Effect of a Standardized Grape Seed Extract on Low-Density Lipoprotein Susceptibility to Oxidation in Heavy Smokers

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The aim of our study was to evaluate the effect of a standardized formulation of a polyphenolic extract of grapes (Leucoselect-Phytosome [LP]) on low-density lipoprotein (LDL) susceptibility to oxidation in a group of heavy smokers. A randomized, double-blind, crossover study was undertaken in 24 healthy male heavy smokers, aged ≥ 50 years. Enrolled subjects were given 2 capsules twice daily for 4 weeks (phase 1). Each capsule contained 75 mg of a grape procyanidin extracts and soy-phosphatidlcholine or placebo consisiting of 75 mg lactose and soy-phosphatidlcholine. A wash out period of 3 weeks was then followed by 4 weeks of the opposite treatment (phase 2). Blood samples were taken at baseline and at the end of each phase and assayed for plasma lipids and LDL susceptibility to oxidation. Compliance was good, and no adverse effects were recorded. Subjects did not show significant modification of total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C) and LDL-C during LP treatment. Among oxidative indices, thiobarbituric acid reactive substances (TBARS) concentration was significantly reduced in subjects taking LP (−14.7% ± 21.1% *v* +5.0% ± 18.1%, $P < .01$), and the lag phase prolonged (+15.4% ± 24.4% *v* −0.1% ± 16.0%, $P < .05$) compared with placebo and basal values. The antioxidant potential of grape seed extract polyphenols may prove effective in a model of oxidative stress (smoking); however more investigational data are needed before use in wider clinical settings.

THE CONSUMPTION of alcohol-containing beverages, in particular, red wine, has been associated with a decreased risk of cardiovascular events.[1-3] Although alcohol per se may be partly responsible for these characteristics (eg, raising high-density lipoprotein-cholesterol [HDL-C]),[4] it has been suggested that other components may be endowed with beneficial cardiac effects.[5]

Flavonoids are plant products, derivatives of 2-phenyl-1-benzopyran (flavane), with several thousands similar compounds found in nature (Fig 1). They exist in food and beverages as free molecules or glycosylated and acylated forms; common sources are fruits, such as apples, pears, and grapes and beverages, such as red wine and tea, as well as chocolate.[6,7] They act as potent free-radical scavengers in vitro and may prevent the oxidation of low-density lipoprotein-cholesterol (LDL-C), thereby delaying atheroma formation,[8-10] perhaps through a sparing action on endogenous lipophilic antioxidant.[11]

Flavonoid intake is inversely related to the occurrence of heart disease in several observational studies.[12-14] In the Zutphen Elderly Study, for example, the relative risk of coronary mortality in the highest versus the lowest tertile of polyphenol intake was 0.42 for flavonols[12] and 0.48 for catechins[15]; the risk was still significant even after adjustment for common cardiovascular risk factors and diet consumption of antioxidant vitamins.

Chromatographic studies in grapes have identified the presence of different flavonoid families, most notably, flavanols (catechin, epicatechin, gallocatechin), flavonols (quercetin, kaempferol), flavones (luteolin), and procyanidins. The latter are also known as condensed tannins and are represented by polymeric flavanols with C4 to C6 or C4 to C8 bonds and an average grade of polymerization between 4 and 11; they are particularly aboundant in grape seeds. The chemical structure and molecular weight of these substance may affect their biologic activity. In general, procyanidins are scarcely absorbed from the gut (particularly highly polymeric molecules), and their bioavailability is further decreased by chemical purification from a natural source.[7,16-18] Therefore, the in vivo effects of grape products on oxidative parameters have not been fully defined, and only scarce and contradictory data are available.[19-25]

We conducted a randomized, placebo-controlled clinical trial to determine if a highly standardized mixture of polyphenols obtained from grape seeds and complexed with phosphatidylcholine to enhance their bioavailability, might improve lipoprotein modification and plasma antioxidant defense in male cigarette smokers.

