MOLECULAR ASPECTS OF PROCYANIDIN BIOLOGICAL ACTIVITY: DISEASE PREVENTATIVE AND THERAPEUTIC POTENTIALS

Hadi Moini[1], Gerald Rimbach[2] and Lester Packer[2*]

[1]*Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA*
[2]*Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA*

CONTENTS

* Author for correspondence:
Lester Packer, Ph.D.
Department of Molecular and Cell Biology
University of California at Berkeley
251 Life Science Addition
Berkeley, CA, 947200, USA
e-mail: packer@socrates.berkeley.edu

SUMMARY

There is a growing interest in the utilization of procyanidins for their dietary and pharmacological properties. A wide spectrum of beneficial activity for human health has been advocated for procyanidins due, in part, to their strong antioxidant activity. More recently the ability of procyanidins to affect gene expression and cell response *in vitro* has been reported, providing a novel mechanistic perspective on the biological activity of these phytochemicals. This article reviews recent cellular and molecular aspects of the biological activity of procyandins and discusses their disease preventative and therapeutic potentials.

KEY WORDS

procyanidins, pycnogenol, antioxidants, protein binding, immunomodulation, cardiovascular disease

1. INTRODUCTION

Procyanidins are generally present in large amounts in the plant kingdom as secondary metabolites and are significant constituents of the human diet. Recently, procyanidins have attracted much attention in the fields of pharmacology and nutrition since they are efficient radical scavengers of reactive oxygen and nitrogen species. Several procyanidins have strong protein binding properties, which provide the biochemical basis for their action in addition to the redox based activity. Cell culture studies have shown that procyanidins are involved in NF-κB dependent signal transduction pathways, thereby they may affect the production of nitric oxide, proinflammatory cytokines and cell adhesion molecules. Procyanidins might have beneficial cardiovascular effects by preventing LDL oxidation, inhibiting platelet aggregation and enhancing endothelial NO secretion.

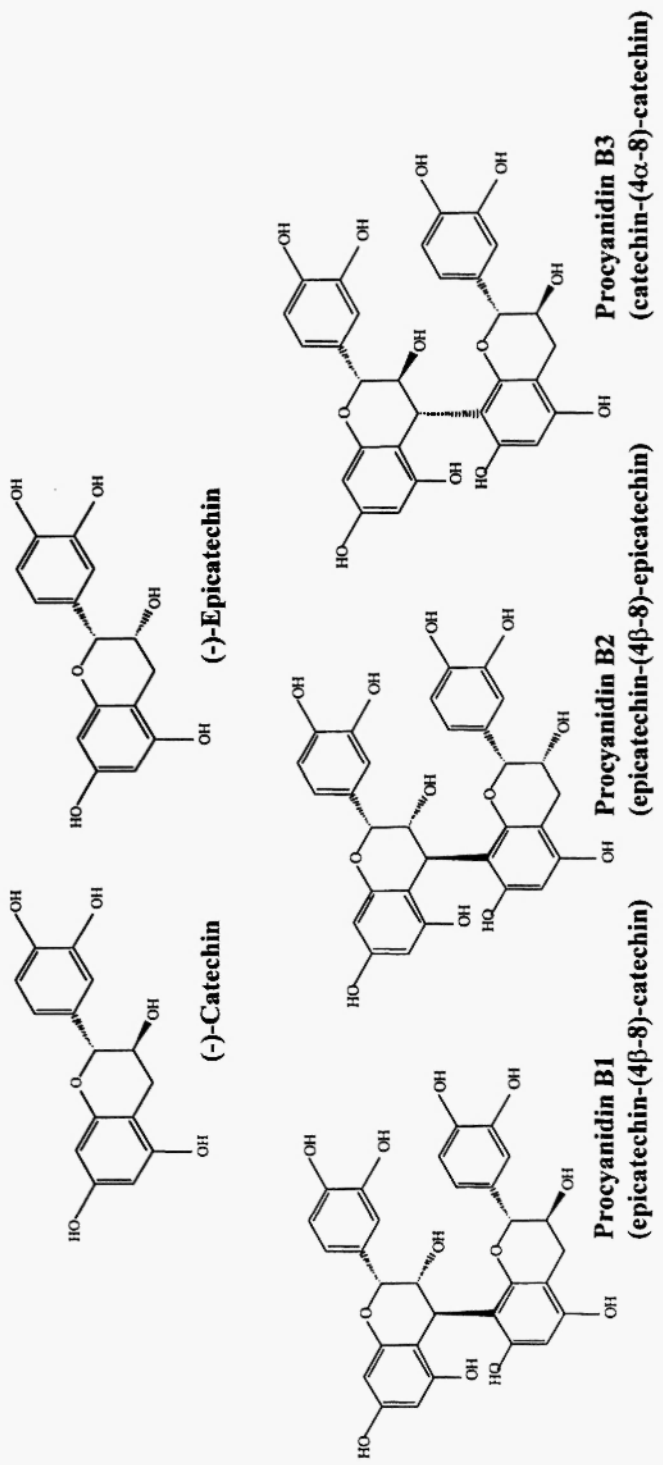
2. CHEMICAL STRUCTURE AND DISTRIBUTION

Procyanidins are high-molecular weight polymers of flavan-3-ol units (catechin, epicatechin). The flavan-3-ol units are linked by C-C bonds, which are established between the C4 of one unit and the C6 or C8 of another unit (Fig. 1) /1/. Since the discovery of procyanidins, several oligomers with a degree of polymerization as high as five have been identified. However, due to the difficulty in analyzing highly polymerized procyanidins, much of the literature on procyanidin content of different plants refer mainly to dimeric and trimeric procyanidins. More recently the introduction of electrospray mass spectrometry techniques coupled with liquid chromatography has led to a more detailed characterization of procyanidins polymers /2,3/ and to the identification of procyanidin molecules with a degree of polymerization of up to seventeen /4/.

Although much progress has been made in defining the content and distribution of polyphenols in food, little information is yet available on procyanidins. Significant concentrations of procyanidins occur in fava beans, sorghum, barley, various berries, grapes, apples and cacao. Due to differences in the methodologies in use for their quantification, it is difficult to compare the amount of procyanidins of various food items. Therefore there is no reliable estimation of dietary intake of procyanidins through the human diet, as reviewed by Santos-Buelga and Scalbert /5/. It should be mentioned that there are also highly standardized procyanidin-rich nutraceuticals available such as those obtained from the bark of *Pinus maritima* (pine bark extract, PBE, pycnogenol®) /6/ and grape seeds /7/. Procyandins have also been found in *Ginkgo biloba* leaf extract EGb761 /8/.

