol inhibits tumor necrosis factor-alpha-induced nuclear factor kappa B activation and adhesion molecule expression in human vascular endothelial cells. *Cell Mol Life Sci* 57: 834–841.
- Peng CC, Glassman PA, Trilli LE, Hayes-Hunter J, Good CB (2004): Incidence and severity of potential drug-dietary supplement interactions in primary care patients: An exploratory study of 2 outpatient practices. *Arch Intern Med* 164: 630–636.
- Polagruto JA, Schramm DD, Wang-Polagruto JF, Lee L, Keen CL (2003): Effects of flavonoid-rich beverages on prostacyclin synthesis in humans and human aortic endothelial cell association with *ex vivo* platelet function. *J Med Foods* 6: 301–308.
- Porter LJ (1994): Flavans and proanthocyanidins. In: Harborne JB, ed., *The Flavonoids: Advances in Research since 1986*. London, Chapman & Hall, pp. 23–55.
- Porter ML, Krueger CG, Wiebe DA, Cunningham DG, Reed JD (2001): Cranberry proanthocyanidins associate with low-density lipoprotein and inhibit *in vitro* Cu²⁺-induced oxidation. *J Sci Food Agric* 81: 1306–1313.
- Preuss HG, Bagchi D, Bagchi M (2002): Protective effects of a novel niacin-bound chromium complex and a grape seed proanthocyanidin extract on advancing age and various aspects of syndrome X. *Ann NY Acad Sci* 957: 250–259.
- Prior RL, Cao G (1999a): Variability in dietary antioxidant related natural product supplements; the need for methods standardization. *J Am Nutraceut Assoc* 2: 46–56.
- Prior RL, Cao G (1999b): Antioxidant capacity and polyphenolic components of teas: Implications for altering *in vivo* antioxidant status. *Proc Soc Exp Biol Med* 220: 255–261.
- Prior RL, Cao G (2000): Analysis of botanicals and dietary supplements for antioxidant capacity: A review. *JAOAC Int* 83: 950–956.
- Qin YZ, Holt RR, Lazarus SA, Ensuna JL, Hammerstone JF, Schmitz HH, Keen CL (2002): Stability of flavan-3-ols epicatechin and catechin and related dimeric procyanidins derived from cocoa. *J Agric Food Chem* 50: 1700–1705.
- Ray S, Bagchi D, Lim PM, Bagchi M, Gross SM, Kothari SC, Preuss HG, Stohs SJ (2001): Acute and long-term safety evaluation of a novel IH636 grape seed proanthocyanidin extract. *Res Commun Mol Pathol Pharmacol* 109: 165–197.
- Rechner AR, Kuhnle G, Bremner P, Hubbard GP, Moore KP, Rice-Evans CA (2002): The metabolic fate of dietary polyphenols in humans. *Free Radic Biol Med* 33: 220–235.

- Reed JD (1995): Nutritional toxicology of tannins and related polyphenols in forage legumes. *J Anim Sci* 73: 1516–1528.
- Reed J (2002): Cranberry flavonoids, atherosclerosis and cardiovascular health. *Crit Rev Food Sci Nutr* 42(Suppl): 301–316.
- Rein D, Paglieroni TG, Pearson DA, Wun T, Schmitz HH, Gosselin R, Keen CL (2000a): Cocoa and wine polyphenols modulate platelet activation and function. *J Nutr* 130 (8S Suppl): 2120S–2126S.
- Rein D, Paglieroni TG, Wun T, Pearson DA, Schmitz HH, Gosselin R, Keen CL (2000b): Cocoa inhibits platelet activation and function. *Am J Clin Nutr* 72: 30–35.
- Rice-Evans CA, Miller NJ, Paganga G (1996): Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 20: 933–956.
- Rios LY, Bennett RN, Lazarus SA, Remesy C, Scalbert A, Williamson G (2002): Cocoa procyanidins are stable during gastric transit in humans. *Am J Clin Nutr* 76: 1106–1110.
- Rios LY, Gonthier MP, Remesy C, Mila I, Lapierre C, Lazarus SA, Williamson G, Scalbert A (2003): Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am J Clin Nutr* 77: 912–918.
- Rohdewald P (2002): A review of the French maritime pine bark extract (pycnogenol), a herbal medication with a diverse clinical pharmacology. *Int J Clin Pharmacol Ther* 40: 158–168.
- Ryu SD, Chung WG (2003): Induction of the procarcinogen-activating CYP1A2 by herbal dietary supplement in rats and humans. *Food Chem Toxicol* 41: 861–866.
- Salunkhe DK, Chavan JK, Kadam SS (1989): *Dietary Tannins: Consequences and Remedies*. Boca Raton, FL, CRC Press, Inc.