The human subjects were intended to represent an oxidative-stress model, because cigarette smoke contains large amount of both carbon- and oxygen-centered free radicals, which can directly initiate and propagate the process of lipid peroxidation.[26,27] In such a context, vitamin E (α-tocopherol) and other lipid-soluble chain-breaking antioxidants may play a role in protecting lipids and proteins from oxidative damage.[28]

SUBJECTS AND METHODS

Polyphenols

A standardized grape seed extract complexed with soy phosphatidylcholine (LEUCOSELECT-Phytosome, EP0275224; US Patent 4,

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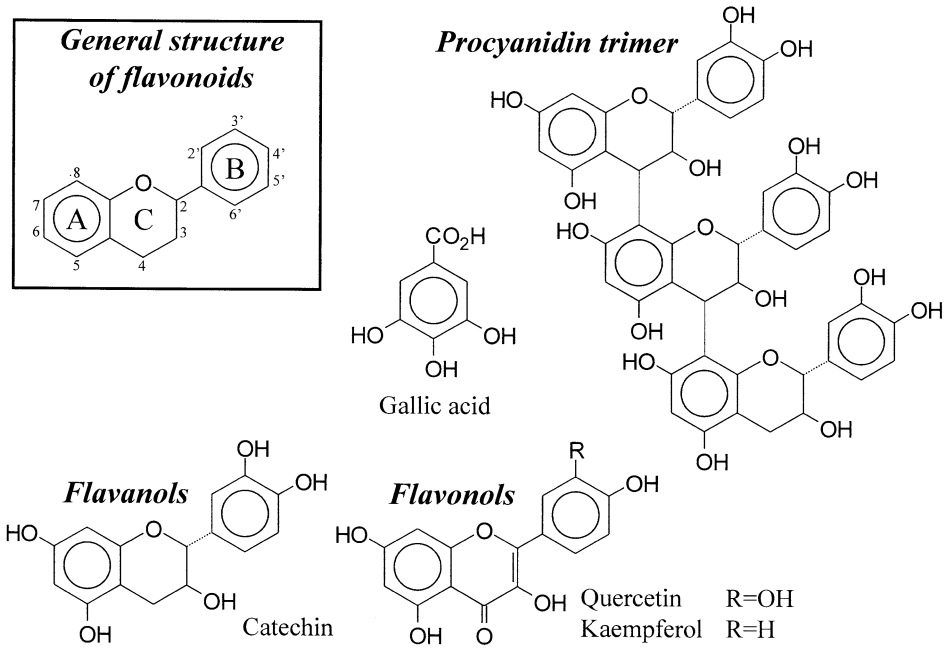

Fig 1. Structure of selected polyphenol flavonoids and gallic acid.

963,527) supplied as a gift by Indena S.p.A. (Milan, Italy) was the source of polyphenols. Its main constituents were represented by a mixture of (+)-catechin, (−)-epicatechin, and gallic acid (15%); procyanidins as (+)-catechin and (−)-epicatechin dimers, trimers, tetramers and their esters with gallic acid (80%), pentamers hexamers, and heptamers and their ester with gallic acid (5%); their mean molecular weight by gel permeation chromatography was 1,800 d, their purity more than 96%.[29,30] Every hard, gelatine capsule contained 75 mg grape seed extract, 192 mg soy phosphatidylcholine, starch, silicon dioxide, and other excipients. Placebo capsules had a similar appearance, but different composition, containing lactose 75 mg instead of the 75-mg flavonoid extract, while phosphatidylcholine and excipients were the same.

Subjects and Experimental Design

Patients. Twenty-four healthy men, aged 50 or more (mean, 54 ± 3; range, 50 to 63 years) were enrolled based on the presence of current smoking habits (>10 cigarettes daily). They were all informed about the risks of smoking and the reasons to stop, but explicitly refused to give it up. None of these subjects were severely dyslipidemic (total cholesterol > 8 mmol/L and/or triglycerides > 3.5 mmol/L) or had received hypolipidemic therapy or any other drug known to interfere with lipid metabolism in the 2 months before this study; none followed a vegetarian diet. All were in apparent good health. In particular, none had acute infection, drug, or alcohol addiction, cholestasis, cholelithiasis, chronic liver disease (or transaminase levels more than twice reference values), diabetes, heart failure, hypothyroidism, renal failure, pancreatitis, or malignant disease; none were obese (body mass index [BMI] ≥ 30 kg/m^2), severely malnourished, or experienced recent bleeding episodes; none were on anticoagulant therapy. Moreover, none had experienced cardiovascular events in the 6 months before this study or had a history of drug hypersensitivity. They were requested not to drink more than 1 glass of wine (but no red wine at all), 2 oranges or 2 glasses of fruit juice or 1 cup of tea per day in the following 2 months to avoid vitamin supplementation, and they were encouraged not to change their diet otherwise. A weekly diary of all food and beverage consumed during the study was reviewed to ensure dietary compliance. The subjects were randomly administered procyanidin capsules or the corresponding placebo.

