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Review

Dietary proanthocyanidins: Occurrence, dietary intake, bioavailability, and protection against cardiovascular disease

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The French have one of the lowest incidences of coronary heart disease in the Western world despite a diet with a relatively high fat content. This phenomenon that has puzzled researchers worldwide for more than a decade is known as the 'French paradox' and has been linked to the high consumption of red wine in France. Red wine is rich in the complex polyphenols, the proanthocyanidins, and these compounds have recently attracted attention as potential cardiac-protective compounds. The present review summarizes the literature on proanthocyanidins with focus on their chemical structure, the occurrence, the daily intake from foods, the bioavailability and metabolism, and the evidence for a protective effect against cardiovascular diseases.

Keywords: Cardiovascular disease / Proanthocyanidins / Red wine / Review

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Abbreviations: DP, degree of polymerization; **LDL**, low-density lipoprotein; **TNFα**, tumor necrosis factor α

1 Introduction

The fact that the French have one of the lowest incidences of coronary heart disease in the Western world despite a diet with a relatively high fat content has been linked to the high consumption of red wine [1]. The French eat 3.8 times as much butter and 2.8 times as much lard as Americans, and they have higher serum cholesterol levels and higher blood pressure than Americans, yet Americans have a 2.5-fold greater death rate due to coronary heart disease than the French population [2]. This has been attributed to the drinking of red wine, since the French consume more wine than Americans, Northern Europeans, and people from the UK [3]. This phenomenon, commonly referred to as the "French paradox", has puzzled researchers worldwide for more than a decade. Epidemiological evidence from many studies overwhelmingly supports the hypothesis that moderate alcohol consumption is significantly associated with a reduction in coronary heart disease mortality [4–6]. Some who have studied this association in a particular population have found that wine, in particular red wine, is more powerful than other alcoholic beverages in decreasing the incidence of coronary heart disease [5, 7] although other studies were not able to show a special epidemiological benefit of wine [4]. Many of the components in wine have during the years been shown to exert cardioprotective properties, sup-

porting the theory of the regular wine intake in France as an explanatory factor of this paradox.

Red wine represents a rich source of polyphenols like anthocyanins, catechins, proanthocyanidins, flavonols, stilbenes, and other phenolics, all potent antioxidants that have been shown to possess biological properties that may protect against cardiovascular disease (for reviews see, *e.g.,* [8 – 11]). However, many of the studies on these compounds show conflicting results and the dietary components responsible for the lower risk of cardiovascular disease in France still remains to be convincingly identified. During the past few years, the proanthocyanidins have been suggested as a candidate to explain the superior effect of red wine as compared to white wine and other alcoholic beverages, since the proanthocyanidins are specifically extracted from the grape seeds and skin during the mash fermentation of red wine.

Research on the proanthocyanidins is however limited and many questions still remain to be answered. The present review will focus on the present knowledge on the proanthocyanidins, their chemical structure, the occurrence, the daily intake from foods, the bioavailability and metabolism, and the evidence for a protective effect against cardiovascular diseases.

2 Dietary proanthocyanidins

2.1 Chemistry and occurrence

Proanthocyanidins are synonymous with condensed tannins and are the second most abundant natural phenolic after lignin [2, 12]. The wide presence of proanthocyanidins in plants makes them an important part of the human diet, and they are found especially in fruits, berries, beans, nuts, cocoa, and wine. Through the formation of complexes with salivary proteins, proanthocyanidins are responsible for the astringent character of fruits (grapes, peaches, apples, pears, berries, *etc.*) and beverages (wine, cider, tea, beer, *etc.*) and for the bitterness of chocolate [2]. This astringency changes over the course of maturation and often disappears when the fruit reaches ripeness. Proanthocyanidins are oligomers or polymers of flavan-3-ols and these units are linked mainly through C4→C8 bond, but the C4→C6 linkage also exists. These linkages are both called B-type linkages. An additional ether bond between C2→C7 resulting in doubly linkage of the flavan-3-ol units is called an A-type linkage. Proanthocyanidins consist of different flavan-3-ol subunits, also called proanthocyanidin monomers or catechins. The most common types are shown in Fig. 1. The proanthocyanidins that exclusively consist of (epi)catechin units are designated procyanidins, the most abundant type of proanthocyanidins in plants. The less common proanthocyanidins containing (epi)afzelechin or (epi)gallocatechin subunits are called propelargonidin or prodelphinidin, respectively. The flavan-3-ol subunits may carry acyl or glycosyl substituents. The most common acyl substituent is gallic acid, which is bound as an ester with the hydroxyl in the C3 position as in tea or wine [2]. Several glycosylated proanthocyanidin oligomers have been identified with the sugar linked to the C3 or the C5 position as the most common glycosylation [2].

The knowledge about the distribution and nature of proanthocyanidins in foods has until recently been very limited. The content of flavonols and proanthocyanidin dimers and trimers in Spanish foods was investigated in 2000 by Pascual-Teresa *et al.* [13]. The same year, Santos-Buelga and Scalbert [2] have reviewed the available data on proanthocyanidins, and large discrepancies were found in the content in food at this time due to different analytical methods or to the nature of the samples analyzed, variety, stage of ripeness, part of food considered, level of processing, *etc.* [2]. Only very recently, the content of proanthocyanidins in human foods have been thoroughly investigated. Gu *et al.* [12, 14] reported in 2003 a screening of 88 different kinds of foods for proanthocyanidin content, including oligomers and polymers, using LC-MS/MS after thiolytic degradation. Out of the 88 plant-based foods investigated, 39 were found to contain proanthocyanidins. The proanthocyanidins were mainly detected in fruits and berries, but also nuts, beans, some minor cereals, such as barley and sorghum, the spices curry and cinnamon, and the beverages wine and beer were found to contain proanthocyanidins. Most of these plant-based foods contained exclusively the homogeneous B-type procyanidins, but few also contained the heterogeneous propelargonidin or prodelphinidin. In some of the investigated plant foods A-type proanthocyanidins were found. The majority of A-type proanthocyanidins found in nature contain only one additional A-type interflavan bond primarily in between the extension units or as an A-type terminal unit. A-type proanthocyanidins was only determined in curry, cinnamon, cranberry, peanut, plums, and avocado in the study by Gu *et al.* [12]. The content of A-type proanthocyanidins was however determined to be very high in these foods, and in curry and cinnamon between 84 – 90% of the total amount of proanthocyanidins were A-type, in cranberry and peanut about 51 – 65%, and in plums and avocado about 29% was A-type proanthocyanidins [12].

Of the 19 vegetables screened, only Indian squash contained proanthocyanidins, and vegetables are therefore not an important source of proanthocyanidins. In addition, no proanthocyanidins were detected in citrus fruits, pineapple, and watermelon, in the cereals oat, rice, and corn, or in 13 other spices [12]. Hence, major, regularly consumed fruit products, like orange juice and grape juice, do not contribute to the intake of proanthocyanidins. In a later study, Gu

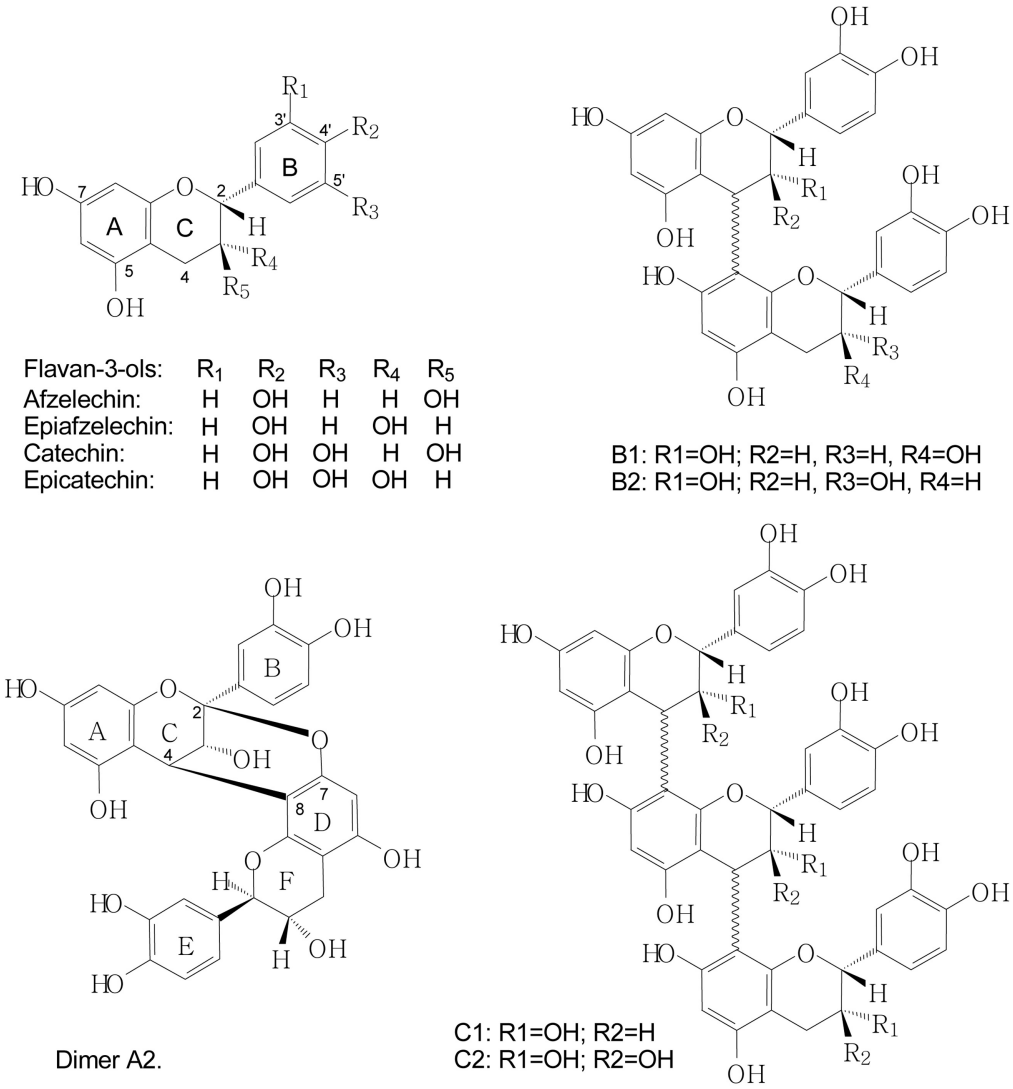

Figure 1. Chemical structures of proanthocyanidins. Structures of the monomeric flavan-3-ols, dimer B1, B2, and trimer C1 and C2 are shown. Dimer A2 is shown as example of the A-type double linkage.

et al. [15] furthermore investigated the concentration of proanthocyanidins in all the foods that were found to contain proanthocyanidins in the first study. Table 1 summarizes the proanthocyanidin content of some of these foods as determined by Gu *et al.* [15] and Fig. 2 shows the content of proanthocyanidins in beverages as determined later by Auger *et al.* [16]. In the studies by Gu *et al.* also the degree of polymerization (DP) was determined in selected foods, and a large variation in the average DP was observed [12, 15]. In black currants, *e.g.*, the average DP was 47.9 ± 5.1, whereas it was only 7.3 ± 0.2 and 2.1 ± 0.0 in wine and beer, respectively (see Table 1).

