Grape Seed Proanthocyanidins Prevent Plasma Postprandial Oxidative Stress in Humans

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Postprandial hyperlipemia is a well-defined risk factor for atherosclerosis. A reasonable contributing mechanism could involve the postprandial increase of plasma lipid hydroperoxides (LPO) affecting the oxidant/antioxidant balance and increasing the susceptibility of LDL to oxidation. Wine has been shown to prevent both these events. The present study was designed to investigate the effect of supplementing a meal with grape seed proanthocyanidins (the main phenolic antioxidant of red wine) on plasma postprandial oxidative stress. In two different sessions, 8 healthy volunteers consumed the same test meal rich in oxidized and oxidizable lipids without (control) or with 300 mg of a proanthocyanidin-rich grape seeds extract (GSE). Lipid hydroperoxide concentration, antioxidant status, and LDL resistance to oxidative modification were measured in postprandial plasma. The content of LPO in chylomicrons was 1.5-fold higher after the control meal than after the GSE-supplemented meal. Plasma LPO increased only after consumption of the control meal. The plasma antioxidant capacity increased in the postprandial phase only following the GSE supplemented meal. LDL isolated 3 h after the control meal tended to be more susceptible to oxidative modification (but the difference did not reach statistical significance). An opposite trend was observed following the GSE supplemented meal. In conclusion, the supplementation of a meal with GSE minimizes the postprandial oxidative stress by decreasing the oxidants and increasing the antioxidant levels in plasma, and, as a consequence, enhancing the resistance to oxidative modification of LDL.

KEYWORDS: Grape seed proanthocyanidins; postprandial oxidative stress; lipid hydroperoxides; LDL oxidation; human

INTRODUCTION

Proanthocyanidins (PAs) are a common component of foods and beverages of plant origin (*1*). PAs, among the most abundant phenolic compounds in grape seeds, are present in substantial amounts in red wine when fermentation takes place in the presence of skin and seeds. Thus, PAs could account, at least in part, for the protective effect of red wine with respect to atherogenesis and cardiovascular disease.

PAs have attracted increasing attention in the fields of nutrition and preventive medicine due to their potential health effects. In vitro, PAs have strong antioxidant activity and scavenge reactive oxygen and nitrogen species (*2−4*), modulate immune function and platelet activation (*5−8*), and produce vasorelaxation by inducing NO release from endothelium (*9*). In vivo, PAs inhibit the progression of atherosclerosis (*10*) and prevent the increase of LDL cholesterol in high-cholesterol-fed rats (*11*). In another study, where cholesterol levels were more carefully controlled, a prevention of atherogenesis in cholesterol-fed rabbits was found to be independent from the cholesterol lowering effect. A decrease in plasma levels of lipid hydroperoxides and an increase of antioxidant capacity was found under these circumstances (*12*).

The bioavailability of PAs is still not completely resolved (*1*) since unequivocal evidence for absorption is missing. However, studies with intestinal cell monolayer indicate that radiolabeled procyanidins dimer and trimer can traverse from the apical to basal side of the cultures and thus may be absorbed (*13*). Furthermore, it has been shown that the colonic microflora degrades PAs to low-molecular-weight aromatic compounds, which are bioavailable. Accordingly, it has been shown that compounds derived from grape seed extracts rapidly appear in rat plasma after single oral administration (*14*). However, a different mechanism not involving bioavailability could account for the protection against cholesterol-induced atherogenesis in rabbits (*10*).

We postulate that PAs taken with food prevent the postprandial oxidative stress in plasma and thus the rise of oxidatively modified, atherogenic lipoproteins (*15*).

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This hypothesis is supported by three sets of evidence:

(1) Postprandial hyperlipemia is a well-defined risk factor. Postprandial levels of triacylglycerols and triacylglycerol-rich lipoproteins correlate with the risk for coronary hearth disease better than the concentrations of triacylglycerols in the post absorptive state (*16−20*). Postprandial levels of chylomicron remnants are related to the rate of progression of coronary lesions in patients with premature coronary artery disease (*21*). Postprandial hyperlipemia induces alterations in metabolism and composition of all major lipoproteins (*22−24*), and LDL isolated during postprandial lipemia is more susceptible to oxidation in vitro than LDL isolated from subjects in fasting condition (*25, 26*). Finally, postprandial LDL induces a greater cholesterol accumulation into cultured macrophages than fasting LDL (*25*).

(2) The plasma level of lipid hydroperoxides increases in the postprandial phase. High levels of lipid oxidation products in the diet produce an increase in the levels of lipid oxidation products in human chylomicrons (*27*) and plasma (*16*) and accelerate the development of atherosclerosis in animals (*28− 30*).