Study protocol. This study was placebo-controlled, conducted in a crossover, double-blind, randomized manner, and lasted 14 weeks (Fig 2). All the subjects underwent a clinical interview and a physical

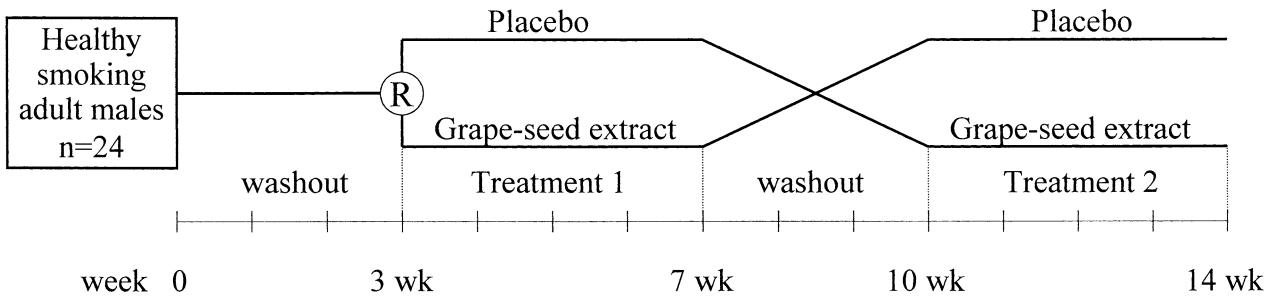

Fig 2. Schematic representation of the crossover trial.

examination; hematochemical safety parameters were checked. After a 3-week run in period, enrolled subjects were given 2 capsules twice daily at the end of their main meals (lunch and dinner), for a total equivalent of 300 mg grape procyanidin extracts, or placebo, daily for 4 weeks (phase 1). A wash out period of 3 weeks was then followed by 4 weeks of the opposite treatment (phase 2). Blood samples were taken at baseline and at the end of each phase and assayed for plasma lipids, LDL susceptibility to oxidation and lipophilic antioxidant vitamins. Drug compliance was evaluated, and all subjects gave their written informed consent. This study was approved by the local Ethics Committee.

Table 1. Age, BMI, Systolic and Diastolic Blood Pressure, and Heart Rate at Enrollment

No. of subjects	24
Age (yr)	54 ± 3 (range, 50–63)
BMI (kg/m^2)	27 ± 3
Systolic blood pressure (mm Hg)	127 ± 13
Diastolic blood pressure (mm Hg)	83 ± 8
Heart rate (bpm)	73 ± 9

Abbreviation: BMI, body mass index.

Analytical Methods

General procedures and lipid determination. After an overnight fast of at least 12 hours., blood samples were drawn from an antecubital vein, with minimal stasis, into tubes containing 1 mmol/L EDTA. They were immediately centrifuged at +4°C and separeted. Total cholesterol (TC), triglycerides (TG), and HDL-C were assayed by standard enzymatic-colorimetric methods. HDL-C was determined after selective precipitation of apolipoprotein (apo) B-containing lipoprotein with phosphotungstic acid in the presence of Mg^{2+}.[31] LDL-C was calculated according to Friedewald's formula.[32] Routine plasma control parameters (complete blood count, plasma glucose and protein determinations, assessment of hepatic and renal function) were determined by standard methods.

Plasma for vitamin and lipid oxidative parameter determination was added to butylated hydroxytoluene (BHT; 20 mmol/L), the aliquot for circulating phenolic marker evaluation was acidified by adding HCl 1N to a final concentration of 10 μL/mL; samples were immediately stored at −80°C. LDL oxidation was evaluated as the susceptibility of LDL to undergo lipid peroxidation and as the native LDL lipid peroxide content as assessed by fluorometrically detected thiobarbituric acid reactive substances (TBARS) and fluorescent products of lipid peroxidation (FPLP).