Gu *et al.* [15] also estimated the daily average intake of proanthocyanidins in the USA and found that in total the daily intake of proanthocyanidins is about 53.6 mg/day excluding the monomers, and 57.7 mg/day including the monomers. The distribution between intake of monomers, dimers, and trimers was found to be almost equal with an intake of 4.1–6.4 mg/day of each group, whereas the intake of oligomers (4 – 10-mers) and polymers (>10-mers) was both around 20 mg/day [15]. Thus, of the estimated total daily intake of proanthocyanidins, about 73% had a DP > 3. Apples (32%), chocolate (17.9%) and grapes (17.8%) was found to be the major contributors to the proanthocyanidin intake in the USA [15]. Earlier estimations of average daily proanthocyanidin intake were based on far more scarce data and ranged from tens to several hundreds of milligrams of proanthocyanidins per day, and the main sources of proanthocyanidins were identified to be apples, pears,

Table 1. Content of proanthocyanidins in common foods[a)]

Food	Monomers	Dimers	Trimers	4–6-mers	7–10-mers	>10-mers	Total	Type[b)]
			(mg/100 g fresh weight foods or mg/L beverages)					
Blueberries	4.0 ± 1.5	7.2 ± 1.8	5.4 ± 1.2	19.6 ± 3.4	14.5 ± 2.0	129.0 ± 47.3	179.8 ± 50.8	PC
Black-currants	0.9 ± 0.2	2.9 ± 0.4	3.0 ± 0.3	10.6 ± 1.7	9.9 ± 1.4	122.4 ± 28.0	147.8 ± 33.0	PC, PD
Cranberries	7.3 ± 1.5	25.9 ± 6.1	18.9 ± 3.4	70.3 ± 13.1	62.9 ± 14.7	233.5 ± 49.1	418.8 ± 75.3	A, PC
Strawberries	4.2 ± 0.7	6.5 ± 1.3	6.5 ± 1.2	28.1 ± 6.5	23.9 ± 3.5	75.8 ± 13.4	145.0 ± 24.9	PP, PC
Apples[c)]	9.6 ± 0.9	13.8 ± 0.6	9.3 ± 0.4	30.2 ± 1.2	25.4 ± 1.2	37.6 ± 2.6	125.8 ± 6.8	PC
Apple juice	1 ± 0	2 ± 0	1 ± 0	4 ± 0	1 ± 0	ND	9 ± 0	PC
Pears	2.7 ± 1.5	2.8 ± 1.3	2.3 ± 0.9	6.5 ± 1.9	4.6 ± 1.0	13.1 ± 11.3	31.9 ± 7.8	PC
Plums	11.4 ± 3.4	31.5 ± 7.4	23.9 ± 5.1	58.0 ± 12.5	33.8 ± 11.9	57.3 ± 24.4	215.9 ± 50.7	A, PC
Peaches	4.7 ± 1.4	7.0 ± 2.2	5.0 ± 1.4	17.7 ± 5.5	10.9 ± 3.7	22.0 ± 7.7	67.3 ± 20.9	PC
Avocados	1.0 ± 0.8	1.5 ± 0.8	1.4 ± 0.4	3.2 ± 0.8	0.4 ± 0.7	ND	7.4 ± 4.3	A, PC
Sorghum, sumac bran	27.8 ± 1.2	78.2 ± 3.4	99.2 ± 7.7	585.5 ± 50.0	734.3 ± 69.3	2440.4 ± 271.0	3965.4 ± 402.5	PC
Barley	11.0 ± 0.3	21.4 ± 1.1	14.6 ± 1.0	27.2 ± 0.6	ND	ND	74.2 ± 3.0	PC
Pinto beans, raw	14.8 ± 0.9	32.0 ± 2.6	28.3 ± 2.1	125.9 ± 9.2	135.6 ± 10.4	459.6 ± 34.2	796.3 ± 58.7	PP, PC
Red kidney beans	21.9 ± 0.2	26.4 ± 0.7	29.1 ± 0.7	117.7 ± 2.8	105.3 ± 2.2	263.4 ± 4.1	563.8 ± 10.4	PP, PC
Hazelnuts	9.8 ± 1.6	12.5 ± 3.8	13.6 ± 3.9	67.7 ± 20.3	74.6 ± 21.9	322.4 ± 102.5	500.7 ± 152.0	PC, PD
Pistachios	10.9 ± 4.3	13.3 ± 1.8	10.5 ± 1.2	42.2 ± 5.2	37.9 ± 4.9	122.5 ± 37.1	237.3 ± 52.0	PC, PD
Almonds	7.8 ± 0.9	9.5 ± 1.6	8.8 ± 1.7	40.0 ± 8.5	37.7 ± 8.4	80.3 ± 28.1	184.0 ± 48.2	PP, PC
Walnuts	6.9 ± 3.4	5.6 ± 0.9	7.2 ± 1.2	22.1 ± 3.3	5.4 ± 0.8	20.0 ± 9.3	67.3 ± 14.7	PC
Peanuts	5.1 ± 1.0	4.1 ± 0.7	3.7 ± 0.5	2.8 ± 0.2	ND	ND	15.6 ± 2.3	A, PC
Peanut butter	2.0 ± 0.9	3.0 ± 0.7	8.1 ± 3.5	ND	ND	ND	13.2 ± 5.2	A, PC
Black chocolate	31.4 ± 0.2	31.2 ± 0.9	21.1 ± 0.8	55.5 ± 3.5	38.5 ± 3.0	68.2 ± 8.8	246.0 ± 0.3	PC
Milk chocolate	26.9 ± 3.0	26.2 ± 2.5	19.3 ± 2.6	51.4 ± 9.8	35.3 ± 7.2	32.8 ± 9.2	192.0 ± 28.8	PC
Beer	4 ± 0	11 ± 1	3 ± 0	4 ± 0	ND	ND	23 ± 2	PC, PD
White wine[d)]	15.1	ND	ND	–	–	–	15.1[d)]	–
Rosé wine[d)]	17.1	ND	ND	–	–	–	17.1[d)]	–
Red wine[d)]	190.0	274.3	93.4	–	–	–	557.7[d)]	–
Red wine	20 ± 1	40 ± 1	27 ± 1	67 ± 2	50 ± 1	110 ± 2	313 ± 5	PC, PD
Grape juice	18 ± 0	34 ± 0	19 ± 0	80 ± 0	69 ± 0	303 ± 2	524 ± 2	PC, PD
Grape seed (dry)	660.3 ± 8.3	417.3 ± 4.8	290.2 ± 4.5	664.0 ± 8.2	400.3 ± 31.3	1100.1 ± 86.3	3532.3 ± 105.8	PC

a) All data are obtained from [15] unless otherwise is stated in a footnote. Values are means ± SD, $n = 4–8$. See [15] for detailed data on food content of proanthocyanidins. ND = not detected

b) Type: PP, propelargonidins, PC, procyanidins, PD, prodelphinidins, A, A-type linkage [15]

c) Red delicious with peel

d) Data obtained from [16]. Mean of 95 different French wines, see original paper for ranges in the wines. Only monomers (catechin and epicatechin) up to trimers were measured. The total given is the sum of monomers to trimers.

grapes, and red wine [2]. It is, however, seen from Fig. 2, that red wine contributes with significant amounts of proanthocyanidins compared to other beverages, and regular drinkers of red wine will thus have a substantial intake of proanthocyanidins. Also regular fruit eaters will easily have a daily intake higher than the average 57.7 mg/day as determined by Gu *et al.* Compared to the mean intake of other flavonoid groups like flavonols, flavones and flavanones, that has been estimated to be totally about 24.2–28.6 mg/d in the populations of Finland, Denmark, and the Netherlands, respectively [17–19], the intake of proanthocyanidins is substantial and adds significantly to the total daily intake of flavonoids.

2.2 Uptake and metabolism

The estimated amount of 57.7 mg/day of proanthocyanidins that we consume daily is about twice the amount of other flavonoids that are present in our food [15]. These compounds are thus an important contributor to the potential health effects of dietary flavonoids, but to produce a biological effect *in vivo*, it is essential that sufficient quantities reach the target tissues. Until very recently the fate of proanthocyanidins in the human body was unknown. However, new analytical techniques have enabled the study of the uptake and metabolism of the complex mixtures of proanthocyanidins present in our food. The proanthocyanidins found in food cover a wide range of DP. As seen in Table 1, the concentration of monomers, dimers, and trimers in foods is by far exceeded by the higher polymerized proanthocyanidins. Recent studies suggest, however, that only the low-molecular-weight oligomers (DP $\leq$ 3) are absorbed intact in the gastrointestinal tract. Déprez *et al.* [20] demonstrated that (+)-catechin and proanthocyanidin dimers and trimers were permeable through the Caco-2 human intestinal cell line. The permeability of a proanthocyanidin polymer with a mean DP of 6 was ~10 times

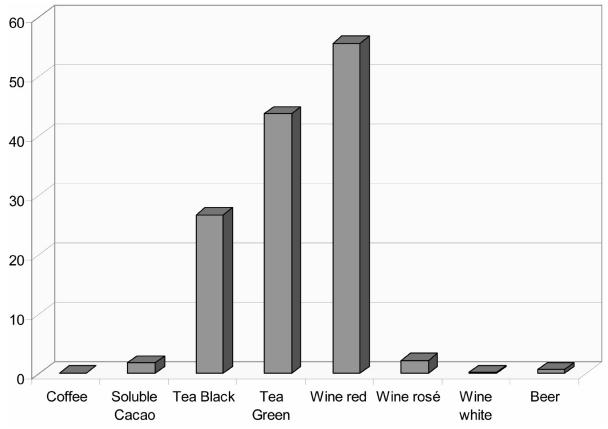

Figure 2. Proanthocyanidin monomer (catechin and epicatechin), dimer and trimer level in beverages, including gallotannins for teas (mg/100 mL). Reproduced from [16], with permission.

lower, suggesting that only the monomers, dimers, and trimers are absorbed and that polymers are not.