3. BIOAVAILABILITY

The bioavailability of plant polyphenols is determined primarily by their basic chemical structure, molecular size, degree of polymerization, and solubility /9/. Numerous factors affect the bioavailability of orally ingested polyphenols such as the dose and the matrix in which they are delivered /10/. While the absorption and plasma concentration of momeric flavonoids, such as catechin /11/ and quercetin /12/, has been established in humans and animals, the metabolic fate of procyanidins is largely unknown. It was recently



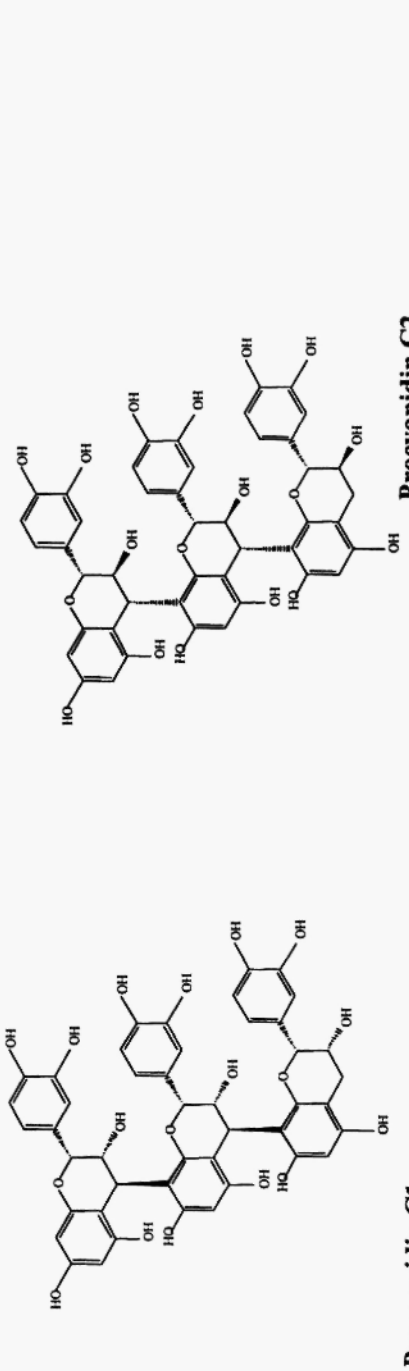


Fig. 1: The structure of monomeric, dimeric and trimeric procyanidins.

demonstrated that the monomer catechin is passively taken up through tight junctions by CaCo-2 cell monolayers, which mimic the intestinal absorptive epithelium and serve as a useful tool for studying trans-epithelial transport. There is more limited uptake of dimeric and trimeric procyanidins by the same route. However, higher oligomers and polymers possibly enter CaCo-2 cells through endocytosis /13/. Additionally it has been suggested that the colonic microflora might depolymerize procyanidins to give catechin subunits which in turn might be absorbed or metabolized /14/. Absorption and distribution of ^{14}C-labeled procyanidins related to sorghum have been investigated in chickens. Analysis of ^{14}C distribution in chicken tissue and excreta suggests that radiolabeled procyanidins from sorghum grain were not absorbed from the digestive tract /15/. However, ^{14}C from non-tannin fractions was absorbed and distributed in various chicken tissues. Grape seed procyanidins obtained from plants grown in a ^{14}C-enriched environment have been used to study the bioavailability and distribution pattern of this family of molecules in mice /16/. Absorption began 10 min after ingestion and slowly declined after 3-7 hours. The distribution of the radioactivity in various organs indicated that procyanidins have a propensity for proline-rich tissues. Thus, the aorta was about 10 times more enriched with procyanidins than the lungs and 5 times more than the liver. Bioavailability of oligomeric procyanidins has also been recently studied in humans /17/. After oral administration of oligomeric procyanidins derived from PBE, the two metabolites δ-(3,4-dihydroxy-phenyl)-γ-valerolactone and δ-(3-methoxy-4-hydroxy-phenyl)-γ-valerolactone conjugated with glucoronic acid or sulfate were identified in the urine. These data indicate that oligomeric procyanidins are possibly absorbed and metabolized by the human body. Although bioavailability of procyanidins is still an open issue, indirect indications suggest that these phytochemicals are, at least partly, bioavailable. Procyanidin supplementation of animal or human diets is associated with biological effects such as enhancement of antioxidant capacity of plasma, anti-inflammatory effects and beneficial effects to the cardiovascular system. Nevertheless the question of absorption of flavonoids in general and procyanidins in particular must be better addressed in the future.

4. PROTEIN BINDING PROPERTIES

One of the best-known properties of procyanidins is probably their capacity to bind to proteins. Procyanidins have a significant affinity for proteins and peptides that possess an open, random coil type conformation and contain a high proportion of proline residues in their sequence. Protein-procyanidin interactions can be attributed to dynamic surface phenomena and are generally reversible. The principal leading forces are hydrophobic interactions, which are reinforced by the establishment of hydrogen bonds. Hydrogen bridges are established between phenolic groups, as proton donors, and the carbonyl groups of the peptide bonds, as proton acceptors /18,19/. Among the proteins that bind to procyanidins most strongly are members of the family of salivary proline-rich proteins (PRPs), collagen, and gelatin /18,20,21/. Complexation of gastrointestinal enzymes and dietary proteins by procyanidins has been considered as a detrimental effect in the digestive tract. It has been suggested that the major function of PRPs is to bind and precipitate dietary procyanidins, thereby counteracting their antinutritive properties /22-24/.

It has been shown, however, that proteins with compact globular structures have less affinity for procyanidins than proteins with random coil structures, such as bovine serum albumin (BSA) or histone F1, by several orders of magnitude. Compact globular proteins which lose their secondary and tertiary structure exhibit higher affinity for procyanidins /20/. It is worth noting that the binding of procyanidins to proteins has been mostly carried out at low pH (between pH 2-5), which further facilitates partial unfolding of proteins /25,26/. Thus, BSA and ovalbumin are only significantly bound by procyanidins when the pH of the incubation mixture is between 3-5 /27/.