LDL isolation, oxidation, and product determination. Plasma stored at −80°C under N_2 was used for LDL isolation within 3 days. Previous studies have shown that plasma storage and freezing/thawing does not affect LDL isolation and its major chemical characteristics.[33]

LDL was isolated by single vertical-spin ultracentrifugation[34] with a discontinuous NaCl/KBr density gradient.[35] Details of this procedure have already been published by one of the investigators.[36] In short, after 1.5 hours at 400,000 × *g* and 7°C in a Centrikon TVF 6513 vertical rotor (Kontron Instruments, Milan, Italy), LDL was recovered from the mid-upper part of the gradient and extensively dialyzed in the dark. LDL protein and LDL-C were determined by established methods. The susceptibility of LDL to undergo lipid peroxidation was assessed spectrophotometrically by continuously monitoring the formation of conjugated dienes at 234 nm after the incubation with 5 mmol/L $CuSO_4$ in phosphate-buffered saline (PBS). All determinations were performed in a computer-assisted spectrophotometer equipped with an automatic sample changer. The lag phase, preceding the formation of conjugated dienes, and the propagation phase, during which time the absorbance at 234 nm rapidly increased to a maximum, was calculated as described.[37] The propagation rate was calculated from the slope of the tangent to the absorbance curve during the propagation phase and using a molar extinction coefficient for conjugated dienes (ϵ_{234}).[36,38]

Lipid peroxidation in native LDL (n-LDL) was assessed by measurement of FPLP,[28] the latter essentially representing the interaction of aldehydic lipid peroxidation products with phospholipids and protein amino groups. In brief, the n-LDL sample was diluted with PBS and mixed with chloroform/methanol plus water. The lipid-containing phase was removed, dried under a stream of N_2 gas at room temperature, resuspended in chloroform, and exposed to ultraviolet (UV) light. Fluorescence values were estimated spectrofluorometrically at 360 nm excitation and 430 nm emission, and the results were expressed as units of relative fluorescence per milligram of LDL-C.[36]

The lipid peroxide content of n-LDL was also evaluated fluorometrically as TBARS.[36,39] Freshly diluted tetramethoxypropane, which yields malondialdehyde, was used as a standard, and results were expressed as nanomoles of malondialdehyde equivalents per milligram of LDL-C.

Antioxidant determinations. Plasma and LDL contents of α-tocopherol, vitamin E, β-carotene, and lycopene were determined by high-performance liquid chromatography (HPLC).[40] Vitamins were separated and quantified by using a Kontron system 450 equipped with a UV-visible wavelength variable Kontron detector 430, and analysis was performed by isocratic elution.[36] Vitamins were expressed as μmol/L (in plasma) and micrograms per milligram of cholesterol (in LDL).

Markers of procyanidins bioavailability. Ferric reducing antioxidant power (FRAP) was used as a measure of plasma reducing (antioxidant) equivalents both before and after treatment. Ferric to ferrous ion reduction at low pH (3.6 in acetate buffer) causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by reading the absorbance change at 593 nm, which are linear over a wide concentration range.[41]

Statistical Analysis

The study was a double-blind, randomized, 2-period crossover design with repeated measurements over time. Treatment effects were tested with the use of repeated measure analysis of variance after excluding significant interactions between treatment sequences (carryover effect). Values are reported as means ± SD. Systat software (Systat, Evanston, IL) was used for statistical analyses.

RESULTS

Pharmacologic compliance was good, and no adverse effects were recorded. All enrolled subjects completed the study. Some general characteristics of the population under investigation are shown in Table 1. The mean age of the participants was 54 ± 3 years; they were normotensive and slightly overweight.

Patients were encouraged to follow a stabilized diet, not changing their mean food intake during the trial: dietary intake of nutrients and antioxidants was not different before and at the end of the study periods in all groups (data not shown). Diet compliance (exclusion of antioxidant supplementation and flavonoid-rich foods and beverages) was good: no one took vitamin pills, no one drank tea or chocolate, and only 5 subjects reported 1 glass of red wine per day in a single week remote from the scheduled blood sampling.