The data based on the absorption and degradation of proanthocyanidins are, however, somewhat conflicting. Spencer *et al.* [21] demonstrated by *in vitro* experiments the possibility that procyanidin oligomers are degraded under *in vitro* conditions that simulated gastric juice (37°C, pH 2.0, 1–4 h, no digestive enzymes) to mixtures of epicatechin monomers and dimers that then could be absorbed in the small intestine. However, a later study by Donovan *et al.* [22] showed that purified procyanidin dimer B3, as well as grape seed proanthocyanidins having a higher degree of polymerization, not were degraded into monomers in rats, and that none of the dimer was identified in plasma or urine. The stability of proanthocyanidins was furthermore investigated in humans by regular analysis of gastric juice sampled with a gastric probe after ingestion of a proanthocyanidin-rich cocoa beverage [23]. This study supports that proanthocyanidins are not degraded under the acidic conditions of the stomach *in vivo*.

Several studies emphasize that the majority of the polymeric proanthocyanidins pass unaltered through the small intestine whereafter they are degraded into small phenolic acids by the colonic microflora in the cecum and large intestine [24]. Déprez *et al.* [25] reported that incubation of polymeric procyanidins with human colonic microflora *in vitro* under anaerobic conditions completely degraded the procyanidins after 48 h into low-molecular-weight phenolic acids. The degradation products included phenylacetic, phenylpropionic, and phenylvaleric acids. Similar metabolic products were observed by Gonthier *et al.* [26] *in vivo* in rats that during five days were provided either catechin monomer, procyanidin dimer B3, trimer C3, or a fraction from willow tree containing a mixture of polymers. In the rats fed the catechin diet, intact catechin and its 3-*O*-methyl derivative excreted in urine accounted for 25.7% of the catechin dose. In the rats provided dimeric, trimeric, or polymeric proanthocyanidins, no monomeric form of catechin or its methylated form was detected. In all groups, phenolic acids including phenylacetic, phenylpropionic, phenylvaleric, and benzoic acids were detected in the urine (in total 16 different metabolites) with the total yield of these phenolic acids expressed as percentages of the catechin equivalent dose decreasing from the catechin monomer (10.6 ± 1.1%), to the dimer (6.5 ± 0.2%), trimer (0.7 ± 0.1%) to the polymer fraction (0.5 ± 0.1%) [26]. Thus, the production of absorbable low-molecular-weight phenolic acids is highest from monomeric and dimeric forms, and negligible from those with a higher degree of polymerization. Similar phenolic acids were observed as the major metabolites in urine collected from healthy human subjects after consumption of chocolate containing high amounts of oligomeric, polymeric proanthocyanidins, and monomeric catechins [27].

A few studies indicate that some procyanidin dimers are able to be absorbed intact in rats and humans [28, 29], whereas others have not been able to detect any intact dimers. In a human study, the procyanidin dimer B2 was detected in the plasma of volunteers ingesting a cocoa beverage; however, the maximal plasma concentration reached 2 h after ingestion was only 0.04 μmol/L compared to a level of 6.0 μmol/L after an equivalent intake of epicatechin [28]. Despite their high abundance in our food, dietary proanthocyanidins are very poorly absorbed and may only exert local effects in the gastrointestinal tract or effects mediated mainly by the monomeric forms and the simple phenolic acids produced by the microbial degradation. Once proanthocyanidins or their fermentation products have crossed the intestinal barrier, they reach the liver through the portal vein, where they are further metabolized. *In vitro* studies have shown that methylated derivatives can be formed from the dimer B3 in fresh liver homogenate obtained from a human biopsy. No *in vivo* study has, however, yet reported on the methylation of proanthocyanidins, but it is very likely that these compounds are being extensively metabolized, hydroxylated, methylated or conjugated to sulfate esters or glucuronides as has been shown for other flavonoids [30 – 33].

This leads to the conclusion that studies on antioxidant and biological effects of proanthocyanidins only make sense if their degree of polymerization and their metabolism is taken into account, since it is evident from the present knowledge that only the monomers and dimers are bioavailable and only to a limited extent, and that it is very unlikely that proanthocyanidins with DP > 2 are able to reach the systemic circulation. Furthermore, only proanthocyanidins with DP ≤ 2 result in the production of significant amounts

of phenolic acid metabolites in the gut [34]. These readily absorbed metabolites may also contribute significantly to the health protective effects observed for proanthocyanidin-rich foods.

3 Cardioprotective properties of proanthocyanidins

3.1 Development of atherosclerosis

Atherosclerosis is the primary cause of many cardiovascular diseases. Mortality from cardiovascular disease is the leading cause of death in the USA numbering 38.5% of all deaths in 2001 [35]. Atherosclerosis is an inflammatory disease that is characterized by the accumulation of lipids in the innermost layer, the intima of the walls of large- and medium-sized arteries (for reviews see [36, 37]). The process initiates by accumulation of modified low-density lipoproteins (LDLs) in the intima and uptake of the modified LDL by macrophages. The LDL may be modified by oxidation by free radicals or by enzyme-mediated oxidation resulting in aggregation within the intima [38]. The trapped oxidized LDL initiates an inflammatory response of the endothelial cells that attracts monocytes to the area. The monocytes adhere to the endothelium, cross into the intima, and differentiate into macrophages. The macrophages engulf oxidized LDL leading to unregulated accumulation of LDL. These lipid-loaded macrophages are called foam cells and are the characteristic components of early atherosclerotic lesions, namely fatty streaks [39]. The foam cells are incapable of escaping the intima and when they undergo cell death (apoptosis) this may result in an oxidative burst that further contributes to the oxidation of LDL, thus aggravating the inflammatory process. The progression of the lesion involves smooth muscle cells, collagen, and platelets in addition to further lipid accumulation [37]. The advanced lesion may also develop a fibrous cap. The atherosclerotic lesions or plaques reduce the endothelial function, limit the effective diameter of the vessels, and thereby restrict the blood flow and the supply of oxygen to tissues. When the plaques grow larger, they have an increased tendency to rupture and cause thrombosis and sudden death by myocardial infarction [39].

3.2 Antioxidant effects and inhibition of LDL oxidation

Highly reactive oxygen species, such as singlet oxygen, $^1O_2$, the superoxide anion radical $O^{2\bullet-}$, the hydroperoxyl radical $OH^\bullet$, the nitrogenoxide radical $NO^\bullet$, and alkyl peroxyl free radicals, are regularly produced in our body. They cause damage to lipids, proteins, and DNA and participate in the pathogenesis and ageing [40]. Humans possess a wide array of endogenous antioxidant defences, which scavenge radicals and repair oxidative damage. The consumption of a diet rich in antioxidants like polyphenols are thought to contribute further to this defence [41].

Polyphenols are able to scavenge reactive oxygen species through electron-donating properties, generating a relatively stable phenoxyl radical. For most proanthocyanidins with an *o*-dihydroxyphenyl group in the B-ring and a fully saturated C-ring, the radical site is at the B-ring and the substitution of the A-ring has only a limited influence on the reduction potentials of the semiquinone radical formed [42]. These semiquinone radicals are quite stable [43], and proanthocyanidins may therefore protect the body against oxidation and by that limit the risk of developing cardiovascular disease.

Several studies have investigated the antioxidative potential of the proanthocyanidins and of proanthocyanidin-rich foods. However, as discussed above, the bioavailability of the proanthocyanidins has only very recently been investigated, and the state-of-the-art is that in addition to the monomers, only the dimers are able to be absorbed in their intact form and only to a very limited extent whereas the polymers are nonbioavailable and transfer unaltered through the gastro-intestinal system. Some of the proanthocyanidin forms are additionally degraded by the gut microflora to low-molecular-weight compounds. This rather new knowledge calls for a reconsideration of the many very promising *in vitro* studies on proanthocyanidins conducted in the past, demonstrating biological and antioxidative effects of proanthocyanidins. None of these studies take into account the metabolism and the limitations in the bioavailability of the proanthocyanidins, and thereby some of the results of these studies may be irrelevant for the *in vivo* situation, implicating oral dosing, unless i.v. doses with proanthocyanidins are used. However, in many of the reported *in vitro* studies, proanthocyanidin-rich extracts or foods are used, like red wine, grape seed extracts, and cocoa powder. These mixtures all additionally contain readily absorbable catechin monomers, proanthocyanidin dimers, and other minor phenolic compounds, and the results of these studies may therefore in part be due to these compounds, rather than of the polymeric proanthocyanidins, and therefore these studies may be reproducible *in vivo* using the same extracts, but this has to be further investigated. Thus, the limits of many of the *in vitro* studies are that the polyphenols tested are different from those present in the body. It is thus not only necessary to know the target and its physicochemical environment but also to know if the compound effectively reaches the target. In the absence of such data it will be difficult to extrapolate results obtained *in vitro* to the *in vivo* situation, and in particular to establish the nature of the dietary phenolic compound providing the most efficient protection against chronic diseases.

Several *in vitro* studies on inhibition of the oxidation of LDL or membrane systems with purified proanthocyanidins have been conducted. Two studies on the antioxidant activity and membrane effects of proanthocyanidins have been performed on liposomes [44, 45]. Both studies conclude that the oligomer chain length influences the antioxidant activity in a manner where increasing protection against oxidation was correlated with increasing chain length of the proanthocyanidins. In another study on cranberry proanthocyanidins, it was likewise observed that only the fractions containing trimers to heptamers or pentamers through nonamers were able to increase the lag time of Cu^{2+} induced oxidation of LDL [46]. A third study also found an increased protection of LDL with increasing chain length of the proanthocyanidins [47]. The relevance of these studies to the *in vivo* antioxidative effects of dietary proanthocyanidin oligomers is, however, questionable since it is very unlikely that these oligomeric and polymeric compounds will reach the systemic circulation in intact form.

In vitro and *in vivo* studies on the protection against oxidation of LDL by food products containing proanthocyanidins, like red wine, cranberry products, grape skin extracts, cocoa, and chocolate, have also been conducted, and the majority of these studies show a protection against LDL oxidation. In these studies the effects may, however, rather be due to the content of monomeric or dimeric proanthocyanidins in the food products. This literature has recently been extensively reviewed [48 – 52].

3.3 Inhibition of the inflammatory response in atherosclerosis

In the past decade there has been a major shift in the paradigm of our understanding of the pathogenesis of atherosclerosis. It is now generally accepted that inflammatory mechanisms play a central role in mediating all phases of the development of atherosclerosis [53]. Since several of the potentially anti-atherogenic compounds in our diet have anti-inflammatory properties, their mechanism of action may be inhibition or blocking of the inflammatory processes of atherosclerosis.