- Sanbongi C, Suzuki N, Sakane T (1997): Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans *in vitro*. *Cell Immunol* 177: 129–136.
- Sanchez-Moreno C, Cao G, Ou B, Prior RL (2003): Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wines obtained from highbush blueberry. *J Agric Food Chem* 51: 4889–4896.
- Sano A, Yamakoshi J, Tokutake S, Tobe K, Kubota Y, Kikuchi M (2003): Procyanidin B1 is detected in human serum after intake of proanthocyanidin-rich grape seed extract. *Biosci Biotechnol Biochem* 67: 1140–1143.
- Santos-Buelga C, Scalbert A (2000): Proanthocyanidins and tannin-like compounds—Nature, occurrence, dietary intake and effects on nutrition and health. *J Sci Food Agric* 80: 1094–1117.
- Sato M, Maulik N, Das DK (2002): Cardioprotection with alcohol: Role of both alcohol and polyphenolic antioxidants. *Ann NY Acad Sci* 957: 122–135.
- Scalbert A, Morand C, Manach C, Rémésy C (2002): Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed Pharmacother* 56: 276–282.
- Schewe T, Kühn H, Sies H (2001a): Flavonoids of cocoa inhibit recombinant human 5-lipoxygenase. *J Nutr* 132: 1825–1829.
- Schewe T, Sadik C, Klotz L-O, Yoshimoto T, Kühn H, Sies H (2001b): Polyphenols of cocoa: Inhibition of mammalian 15-lipoxygenase. *Biol Chem* 382: 1687–1696.
- Schlesinger M, Weiss EI, Hochman N, Ofek I, Zakay-Rones Z (2003): Effect of cranberry juice constituents on haemagglutination and infectivity of influenza virus. *Antiviral Res* 57: A82 (Abstract #140).
- Schmitz HH, Romanczyk L (2000): Chocolate flavonoids: *In vitro* evidence suggesting a cardiovascular health benefit. AAAS Annual Meeting and Science Innovation Exposition, Feb 17–22, Washington, DC.
- Schneider C, Bord C, Misse P, Arnaud B, Schmitt-Bernard C (2002): [Spontaneous hyphema caused by *Ginkgo biloba* extracts.] *J Fr Ophthalmol* 25: 731–732. [Article in French, information taken from English abstract.]
- Schonlau F, Rohdewald P (2001): Pycnogenol for diabetic retinopathy. A review. *Int Ophthalmol* 24: 161–171.
- Schramm DD, Wang JF, Holt RR, Ensunsa JL, Gonsalves JL, Lazarus SA, Schmitz HH, German JB, Keen CL (2001): Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. *Am J Clin Nutr* 73: 36–40.
- Schramm DD, Karim M, Schrader HR, Holt RR, Kirkpatrick NJ, Ploagruo JA, Ensunsa JL, Schmitz HH, Keen CL (2003): Food effects on the absorption and pharmacokinetics of cocoa flavanols. *Life Sci* 73: 857–869.
- Shahat AA, Cos P, De Bruyne T, Apers S, Hammouda FM, Ismail SI, Azzam S, Claeys M, Goovaerts E, Pieters L, Vanden Berghe D, Vlietinck AJ (2002): Antiviral and antioxidant activity of flavonoids and proanthocyanidins from *Crataegus sinaica*. *Planta Med* 68: 539–541.
- Shand B, Strey C, Scott R, Morrison Z, Gieseg S (2003): Pilot study on the clinical effects of dietary supplementation with enzogenol, a flavonoid extract of pine bark and vitamin C. *Phytother Res* 17: 490–494.
- Shanmuganayagam D, Beahm MR, Osman HE, Krueger CG, Reed JD, Folts JD (2002): Grape seed and grape skin extracts elicit a greater antiplatelet effect when used in combination than when used individually in dogs and humans. *J Nutr* 132: 3592–3598.
- Sharon N, Ofek I (2002): Fighting infectious diseases with inhibitors of microbial adhesion to host tissues. *Crit Revs Food Sci Nutr* 42 (Suppl): 267–272.
- Singletary KW, Meline B (2001): Effect of grape seed proanthocyanidins on colon aberrant crypts and breast tumors in a rat dual-organ tumor model. *Nutr Cancer* 39: 252–258.
- Steinberg FM, Holt RR, Schmitz HH, Keen CL (2002): Cocoa procyanidin chain length does not determine

- the ability to protect LDL from oxidation when monomer units are controlled. *J Nutr Biochem* 13: 645–652.
- Steinberg FM, Bearden MM, Keen CL (2003): Cocoa and chocolate flavonoids: Implications for cardiovascular health. *J Am Diet Assoc* 103: 215–223.