(3) Wine antioxidants prevent the postprandial rise of lipid hydroperoxides in plasma. The postprandial increase of plasma lipid hydroperoxides is reduced when the meal is consumed with red wine (*16*), and red wine prevents the increased susceptibility to oxidative modification of postprandial LDL (*26*). A possible mechanism is envisaged from the chemistry of lipid hydroperoxides during gastric digestion. Lipid hydroperoxides present in food are decomposed in the stomach in the presence of myoglobin or other sources of transition metals, and this leads to multiplication of their concentration through radical chain reactions. In the presence of suitable phenolic antioxidants, not only is peroxidation prevented but also the original hydroperoxides disappear since they are decomposed in a peroxidatic reaction where polyphenols act as hydrogen donors (*31*). This effect is particularly evident when the source of antioxidants is wine, which contains PAs that are active at acidic pH by means of a hydrogen-transfer reaction mechanism (*32*).

In this nutritional study, we further tested the hypothesis that postprandial oxidative stress can be inhibited by PAs taken with food. The PAs were used in the form of a grape seed extract (GSE) that is rich in PAs but mimics the effect of wine without the complication of other wine components.

MATERIALS AND METHODS

Subjects and Test Meal. Eight healthy men (25−40 years old) selected from the laboratory staff were studied after informed consent was obtained. The subjects were asked to keep their diet as constant as possible during the study period, and none of them was taking any drugs or vitamin supplement. The Institutional ethics committee approved the study protocol.

The eight subjects ate the same test meal in two different sessions (2 weeks apart) after a 16-h fasting interval. The test meal consisted of "Milanese" meat (beef, egg, and breadcrumbs, fried in corn oil) and fried potatoes. This meal was eaten either alone or with 300 mg of a mixture of polyphenols from grape seed extract that is rich in proanthocyanidins. The extract was in the form of capsules that are commercially available under the trade name Leucoselect, and four capsules were taken just before and during the meal. Leucoselect (provided by Indena Spa, Milano, Italy) contains approximately 15% (+)-catechin and (−)-epicatechin, 80% (−)-epicatechin 3-*O*-gallate, dimers, trimers, tetramers, and their gallates, and 5% pentamers, hexamers, heptamers, and their gallates (*33*).

The energy content of the meal was 40% of the estimated daily energy need, calculated as 1,5× basal metabolic rate. The percentages of total energy intake derived from protein, fat, and carbohydrate were

Table 1. Plasma Concentration of Metabolic Parameters (Mean ± SE of 8 Subjects)

	control			grape seed extract		
	baseline	1 h	3 h	baseline	1 h	3 h
tot. cholesterol, mg/dL	159 ± 8	164 ± 9^a	164 ± 10	164 ± 12	169 ± 12^b	167 ± 12
triacylglycerol, mg/dL	84 ± 15	113 ± 15^a	120 ± 18^a	71 ± 9	99 ± 13^a	122 ± 21^a

^{a} P < 0.05 and ^{b}P < 0.005 from baseline by paired *t*-test.

18, 32, and 50%, respectively. Saturated, monounsaturated, and polyunsaturated fatty acids represented 19, 33, and 48% of total fat, respectively (*34*).

Plasma Treatment and Analyses. Blood was collected in EDTA (1 mg/mL) before and at 1- and 3-h intervals after the test meal. Plasma total antioxidant capacity and ascorbic acid were analyzed immediately after blood centrifugation. Plasma samples for metabolic and antioxidant analyses (total cholesterol, triacylglycerols, SH groups, and uric acid) were stored at −80 °C until the analysis was performed.

Plasma total cholesterol, triacylglycerols, and urate were measured by commercial kits purchased from SIGMA Chemical Co (St. Louis, MO). SH groups were measured according to Ellmann (*35*). Ascorbic acid was measured by EC-HPLC, according to Kutnink (*36*); dehydroascorbic acid was indirectly estimated by converting it to ascorbic acid after reduction with dl-homocysteine (*37*). α-Tocopherol was measured by reversed phase HPLC with fluorescence detection after extraction from plasma (*38*). Reduced and oxidized glutathione were measured by high-performance liquid chromatography with fluorometric detection after derivatization with *o*-phthaldialdehyde (*39*). The total antioxidant capacity of plasma (TRAP) was measured as described previously by Ghiselli et al. (*40*). TRAP measures the amount of peroxyl radicals that are trapped by 1 L of plasma and is expressed in millimoles. The concentration of lipid hydroperoxides was measured by kinetic analysis of photon emission as described previously (*41*).

Chylomicron-VLDL Fraction and LDL Preparation. Chylomicron-VLDL fraction (*d* < 1.0063) and LDL (*d* = 1.019−1.063 g/mL) were isolated from plasma (at time 3 and at times 0 and 3, respectively) by sequential ultracentrifugation in salt solutions, according to Havel et al. (*42*), using a Beckman T-100 benchtop ultracentrifuge (T-100.3 rotor). Protein was measured by the method of Lowry et al. (*43*), using bovine serum albumin as a standard.

Cholesterol, triacylglycerols, and lipid hydroperoxides levels were measured in chylomicron-VLDL fractions, while cholesterol, triacylglycerols, and α-tocopherol concentrations were measured in LDL, as described above. For oxidation experiments, freshly isolated LDL was dialyzed in the dark for 18 h at 4 °C against two changes of 0.01 M phosphate buffered saline (PBS), 0.15 M NaCl, pH 7.4 (2 L each).