Proanthocyanidins have been shown to mediate several anti-inflammatory mechanisms involved in the development of cardiovascular disease. Studies indicate that they, *e.g.*, are implicated in the modulation of the monocyte adhesion in the inflammatory process of atherosclerosis. The TNFα-induced expression of vascular adhesion molecule-1 (VCAM-1), playing a pivotal role in the inflammatory response, was for example shown to be mediated by a grape seed proanthocyanidin extract in human endothelial cells [54]. However, like for the *in vitro* antioxidative effects of proanthocyanidins discussed in Section 3.2, care must be taken when interpreting the effects of proanthocyanidins in cellular systems. Here, the observed effects may result from adherence of the proanthocyanidin molecules to the cellular surface rather than to absorption of the compounds into the cell. It may be difficult to elucidate by which mechanism the compounds exert their effects in a cellular assay, since the polyphenols are known to bind strongly to proteins, and thus also to cellular surface membranes [55, 56]. Thus, as discussed above, observed cardiac-protective effects of oligomeric proanthocyanidins in cell systems may be rather irrelevant for the *in vivo* situation due to the very limited uptake of the intact forms of these large compounds. Any observed effects of proanthocyanidin rich fractions may therefore be due to either cellular surface interaction with the proanthocyanidins or to the absorbance of the low-molecular-weight compounds present in the fraction.

However, some studies have taken the metabolism of the compounds into account. Koga *et al.* [57], *e.g.*, investigated the effects of plasma metabolites of (+)-catechin on the modulation of monocyte adhesion to human aortic endothelial cells, and found that the plasma metabolites of catechin, was potent inhibitors. A very recent study employed another set up to overcome this problem when studying the effects of wine polyphenols on the adhesion of monocytes to endothelial cells [58]. Here, monocytes were isolated from healthy men after 28 days of consumption of either red wine or gin. The TNFα-induced adhesion of the isolated monocytes to an endothelial cell line was then studied, and the red wine consumption virtually abolished the adhesion, whereas it was only partially reduced after gin consumption. Another recent human study confirms the ability of red wine to affect inflammatory markers of atherosclerosis. Estruch *et al.* [59] report on the reduction of a number of inflammatory biomarkers by red wine compared to gin consumption in a randomized crossover trial with 40 healthy men. Among other markers, the adhesion molecules VCAM-1, ICAM-1, interleukin-1α and C-reactive protein were significantly reduced by the intervention with red wine. In a study by Kalin *et al.* [60] the effects of a proanthocyanidin-rich extract was investigated in systemic sclerosis patients having elevated expression of soluble adhesion molecules including ICAM-1, VCAM-1, P- and E-selctin, and the grape seed extract used significantly attenuated the increased expression of these adhesion molecules.

3.4 Improvement of endothelial function

The endothelial function plays an important role in regulating the vascular function, and endothelial dysfunction is associated with increased cardiovascular disease risk. Vascular endothelium synthesizes and releases nitric oxide

(NO), which in turn promotes vasorelaxation (endothelium-dependent), reduces platelet aggregation, and limits the flux of atherogenic plasma proteins into the arterial wall. Animal and human studies have shown that proanthocyanidins may have favorable effects on the vascular endothelial function as recently reviewed by Duffy *et al.* [61] and Dell' Agli *et al.* [10]. *In vivo* red wine polyphenolics were shown to reduce blood pressure in normo- and hypertensive rats [62–64]. The administration of purple grape juice improved the endothelium-dependent, flow-mediated vasodilation in coronary artery disease patients with impaired endothelial function [65]. Red wine was shown to reverse acute endothelial dysfunction caused by saturated fat intake or by cigarette smoking in healthy volunteers [66, 67]. A placebo-controlled 2-week study with chocolate high or low in cocoa flavonoids improved endothelial function and increased epicatechin plasma concentration in healthy subjects [68]. None of these studies were, however, conducted with purified proanthocyanidins, so the observed effects are rather a result of the combination of the phenolic compounds present in the investigated products or extracts.

The investigations of the vasorelaxation of red wine and grape polyphenols are numerous as reviewed by Dell'Agli *et al.* [10], and the results convincingly demonstrate that red wine polyphenols have beneficial vasodilatatory effects. The underlying mechanisms seem to involve an increase of intracellular Ca^{2+} as the critical step for the activation of NO-synthase [69, 70]. Attempts to characterize the active compounds in red wine polyphenolic fractions have only been performed *in vitro* using rat aorta [71–74], and surprisingly show that monomeric catechins and simple phenols (benzoic acid, gallic acid, and hydroxycinnamic acids) are devoid of effect. On the contrary, it was shown that the compounds exhibiting the most endothelium-dependent relaxation were the anthocyanins and the proanthocyanidin trimers, tetramers, pentamers, polymers, and their gallates [71–74]. Bearing in mind the bioavailability of proanthocyanidins (see Section 2.2), these *in vitro* studies seem not to have identified the compounds accountable for the vasodilatory effects *in vivo*, since the oligomeric forms of proanthocyanidins are nonabsorbable. These studies are thus only relevant for therapeutic use of i.v. doses of oligomeric proanthocyanidins. Consequently, the active compounds that are responsible for the observed vasorelaxation of red wine polyphenols thus still remain to be identified and could very well be a combination of the metabolic products of all the different polyphenols present.

Vascular smooth muscle cells are involved in the pathogenesis of atherosclerosis, since their proliferation and migration are critical steps in the progression of the thickening of the aortic intima and the development of arterial sclerosis. In the lesion formed, the release of platelet-derived growth factor (PDGF) by platelets, endothelial or vascular cells is a potent mitogenic and chemotactic agent involved in all phases of the atherogenesis [75]. Rosenkranz *et al.* [75] recently demonstrated that red wine polyphenols, and not white wine polyphenols, are able to inhibit the PDGF receptor and thus abrogate the PDGF-dependent signalling pathways and cellular responses in vascular smooth muscle cells. The authors furthermore reported, that when enriching the white wine with grape seed polyphenols, mimicking the mash fermentation of red wine, inhibition of PDGF was also observed. This suggests that the mash fermentation of red wine results in the extraction of the active compounds from grape seeds and skin. The differences in the phenolic profiles in the wines employed was investigated and showed that red wine contained far more proanthocyanidins, gallic acid, and catechins [75]. Other studies on red wine polyphenols have been thoroughly reviewed by Dell'Agli *et al.* [10].

3.5 Platelet aggregation

Aggregation of platelets is known to contribute to the development of atherosclerosis by several mechanisms [76] and the inhibition of platelet aggregation is thus regarded as beneficial. Platelets produce the pro-inflammatory mediators, such as thromboxane A2, PAF, and serotonin, and are thus key participants in the atherogenesis [77]. Recent studies on the polyphenols in cocoa have shown that epicatechin and proanthocyanidins have potent anti-inflammatory properties [78]. The low-molecular weight-proanthocyanidins and epicatechin itself were shown to be potent inhibitors of human 5-lipoxygenase [79] and proanthocyanidins from cocoa were demonstrated to decrease platelet function significantly *in vivo* in humans [80]. Furthermore, another study showed that the combination of quercetin and catechin synergistically inhibited platelet function in collagen-induced platelet aggregation by antagonizing the intracellular production of hydrogen peroxide [81].

Several studies have reported beneficial effects of red wine and other polyphenol rich fractions from grapes on platelet aggregation as reviewed by Ruf [82], and Santos-Buelga and Scalbert [2]. Other cardioprotective effects of proanthocyanidins have additionally been reviewed by Bagchi *et al.* [83], Reed *et al.* [84], Steinberg *et al.* [85], and Yilmaz *et al.* [86]. Keevil *et al.* [87] found reduced platelet aggregation after one week of treatment with red grape juice, but not after treatment with orange or grapefruit juice. Further indications of beneficial effects of proanthocyanidin-rich foods were observed by Folts [88] who found inhibitory effects on platelet aggregation in dogs and humans by red wine and red grape juice, but not by white wine. In rats, red wine and alcohol-free red wine increased the bleeding time and reduced the thrombus weight as well as the platelet adhesion to collagen [89]. No effect was

observed with either white wine or ethanol. Furthermore, administration of red wine or grape juice intragastrically eliminated the cyclic flow reductions in the stenosed coronary arteries of dogs and also inhibited the aggregation of platelets measured *ex vivo* after induction with collagen [90]. Again, white wine showed no effect. In rats, red wine, but not white wine or ethanol, inhibited the rebound effect of thrombin-induced platelet aggregation 18 h after withdrawing the alcoholic beverage from the diet [91]. Grape juice, but not orange or grapefruit juice fed to monkeys for 7 days inhibited the collagen-induced *ex vivo* platelet aggregation [92]. Finally, a crude preparation of grape polyphenols fed to rats inhibited the thrombin-induced platelet aggregation [93]. All these animal data demonstrate that red wine polyphenols are able to inhibit platelet aggregation.

4 Pycnogenol, a proanthocyanidin-rich extract from French maritime pine

Although this review focuses on the dietary proanthocyanidins, we find it relevant to include an overview of the literature on the dietary supplement Pycnogenol, since a large part of the present proanthocyanidin studies are performed with this extract. Pycnogenol is a standardized water extract of the bark from the French maritime pine, *Pinus maritime*, containing polyphenolic monomers (catechin, epicatechin, and taxifolin), proanthocyanidins and phenolic acids (*p*-hydroxybenzoic acid, caffeic acid, and ferulic acid) [94]. Pycnogenol has been claimed to have numerous health beneficial properties, *e.g.*, increase the body's resistance to inflammation, protect against blood vessel and skin damage, improve the general function of the brain, relieve the distresses of arthritis, diabetes, and stroke, relieve premenstrual symptoms, act as a powerful antioxidant, and work synergistically with vitamin C, protecting it from degradation [94 – 96]. The majority of the studies published to date are, however, *in vitro* or cell culture studies. Studies on the bioavailability of the components in Pycnogenol have been very limited, only a single study has demonstrated that ferulic acid is excreted into urine after ingestion of 200 mg pinebark extract [97]. The potential of Pycnogenol to protect against cardiovascular diseases has been reported in several *in vitro* studies using cellular systems or *ex vivo* oxidation studies [98 – 105]. However, the number of human studies attempting to confirm these results have been very limited [106 – 109], and only few are well conducted with, *e.g.*, high numbers of subjects, placebo-control, blinding, and randomization of treatments and groups. Some of these studies show conflicting results, it has, *e.g.*, been shown that Pycnogenol acts as an antioxidant *in vivo* by significantly increasing the oxygen radical absorbance capacity (ORAC) in plasma [110], whereas another placebo-controlled study was unable to confirm these results [111]. The latter study was also unable to confirm earlier *in vitro* results indicating an ability of Pycnogenol to protect and regenerate vitamin C stores [112]. Furthermore, some of the studies show that Pycnogenol is able to improve the endothelial function of hypertensive patients [108], and to inhibit platelet aggregation [107], whereas a study on the modification of blood pressure in mildly hypertensive patients failed to show any effects different from that of the placebo treatment [109]. In a study by Silliman *et al.* [111] on the antioxidant potential of Pycnogenol it was concluded that, determined as ORAC units, the daily dose of 200 mg Pycnogenol only compares to about a single serving of fruits or vegetables, and the authors of this study therefore question the superior health effects of Pycnogenol compared to a diet rich in fruits and vegetables.