The FRAP test indicated effective absorption of ingested procyanidins (Table 2), even if split-up data showed significant difference from baseline only for the subgroup "grape seed

Table 2. FRAP Analysis of Reducing (antioxidant) Plasma Capacity in Patients at Basal Time and After 4 Weeks of Therapy

		FRAP (μmol Fe^{2+})				
	No.	Basal	4 Weeks	P	Δ%	*P*
First treatment period (weeks 3 to 7)						
Subgroup 1 (**grape seed extract** → placebo)	12	669 ± 82	732 ± 82	.001	9.8 ± 7.2	.013
Subgroup 2 (**placebo** → grape seed extract)	9	707 ± 78	711 ± 117	.866	0.4 ± 10.8	
Second treatment period (weeks 10 to 14)						
Subgroup 1 (grape seed extract → **placebo**)	12	712 ± 50	725 ± 69	.383	1.7 ± 5.8	.285
Subgroup 2 (placebo → **grape seed extract**)	9	705 ± 100	738 ± 92	.170	5.6 ± 10.8	
Cumulative						
Grape seed extract	21	684 ± 94	735 ± 84	.001	8.0 ± 8.9	.014
Placebo	21	709 ± 66	717 ± 97	.584	1.0 ± 8.8	

NOTE. Data are mean ± SD. Cumulative (grape seed extract and placebo) and split-up data are presented (for each of the 2 crossover periods, considering the subgroup initially on grape seed extract, group 1, or placebo, group 2). Three samples were lost to laboratory determination, explaining differences in subject number.

extract → placebo" (n = 12, $P < .001$), while for the subgroup "placebo → grape seed extract," partly due to lacking data (n = 9, 3 samples missed), it did not reach statistical significance ($P = .170$). No significant modification of TC, TG, HDL-C, and LDL-C was apparent during procyanidin treatment (Table 3).

Among oxidative indices, fluorescence lipid products and the propagation rate showed a favorable, statistically nonsignificant trend after grape seed extract ingestion (−2.9% and −3.6%, respectively), but not after placebo. On the other hand, compared with basal values, TBARS concentration was significantly reduced by active treatment, and this effect clearly differentiated procyanidin-treated (−14.7% ± 21.1%) and placebo-treated subjects (+5.0% ± 18.1%, $P < .01$). The lag phase of LDL oxidation was similarly prolonged with respect to baseline (+15.4% ± 24.4% after procyanidins and −0.1% ± 16.0% after placebo, $P < .05$) (Table 4, Fig 3). All of these oxidative parameters acted in the expected direction, congruous to procyanidin treatment, and they were not influenced, or accompanied, by a concomitant variation in plasma or LDL-bound α-tocopherol, retinol, β-carotene, or γ-lycopene (Table 5).

DISCUSSION

In this randomized, crossover study, we demonstrated that protracted consumption of a highly standardized phenolic extract of grape seeds may be effective and reduce LDL oxidation in smoking subjects. All investigated indexes of LDL oxidation showed a favorable modification, even if only lag phase and a reduced production of TBARS reached a statistically significant level. The effects were not related to a treatment sequence-effect ('carry-over'), thus confirming the validity of the study. They did not seem dependent on vitamin E and other lipid-soluble–reducing substances present in blood (lycopene, β-carotene, retinol) or to their preferential distribution to LDL particles, but were probably related to direct antioxidant properties or to mechanisms involving ion (copper) chelation.

In a previous report from our laboratory, the plasma and tissue levels of vitamin E, vitamin C, and lipid peroxides were evaluated in smoking men undergoing coronary bypass surgery,[28] and some of these parameters seemed related to the seriousness of coronary atherosclerosis. Therefore, we chose smoking as an exemplary condition of "oxidative stress" to test the possible protective effects of flavonoids.