LDL Resistance to Oxidative Modification. Dialyzed LDL (200 μg of protein/mL) was oxidized in PBS at 37 °C for 4 h in the presence of 5 μM CuCl$_2$. Oxidation was stopped by addition of 3 mM EDTA and 100 μM BHT and refrigeration. LDL electrophoresis was performed at pH 8.6 in 0.05 M barbital buffer on 0.5% agarose gels. The gels were stained with Sudan B black. Electrophoretic mobility of LDL was expressed relative to the mobility (REM) of a native LDL standard. Tryptophan residues were determined by the measurement of intrinsic fluorescence in cetyltrimethylammonium bromide (*44*).

Statistical Analysis. Data are presented as mean and standard errors for the eight subjects. Comparisons between control and PAs-supplemented meals were performed using the paired Student *t*-test.

RESULTS

In the postprandial phase following the intake of the test meals, an expected increase of plasma cholesterol and triacylglycerols was observed (**Table 1**). The concentration of plasma cholesterol rose on average by approximately 3% over baseline after 3 h, while the increase of triacylglycerol was more considerable (30−40%). These changes are within the normal range for normal subjects.
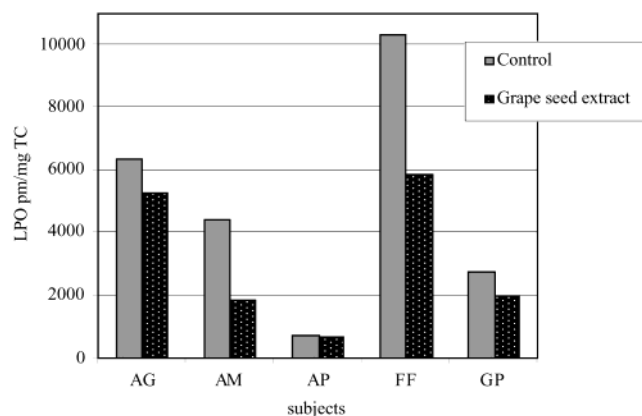
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Figure 1. Lipid hydroperoxides in chylomicrons. Data represent the LPO concentration (pmol of ROOH/mg of cholesterol) in chylomicrons of 5 subjects 3 h after the meals: gray bar, control meal; black bar, GSE-supplemented meal.

In agreement with previous reports indicating that minimal amounts of lipid hydroperoxides may be absorbed after a meal, lipid hydroperoxides were detected in chylomicrons (**Figure 1**) although at variable concentrations among different subjects. The intake by the same subjects of the GSE-supplemented meal reduced the specific lipid hydroperoxide content in chylomicrons while the rise in cholesterol and triglycerides was not affected.

The postprandial rise of lipid hydroperoxides was also apparent in plasma (**Figure 2**). Remarkably, the increase was fully prevented when test meal was supplemented with GSE. Indeed, GSE supplementation produced a small but significant decrease of plasma lipid hydroperoxides at the 1 h postprandial interval.

Besides providing lipid hydroperoxides, food intake is expected to alter the plasma oxidant/antioxidant status by affecting the concentration of different antioxidants. This aspect was analyzed by measuring the concentration of different antioxidants and the total antioxidant capacity as TRAP (**Tables 2** and **3**).

α-Tocopherol and ascorbic acid levels slightly increase after both test meals, although a statistical significance was reached only in the GSE group. It is noteworthy that the reduced form of ascorbic acid (expressed as % of total ascorbic acid) tended to decrease only after the control meal, although the difference from baseline did not reach statistical significance ($P = 0.063$).

A statistically significant increase in the concentration of titratable SH groups in plasma was observed 3 h after the GSE-supplemented meal, while no modification was observed following the control meal.

Urate concentrations increased after both meals. A decrease in total GSH was detectable in the postprandial plasma of both groups. Nevertheless, an effect of GSE on redox status of glutathione was evident as the percentage of the reduced form tended to increase after the GSE meal, while it decreased significantly after the control meal.

In agreement with the minor changes in the plasma levels of different antioxidants, the results for the TRAP analysis indicate that the antioxidant capacity of postprandial plasma significantly increased only when the food was supplemented with GSE. Moreover, while experimental TRAP values for the test meal fit the values calculated from the concentration of major plasma antioxidants, the measured TRAP values exceed the theoretical values following the GSE supplemented meal (**Table 3**). Thus, some other antioxidants appear to be contributing to the plasma

antioxidant capacity following the intake of the GSE-supplemented meal.

These data describe a postprandial oxidative stress following the test meal, associated with a rise of lipid hydroperoxides while the total plasma antioxidant capacity remains functionally unchanged. Supplementation of the meal with GSE blunts this oxidative stress by preventing the rise of lipid hydroperoxides and increasing the plasma antioxidant capacity.