5 Effect of proanthocyanidins on atherosclerosis development in animal models

The effect of oral doses of proanthocyanidins on atherosclerosis as end-point has only been investigated very recently, and the studies are limited to grape proanthocyanidins either provided as wine, grape juice, or a grape/wine extract. A few studies have been conducted in hamsters fed an atherogenic diet rich in saturated fat and cholesterol, resulting in hypercholesterolemic hamsters with a similar lipid profile as hypercholesterolemic humans. Auger *et al.* [113] used this model to investigate the early effects on atherosclerosis of a red wine phenolic extract containing in total 471 mg/g phenols (expressed as gallic acid equivalents) including: 17.3 mg/g (epi)catechin, 36.3 mg/g dimeric proanthocyanidins, 12.4 mg/g anthocyanins, and 20 mg/g phenolic acids. The hamsters were provided the phenolic extract in either ethanol or water and with ethanol and water as the respective controls. This revealed that after 8 weeks, both groups receiving the phenolic extract with or without ethanol, had lowered plasma cholesterol and triglycerides, and aortic fatty streak area was reduced by 32% and 29%, respectively, in comparison with their respective controls. Also ethanol it self was observed to significantly reduce fatty streak area suggesting that ethanol is a complementary component of phenolics in the health benefits of red wine [113]. Another study in hamsters compared the effect of red wine, dealcoholized red wine, and grape juice on atherosclerosis, and here it was on the contrary observed, that when compared with dealcoholized wine and normalized to polyphenol dose, red wine's beneficial effects could be attributed entirely to the polyphenols [114]. Grape juice was calculated to be much more effective than red wine or dealcoholized red wine at the same polyphenol dose in inhibiting atherosclerosis and improving lipids and antioxidant

parameters. This study thus suggests that grape juice or nonalcoholic red wine are an excellent alternative to red wine in this model of atherosclerosis [114]. A grape seed proanthocyanidin-rich extract has also been investigated in the hamster model for atherosclerosis, and here the atherosclerosis was reduced by 50% and 63%, after dosing for 10 weeks with 50 mg/kg and 100 mg/kg, respectively [115].

However, a study in apolipoprotein E-deficient mice failed to show any protective effects against mature atherosclerosis by red wine or red wine powder in water [116], nor were there any differences in plaque stability measured as the collagen content between the groups receiving red wine or their respective control groups. Nevertheless, three other recent studies in apolipoprotein E deficient mice conducted with either red wine or dealcoholized red wine confirmed the protection against atherosclerosis in aorta by the red wine polyphenols [117 – 119]. In the study by Waddington *et al.* [118] markers of fatty acid peroxidation, such as the F2-isoprostanes and hydroxyeicosatetraenoic acids, were also determined. Red wine polyphenols had, however, no effect on these markers of lipid peroxidation, which led the authors to conclude that the ability of polyphenols to reduce aortic lipid deposition may be independent of the inhibition of lipid peroxidation in this animal model. On the contrary, a study by Aviram and Fuhrman [52] showed a 40% reduction in the LDL oxidation demonstrating that the red wine polyphenols are able to protect the lipoproteins from oxidation as also shown in numerous *in vitro* and *ex vivo* studies as discussed in Section 3.2.

A single study has investigated the effect of grape polyphenols in ovariectomized Guinea pigs, a model for postmenopausal women, and also here atherosclerosis-lowering effects were observed of the grape preparation used [120]. Plasma triglycerides and very-low-density lipoprotein (VLDL) cholesterol were 39% and 50% lower, respectively, in the pigs fed the grape diet compared with controls, and concentrations of cholesterol in the aorta were 33% lower in Guinea pigs fed the grape diet. In addition, hepatic acyl CoA : cholesteryl acyltransferase activity was 27% lower in the grape diet-fed group compared with controls. The authors conclude that in ovariectomized Guinea pigs, grape intake apparently alters the hepatic cholesterol metabolism, which may affect VLDL secretion rates and result in less accumulation of cholesterol in the aorta [120].

The effect of purified proanthocyanidins on the development of atherosclerosis still remains to be investigated. One could speculate that since a major part of the proanthocyanidins are metabolized into simple phenolic acids in the intestine, studies on phenolic acids may give a hint about the effects of purified proanthocyanidins on atherosclerosis. Auger *et al.* [121] studied the effect of hydroxycinnamic acids, *i. e.*, caffeic acid and sinapic acid present in wine and chlorogenic acid present in apple compared to a red wine phenolic extract in the hypercholesterolemic hamster model. Plasma cholesterol concentration was lower in the group that received red wine phenolic extract (–22%) whereas the hydroxycinnamic acids had no effect. Consumption of red wine phenolic extract decreased Apo-B concentration (-46%) and significantly reduced aortic fatty streak area (–30%) in comparison with controls and hydroxycinnamic acids. This demonstrates that chronic ingestion of the nonalcoholic components of red wine, mainly polyphenols, prevent the development of atherosclerosis in hamster and that wine hydroxycinnamic acids are not the phenolic compounds involved in this beneficial effect. The phenolic acid metabolites produced in the gut by metabolism of proanthocyanidins are, however, somewhat structurally different from the hydroxycinnamates investigated in this study.

The catechins are the monomeric forms of proanthocyanidins and some studies suggest that the oligomers can be degraded into the monomeric forms, whereas others do not report this finding [24]. Nevertheless, the monomeric catechin content in proanthocyanidin-rich foods is substantial (see Table 1 and Fig. 2), so the beneficial effects of these foods may also be due to the high catechin content. The studies on atherosclerosis prevention of pure catechins is also very limited but at single study on tea catechins, provided as a tea extract from green tea containing primarily epigallocatechin gallate and epicatechin, has been performed in apolipoprotein E-deficient mice. This study showed a protective effect of the catechins present in tea [122], which are in part structurally different from those found in wine, due to the various gallic acid substituents. The tea ingestion did, however, not influence plasma cholesterol or triglyceride concentrations. The plasma lipid peroxides were reduced in the tea group at week 8, suggesting that the *in vivo* oxidative state was improved by the tea. Atheromatous areas in the aorta was also significantly attenuated in the tea group compared with the control group, and aortic cholesterol and triglyceride contents were 27% and 50% lower, respectively, in the tea group [122]. These results suggest that catechins may be important contributors to the anti-atherogenic effects observed of proanthocyanidin-rich foods, due to the concomitant content of catechins in these products. Further studies on purified proanthocyanidins, monomeric catechins and their metabolites, the simple phenolic acids, are therefore greatly warranted, and will provide more insight in the nature of the beneficial phenolic constituents in grapes.

All animal studies reported on proanthocyanidins and atherosclerosis development are limited to few different animal models, so very little information on the mechanisms of protection is gained from these studies. Within the past years, hundreds of inbred atherosclerosis mouse mod-

els have been established, with relatively well-defined genetic maps facilitating genetic experimentation. These animal models are designed to be preliminary tools for a better understanding of the pathogenesis, mechanisms of prevention, and therapy of arteriosclerosis in humans [123]. Future studies implicating different animal models may thus provide new important information on the mechanisms underlying the prevention of atherosclerosis development by proanthocyanidin-rich foods and extracts.

6 Prevention of cardiovascular disease

Only few and all very recent human intervention studies have been conducted with proanthocyanidins to investigate the preventive effects on risk markers associated with development of cardiovascular disease. These studies support a beneficial effect of proanthocyanidin-rich foods or extracts but none have been conducted with pure proanthocyanidins. Two studies investigated the effects of grape seed extracts, one in hypercholesterolemic subjects provided grape seed extract, niacin-bound chromium or a combination of both or placebo. This study showed that the combination of grape seed extract and chromium decreased total cholesterol and LDL levels, and a trend to decrease oxidized LDL was also observed [124]. In the other study, Vigna *et al.* [125] studied the antioxidative potential of a grape seed extract in heavy smokers, a model of oxidative stress, and found that the oxidative measures, thiobarbituric acid reactive substances (TBARS) and the lag phase for LDL oxidation was significantly reduced. In this study, no effects on cholesterol or plasma lipoproteins were observed.

The effects of cocoa products have also been investigated recently in two human intervention studies. Cocoa corresponding to ~651 mg proanthocyanidins/day was provided to healthy subjects for 6 weeks [126]. Compared to the baseline, LDL oxidizability was reduced but no effect on urinary F_2-isoprostanes or markers of inflammation (cytokines, interleukin-1 β, interleukin-6, TNFα, C-reactive protein, and *p*-selectin) was observed. However, when inducing oxidative stress by strenuous physical exercise, as was done in the study by Wiswedel *et al.* [127], cocoa was found to lower the lipid peroxidation determined as the plasma level of F_2-isoprostanes. Thus, in subjects prone to oxidative stress, proanthocyanidin-rich products exert antioxidative properties, supporting that intake of proanthocyanidin- and catechin-rich foods may lower the risk of developing cardiovascular diseases.

The association between risk of cardiovascular diseases and proanthocyanidin consumption has never been examined in epidemiological studies but several studies have investigated the relationship between intake of proanthocyanidin-rich foods, like wine and tea, on cardiovascular diseases. The early studies by Hertog *et al.* [19, 128, 129] showed a highly protective effect of tea consumption against cardiovascular disease, but some of the later and larger cohort studies, trying to confirm these early studies, found no association or even aggravating effects of tea intake [130 – 133]. The association of tea and incidences of cardiovascular diseases was later further investigated in several cohort studies. These studies have all been reviewed in a recent meta-analysis investigating the relationship between tea consumption and stroke, myocardial infarction, and all coronary heart disease in ten cohort studies and seven case-control studies [134]. The incidence rate of myocardial infarction was concluded to be weakly inversely associated (11%) with a tea consumption of three cups per day. However, the authors stress that the heterogeneity of the studies and the risk of bias due to the larger number of smaller studies showing a protective effect urge caution in interpreting this result. A recent study on the mechanisms of the protective effect of tea does, however, support a beneficial effect of tea intake, since consumption of 900 mL black tea for 4 weeks was shown to reverse the endothelial vasomotor dysfunction in patients with proven coronary artery disease. Several researchers suggested that the catechin content in tea could be the protective factor. Arts *et al.* [135] thus estimated the catechin intake to 72 ± 47.8 mg/day in the Zutphen Elderly Study and found a significant negative association between ischemic heart disease and intake of tea catechins. However, in another study on catechin intake by the same author with postmenopausal women from Iowa, there was only seen a protective effect of catechins from other dietary sources than tea [136].