Procyanidins have been shown to inhibit rat liver mitochondrial respiratory complex I-III /28/ as well as the activities of various enzymes, such as β-glucoronidase, hyaluronidase, xanthine oxidase /29/, angiotensin-converting enzyme /30/ and various protein kinases /31,32/, which are involved in signal transduction pathways. Recently, we have applied PAGE to directly assess the binding of PBE and purified procyanidins to some enzymes which are involved in scavenging and generating reactive oxygen species /33/. PBE readily binds and inhibits xanthine oxidase and catalase while it did not bind or

affect the activity of glucose oxidase or superoxide dismutase /34/. The possible structural differences that cause the binding of PBE to some proteins but not to others are not known; however, overall selectivity has been observed for the binding of PBE to the selected enzymes. The selectivity in the binding of procyanidins to proteins has been studied by Zhu *et al.* /35/, who used radioligand-receptor binding assays to assess the selectivity of binding of procyanidins to various receptors such as adrenoceptors, serotonin, opiate and muscarinic receptors. It was found that procyanidin B3 selectively binds to a single receptor whereas procyanidin B2 binds to two receptors out of 16 receptors tested. Procyanidin B4 inhibited ligand-receptor binding for 5 of the 16 receptors tested, showing less selectivity when compared to procyanidin B2 and B3. These data indicate that various procyanidins might be quite selective in binding to a particular protein.

5. ANTIOXIDANT PROPERTIES

The antioxidant properties of small phenolics including flavonoids and phenolic acids have been extensively investigated and suggested to have potential health benefits for humans /36/. However, more needs to be known about the antioxidant activities of procyanidins. This might be related to the fact that few procyanidins are yet to be available in a purified form. It has been demonstrated *in vitro* that procyanidins are potent antioxidants against reactive oxygen species such as superoxide anion, hydroxyl, and nitric oxide radicals /37,38/. Furthermore, procyanidin oligomers of different sizes (monomer through nonamer) isolated from the seeds of *Theobroma cacao* have been found to be protective against peroxynitrite-dependent oxidation /39/. Procyanidins are able to scavenge reactive oxygen and nitrogen species through electron donating properties to generate a relatively stable phenoxyl radical, which acts to terminate radical propagation by reacting with other free radicals /40/. Beside the scavenging effects against reactive oxygen and nitrogen species, procyanidins may act as antioxidants by chelating transition metals such as iron and copper, which are known to be involved in the initiation of peroxidative processes /29,41/. However, it should be taken into account that under certain conditions, such as high concentrations of phenolic antioxidants, high pH and in the presence of iron, phenolic compounds

might initiate an autooxidative process, thereby acting as pro-oxidants /40/. Recently, formation of intramolecular hydrogen bonds between the two B-rings of procyanidin B3 has been reported during its oxidation in the presence of horseradish peroxidase and hydrogen peroxide, leading to stable free radical EPR signals /42/.

The antioxidant properties of procyanidins are likely also dependent upon factors such as degree of polymerization (which is still controversial), galloylation, complexation with proteins, and partitioning between the lipid and aqueous phase. The radical scavenging activity of procyanidins has been reported to be increased for trimers and then decreased for higher molecular weight procyanidins /43/, while in other studies no differences in antioxidant properties were observed between monomers, dimers and trimers /38/. Interestingly, antioxidant activity of procyanidins in the lipid phase decreased with the degree of polymerization; in contrast, antioxidant action in the aqueous phase increased from monomer to trimer and then decreased from trimer to tetramer. In addition, galloylation of catechin and dimeric procyanidins decreased lipid phase activity and increased the aqueous phase antioxidant activity /44/. Complexation with proteins might also affect antioxidant properties of procyanidins. Procyanidin-protein complex was reported to retain at least half the antioxidant activity of the free procyanidins /45/. Interestingly, ethanol was proposed to reduce the binding of procyanidins to proteins and thereby indirectly contribute to the antioxidant capacity of procyanidins by increasing their bioavailability /46/. Finally, procyanidins might participate in the redox antioxidant network as indicated by their ability to regenerate the ascorbyl radical /47/ and to protect endogenous cellular vitamin E /48/ and glutathione /49/ from loss due to oxidative stress.

6. IMMUNOMODULATORY EFFECTS

Many of the basic events of cellular regulation are sensitive to the balance between oxidants and antioxidants. Critical steps in signal transduction, such as protein phosphorylation and binding of transcription factors to consensus sites on DNA, are modulated by the intracellular redox status. Experimental data regarding procyanidins show their ability to differentially affect gene expression in cultured cells /50/. At least one well-defined transcription factor, nuclear factor

kappa B (NF-κB), has been identified to be regulated by the intracellular redox state. The transcription factor NF-κB in cooperation with others has been suggested to coordinate the expression of genes encoding proteins which are involved in inflammatory processes. In particular, NF-κB contributes to the production of interleukin-1, interleukin-6, tumor necrosis factor alpha (TNF-α), lymphotoxin, GM-CSF, interferon gamma (IFN-γ) and inducible nitric oxide synthase (iNOS). Furthermore, some of the cytokines, e.g. interleukin (IL)-1 and TNF-α, activate NF-κB, thus initiating an auto-regulatory feedback loop /51/.

6.1 Nuclear factor kappa B

NF-κB activation by various stimuli occurs upon its dissociation from the inhibitory protein IκB and its subsequent nuclear translocation. /52/. Because of the pivotal role in inflammatory response a significant effort has focused on developing therapeutic agents that regulate NF-κB activity. The effect of purified procyanidins and PBE on NF-κB-dependent gene expression in RAW 264.7 macrophages has been studied by Park *et al.* /53/ using a dual-luciferase reporter gene assay. Nearly a two-fold increase in luciferase activity was observed when macrophages were stimulated with IFN-γ in comparison to unstimulated control cells. The monomeric procyanidins, catechin and epicatechin, completely counteracted IFN-γ-induced NF-κB transactivation. Pretreatment of macrophages with procyanidin B1 and procyanidin B2 resulted in a slight decrease in the luciferase activity. However, in contrast to the monomers and dimers, procyanidin C2 and PBE significantly increased luciferase activity in IFN-γ-stimulated macrophages, which was completely inhibited by an anti-murine TNF-α-neutralizing antibody. These results indicate that procyanidin C2 or PBE-induced TNF-α secretion is crucial for NF-κB activation in macrophages. Interestingly, in macrophages stimulated with IFN-γ plus lipopolysaccharide (LPS), PBE had no effect on the DNA binding of NF-κB /54/. However, when macrophages were stimulated with LPS alone, PBE blocked the binding of NF-κB to its DNA consensus sequence /55/. It is apparent that the nature of the activating signal could be important in the modulation of NF-κB dependent signal transduction pathways in macrophages. Since procyanidins modulate NF-κB activity, they would be expected to affect NF-κB-dependent gene expression of mediators of inflammation such

as nitric oxide and proinflammatory cytokines in other cell types. Interestingly, PBE was shown to inhibit UVR-induced NF-κB-dependent gene expression in human keratinocytes /56/.