The chemically standardized grape seed extract we used is particularly rich in procyanidins. It had been tested in several experimental models[42] and showed an improved plasma antioxidant profile both in young and old rats[43] and its capability to protect isolated rabbit heart Langendorff preparation from ischemia/reperfusion injury.[44] More recently, we showed that this extract may reduce plaque formation in the aortic arc or carotid intima/media ratio of rabbits fed an atherogenic diet (unpublished data). In man it seems particularly well tolerated, and 300 mg procyanidin-equivalent per day may increase plasma total antioxidant power rapidly and persistently without significant variation of vitamin C and vitamin E plasma levels.[45]

The absorption of flavonoids is a debated issue; it depends on their chemical form, molecular weight, and degree of polymerization and probably on alcohol coadministration and individual variation in absorptive response. Anthocyanins glycosides are the native forms in plants; they have limited, but definite, absorption[46] or may be hydrolyzed by gut microorganisms

Table 3. Total Cholesterol, Tryglicerides, LDL- and HDL-Cholesterol in 24 Smoking Patients at Basal Time and After 4 Weeks of Therapy

	Grape Seed Extract		Placebo	
Lipid Parameter	Basal (n = 24)	4 Weeks (n = 24)	Basal (n = 24)	4 Weeks (n = 24)
Total cholesterol (mmol/L)	5.90 ± 1.01	6.09 ± 0.96	6.09 ± 0.91	6.06 ± 0.88
LDL cholesterol (mmol/L)	4.04 ± 0.85	4.15 ± 0.93	4.15 ± 0.88	4.15 ± 0.85
HDL cholesterol (mmol/L)	1.11 ± 0.28	1.11 ± 0.28	1.09 ± 0.28	1.09 ± 0.26
Triglycerides (mmol/L)	1.66 ± 0.62	1.84 ± 0.88	1.91 ± 0.75	1.81 ± 0.63

NOTE. Data are mean ± SD.

Table 4. FPLP, Lag Phase, Propagation Rate, and TBARS in 24 Smoking Patients: Basal and After 4 Weeks of Treatment

	Grape Seed Extract			Placebo		
	Basal (n = 24)	4 Weeks (n = 24)	Δ %	Basal (n = 24)	4 Weeks (n = 24)	Δ %
FPLP (RFU/ml Plasma)	22.1 ± 5.1	20.8 ± 6.9	−2.9 ± 33.4	20.2 ± 6.0	20.3 ± 3.9	+4.6 ± 22.2
Lag phase (min)	52.9 ± 7.6	61.1 ± 15.6*	+15.4 ± 24.4†	59.0 ± 13.0	57.7 ± 9.8	−0.13 ± 16.0
Propagation rate (nmol/min/mg LDL-C)	7.9 ± 1.5	7.5 ± 1.6	−3.6 ± 22.2	7.2 ± 1.3	7.5 ± 1.3	+5.6 ± 14.4
TBARS (nmol/mg protein)	0.64 ± 0.11	0.54 ± 0.13*	−14.7 ± 21.1‡	0.56 ± 0.10	0.57 ± 0.08	+5.0 ± 18.1

Abbreviation: RFU, relative flurescence units.
*$P < .005$ v basal; †$P < .05$ v placebo; ‡$P < .005$ v placebo.

before. Procyanidin polymers (condensed tannins) are perhaps not easily absorbed in the intestine, but evidence for absorption has been reported.[7] The chemical detection of polyphenols in plasma is difficult, because thousands of different compounds have been described, and metabolites are ill-defined; the widespread Folin-Ciocalteau identification of "phenolic markers" seems unreliable.[22] Indeed in the absence of a reference technique, we tested the pharmacologic compliance of our smoking subjects with another validated, functional method, the FRAP[41]; at the same time these results confirmed the bioavail-