The outcome of this effect on the balance between plasma oxidant/antioxidant status was evaluated on the basis of the oxidative stability of LDL, an accepted index for the occurrence of oxidative modifications to particle structure and composition. No statistically significant differences were observed in LDL levels of cholesterol, triacylglycerols, and α-tocopherol following the test meal and the GSE-supplemented meal (**Table 4**).

The oxidative resistance of postprandial LDL was tested using copper-catalyzed oxidation, by measuring the loss of tryptophan residues in apoB-100, and by the increase of the electrophoretic motility (**Table 5**). Although the observed differences did not reach statistical significance, the results suggest that LDL isolated after the control meal tended to be more susceptible to oxidative modification than baseline LDL, in agreement with previous reports, while the opposite trend was observed in the case of the GSE-supplemented meal.

DISCUSSION

This study provides evidence that a single high-fat fried meal induces a transient unbalance between lipid hydroperoxide level and antioxidant status in plasma, and that this change can be prevented when the meal is supplemented with a PAs-rich grape seed extract (GSE).

Different studies have shown that in rats (*45−47*), rabbits (*48*), and humans (*16*, *27*, *49*) dietary oxidized lipids or their degradation products (*50−55*) can reach plasma lipoproteins.

In agreement with these studies, we detected a postprandial increase of lipid peroxides in plasma, following the intake of a high polyunsaturated fat test meal. Consistently, lipid hydroperoxides in chylomicrons were higher following the control meal than the GSE-supplemented meal, the specific content of lipid hydroperoxides/mg of cholesterol in chylomicrons being higher in all subjects. Moreover, following the GSE-supplemented meal, the average plasma peroxide concentration was comparable to fasting levels.

The most likely mechanism of the protective effect of GSE is related to the decomposition of hydroperoxides during gastric digestion. Lipid hydroperoxides present in food are decomposed in the presence of catalysts such as the myoglobin of meat, producing further peroxidation of lipids and thus increasing the chance of absorption. As a prototypical hemoprotein, myoglobin catalyzes the decomposition of hydroperoxides to peroxyl radicals and other reactive products (*31*). In meals without meat, it is possible that other heme proteins may also catalyze similar reactions, albeit with differing efficacy. In the presence of a suitable hydrogen donor, the decomposition of lipid hydroperoxides can take place without peroxidative chain propagation since peroxidation-driving radicals are quenched (*31*). This mechanism is reasonably more efficient for PAs among other catechol bearing polyphenols due to the hydrogen transfer mechanism of the redox transition that is not inhibited by acidic pH (*32*). Remarkably, during the peroxidatic cycle the interaction between the heme compound and a suitable hydrogen donor not only prevents peroxidation but also eliminates the hydroperoxides present in the food.
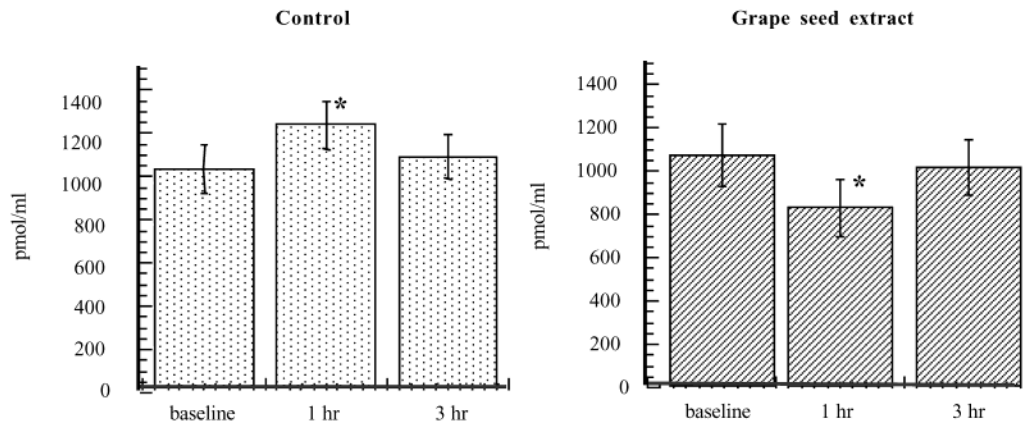
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Control

Grape seed extract

Figure 2. Effect of GSE on plasma postprandial lipid hydroperoxides, showing the time course of the concentration of plasma LPO (pmol/mL) after administration of the two test meals. Data represent mean ± se of the 8 subjects for each test meal.