6.2 Inducible nitric oxide

In macrophages, nitric oxide (NO) is a cytotoxic mediator and contributes to the antimicrobial and tumoricidal activity of the cells /57/. However, high NO production has been associated with oxidative stress and with the pathophysiology of various diseases such as arthritis, diabetes mellitus, septic shock, and autoimmune and chronic inflammation as in neurodegenerative and circulatory disorders. Expression of the iNOS gene is regulated by endotoxins such as LPS and cytokines such as IFN-γ and interleukin-2. Two upstream DNA regions of the iNOS promoter, the RI and RII domains, are required for maximal promoter activation by LPS, and the RII domain mediates promoter *trans*-activation of IFN-γ. Both of these domains comprise multiple sequences homologous to those of *cis* elements involved in transcription activation, such as NF-κB binding sites, IFN-γ response elements, and IFN-γ-activated factor binding sequence /58/.

Virgili *et al.* /54/ investigated the effect of procyanidins extracted from *Pinus maritima* on NO metabolism in macrophages. RAW 264.7 cells were stimulated by the bacterial wall component LPS and IFN-γ, which induce the production of large amounts of nitric oxide. Preincubation of LPS plus IFN-γ stimulated cells with PBE significantly decreased NO production. It was found that the effect was due to the combination of several biological effects, such as NO radical scavenging, inhibition of iNOS activity, and inhibition of iNOS mRNA expression. In addition, possible direct binding of PBE to iNOS protein should be taken into account for the decrease of NO generation by PBE. Stimulation of macrophages with LPS in the presence of procyanidins could be problematic since procyanidin-rich extracts have been found to suppress the activity of LPS and lipid A preparations. Inhibition of the production of NO is not exclusively mediated by the cellular effects of procyanidins *per se* but might be partially caused by a direct interaction of these compounds with the LPS molecule /59/. Therefore in our recent studies macrophages have been stimulated with IFN-γ alone. Pretreatment of macrophages with monomeric procyanidins, catechin and epicatechin, significantly decreased IFN-γ-induced NO production by 40%, 60%, and 75%,

respectively, as compared to IFN-γ-treated cells /53/. Similar to NF-κB transactivation activity, the dimers procyanidin B1 and procyanidin B2 showed a moderate inhibitory effect on NO production, whereas procyanidin C2 and PBE significantly increased IFN-γ-induced NO production. These findings suggest that the degree of polymerization of procyanidins might be important in determining the mechanism(s) by which procyanidins modulate NO production.

6.3 Proinflammatory cytokines

Proinflammatory cytokines have beneficial or detrimental effects, depending on the context and amount in which they are produced. During infection, they are mostly beneficial but in cancer and chronic inflammatory diseases they may be detrimental. Therefore, cellular manipulation of cytokine production is of importance in determining the outcome of the inflammatory response. The effect of purified procyandins prepared from cocoa on the expression and secretion of IL-1β has been recently studied in peripheral blood mononuclear cells. Interestingly, the small fractions of cocoa containing oligomers up to tetramers consistently reduced IL-1β gene expression, while the larger oligomers (pentamer-decamer) significantly increased IL-1β mRNA levels of phytohemagglutinin (PHA)-stimulated cells. A structure related activity of procyanidins has also been reported in the case of TNF-α in murine macrophages /53/. Furthermore, pretreatment of LPS-stimulated RAW 264.7 cells with PBE has been shown to reduce both the production of IL-1β and its mRNA level in a dose-dependent manner, possibly by down-regulation of DNA binding activity of NF-κB and activator protein-1 /55/. Sangbongi *et al.* /60/ studied the effects of cocoa liquor polyphenols (CLP) on human immune function *in vitro*. CLP inhibited lymphocyte proliferation and immunglobulin G production in response to the mitogen PHA. It is hypothesized that the inhibition was, at least partly, due to reduced gene expression and secretion of IL-2. PBE was shown to suppress IFN-γ induced adhesion of lymphocytes to keratinocytes via inhibition of the expression of ICAM-1 on keratinocytes. The effect of PBE on cell adhesion molecule expression may represent an alternative mechanism that contributes to the anti-inflammatory properties of procyanidins /61/.

Immunomodulatory effects of dietary PBE have been studied in ethanol-fed or LP-BM5 retrovirus infected mice, which is an accepted model for HIV /62/. Although the infection caused by retrovirus and

chronic ethanol consumption are two different pathophysiological conditions, they share the common feature of inducing abnormalities in the function of cells involved in cellular and humoral immunity, resulting in an increased susceptibility to infectious agents. Dietary PBE reduced elevated levels of IL-6 produced *in vitro* by cells from retrovirus infected mice and IL-10 secreted by spleen cells from mice consuming ethanol. Furthermore, natural killer cell cytotoxicity was increased by PBE treatment, suggesting that PBE might at least partially normalize retrovirus-induced immune dysfunction.

7. CARDIOVASCULAR EFFECTS

Epidemiological studies suggest that the consumption of alcohol-containing beverages reduces mortality from coronary artery disease (CAD) /63,64/. The low CAD mortality in the French population despite a diet high in saturated fat, the so-called "French paradox", has been explained by the high consumption of wine, particularly red wine, by the population /65/. This cardioprotective effect has been attributed to the polyphenolic fraction of red wine, which contains a variety of antioxidants such as catechin, epicatechin, proantho-cyanidins, and resveratol /66/. The most extensively studied mecha-nisms, which might be involved in the protective effect of red wine polyphenols, are prevention of LDL oxidation, inhibition of platelet aggregation, and stimulation of endothelial NO secretion.