The association between intake of red wine and cardiovascular diseases has also been investigated in several epidemiological studies, and several of these studies support that wine drinkers have a lower mortality from cardiovascular disease than others [1, 5, 7, 137]. Other cohort studies have, however, found equally beneficial effects of all alcoholic beverages, and there is no general agreement on this matter as stated in the review by Rimm *et al.* [4] and recently by Ruf [138]. It has been proposed that the possible lower mortality by cardiovascular disease in wine drinkers could be due in part to differences in lifestyle, *e.g.*, in dietary habits and exercise, since factors, such as dietary fat composition, little exercise, and hypertension, also are major risk factors on the development of atherosclerosis [139].

7 Conclusions

As a consequence of the 'French paradox', researchers have searched for potentially health-protective compounds in red wine for the past decade. Only very recently, it was sug-

gested that the proanthocyanidin content in red wine could be explanatory of the 'French paradox', and focus was turned to these complex polyphenols that originate from the grape seed and skin and are extracted into the red wine during the mash fermentation. Numerous *in vitro* and animal studies have demonstrated a cardioprotective potential of these compounds.

Knowledge on both the intake and bioavailability of proanthocyanidins in humans are, however, limited and have only very recently been investigated by few research groups owing to analytical limits imposed by the complexity of their chemical structures. The available studies indicate that the bioavailability of the polymerized proanthocyanidins is very limited, and that the biological active compounds rather should be sought in the monomeric forms of the proanthocyanidins, the catechins, and in the pool of low-molecular-weight metabolic products generated by degradation of the proanthocyanidins in the intestine. More studies on the bioavailability of proanthocyanidins are, however, necessary to definitively establish the mechanisms of absorption and metabolism of these highly complex compounds. The indications of a limited bioavailability of the oligomeric and polymeric forms of the proanthocyanidins do, however, advocate caution when interpreting *in vitro* studies conducted in the past, demonstrating biological and antioxidative effects of high-molecular-weight proanthocyanidins.

Several animal studies have nevertheless confirmed the *in vitro* indications of a cardioprotective potential of the proanthocyanidins, and have, *e.g.*, shown significantly decreases in plasma cholesterol levels and of the extent of atherosclerosis after feeding with proanthocyanidins. Only few human studies have yet been conducted but they indicate an ability of proanthocyanidin-rich extracts to lower the oxidation of LDL. Epidemiological studies on the association between intake of proanthocyanidins and risk of cardiovascular diseases are lacking but the recently reported surveys of the content of proanthocyanidins in foods form the basis for future studies on this matter. Furthermore, the cardioprotective properties of purified proanthocyanidins also still remain to be investigated. Such studies are greatly warranted to determine the biological effects *in vivo* of the proanthocyanidins *per se* instead of the poorly defined polyphenol mixtures contained in the majority of the proanthocyanidin-rich extracts or foods used until now in animal and human studies on proanthocyanidins. Several questions thus await to be answered before the complete picture of the role of proanthocyanidins in dietary disease protection is resolved but at present the evidence for a cardioprotective potential of proanthocyanidin-rich foods or extracts seems promising.

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8 References

[1] Renaud, S., de Lorgeril, M., Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* 1992, *339*, 1523 – 1526.

[2] Santos-Buelga, C., Scalbert, A., Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.* 2000, *80*, 1094 – 1117.

[3] Porter, L. J., Flavans and proanthocyanidins, in: Harborne J. B. (Ed.), *The Flavonoids – Advances in Research since 1986*, Chapman and Hall, London 1994, pp. 23 – 55.

[4] Rimm, E. B., Klatsky, A., Grobbee, D., Stampfer, M. J., Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits. *BMJ* 1996, *312*, 731 – 736.

[5] Gronbaek, M., Deis, A., Sorensen, T. I., Becker, U., Schnohr, P., Jensen, G., Mortality associated with moderate intakes of wine, beer, or spirits. *BMJ* 1995, *310*, 1165 – 1169.

[6] Constant, J., Alcohol, ischemic heart disease, and the French paradox. *Coronary Artery Disease* 1997, *8*, 645 – 649.

[7] Theobald, H., Bygren, L. O., Carstensen, J., Engfeldt, P., A moderate intake of wine is associated with reduced total mortality and reduced mortality from cardiovascular disease. *J. Stud. Alcohol* 2000, *61*, 652 – 656.

[8] Rasmussen, S. E., Functional foods, cardiovascular disease and diabetes, in: Arnoldi, A. (Ed.), *Flavonoids and Cardiovascular disease.* Woodhead Publishing, 2004.

[9] Pervaiz, S., Resveratrol: from grapevines to mammalian biology. *FASEB J.* 2003, *17*, 1975 – 1985.

[10] Dell'Agli, M., Busciala, A., Bosisio, E., Vascular effects of wine polyphenols. *Cardiovasc. Res.* 2004, in press.

[11] Williams, C. A., Grayer, R. J., Anthocyanins and other flavonoids. *Nat. Prod. Rep.* 2004, *21*, 539 – 573.

[12] Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., *et al.*, Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* 2003, *51*, 7513 – 7521.

[13] Pascual-Teresa, S., Santos-Buelga, C., Rivas-Gonzalo, J. C., Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. *J. Agric. Food Chem.* 2000, *48*, 5331 – 5337.

[14] Gu, L., Kelm, M. A., Hammerstone, J. F., Zhang, Z., *et al.*, Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. *J. Mass Spectrom.* 2003, *38*, 1272 – 1280.

[15] Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., *et al.*, Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Nutr.* 2004, *134*, 613 – 617.

[16] Auger, C., Al Awwadi, N., Bornet, A., Rouanet, J. M., *et al.*, Catechins and procyanidins in Mediterranean diets. *Food Res. Int.* 2004, *37*, 233 – 245.

[17] Knekt, P., Kumpulainen, J., Jarvinen, R., Rissanen, H., *et al.*, Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.* 2002, *76*, 560 – 568.

[18] Geleijnse, J. M., Launer, L. J., Van der Kuip, D. A., Hofman, A., Witteman, J. C., Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. *Am. J. Clin. Nutr.* 2002, *75*, 880 – 886.

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

[20] Deprez, S., Mila, I., Huneau, J. F., Tome, D., Scalbert, A., Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxid. Redox. Signal.* 2001, *3*, 957 – 967.

[21] Spencer, J. P. E., Chaudry, F., Pannala, A. S., Srai, S. K., *et al.*, Decomposition of Cocoa Procyanidins in the Gastric Milieu. *Biochem. Biophys. Res. Commun.* 2000, *272*, 236 – 241.

[22] Donovan, J. L., Manach, C., Rios, L., Morand, C., *et al.*, Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B3. *Br. J. Nutr.* 2002, *87*, 299 – 306.

[23] Rios, L. Y., Bennett, R. N., Lazarus, S. A., Remesy, C., *et al.*, Cocoa procyanidins are stable during gastric transit in humans. *Am. J. Clin. Nutr.* 2002, *76*, 1106 – 1110.

[24] Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L., Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 2004, *79*, 727 – 747.

[25] Deprez, S., Brezillon, C., Rabot, S., Philippe, C., *et al.*, Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J. Nutr.* 2000, *130*, 2733 – 2738.

[26] Gonthier, M. P., Donovan, J. L., Texier, O., Felgines, C., *et al.*, Metabolism of dietary procyanidins in rats. *Free Radic. Biol. Med.* 2003, *35*, 837 – 844.

[27] Rios, L. Y., Gonthier, M. P., Remesy, C., Mila, I., *et al.*, Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am. J. Clin. Nutr.* 2003, *77*, 912 – 918.

[28] Holt, R. R., Lazarus, S. A., Sullards, M. C., Zhu, Q. Y., *et al.*, Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. *Am. J. Clin. Nutr.* 2002, *76*, 798 – 804.

[29] Baba, S., Osakabe, N., Natsume, M., Terao, J., Absorption and urinary excretion of procyanidin B2 [epicatechin-(4[beta]-8)-epicatechin] in rats. *Free Radic. Biol. Med.* 2002, *33*, 142 – 148.

[30] Gee, J. M., DuPont, M. S., Day, A. J., Plumb, G. W., *et al.*, Intestinal transport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway. *J. Nutr.* 2000, *130*, 2765 – 2771.

[31] Nielsen, S. E., Breinholt, V., Justesen, U., Cornett, C., Dragsted, L. O., *In vitro* biotransformation of flavonoids by rat liver microsomes. *Xenobiotica* 1998, *28*, 389 – 401.

[32] Nielsen, S. E., Breinholt, V., Cornett, C., Dragsted, L. O., Biotransformation of the citrus flavone tangeretin in rats. Identification of metabolites with intact flavane nucleus. *Food Chem. Toxicol.* 2000, *38*, 739 – 746.

[33] Zhu, B. T., Ezell, E. L., Liehr, J. G., Catechol-*O*-methyltransferase-catalyzed rapid *O*-methylation of mutagenic flavonoids. *J. Biol. Chem.* 1994, *269*, 292 – 299.

[34] Cremin, P., Kasim-Karakas, S., Waterhouse, A. L., LC/ES-MS detection of hydroxycinnamates in human plasma and urine. *J. Agric. Food Chem.* 2001, *49*, 1747 – 1750.

[35] Eyre, H., Kahn, R., Robertson, R. M., the ACS/ADA/AHA Collaborative Writing Committee, ACS/ADA/AHA Collaborative Writing Committee, Clark, N. G., Doyle, C., Hong, Y., Gansler, T., Glynn, T., Smith, R. A., Taubert, K., Thun, M. J., Preventing Cancer, Cardiovascular Disease, and Diabetes: A Common Agenda for the American Cancer Society, the American Diabetes Association, and the American Heart Association. *Circulation* 2004, *109*, 3244 – 3255.

[36] Thompson, G. R., Pathogenesis of atherosclerosis, in: *A Handbook of Hyperlipidemia*, Second Edition, Current Science Ltd., London 1994, pp. 87 – 99.

[37] Steinberg, D., Low density lipoprotein oxidation and its pathobiological significance. *J. Biol. Chem.* 1997, *272*, 20963 – 20966.

[38] Brown, M. S., Goldstein, J. L., Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Rev. Biochem.* 1983, *52*, 223 – 261.

[39] Fuster, V., Lewis, A., Conner Memorial Lecture. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. *Circulation* 1994, *90*, 2126 – 2146.