Table 2. Plasma Concentration of Indicators of Antioxidant Status (Mean ± SD of 8 Subjects)

	control			grape seed extract		
	baseline	1 h	3 h	baseline	1 h	3 h
α-tocopherol, μg/mL	9.6 ± 1.4	9.9 ± 1.4	9.9 ± 1.5	9.1 ± 1.2	9.4 ± 1.3	9.4 ± 1.3
SH group, μM	465 ± 20	454 ± 26	467 ± 28	437 ± 11	438 ± 11	462 ± 11[a]
uric acid, mg/dL	5.8 ± 0.2	6.4 ± 0.2[b]	6.6 ± 0.3[b]	6.0 ± 0.3	6.4 ± 0.3[b]	6.6 ± 0.6[a]
glutathione tot., μM	6.3 ± 0.3	5.7 ± 0.4[a]	5.5 ± 0.4[a]	6.1 ± 0.6	5.5 ± 0.4	5.7 ± 0.4
glutathione red, % tot.	26 ± 2	21 ± 1[a]	23 ± 2[a]	21 ± 2	24 ± 1	25 ± 3
ascorbic acid tot., μM	67.0 ± 7.7	69.6 ± 8.4	71.1 ± 9.5	72.0 ± 9.5	75.6 ± 10.0[b]	73.0 ± 9.2
ascorbic acid red, % tot.	95 ± 2	93 ± 3	90 ± 1	96 ± 1.5	95 ± 2.4	95 ± 2[c]

[a] $P < 0.05$ and [b] $P < 0.005$ from baseline by paired *t*-test. [c] $P < 0.05$ from control at 3 h by paired *t*-test.

Table 3. Comparison between "TRAP Measured" and "TRAP Calculated"[a]

	control			grape seed extract		
	baseline	1 h	3 h	baseline	1 h	3 h
TRAP measd, mM	1.22 ± 0.04	1.26 ± 0.05	1.26 ± 0.05	1.19 ± 0.07	1.30 ± 0.08[b]	1.31 ± 0.06[c,d]
TRAP calcd, mM	1.22 ± 0.04	1.26 ± 0.04	1.28 ± 0.04	1.19 ± 0.07	1.23 ± 0.07	1.26 ± 0.06[d]

[a] TRAP calculated was obtained adding to the basal (measured) TRAP, the value resulting from the changes in the concentration of each TRAP component, multiplied by its specific stoichiometric factor (40). [b] $P < 0.05$ and [c] $P < 0.005$ from baseline by paired *t*-test. [d] $P < 0.01$ "TRAP measured" from "TRAP calculated" by paired *t*-test.

Table 4. Concentration of Metabolic Parameters in LDL (Mean ± SD of 8 Subjects)

	control		grape seed extract	
	baseline	3 h	baseline	3 h
cholesterol, mg/mg of protein	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.7	1.5 ± 0.7
triacylglycerol, mg/mg of protein	0.32 ± 0.06	0.33 ± 0.07	0.33 ± 0.08	0.28 ± 0.07
α-tocopherol, μg/mg of protein	5.4 ± 0.4	5.6 ± 0.6	5.7 ± 0.7	5.5 ± 0.7

Table 5. Time Course of in Vitro LDL Oxidation by 5 μM Cu(II) (Mean ± SE of 8 Subjects)

		control		grape seed extract	
	time (h)	baseline 0 h	postprandial 3 h	baseline 0 h	postprandial 3 h
tryptophan residues, %	0	100	100	100	100
	2	60 ± 6	57 ± 5	65 ± 8	64 ± 5
	4	39 ± 6	32 ± 2	39 ± 9	41 ± 5
REM[a]	0	1	1	1	1
	4	3.1 ± 0.3	3.8 ± 0.2	3.5 ± 0.5	3.1 ± 0.3

[a] Relative electrophoretic mobility.

As an alternative or additive mechanism, it is possible that some polyphenols present in GSE or produced during metabolism prevent the postprandial increase in lipid hydroperoxides by activating serum paraoxonase, a HDL-enzyme decomposing lipoprotein peroxides (56−58). Such a mechanism would require bioavailability of some PAs metabolites; our study seems to indirectly confirm that PAs (likely the low-weight polymer) or their microbial metabolites are absorbed. In fact, we observe an increase in plasma antioxidant capacity, which could not be explained by postprandial increase in the endogenous antioxidants.

Finally, PAs or their metabolites could indirectly reduce dietary lipid hydroperoxides in gut or in liver inducing detoxifying enzyme.

The postprandial plasma antioxidant status depends on factors such as the intake and consumption of compounds that are either oxidants or antioxidants and the sparing effect of some antioxidants on other antioxidants that have higher redox potentials. Thermodynamic constraints and kinetics of reactions involved prevent an accurate prediction of the outcome of these multiple pathways in the network of oxidant/antioxidant reactions taking place before and after absorption. Nevertheless, the present data indicate that a postprandial oxidative stress is imposed in plasma through the concurrent increase in lipid hydroperoxides with only minor changes in the antioxidant status. When the meal is supplemented with GSE, the plasma

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level of lipid hydroperoxides is prevented and net plasma antioxidant capacity increases. This is apparently accounted for by increased ascorbate, thiols, and urate along with a yet undefined antioxidant (probably a PAs metabolite). The presence of the latter is suggested by a discrepancy between the theoretical and experimental values of TRAP.