7.1 LDL oxidation

Intake of red wine /67,68/, alcohol-free red wine /69/, or red wine polyphenols /70/ by volunteers increased either the antioxidant capacity (AOC) of plasma in the hours following ingestion or the basal AOC after regular ingestion for up to 4 weeks, reflecting higher capacity of the plasma to scavenge reactive oxygen and nitrogen species. Furthermore, several studies have shown an inhibitory effect of red wine on LDL oxidation *in vitro* /71,72/. This antioxidative effect has been attributed to the presence of polyphenols mainly originating from the skin and seeds of the grapes /73/. Therefore, it would be expected that red wine, by increasing plasma AOC, might diminish oxidation of LDL and thereby contribute to the prevention of CAD. Although the effect of red wine on the susceptibility of LDL to

oxidation has been extensively studied, the outcomes are conflicting. Two hours after the consumption of red wine, *ex vivo* LDL oxidation was found to be significantly inhibited /74/, while no change in the susceptibility of LDL to oxidation was observed after 4 hours /75/. Consumption of red wine, but not white wine, by healthy volunteers for up to 2 weeks was reported to prolong the lag time for copper-catalyzed oxidation of LDL /76,77/. However, de Rijke *et al.* /78/ observed no change in the susceptibility of LDL to oxidation after consumption of either red wine or white wine for 4 weeks. The discrepancies among the outcomes of these studies have been suggested to be due to the EDTA content of LDL and the methods used for its removal before oxidation experiments. Moreover, partitioning of absorbed phenolic compounds in the aqueous phase of plasma has been suggested as a possible reason for the lack of the effect of wine consumption on LDL oxidizability *ex vivo* /70,76/.

7.2 Platelet aggregation

The effect of red wine supplementation on platelet aggregation and haemostatic parameters has been evaluated in various animal models. It has been shown that supplementation of the diets of dogs, monkeys, and rats with grape juice or crude preparations of grape polyphenols inhibited the aggregation of platelets measured *ex vivo* after induction with either collagen or thrombin /79-81/. Furthermore, red wine supplementation inhibited the rebound effect of thrombin-induced platelet aggregation in rats after withdrawal of the alcoholic beverage from the diet. This protective effect was also observed with procyanidins extracted from grape seeds or from wine itself /82/. Red wine and alcohol-free red wine supplementation to rats prolonged bleeding time, reduced thrombus weight, and decreased platelet adhesion to fibrillar collagen, while no change in these parameters was observed after white wine or ethyl alcohol supplementation /83/. In spite of these findings, no effect on *ex vivo* platelet aggregation was observed after moderate consumption of red wine or white wine by humans. Although the reason for such interspecies differences is not known, the positive effect of a moderate consumption of red wine on haemostasis in humans seems to be due to alcohol and not to the non-alcoholic fraction present in red wine /84-86/.

7.3 Endothelium dependent vasorelaxation

Various grape products, including red wine and red wine polyphenolic compounds, as well as PBE have been demonstrated to cause endothelium-dependent relaxation (EDR) in isolated rat aortic rings in vitro /87-90/. Furthermore, oligomeric procyanidins and anthocyanins were identified as the classes of compounds which might be responsible for the observed EDR induced by the original red wine polyphenolic compounds (RWPC) /91/. The antioxidant activity of procyanidins might play a significant role in their vasorelaxing properties. Procyanidins, by scavenging superoxide radicals, might prevent the formation of peroxynitrite and thereby maintain higher levels of NO, which is known to induce vasorelaxation. However, it has been suggested that RWPC- and PBE-induced EDR is due to an increase in NO synthesis /89-90/. RWPC-induced NO secretion was proposed to be mediated by influx of extracellular Ca^{2+} via an N-ethyl-maleimide sensitive pathway /92/. A few studies investigated the effect of red wine, white wine, or procyanidin-rich extracts on animal models of atherosclerosis. Red wine was reported to significantly reduce aortic atherosclerosis in cholesterol-fed rabbits in contrast to white wine or spirits /93/. Recently, it was shown that oral supplementation with a proanthocyanidin-rich extract from grape seeds reduced severe atherosclerosis in the aorta of cholesterol-fed rabbits /94/. While the proanthocyanidin-rich extract did not affect the changes in serum lipid profile of cholesterol-fed rabbits, a marked decrease was observed in the number of oxidized LDL-positive foam cells. Antioxidant activity of proanthocyanidins in aqueous phase such as plasma and interstitial fluid of the arterial wall was proposed to be responsible for the observed inhibition of LDL oxidation and anti-atherosclerotic activity.

8. CONCLUDING REMARKS

Procyanidins exert various important biological effects in cultured cells, laboratory animals, and in humans. Current data are promising, justifying further research, which should investigate in humans the bioavailability, antioxidant and cell regulatory activity to reveal the underlying molecular mechanisms for the anti-inflammatory and protective cardiovascular effects of procyanidins. Procyanidin action in the human body appears to be complex and likely results from

continuous intake of these phytochemicals that provides health benefits over the long term. Differential changes in the expression of several groups of genes might be a key point underlying their complex behavior *in vivo*. Therefore determining a global picture of the effects of procyanidins on gene expression through genomic techniques could provide a better understanding of their action on the molecular level. Using this approach the effects of procyanidins derived from PBE on gene expression in human keratinocytes have been investigated /95/. PBE treatment of HaCaT cells resulted in a dramatic decrease in the expression of a group of genes, known to be detected in high levels in psoriasis and various inflammatory dermatoses. Moreover, using DNA array technology our laboratory has recently demonstrated that *Ginkgo biloba* extract, EGb761, modulates the global gene expression profile of a human cancer line /96/. Functional classification of the affected mRNAs showed the largest changes in the abundance of mRNAs for intracellular vesicular transport, mitochondria, transcription factors, and antioxidants. Overall c-DNA microarrays open up new avenues in discovering redox-sensitive genes, transcription factors and signal transduction pathways. This approach might help to gain better insight into the molecular mechanisms of procyandins, thereby offering a novel strategy in phytochemical research.

ACKNOWLEDGEMENTS

G.R. is supported by a grant from the German Research Society (DFG Forschungs-stipendium Ri-884/3-1). The authors thank Gladys Lee for her editorial help and extend their gratitude to Dr. Claude Saliou for providing the figure.