[40] Kehrer, J. P., Smith, C. V., Free radicals in biology: sources, reactivities, and roles in the etiology of human diseases, in: Frei, B. (Ed.), *Natural Oxidants*, Academic Press, New York 1994, pp. 25 – 62.

[41] Gutteridge, J. M. C., Halliwell, B., *Antioxidants in Nutrition, Health, and Disease*, Oxford University Press, Oxford 1994.

[42] Jovanovic, S. V., Steenken, S., Simic, M. G., Hara, Y., Antioxidant properties of flavonoids: reduction potentials and electron transfer reactions of flavonoid radicals, in: Rice-Evans, C., Packer, L. (Eds.), *Flavonoids in Health and Disease*. Marcel Dekker, New York 1998, pp. 137 – 161.

[43] Bors, W., Saran, M., Radical scavenging by flavonoid antioxidants. *Free Radic. Res. Commun.* 1987, *2*, 289 – 294.

[44] Lotito, S. B., Actis-Goretta, L., Renart, M. L., Caligiuri, M., *et al.*, Influence of Oligomer Chain Length on the Antioxidant Activity of Procyanidins. *Biochem. Biophys. Res. Commun.* 2000, *276*, 945 – 951.

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

[46] Porter, M. L., Krueger, C. G., Wiebe, D. A., Cunningham, D. G., Reed, J. D., Cranberry proanthocyanidins associate with low-density lipoproteins and inhibit *in vitro* Cu^{2+}-induced oxidation. *J. Sci. Food Agric.* 2001, *81*, 1306 – 1313.

[47] da Silva Porto, P. A. L., Laranjinha, J. A. N., de Freitas, V. A. P., Antioxidant protection of low density lipoprotein by procyanidins: structure/activity relationships. *Biochem. Pharmacol.* 2003, *66*, 947 – 954.

[48] Kromhout, D., Diet and cardiovascular diseases. *J. Nutr. Health Aging* 2001, *5*, 144 – 149.

[49] World Health Organization, Natural Antioxidants and Lipid Peroxidation in Atherosclerosis and Cancer. *Alpac*, Helsinki, Finland, Aug. 22 – 25, 1995.

[50] Weisburger, J. H., Chemopreventive effects of cocoa polyphenols on chronic diseases. *Exp. Biol. Med. (Maywood)* 2001, *226*, 891 – 897.

[51] Fuller, C. J., Jialal, I., Effects of antioxidants and fatty acids on low-density-lipoprotein oxidation. *Am. J. Clin. Nutr.* 1994, *60*, 1010S – 1013S.

[52] Aviram, M., Fuhrman, B., Wine flavonoids protect against LDL oxidation and atherosclerosis. *Ann. N. Y. Acad. Sci.* 2002, *957*, 146 – 161.

[53] Blake, G. J., Ridker, P. M., Inflammatory biomarkers and cardiovascular risk prediction. *J. Int. Med.* 2002, *252*, 283 – 294.

[54] Sen, C. K., Bagchi, D., Regulation of inducible adhesion molecule expression in human endothelial cells by grape seed proanthocyanidin extract. *Mol. Cell Biochem.* 2001, *216*, 1 – 7.

[55] Walle, T., Vincent, T. S., Walle, U. K., Evidence of covalent binding of the dietary flavonoid quercetin to DNA and protein in human intestinal and hepatic cells. *Biochem. Pharmacol.* 2003, *65*, 1603 – 1610.

[56] Hatano, T., Hori, M., Hemingway, R. W., Yoshida, T., Size exclusion chromatographic analysis of polyphenol-serum albumin complexes. *Phytochemistry* 2003, *63*, 817 – 823.

[57] Koga, T., Meydani, M., Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells. *Am. J. Clin. Nutr.* 2001, *73*, 941 – 948.

[58] Badia, E., Sacanella, E., Fernandez-Sola, J., Nicolas, J. M., *et al.*, Decreased tumor necrosis factor-induced adhesion of human monocytes to endothelial cells after moderate alcohol consumption. *Am. J. Clin. Nutr.* 2004, *80*, 225 – 230.

[59] Estruch, R., Sacanella, E., Badia, E., Antunez, E., *et al.*, Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial: Effects of wine on inflammatory markers. *Atherosclerosis* 2004, *175*, 117 – 123.

[60] Kalin, R., Righi, A., Del Rosso, A., Bagchi, D., *et al.*, Activin, a grape seed-derived proanthocyanidin extract, reduces plasma levels of oxidative stress and adhesion molecules (ICAM-1, VCAM-1 and E-selectin) in systemic sclerosis. *Free Radic. Res.* 2002, *36*, 819 – 825.

[61] Duffy, S. J., Vita, J. A., Effects of phenolics on vascular endothelial function. *Curr. Opin. Lipidol.* 2003, *14*, 21 – 27.

[62] Mizutani, K., Ikeda, K., Kawai, Y., Yamori, Y., Extract of wine phenolics improves aortic biomechanical properties in stroke-prone spontaneously hypertensive rats (SHRSP). *J. Nutr. Sci. Vitaminol.* 1999, *45*, 95 – 106.

[63] Diebolt, M., Bucher, B., Andriantsitohaina, R., Wine polyphenols decrease blood pressure, improve NO vasodilatation, and induce gene expression. *Hypertension* 2001, *38*, 159 – 165.

[64] Bernatova, I., Pechanova, O., Babal, P., Kysela, S., *et al.*, Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. *Am. J. Physiol. Heart Circul. Physiol.* 2002, *282*, H942 – H948.

[65] Stein, J. H., Keevil, J. G., Wiebe, D. A., Aeschlimann, S., Folts, J. D., Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* 1999, *100*, 1050 – 1055.

[66] Koulouris, S., Marousis, P., Karabinos, I., Triantafyllou, K., *et al.*, W12.300 Saturated fat administration in healthy volunteers is associated with postprandial endothelial dysfunction which is totally reversed by the concomitant intake of red wine. *Atherosclerosis Suppl.* 2004, *5*, 69 – 70.

[67] Papamichael, C., Karatzis, E., Karatzi, K., Aznaouridis, K., *et al.*, Red wine's antioxidants counteract acute endothelial dysfunction caused by cigarette smoking in healthy nonsmokers. *Am. Heart J.* 2004, *147*, 274.

[68] Engler, M. B., Engler, M. M., Chen, C. Y., Malloy, M. J., *et al.*, Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J. Am. Coll. Nutr.* 2004, *23*, 197 – 204.

[69] Andriambeloson, E., Stoclet, J. C., Andriantsitohaina, R., Mechanism of endothelial nitric oxide-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. *J. Cardiovasc. Pharmacol.* 1999, *33*, 248 – 254.

[70] Martin, S., Andriambeloson, E., Takeda, K., Andriantsitohaina, R., Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signalling pathways leading to nitric oxide production. *Brit. J. Pharmacol.* 2002, *135*, 1579 – 1587.

[71] Fitzpatrick, D. F., Bing, B., Maggi, D. A., Fleming, R. C., O'Malley, R. M., Vasodilating procyanidins derived from grape seeds. *Ann. N. Y. Acad. Sci.* 2002, *957*, 78 – 89.

[72] Andriambeloson, E., Magnier, C., Haan-Archipoff, G., Lobstein, A., *et al.*, Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. *J. Nutr.* 1998, *128*, 2324 – 2333.

[73] Andriambeloson, E., Kleschyov, A. L., Muller, B., Beretz, A., *et al.*, Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. *Brit. J. Pharmacol.* 1997, *120*, 1053 – 1058.

[74] Fitzpatrick, D. F., Fleming, R. C., Bing, B., Maggi, D. A., O'Malley, R. M., Isolation and characterization of endothelium-dependent vasorelaxing compounds from grape seeds. *J. Agric. Food Chem.* 2000, *48*, 6384 – 6390.

[75] Rosenkranz, S., Knirel, D., Dietrich, H., Flesch, M., *et al.*, Inhibition of the PDGF receptor by red wine flavonoids provides a molecular explanation for the "French paradox". *FASEB J.* 2002, *16*, 1958 – 1960.

[76] Fuster, V., Badimon, L., Badimon, J. J., Chesebro, J. H., The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N. Engl. J. Med.* 1992, *326*, 242 – 250.

[77] Ross, R., The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993, *362*, 809.

[78] Steinberg, F. M., Bearden, M. M., Keen, C. L., Cocoa and chocolate flavonoids: implications for cardiovascular health. *J. Am. Diet. Assoc.* 2003, *103*, 215 – 223.

[79] Schewe, T., Kuhn, H., Sies, H., Flavonoids of cocoa inhibit recombinant human 5-lipoxygenase. *J. Nutr.* 2002, *132*, 1825 – 1829.

[80] Murphy, K. J., Chronopoulos, A. K., Singh, I., Francis, M. A., *et al.*, Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. *Am. J Clin. Nutr.* 2003, *77*, 1466 – 1473.

[81] Pignatelli, P., Pulcinelli, F. M., Celestini, A., Lenti, L., *et al.*, The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. *Am. J. Clin. Nutr.* 2000, *72*, 1150 – 1155.

[82] Ruf, J. C., Wine and polyphenols related to platelet aggregation and atherothrombosis. *Drugs Exp. Clin Res.* 1999, *25*, 125 – 131.

[83] Bagchi, D., Sen, C. K., Ray, S. D., Das, D. K., *et al.*, Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. *Mutat. Res.* 2003, *523 – 524*, 87 – 97.

[84] Reed, J., Cranberry flavonoids, atherosclerosis and cardiovascular health. *Crit. Rev. Food Sci. Nutr.* 2002, *42*, 301 – 316.

[85] Steinberg, F. M., Bearden, M. M., Keen, C. L., Cocoa and chocolate flavonoids: Implications for cardiovascular health. *J. Am. Diet. Assoc.* 2003, *103*, 215 – 223.

[86] Yilmaz, Y., Toledo, R. T., Health aspects of functional grape seed constituents. *Trends Food Sci. Technol.* 2004, in press.

[87] Keevil, J. G., Osman, H. E., Reed, J. D., Folts, J. D., Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. *J. Nutr.* 2000, *130*, 53 – 56.

[88] Folts, J. D., Antithrombotic potential of grape juice and red wine for preventing heart attacks. *Pharmaceut. Biol.* 1998, *36*, suppl. 1, 21 – 27.

[89] Wollny, T., Aiello, L., Di Tommaso, D., Bellavia, V., *et al.*, 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.

[90] Demrow, H. S., Slane, P. R., Folts, J. D., Administration of wine and grape juice inhibits *in vivo* platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation* 1995, *91*, 1182 – 1188.

[91] Ruf, J. C., Berger, J. L., 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.