The presence of lipid hydroperoxides in plasma lipoproteins and the plasma antioxidant status appears also to affect the oxidative stability of LDL. In a recent study we demonstrated that red wine prevented the postprandial increase in LDL oxidative susceptibility (*26*). The effect was similar, although less straightforward, in this study when the meal was supplemented with GSE enriched with PA, instead of wine. The possible role of ethanol in enhancing the effect of polyphenols therefore emerges as an appealing hypothesis

It has been recently reported that during the postprandial phase the percentage of atherogenic LDL− increases. In this mildly modified LDL the content of lipid hydroperoxides and lysophospholipids are higher than in the remainder of the unmodified LDL. Moreover, the apo B of LDL− is unfolded (*59*).

The present findings regarding the postprandial increase in LDL oxidizability fits well with the increase in LDL− since several groups have shown that LDL− is more susceptible to oxidation and that the presence of LDL− enhances the oxidative susceptibility of normal LDL.

The protection afforded by GSE is evident in light of the prevention of a postprandial plasma rise of lipid hydroperoxides both in the pre- and postabsorptive phase, in turn due to decomposition of lipid peroxides and inhibition of lipid peroxidation in the stomach, providing further direct/indirect antioxidant protection in plasma.

ABBREVIATIONS USED

BHT, butylated hydroxytoluene; EDTA, ethylenediaminetetraacetic acid; GSE, grape seed extract; GSH, glutathione; LDL, low-density lipoprotein; LPO, lipid hydroperoxide; PA, proanthocyanidin; REM, relative electrophoretic mobility; TRAP, total radical trapping antioxidant parameter.

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LITERATURE CITED

(1) Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds in human nutrition. *J. Food Sci. Agric.* **2000**, *80*, 1094−1117.

(2) Arteel, G. E.; Sies, H. Protection against peroxynitrite by cocoa polyphenol oligomers. *FEBS Lett* **1999**, *462*, 167−70.

(3) Ariga, T.; Hamano, M. Radical scavenging action and its mode in procyanidins B-1 and B-3 from Azuki beans to peroxyl radicals. *Agric. Biol. Chem.* **1990**, *54*, 2499−2504.

(4) Hagerman, A. E.; Riedl, K. M.; Jones, G. A.; Sovik, K. N.; Ritchard, N. T.; Hartzfeld, P. W.; Riechel, T. L. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem.* **1998**, *46*, 1887−1892.

(5) Mao, T. K.; Powell, J. J.; van de Water, J.; Keen, C. L.; Schmitz, H. H.; Gershwin, M. E. The influence of cocoa procyanidins on the transcription of interleukin-2 in peripheral blood mononuclear cells. *Int. J. Immunother.* **1999**, *15*, 23−29.

(6) Rein, D.; Paglieroni, T. G.; Wun, T.; Pearson, D. A.; Schmitz, H. H.; Gosselin, R.; Keen, C. L. Cocoa inhibits platelet activation and function. *Am. J. Clin. Nutr.* **2000**, *72*, 30-5.

(7) Packer, L.; Rimbach, G.; Virgili, F. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (Pinus maritima) bark, pycnogenol. *Free Radical Biol. Med.* **1999**, *27*, 704−24.

(8) Sanbongi, C.; Suzuki, N.; Sakane, T. Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans in vitro. *Cell Immunol.* **1997**, *177*, 129−36.

(9) 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*, 6384−90.

(10) Yamakoshi, J.; Kataoka, S.; Koga, T.; Ariga, T. Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* **1999**, *142*, 139−49.

(11) Tebib, K.; Rouanet, J. M.; Besancon, P. Effect of grape seed tannins on the activity of some rat intestinal enzyme activities. *Enzyme Protein* **1994**, *48*, 51−60.

(12) Ursini, F.; Tubaro, F.; Rong, J.; Sevanian, A. Optimization of nutrition: polyphenols and vascular protection. *Nutr. Rev.* **1999**, *57*, 241−9.

(13) Deprez, S.; Mila, I.; Scalbert, A. Carbon-14 biolabeling of (+)-catechin and proanthocyanidin oligomers in willow tree cuttings. *J. Agric. Food Chem.* **1999**, *47*, 4219−30.

(14) Koga, T.; Moro, K.; Nakamori, K.; Yamakoshi, J.; Hosoyama, H.; Kataoka, M.; Ariga, T. Increase of oxidative potential of rat plasma by oral administration of proanthocyanidin-rich extract from grape seeds. *J. Agric. Food Chem.* **1999**, *47*, 1892−1897.

(15) Ursini, F.; Sevanian, A. Postprandial oxidative stress. *Biol. Chem.* **2002**, in press.

(16) Ursini, F.; Zamburlini, A.; Cazzolato, G.; Maiorino, M.; Bon, G. B.; Sevanian, A. Postprandial plasma lipid hydroperoxides: a possible link between diet and atherosclerosis. *Free Radical Biol. Med.* **1998**, *25*, 250−2.

(17) Staprans, I.; Hardman, D. A.; Pan, X. M.; Feingold, K. R. Effect of oxidized lipids in the diet on oxidized lipid levels in postprandial serum chylomicrons of diabetic patients. *Diabetes Care* **1999**, *22*, 300−6.