REFERENCES

1. Haslam E. Symmetry and promiscuity in procyanidin biochemistry. Phytochemistry 1977; 16: 1625-1640.
2. deFreitas VAP, Glories Y, Bourgeois G, Vitry C. Characterisation of oligomeric and polymeric procyanidins from grape seeds by liquid secondary ion mass spectrometry. Phytochemistry 1998; 49: 1435-1441.
3. Cheynier V, Doco T, Fulcrand H, Guyot S, LeRoux E, Souquet JM, Rigaud J, Moutounet M. ESI-MS analysis of polyphenolic oligomers and polymers. Analysis 1997; 25: M32-M37.

4. Guyot S, Doco T, Souquet JM, Moutounet M, Drilleau JF. Characterization of highly polymerized procyanidins in cider apple (*Malus sylvestris var kermerrien*) skin and pulp. Phytochemistry 1997; 44: 351-357.
5. Santos-Buelga C, Scalbert A. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. J Sci Food Agr 2000; 80: 1094-1117.
6. Packer L, Rimbach G, Virgili F. Antioxidant activity and biological properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. Free Radical Biol Med 1999; 27: 704-724.
7. Shrikhande AJ. Wine by-products with health benefits. Food Res Int 2000; 33: 469-474.
8. DeFeudis F. *Ginkgo biloba* Extract (EGb 761). From the Chemistry to the Clinic. Wiesbaden: Ullstein Medical, 1998.
9. Wollgast J, Anklam E. Polyphenols in chocolate: is there a contribution to human health? Food Res Int 2000; 33: 449-459.
10. Donovan JL, Bell JR, Kasim-Karakas S, German JB, Walzem RL, Hansen RJ, Waterhouse AL. Catechin is present as metabolite in human plasma after consumption of red wine. J Nutr 1999; 129: 1662-1668.
11. Bell JR, Donovan JL, Wong R, Waterhouse AL, German JB, Walzem RL, Kasim-Karakas SE. (+)-Catechin in human plasma after ingestion of a single serving of reconstituted red wine. Am J Clin Nutr 2000; 71: 103-108.
12. Ader P, Wessman A, Wolffram S. Bioavailability and metabolism of the flavonol quercetin in the pig. Free Radical Biol Med 2000; 28: 1056-1067.
13. Clifford MN. CPT and Health. Intermediate Report, FAIR Project Contract No. PL95-0653. 1999.
14. Heilmann J, Merfort I. Aktueller Kenntnisstand zu Metabolismus von Flavonoiden. II Resorption und Metabolismus von Flavonen, Flavanonen, Proanthocyanidinen and Isoflavanoiden. Pharmazie in unserer Zeit 1998; 27: 173-183.
15. Jimenezramsey LM, Rogler JC, Housley TL, Butler LG, Elkin RG. Absorption and distribution of C-14-labeled condensed tannins and related sorghum phenolics in chickens. J Agric Food Chem 1994; 42: 963-967.
16. Laparra J, Michaud J, Masquelier J. A pharmacokinetic study on oligomeric flavonoids. Plant Med Phytother 1977; 11: 133-142:
17. Duweler KG, Rohdewald P. Urinary metabolites of French maritime pine bark extract in humans. Pharmazie 2000; 55: 364-368.
18. Haslam E. Natural polyphenols (vegetable tannins) as drugs - possible modes of action. J Nat Prod 1996; 59: 205-215.
19. Spencer CM, Cai Y, Martin R, Gaffney SH, Goulding PN, Magnolato D, Lilley TH, Haslam E. Polyphenol complexation - some thoughts and observations. Phytochemistry 1988; 27: 2397-2409.
20. Hagerman AE, Butler LG. The specificity of proanthocyanidin-protein interactions. J Biol Chem 1981; 256: 4494-4497.
21. Baxter NJ, Lilley TH, Haslam E, Williamson MP. Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. Biochemistry 1997; 36: 5566-5577.

22. Mehansho H, Ann DK, Butler LG, Rogler J, Carlson DM. Induction of proline-rich proteins in hamster salivary glands by isoproterenol treatment and an unusual growth inhibition by tannins. J Biol Chem 1987; 262: 12344-12350.
23. Scalbert A. Antimicrobial properties of tannins. Phytochemistry 1991; 30: 3875-3883.
24. McArthur C, Sanson GD, Beal AM. Salivary proline-rich proteins in mammals: Roles in oral homeostasis and counteracting dietary tannin. J Chem Ecol 1995; 21: 663-691.
25. Hagerman AE, Butler LG. Condensed tannin purification and characterization of tannin-associated proteins. J Agric Food Chem 1980; 28: 947-952.
26. Saito M, Hosoyama H, Ariga T, Kataoka S, Yamaji N. Antiulcer activity of grape seed extract and procyanidins. J Agric Food Chem 1998; 46: 1460-1464.
27. Hagerman AE, Butler LG. Protein precipitation method for the quantitative determination of tannins. J Agric Food Chem 1978; 26: 809-812.
28. Moini H, Arroyo A, Vaja J, Packer L. Bioflavonoid effects on the mitochondrial respiratory electron transport chain and cytochrome c redox state. Redox Rep 1999; 4: 37-41.
29. Facino RM, Carini M, Aldini G, Berti F, Rossoni G, Bombardelli E, Morazzoni P. Procyanidines from *Vitis vinifera* seeds protect rabbit heart from ischemia reperfusion injury: Antioxidant intervention and/or iron and copper sequestering ability. Planta Med 1996; 62: 495-502.
30. Blaszo G, Gaspar R, Gabor M, Rueve HJ, Rohdewald P. ACE inhibition and hypotensive effect of a procyanidin containing extract from the bark of *Pinus pinaster* Sol. Pharm Pharmacol Lett 1996; 6: 8-11.
31. Nardini M, Scaccini C, Packer L, Virgili F. In vitro inhibition of the activity of phosphorylase kinase, protein kinase C and protein kinase A by caffeic acid and a procyanidin-rich pine bark (*Pinus marittima*) extract. Biochim Biophys Acta 2000; 1474: 219-225.
32. Wang BH, Foo LY, Polya GM. Differential inhibition of eukaryote protein kinases by condensed tannins. Phytochemistry 1996; 43: 359-365.
33. Moini H, Guo Q, Packer L. Protein binding action of the procyanidins from French maritime pine bark extract: application of polyacrylamide gel electrophoresis. Meth Enzymol 2000; in press.
34. Moini H, Guo Q, Packer L. Antioxidant activity and protein binding action of procyanidin-rich French maritime pine bark extract - pycnogenol: effect on xanthine oxidase. J Agric Food Chem 2000; in press.
35. Zhu M, Phillipson JD, Greengrass PM, Bowery NE, Cai Y. Plant polyphenols: biologically active compounds or non-selective binders to protein? Phytochemistry 1997; 44: 441-447.
36. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids [published erratum appears in Free Radical Biol Med 1996; 21: 417]. Free Radical Biol Med 1996; 20: 933-956.