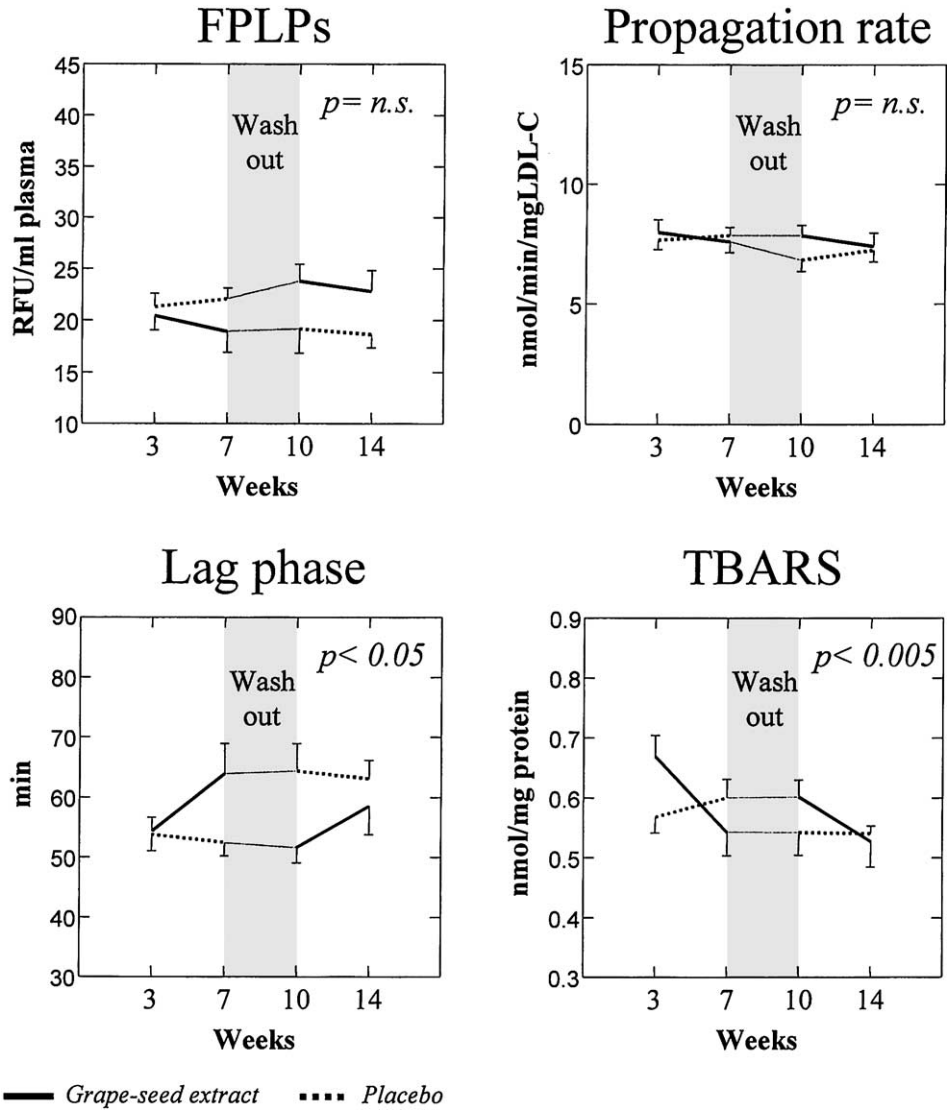

Fig 3. Peroxidative lipid parameters during the trial (mean ± SEM.). *P* values are intended for whole trial results.

Table 5. Plasma and LDL Retinol, Tocopherol, Lycopene, and β-Carotene in 24 Smoking Patients: Basal and After 4 Weeks of Treatment

	Grape Seed Extract			Placebo		
	Basal (n = 24)	4 Weeks (n = 24)	Δ %	Basal (n = 24)	4 Weeks (n = 24)	Δ %
Plasma retinol (μmol/L)	1.51 ± 0.31	1.39 ± 0.31	−5.8 ± 20.3	1.37 ± 0.46	1.46 ± 0.49	+13.3 ± 39.8
Plasma tocopherol (μmol/L)	18.2 ± 5.5	16.1 ± 4.8	−7.9 ± 24.0	15.6 ± 5.9	17.1 ± 6.0	+18.4 ± 47.6
Plasma lycopene (μmol/L)	0.44 ± 0.19	0.46 ± 0.16	+9.9 ± 48.3	0.35 ± 0.17	0.45 ± 0.17	+60.8 ± 108.9
Plasma β-carotene (μmol/L)	0.67 ± 0.53	0.75 ± 0.35	+60.4 ± 120.2	0.79 ± 0.46	0.83 ± 0.38	+25.9 ± 58.2
LDL tocopherol (μg/mg chol)	3.76 ± 0.90	3.80 ± 0.74	+3.8 ± 29.6	3.75 ± 0.72	3.62 ± 0.76	−2.4 ± 18.2
LDL lycopene (μg/mg chol)	0.31 ± 0.37	0.25 ± 0.10	+22.9 ± 58.0	0.21 ± 0.08	0.29 ± 0.16	+41.3 ± 92.8
LDL β-carotene (μg/mg chol)	0.48 ± 0.22	0.43 ± 0.24	+0.7 ± 45.8	0.41 ± 0.18	0.42 ± 0.18	+12.8 ± 58.9

ability of the grape seed extract, as we have discussed elsewhere.[16,45]