[92] Osman, H. E., Maalej, N., Shanmuganayagam, D., Folts, J. D., Grape juice but not orange or grapefruit juice inhibits platelet activity in dogs and monkeys. *J. Nutr.* 1998, *128*, 2307 – 2312.

[93] Xia, J., Allenbrand, B., Sun, G. Y., Dietary supplementation of grape polyphenols and chronic ethanol administration on LDL oxidation and platelet function in rats. *Life Sci.* 1998, *63*, 383 – 390.

[94] Rohdewald, P., A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. *Int. J. Clin. Pharmacol. Ther.* 2002, *40*, 158 – 168.

[95] Schonlau, F., Rohdewald, P., Pycnogenol for diabetic retinopathy. A review. *Int. Ophthalmol.* 2001, *24*, 161 – 171.

[96] Packer, L., Rimbach, G., Virgili, F., Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (Pinus maritima) bark, pycnogenol. *Free Radic. Biol. Med.* 1999, *27*, 704 – 724.

[97] Virgili, F., Pagana, G., Bourne, L., Rimbach, G., *et al.*, Ferulic acid excretion as a marker of consumption of a French maritime pine (Pinus maritima) bark extract. *Free Radic. Biol. Med.* 2000, *28*, 1249 – 1256.

[98] Bayeta, E., Lau, B. H. S., Pycnogenol inhibits generation of inflammatory mediators in macrophages. *Nutr. Res.* 2000, *20*, 249 – 259.

[99] Bito, T., Roy, S., Sen, C. K., Packer, L., Pine bark extract pycnogenol downregulates IFN-gamma-induced adhesion of T cells to human keratinocytes by inhibiting inducible ICAM-1 expression. *Free Radic. Biol. Med.* 2000, *28*, 219 – 227.

[100] Liu, F. J., Zhang, Y. X., Lau, B. H., Pycnogenol enhances immune and haemopoietic functions in senescence-accelerated mice. *Cellular And Molecular Life Sciences: CMLS* 1998, *54*, 1168 – 1172.

[101] Rong, Y., Li, L., Shah, V., Lau, B. H., Pycnogenol protects vascular endothelial cells from t-butyl hydroperoxide induced oxidant injury. *Biotechnol. Therapeut.* 1994, *5*, 117 – 126.

[102] 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.

[103] Cho, K. J., Yun, C. H., Yoon, D. Y., Cho, Y. S., *et al.*, Effect of Bioflavonoids Extracted from the Bark of Pinus maritima on Proinflammatory Cytokine Interleukin-1 Production in Lipopolysaccharide-Stimulated RAW 264.7. *Toxicol. Appl. Pharmacol.* 2000, *168*, 64 – 71.

[104] Liu, F., Lau, B. H., Peng, Q., Shah, V., Pycnogenol protects vascular endothelial cells from beta-amyloid-induced injury. *Biol. Pharmaceut. Bull.* 2000, *23*, 735 – 737.

[105] Peng, Q., Wei, Z., Lau, B. H., Pycnogenol inhibits tumor necrosis factor-alpha-induced nuclear factor kappa B activation and adhesion molecule expression in human vascular endothelial cells. *Cellular And Molecular Life Sciences: CMLS* 2000, *57*, 834 – 841.

[106] Spadea, L., Balestrazzi, E., Treatment of vascular retinopathies with Pycnogenol. *Phytother. Res.* 2001, *15*, 219 – 223.

[107] Putter, M., Grotemeyer, K. H., Wurthwein, G., Araghi-Niknam, M., *et al.*, Inhibition of smoking-induced platelet aggregation by aspirin and pycnogenol. *Thromb. Res.* 1999, *95*, 155 – 161.

[108] Liu, X., Wei, J., Tan, F., Zhou, S., *et al.*, Pycnogenol, French maritime pine bark extract, improves endothelial function of hypertensive patients. *Life Sci.* 2004, *74*, 855 – 862.

[109] Hosseini, S., Lee, J., Sepulveda, R. T., Rohdewald, P., Watson, R. R., A randomized, double-blind, placebo-controlled, prospective, 16 week crossover study to determine the role of Pycnogenol in modifying blood pressure in mildly hypertensive patients. *Nutr. Res.* 2001, *21*, 1251 – 1260.

[110] Devaraj, S., Vega-Lopez, S., Kaul, N., Schonlau, F., *et al.*, Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. *Lipids* 2002, *37*, 931 – 934.

[111] Silliman, K., Parry, J., Kirk, L. L., Prior, R. L., Pycnogenol does not impact the antioxidant or vitamin C status of healthy young adults. *J. Am. Diet. Assoc.* 2003, *103*, 67 – 72.

[112] Cossins, E., Lee, R., Packer, L., ESR studies of vitamin C regeneration, order of reactivity of natural source phytochemical preparations. *Biochem. Molec. Biol. Int.* 1998, *45*, 583 – 597.

[113] Auger, C., Caporiccio, B., Landrault, N., Teissedre, P. L., *et al.*, Red wine phenolic compounds reduce plasma lipids and apolipoprotein B and prevent early aortic atherosclerosis in hypercholesterolemic golden Syrian hamsters (Mesocricetus auratus). *J. Nutr.* 2002, *132*, 1207 – 1213.

[114] Vinson, J. A., Teufel, K., Wu, N., Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis* 2001, *156*, 67 – 72.

[115] Vinson, J. A., Mandarano, M. A., Shuta, D. L., Bagchi, M., Bagchi, D., Beneficial effects of a novel IH636 grape seed proanthocyanidin extract and a niacin-bound chromium in a hamster atherosclerosis model. *Mol. Cell Biochem.* 2002, *240*, 99 – 103.

[116] Bentzon, J. F., Skovenborg, E., Hansen, C., Moller, J., *et al.*, Red wine does not reduce mature atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2001, *103*, 1681 – 1687.

[117] Stocker, R., O'Halloran, R. A., Dealcoholized red wine decreases atherosclerosis in apolipoprotein E gene-deficient mice independently of inhibition of lipid peroxidation in the artery wall. *Am. J. Clin. Nutr.* 2004, *79*, 123 – 130.

[118] Waddington, E., Puddey, I. B., Croft, K. D., Red wine polyphenolic compounds inhibit atherosclerosis in apolipoprotein E-deficient mice independently of effects on lipid peroxidation. *Am. J. Clin. Nutr.* 2004, *79*, 54 – 61.

[119] Aviram, M., Fuhrman, B., Wine flavonoids protect against LDL oxidation and atherosclerosis. *Ann. N. Y. Acad. Sci.* 2002, *957*, 146 – 161.

[120] Zern, T. L., West, K. L., Fernandez, M. L., Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. *J. Nutr.* 2003, *133*, 2268 – 2272.

[121] Auger, C., Laurent, N., Laurent, C., Besancon, P., *et al.*, Hydroxycinnamic acids do not prevent aortic atherosclerosis in hypercholesterolemic golden Syrian hamsters. *Life Sci.* 2004, *74*, 2365 – 2377.

[122] Miura, Y., Chiba, T., Tomita, I., Koizumi, H., *et al.*, Tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice. *J. Nutr.* 2001, *131*, 27 – 32.

[123] Xu, Q., Mouse models of arteriosclerosis: from arterial injuries to vascular grafts. *Am. J. Pathol.* 2004, *165*, 1 – 10.

[124] Preuss, H. G., Wallerstedt, D., Talpur, N., Tutuncuoglu, S. O., *et al.*, Effects of niacin-bound chromium and grape seed proanthocyanidin extract on the lipid profile of hypercholesterolemic subjects: a pilot study. *J. Med.* 2000, *31*, 227 – 246.

[125] Vigna, G. B., Costantini, F., Aldini, G., Carini, M., *et al.*, Effect of a standardized grape seed extract on low-density lipoprotein susceptibility to oxidation in heavy smokers. *Metabolism* 2003, *52*, 1250 – 1257.

[126] Mathur, S., Devaraj, S., Grundy, S. M., Jialal, I., Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. *J. Nutr.* 2002, *132*, 3663 – 3667.

[127] Wiswedel, I., Hirsch, D., Kropf, S., Gruening, M., *et al.*, Flavanol-rich cocoa drink lowers plasma F2-isoprostane concentrations in humans. *Free Radic. Biol. Med.* 2004, *37*, 411 – 421.

[128] Hertog, M. G., Kromhout, D., Aravanis, C., Blackburn, H., *et al.*, Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. *Arch. Int. Med.* 1995, *155*, 381 – 386.

[129] Keli, S. O., Hertog, M. G., Feskens, E. J., Kromhout, D., Dietary flavonoids, antioxidants vitamins, and incidence of stroke: the Zutphen study. *Arch. Int. Med.* 1996, *156*, 637 – 642.

[130] Rimm, E. B., Katan, M. B., Ascherio, A., Stampfer, M. J., Willett, W. C., Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Ann. Int. Med.* 1996, *125*, 384 – 389.

[131] Hertog, M. G., Sweetnam, P. M., Fehily, A. M., Elwood, P. C., Kromhout, D., Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am. J. Clin. Nutr.* 1997, *65*, 1489 – 1494.

[132] Hirvonen, T., Virtamo, J., Korhonen, P., Albanes, D., Pietinen, P., Intake of flavonoids, carotenoids, vitamins C and E, and risk of stroke in male smokers. *Stroke* 2000, *31*, 2301 – 2306.

[133] Sesso, H. D., Gaziano, J. M., Liu, S., Buring, J. E., Flavonoid intake and the risk of cardiovascular disease in women. *Am. J. Clin. Nutr.* 2003, *77*, 1400 – 1408.

[134] Peters, U., Poole, C., Arab, L., Does tea affect cardiovascular disease? A meta-analysis. *Am. J. Epidemiol.* 2001, *154*, 495 – 503.

[135] Arts, I. C., Hollman, P. C., Feskens, E. J., Bueno de Mesquita, H. B., Kromhout, D., Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study. *Am. J. Clin. Nutr.* 2001, *74*, 227 – 232.

[136] Arts, I. C., Jacobs, D. R., Jr., Harnack, L. J., Gross, M., Folsom, A. R., Dietary catechins in relation to coronary heart disease death among postmenopausal women. *Epidemiology* 2001, *12*, 668 – 675.

[137] Nanji, A. A., Alcohol and ischemic heart disease: wine, beer or both? *Int. J. Cardiol.* 1985, *8*, 487 – 489.

[138] Ruf, J. C., Overview of epidemiological studies on wine, health and mortality. *Drugs Exp. Clin. Res.* 2003, *29*, 173 – 179.

[139] Tjonneland, A., Gronbaek, M., Stripp, C., Overvad, K., Wine intake and diet in a random sample of 48763 Danish men and women. *Am. J. Clin. Nutr.* 1999, *69*, 49 – 54.