37. Noda Y, Anzai K, Mori A, Kohno M, Shinmei M, Packer L. Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectrometer system. Biochem Mol Biol Int 1997; 42: 35-44.
38. Dasilva JMR, Darmon N, Fernandez Y, Mitjavila S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. J Agric Food Chem 1991; 39: 1549-1552.
39. Arteel GE, Briviba K, Sies H. Protection against peroxynitrite by cocoa polyphenol oligomers. FEBS Lett 1999; 445: 226-230.
40. Shahidi F, Janitha PK, Wanasundara PD. Phenolic antioxidants. Crit Rev Food Sci Nutr 1992; 32: 67-103.
41. Brown JE, Khodr H, Hider RC, Rice-Evans CA. Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. Biochem J 1998; 330: 1173-1178.
42. Guo Q, Zhao B, Packer L. Electron spin resonance study of free radicals formed from a procyanidin-rich pine (*Pinus maritima*) bark extract, pycnogenol. Free Radical Biol Med 1999; 27: 1308-1312.
43. de Gaulejac NSC, Vivas N, de Freitas V, Bourgeois G. The influence of various phenolic compounds on scavenging activity assessed by an enzymatic method. J Sci Food Agr 1999; 79: 1081-1090.
44. Plumb GW, De Pascual-Teresa S, Santos-Buelga C, Cheynier V, Williamson G. Antioxidant properties of catechins and proanthocyanidins: effect of polymerisation, galloylation and glycosylation. Free Radical Res 1998; 29: 351-358.
45. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 1998; 46: 1887-1892.
46. Serafini M, Maiani G, FerroLuzzi A. Effect of ethanol on red wine tannin-protein (BSA) interactions. J Agric Food Chem 1997; 45: 3148-3151.
47. Cossins E, Lee R, Packer L. ESR studies of vitamin C regeneration, order of reactivity of natural source phytochemical preparations. Biochem Mol Biol Int 1998; 45: 583-597.
48. Virgili F, Kim D, Packer L. Procyanidins extracted from pine bark protect alpha-tocopherol in ECV 304 endothelial cells challenged by activated RAW 264.7 macrophages: role of nitric oxide and peroxynitrite. FEBS Lett 1998; 431: 315-318.
49. Rimbach G, Virgili F, Park YC, Packer L. Effect of procyanidins from *Pinus maritima* on glutathione levels in endothelial cells challenged by 3-morpholinosyndonimine or activated macrophages. Redox Rep 1999; 4: 171-177.
50. Gohil K, Roy S, Packer L, Sen CK. Antioxidant regulation of gene expression: analysis of differentially expressed mRNAs. Meth Enzymol 1999; 300: 402-410.
51. Kopp EB, Ghosh S. NF-κB and rel proteins in innate immunity. Adv Immunol 1995; 58: 1-27.
52. Flohe L, Brigelius-Flohe R, Saliou C, Traber MG, Packer L. Redox regulation of NF-κB activation. Free Radical Biol Med 1997; 22: 1115-1126.

53. Park YC, Rimbach G, Saliou C, Valacchi G, Packer L. Activity of monomeric, dimeric, and trimeric flavonoids on NO production, TNFα secretion, and NF-κB-dependent gene expression in RAW 264.7 macrophages. FEBS Lett 2000; 465: 93-97.
54. Virgili F, Kobuchi H, Packer L. Procyanidins extracted from *Pinus maritima* (pycnogenol): scavengers of free radical species and modulators of nitrogen monoxide metabolism in activated murine RAW 264.7 macrophages. Free Radical Biol Med 1998; 24: 1120-1129.
55. Cho KJ, Yoon DY, Cho YS, Rimbach G, Packer L, Chung AS. Effect of bioflavonoids from the bark of *Pinus maritima* on proinflamatory cytokine interleukin-1 production in RAW 264.7 macrophages. Toxicol Appl Pharmacol 2000; 168: 64-77.
56. Saliou C, Rimbach G, Moini H, McLaughlin L, Hosseini S, Lee J, Watson RR, Packer L. Solar ultraviolet-induced erythema in human skin and nuclear factor-κB dependent gene expression in keratinocytes are modulated by a French maritime pine bark extract. Free Radical Biol Med 2000; in press.
57. Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. J Biol Chem 1994; 269: 13725-13728.
58. Mizutani A, Maki H, Torii Y, Hitomi K, Tsukagoshi N. Ascorbate-dependent enhancement of nitric oxide formation in activated macrophages. Nitric Oxide 1998; 2: 235-241.
59. Azumi S, Tanimura A, Tanamoto K. A novel inhibitor of bacterial endotoxin derived from cinnamon bark. Biochem Biophys Res Comm 1997; 234: 506-510.
60. Sanbongi C, Suzuki N, Sakane T. Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans in vitro. Cell Immunol 1997; 177: 129-136.
61. Bito T, Roy S, Sen CK, Packer L. Pine bark extract pycnogenol downregulates IFNγ-induced adhesion of T cells to human keratinocytes by inhibiting inducible ICAM-1 expression. Free Radical Biol Med 2000; 28: 219-227.
62. Cheshier JE, Ardestani-Kaboudanian S, Liang B, Araghiniknam M, Chung S, Lane L, Castro A, Watson RR. Immunomodulation by pycnogenol in retrovirus-infected or ethanol-fed mice. Life Sci 1996; 58: 87-96.
63. St Leger AS, Cochrane AL, Moore F. Factors associated with cardiac mortality in developed countries with particular reference to consumption of wine. Lancet 1979; i (8124): 1017-1020.
64. Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: Is the effect due to beer, wine, or spirits [see comments]. BMJ 1996; 312: 731-736.
65. Renaud S, Delorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 1992; 339: 1523-1526.
66. Das DK, Sato M, Ray PS, Maulik G, Engelman RM, Bertelli AA, Bertelli A. Cardioprotection of red wine: role of polyphenolic antioxidants. Drug Exp Clin Res 1999; 25: 115-120.
67. Maxwell S, Cruickshank A, Thorpe G. Red wine and antioxidant activity in serum. Lancet 1994; 344: 193-194.