The pharmacologic bases of flavonoid action may be primarily found in radical scavenging,[47,48] even if antiplatelet actions,[25] a modulation of endothelial key-enzymes[22] and extracellular matrix effect seem also to be relevant.[30] Antioxidant capacity relates to phenolic group interactions with hydrophobic and hydrophilic lipoprotein (LDL) constituents and perhaps to the ability to inhibit the activity of enzymes, such as lypo- and cyclo-oxigenase, phospholypase A_2, glutation reductase, xantine oxidase, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[8] According to Frankel et al,[8] incubation of human plasma with red wine polyphenols resulted in lipid peroxidation and conjugated diene inhibition, with vitamin E showing only about half this capability. Kelly and Abbey[49] studied the antioxidant properties of different phenolic compounds present in red wine. Among several investigated classes, they showed a major effect exerted by catechins and monomeric antocyanidins, whereas flavonols were the least active; polymeric procyanidins had an intermediate effect, but were particularly important, because they represented the most abundant flavonoids in the beverage.[49]

In vivo experience provided less clear results. Some investigators could not find any significant effect by red wine on LDL oxidative parameters, vitamin C, and glutathion or on LDL-bound vitamin E and ubichinon.[20] In contrast, others demonstrated that daily consumption of red wine,[19] red wine polyphenols,[22] or red grape juice[50] reduced LDL susceptibility to lipid peroxidation, an effect related to attenuation in atherosclerosis development in animals.[51] These latter results substantiate our findings, attained in a different context of subjects with distinct chemical extract and a longer treatment period (4 weeks) and may allow some speculation about clinical relevance. In the present experience, sparing plasma vitamin E (tocopherol) or other circulating antioxidants does not seem responsible for these effects. Also Nigdikar et al,[22] Fuhrman et al,[19] and a small trial we have previously performed[45] confirm this view. On the other hand, Viana et al,[11] in an in vitro experiment, related the antioxidant ability of flavonoids to a delayed consumption of vitamin E, while Carbonneau et al[52] described an increase of plasma antioxidant capacity and LDL-tocopherol concentration despite a lack of protection against Cu^{2+}-LDL oxidation.[52] These results are rather puzzling when compared with ours in which LDL-tocopherol, lycopene, β-carotene, and retinol, in addition to corresponding total plasma oncentration, were unchanged after procyanidin treatment. According to Carbonneau et al,[52] the phenolic compounds may be loosely bound to LDLs, and extensive LDL dialysis totally removes these substances. Our data seem to indicate that LDL polyphenol interaction is more stable, depending on the molecular species involved or, alternatively, that polyphenols exert their antioxidant effect through an undefined chemical.

It has been shown that flavonoids differ in their potential eulipidemic action. Some may be helpful through a reduction in LDL-C or TG,[53] while HDL-C may also be increased.[54] Alcohol in wine may directly mediate this metabolic action, which mainly consists of an increase in plasma TG and HDLs[5]; dealcoholization of wine, grape juice, or polyphenol extracts has never been described to modify lipid levels. Also, our study did not show any modification of plasma lipoprotein levels. In particular, neither LDL-C nor HDL-C nor their ratio varied during grape seed extract treatment.

A final comment is addressed to the significance of an antioxidant-rich diet. It has been demonstrated that vitamin E consumption through diet will prevent cardiovascular mortality, while pill supplementation seems less effective.[55] It is possible that the link between foods rich in vitamin E and mortality is mediated by other substances, such as flavonoids, which are present in these same foods, but not in vitamin E pills. At the same time, epidemiologic surveys support the relationships between high polyphenol intake and low mortality,[12-14] while new favorable vascular effects have been described (eg, an improved endothelial function and diminished platelet function).[22,25] It is hoped that this hypothesis can be soon tested with an appropriate trial.

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