(18) Zilversmit, D. B. Atherogenesis: a postprandial phenomenon. *Circulation* **1979**, *60*, 473−85.

(19) Patsch, J. R.; Miesenbock, G.; Hopferwieser, T.; Muhlberger, V.; Knapp, E.; Dunn, J. K.; Gotto, A. M., Jr.; Patsch, W. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler. Thromb.* **1992**, *12*, 1336−45.

(20) Cohn, J. S. Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. *Can. J. Cardiol.* **1998**, *14 Suppl B*, 18B−27B.

(21) Karpe, F.; Steiner, G.; Uffelman, K.; Olivecrona, T.; Hamsten, A. Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* **1994**, *106*, 83−97.

(22) Cohn, J. S.; McNamara, J. R.; Cohn, S. D.; Ordovas, J. M.; Schaefer, E. J. Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. *J. Lipid Res.* **1988**, *29*, 925−36.

(23) Ebenbichler, C. F.; Kirchmair, R.; Egger, C.; Patsch, J. R. Postprandial state and atherosclerosis. *Curr. Opin. Lipidol.* **1995**, *6*, 286−90.

(24) Chung, B. H.; Hennig, B.; Cho, B. H.; Darnell, B. E. Effect of the fat composition of a single meal on the composition and cytotoxic potencies of lipolytically releasable free fatty acids in postprandial plasma. *Atherosclerosis* **1998**, *141*, 321−32.

(25) Lechleitner, M.; Hoppichler, F.; Foger, B.; Patsch, J. R. Low-density lipoproteins of the postprandial state induce cellular cholesteryl ester accumulation in macrophages. *Arterioscler. Thromb.* **1994**, *14*, 1799−807.

(26) Natella, F.; Ghiselli, A.; Guidi, A.; Ursini, F.; Scaccini, C. Red wine mitigates the postprandial increase of LDL susceptibility to oxidation. *Free Radical Biol. Med.* **2001**, *30*, 1036−44.

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(27) Staprans, I.; Rapp, J. H.; Pan, X. M.; Kim, K. Y.; Feingold, K. R. Oxidized lipids in the diet are a source of oxidized lipid in chylomicrons of human serum. *Arterioscler. Thromb.* **1994**, *14*, 1900−5.

(28) Staprans, I.; Rapp, J. H.; Pan, X. M.; Hardman, D. A.; Feingold, K. R. Oxidized lipids in the diet accelerate the development of fatty streaks in cholesterol-fed rabbits. *Arterioscler. Thromb. Vasc. Biol.* **1996**, *16*, 533−8.

(29) Staprans, I.; Pan, X. M.; Rapp, J. H.; Feingold, K. R. Oxidized cholesterol in the diet accelerates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Arterioscler. Thromb. Vasc. Biol.* **1998**, *18*, 977−83.

(30) Staprans, I.; Pan, X. M.; Rapp, J. H.; Grunfeld, C.; Feingold, K. R. Oxidized cholesterol in the diet accelerates the development of atherosclerosis in LDL receptor- and apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **2000**, *20*, 708−14.

(31) Kanner, J.; Lapidot, T. The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biol. Med.* **2001**, *31*, 1388−95.

(32) Ursini, F.; Rapuzzi, I.; Toniolo, R.; Tubaro, F.; Bontempelli, G. Characterization of antioxidant effect of procyanidins. *Methods Enzymol.* **2001**, *335*, 338−50.

(33) Gabetta, B.; Fuzzati, N.; Griffini, A.; Lolla, E.; Pace, R.; Ruffilli, T.; Peterlongo, F. Characterization of proanthocyanidins from grape seeds. *Fitoterapia* **2000**, *71*, 162−75.

(34) *INN Tabelle di Composizione degli Alimenti*; Istituto Nazionale della Nutrizione: Roma, 1997.

(35) Ellmann, G. L. Tissue sulphydriyl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70−77.

(36) Kutnink, M. A.; Hawkes, W. C.; Schaus, E. E.; Omaye, S. T. An internal standard method for the unattended high-performance liquid chromatographic analysis of ascorbic acid in blood components. *Anal. Biochem.* **1987**, *166*, 424−30.

(37) Behrens, W. A.; Madere, R. A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids, and foods. *Anal. Biochem.* **1987**, *165*, 102−7.

(38) Bieri, J. G.; Talliver, G. T.; Catignani, L. G. Simultaneous determination of α-tocopherol and retinol in plasma or red cells by high-pressure liquid chromatography. *Am. J. Clin. Nutr.* **1979**, *32*, 2143−2149.

(39) Neuschwander-Tetri, B. A.; Roll, F. J. Glutathione measurement by high-performance liquid chromatography separation and fluorometric detection of the glutathione-orthophthalaldehyde adduct. *Anal. Biochem.* **1989**, *179*, 236−41.

(40) Ghiselli, A.; Serafini, M.; Maiani, G.; Azzini, E.; Ferro-Luzzi, A. A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radical Biol. Med.* **1995**, *18*, 29−36.