- Stevens JF, Miranda CL, Wolthers KR, Schimerlik M, Deinze ML, Buhler DR (2002): Identification and in vitro biological activities of hop proanthocyanidins; inhibition of nNOS activity and scavenging of reactive nitrogen species. *J Agric Food Chem* 50: 3435–3443.
- Stromgaard K, Nakanishi K (2004): Chemistry and biology of terpene trilactones from *Ginkgo biloba*. *Angew Chem Int Ed Engl* 43: 1640–1658.
- Tanaka N, Sekiya N, Hattori M, Goto H, Shibahara N, Shimada Y, Terasawa K (2003): Measurement of plasma procyanidin B-2 and procyanidin B-3 levels after oral administration in rat. *Phytomedicine* 10: 122–126.
- Ursini F, Sevanian A (2002): Wine polyphenols and optimal nutrition. *Ann NY Acad Sci* 957: 200–209.
- Valdez LB, Actis-Goretta L, Boveris A (2002): Polyphenols in red wines prevent NADH oxidation induced by peroxynitrite. *Ann NY Acad Sci* 957: 274–278.
- van Beck TA (2002): Chemical analysis of *Ginkgo biloba* leaves and extracts. *J Chromatogr A* 967: 21–55.
- Varga E (2002): [The protective effect of EGb 761 in isolated isochemical/reperfused rat hearts.] *Acta Pharm Hung* 72: 265–271. [Article in Hungarian; information taken from English abstract.]
- Verstraeten S, Keen CL, Schmitz HH, Fraga CG, Oteiza P (2003): Flavan-3-ols and procyanidins protect liposomes against lipid oxidation and disruption of the bilayer structure. *Free Rad Biol Med* 34: 84–92.
- Waghorn GC, McNabb WC (2003): Consequences of plant phenolic compounds for productivity and health of ruminants. *Proc Nutr Soc* 62: 383–392.
- Wan J, Vinson JA, Etherton TD, Proch J, Lazarus SA, Kris-Etherton PM (2001): Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *Am J Clin Nutr* 74: 596–602.
- Weiss EI, Lev-Dor R, Sharon N, Ofek I (2002): Inhibitory effect of a high-molecular weight constituent of cranberry on adhesion of oral bacteria. *Crit Revs Food Sci Nutr* 42(Suppl): 285–292.
- White E, Patterson RE, Kristal AR, Thornquist M, King I, Shattuck AL, Evans I, Satia-Abouta J, Littman AJ, Potter JD (2004): Vitamins and Lifestyle cohort study: Study design and characteristics of supplement users. *Am J Epidemiol* 159: 83–93.
- Winston D, Graff A, Brinckmann J, Langer R, Turner A, Reich E, Bieber A, Howell A, Romm AJ (2002): Cranberry fruit. In: Upton R, ed., *American Herbal Pharmacopoeia and Therapeutic Compendium*. Santa Cruz, CA, American Herbal Pharmacopoeia, pp. 12–24.
- Wu X, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL (2004): Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agric Food Chem* 52: 4026–4037.
- Yamagishi M, Natsume M, Osakabe N, Nakamura H, Furuka F, Imazawa T, Nishikawa A, Hirose M (2002): Effects of cacao liquor proanthocyanidins on PhIP-induced mutagenesis *in vitro*, and *in vivo* mammary and pancreatic tumorigenesis in female Sprague-Dawley rats. *Cancer Lett* 185: 123–130.
- Yamakoshi J, Saito M, Kataoka S, Kikuchi M (2002): Safety evaluation of proanthocyanidin-rich extract from grape seeds. *Food Chem Toxicol* 40: 599–607.
- Yang C, Yang YR, Yao WX (2002): Extraction of pharmaceutical components from *Ginkgo* leaves using supercritical carbon dioxide. *J Agric Food Chem* 50: 846–849.
- Ye X, Krohn RL, Liu W, Joshi SS, Kuszynske CA, McGinn TR, Bagchi M, Preuss HG, Shohs SJ, Bagchi D (1999): The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultured human cancer cells. *Mol Cell Biochem* 196: 99–108.
- Yu SM, Ghandour RM, Huang SJ (2004): Herbal supplement use among US women. *J Am Med Womens Assoc* 59: 17–24.
- Zhang J, Fu S, Liu S, Mao T, Xiu R (2000): The therapeutic effect of *Ginkgo biloba* extract in SHR rats and its possible mechanisms based on cerebral microvascular flow and vasomotion. *Clin Hemorheol Microcirc* 23: 133–138.

Copyright of Pharmaceutical Biology is the property of Swets & Zeitlinger, BV and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.