68. Whitehead TP, Robinson D, Allaway S, Syms J, Hale A. Effect of red wine ingestion on the antioxidant capacity of serum [see comments]. Clin Chem 1995; 41: 32-35.
69. Serafini M, Maiani G, Ferro-Luzzi A. Alcohol-free red wine enhances plasma antioxidant capacity in humans. J Nutr 1998; 128: 1003-1007.
70. Carbonneau MA, Léger CL, Monnier L, Bonnet C, Michel F, Fouret G, Dedieu F, Descomps B. Supplementation with wine phenolic compounds increases the antioxidant capacity of plasma and vitamin E of low-density lipoprotein without changing the lipoprotein Cu^{2+}-oxidizability: possible explanation by phenolic location. Eur J Clin Nutr 1997; 51: 682-690.
71. Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 1993; 341: 454-457.
72. Struck M, Watkins T, Tomeo A, Halley J, Bierenbaum M. Effect of red and white wine on serum lipids, platelet aggregation, oxidation products and antioxidants: A preliminary report. Nutr Res 1994; 14: 1811-1819.
73. Soleas GJ, Diamandis EP, Goldberg DM. Wine as a biological fluid: history, production, and role in disease prevention. J Clin Lab Anal 1997; 11: 287-313.
74. Miyagi Y, Miwa K, Inoue H. Inhibition of human low-density lipoprotein oxidation by flavonoids in red wine and grape juice. Am J Cardiol 1997; 80: 1627-1631.
75. Caccetta RA, Croft KD, Beilin LJ, Puddey IB. Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vivo lipoprotein oxidizability. Am J Clin Nutr 2000; 71: 67-74.
76. Nigdikar SV, Williams NR, Griffin BA, Howard AN. Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation in vivo. Am J Clin Nutr 1998; 68: 258-265.
77. Fuhrman B, Lavy A, Aviram M. Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation [see comments]. Am J Clin Nutr 1995; 61: 549-554.
78. de Rijke YB, Demacker PN, Assen NA, Sloots LM, Katan MB, Stalenhoef AF. Red wine consumption does not affect oxidizability of low-density lipoproteins in volunteers. Am J Clin Nutr 1996; 63: 329-334.
79. Demrow HS, Slane PR, Folts JD. Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. Circulation 1995; 91: 1182-1188.
80. Osman HE, Maalej N, Shanmuganayagam D, Folts JD. Grape juice but not orange or grapefruit juice inhibits platelet activity in dogs and monkeys. J Nutr 1998; 128: 2307-2312.
81. Xia J, Allenbrand B, Sun GY. Dietary supplementation of grape polyphenols and chronic ethanol administration on LDL oxidation and platelet function in rats. Life Sci 1998; 63: 383-390.

82. Ruf JC, Berger JL, Renaud S. Platelet rebound effect of alcohol withdrawal and wine drinking in rats. Relation to tannins and lipid peroxidation. Arterioscler Thromb Vasc Biol 1995; 15: 140-144.
83. Wollny T, Aiello L, Di Tommaso D, Bellavia V, Rotilio D, Donati MB, de Gaetano G, Iacoviello L. Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production. Br J Pharmacol 1999; 127: 747-755.
84. Pace-Asciak CR, Rounova O, Hahn SE, Diamandis EP, Goldberg DM. Wines and grape juices as modulators of platelet aggregation in healthy human subjects. Clin Chim Acta 1996; 246: 163-182.
85. Pellegrini N, Pareti FI, Stabile F, Brusamolino A, Simonetti P. Effects of moderate consumption of red wine on platelet aggregation and haemostatic variables in healthy volunteers. Eur J Clin Nutr 1996; 50: 209-213.
86. Lavy A, Fuhrman B, Markel A, Dankner G, Ben-Amotz A, Presser D, Aviram M. Effect of dietary supplementation of red or white wine on human blood chemistry, hematology and coagulation: Favorable effect of red wine on plasma high-density lipoprotein. Ann Nutr Metabol 1994; 38: 287-294.
87. Fitzpatrick DF, Hirschfield SL, Coffey RG. Endothelium-dependent vaso-relaxing activity of wine and other grape products. Am J Physiol 1993; 265: H774-H778.
88. Fitzpatrick DF, Hirschfield SL, Ricci T, Jantzen P, Coffey RG. Endothelium-dependent vasorelaxation caused by various plant extracts. J Cardiovasc Pharmacol 1995; 26: 90-95.
89. Fitzpatrick DF, Bing B, Rohdewald P. Endothelium-dependent vascular effects of pycnogenol. J Cardiovasc Pharmacol 1998; 32: 509-515.
90. Andriambeloson E, Kleschyov AL, Muller B, Beretz A, Stoclet JC, Andriantsitohaina R. Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. Br J Pharmacol 1997; 120: 1053-1058.
91. Andriambeloson E, Magnier C, Haan-Archipoff G, Lobstein A, Anton R, Beretz A, Stoclet JC, Andriantsitohaina R. Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. J Nutr 1998; 128: 2324-2333.
92. Andriambeloson E, Stoclet JC, Andriantsitohaina R. Mechanism of endothelial nitric oxide-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. J Cardiovasc Pharmacol 1999; 33: 248-254.
93. Klurfield DM, Kritchevsky D. Differential effects of alcohol beverages on experimental atherosclerosis in rabbits. Exp Mol Pathol 1981; 34: 62-71.
94. Yamakoshi J, Kataoka S, Koga T, Ariga T. Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. Atherosclerosis 1999; 142: 139-149.
95. Rihn B, Saliou C, Bottin MC, Keith G, Packer L. From ancient remedies to modern therapeutics: pine bark uses in skin disorders revisted. Phytother Res 2000; in press.

96. Gohil K, Moy RK, Farzin S, Maguire JJ, Packer L. *Ginkgo biloba* extract alters the mRNA expression profile of a human cell line: induction of antioxidant response and the Golgi system. Free Radical Res 2000; in press.