(41) Zamburlini, A.; Maiorino, M.; Barbera, P.; Roveri, A.; Ursini, F. Direct measurement by single photon counting of lipid hydroperoxides in human plasma and lipoproteins. *Anal. Biochem.* **1995**, *232*, 107−13.

(42) Havel, R. J.; Eder, H. A.; Bragdon, J. H. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **1955**, *34*, 1345−1353.

(43) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

(44) Reyftmann, J. P.; Santus, R.; Maziere, J. C.; Morliere, P.; Salmon, S.; Candide, C.; Maziere, C.; Haigle, J. Sensitivity of tryptophan and related compounds to oxidation induced by lipid autoperoxidation. Application to human serum low- and high- density lipoproteins. *Biochim. Biophys. Acta* **1990**, *1042*, 159−67.

(45) Staprans, I.; Rapp, J. H.; Pan, X. M.; Feingold, K. R. The effect of oxidized lipids in the diet on serum lipoprotein peroxides in control and diabetic rats. *J. Clin. Invest.* **1993**, *92*, 638−43.

(46) Aw, T. Y.; Williams, M. W.; Gray, L. Absorption and lymphatic transport of peroxidized lipids by rat small intestine in vivo: role of mucosal GSH. *Am. J. Physiol.* **1992**, *262*, G99−106.

(47) Staprans, I.; Pan, X. M.; Miller, M.; Rapp, J. H. Effect of dietary lipid peroxides on metabolism of serum chylomicrons in rats. *Am. J. Physiol.* **1993**, *264*, G561−8.

(48) Nakatsugawa, K.; Kameda, T. Absorption of methyl linoleate hydoperoxides in rabbits. *Yukagaku (J. Jpn. Oil Chem. Soc.)* **1980**, *32*, 361−366.

(49) Naruszewicz, M.; Wozny, E.; Mirkiewicz, E.; Nowicka, G.; Szostak, W. B. The effect of thermally oxidized soya bean oil on metabolism of chylomicrons. Increased uptake and degradation of oxidized chylomicrons in cultured mouse macrophages. *Atherosclerosis* **1987**, *66*, 45−53.

(50) Grootveld, M.; Atherton, M. D.; Sheerin, A. N.; Hawkes, J.; Blake, D. R.; Richens, T. E.; Silwood, C. J.; Lynch, E.; Claxson, A. W. In vivo absorption, metabolism, and urinary excretion of alpha, beta-unsaturated aldehydes in experimental animals. Relevance to the development of cardiovascular diseases by the dietary ingestion of thermally stressed polyunsaturate-rich culinary oils. *J. Clin. Invest.* **1998**, *101*, 1210−8.

(51) Kanazawa, K.; Kanazawa, E.; Natake, M. Uptake of secondary autoxidation products of linoleic acid by the rat. *Lipids* **1985**, *20*, 412−9.

(52) Kanazawa, K.; Ashida, H. Dietary hydroperoxides of linoleic acid decompose to aldehydes in stomach before being absorbed into the body. *Biochim. Biophys. Acta* **1998**, *1393*, 349−61.

(53) Vine, D. F.; Croft, K. D.; Beilin, L. J.; Mamo, J. C. Absorption of dietary cholesterol oxidation products and incorporation into rat lymph chylomicrons. *Lipids* **1997**, *32*, 887−93.

(54) Osada, K.; Sasaki, E.; Sugano, M. Lymphatic absorption of oxidized cholesterol in rats. *Lipids* **1994**, *29*, 555−9.

(55) Emanuel, H. A.; Hassel, C. A.; P. B., A.; Bergmann, S. D.; Zavoral, J. H. Plasma cholesterol oxidation products (oxysterols) in human subjects fed a meal rich in oxysterols. *J. Food Sci.* **1991**, *56*, 843−847.

(56) Aviram, M.; Rosenblat, M.; Bisgaier, C. L.; Newton, R. S.; Primo-Parmo, S. L.; La Du, B. N. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin. Invest.* **1998**, *101*, 1581−90.

(57) Aviram, M.; Dornfeld, L.; Rosenblat, M.; Volkova, N.; Kaplan, M.; Coleman, R.; Hayek, T.; Presser, D.; Fuhrman, B. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *Am. J. Clin. Nutr.* **2000**, *71*, 1062−76.

(58) Hayek, T.; Fuhrman, B.; Vaya, J.; Rosenblat, M.; Belinky, P.; Coleman, R.; Elis, A.; Aviram, M. Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arterioscler. Thromb. Vasc. Biol.* **1997**, *17*, 2744−52.

(59) Parasassi, T.; Bittolo-Bon, G.; Brunelli, R.; Cazzolato, G.; Krasnowska, E. K.; Mei, G.; Sevanian, A.; Ursini, F. Loss of apoB-100 secondary structure and conformation in hydroperoxide rich, electronegative LDL(−). *Free Radical Biol. Med.* **2001**, *31*, 82−9.