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Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol–vitamin E-deficient diet

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Effects of dietary monomeric and polymeric grape seed tannins on the activity of antioxidant enzymes, total glutathione and level of lipid peroxidation in various tissues were investigated in rats fed a high cholesterol diet poor in vitamin E. They were compared with those in rats receiving a high cholesterol–vitamin E-sufficient diet without addition of tannins. Four groups of rats were studied for 10 weeks: Group 1, sufficient vitamin E diet; Group 2, deficient vitamin E diet; Group 3, deficient vitamin E diet + monomeric tannins (71 mg/kg); Group 4, deficient vitamin E diet + polymeric tannins (71 mg/kg). Compared with a normal vitamin E diet (Group 1), aortic, cardiac, hepatic, intestinal, muscular and renal catalase, glutathione peroxidase and superoxide dismutase activities were significantly lower in rats receiving the deficient vitamin E diet (Group 2); polymeric tannins (Group 4), but not monomeric tannins, were able to restore all these enzymic activities. In all tissues and in blood, total glutathione concentration, which was significantly lowered by vitamin E deficiency, was brought to the normal level only with polymeric tannins. Furthermore, the lipid peroxidation in plasma and tissues was significantly reduced in the presence of supplemented polymeric tannins as much as in the presence of vitamin E. It is therefore likely that polymeric grape seed tannins function as antioxidants *in vivo*, negating the effects of the oxidative stress induced by both vitamin E deficiency and atherogenic diet.

INTRODUCTION

Free radical-mediated lipid peroxidation has been implicated in a variety of pathological processes (Kehrer, 1993), especially in both the initiation and promotion of atherosclerosis (Henning & Chow, 1988; Henriksson *et al.*, 1985; Prasad & Kalra, 1993; Steinberg *et al.*, 1989; Steinberg, 1992). Free radical involvement in such pathological states occurs whenever a disturbance in the pro-oxidant–antioxidant balances is in favour of the former, leading to potential damage. Nutrition plays an important role in determining the cellular antioxidative defence mechanisms (Chow, 1988); vitamin E, selenium and copper are the major dietary factors that are known to affect the antioxidative ability of an organism (Capel, 1988). Increased superoxide anion ($O_2^{\cdot -}$) production in hypercholesterolaemic vessels contributes to the atherosclerotic process (Ohara *et al.*, 1993). It has been reported that hypercholesterolaemic atherosclerosis is associated with increased tissue content of a lipid peroxidation product, malondialdehyde (MDA). A number of reports have suggested that the oxidation of LDL induced by free radicals is an important initial event in pathogenesis of atherosclerosis in humans (Esterbauer *et al.*, 1992; Steinberg *et al.*, 1989); these studies suggested that oxygen free radicals are increased in hypercholesterolaemia. Vitamin E prevents oxidative modification of LDL (Steinbrecher *et al.*, 1984) and oxidation of LDL could not be detected until all endogenous vitamin E was consumed (Jessup *et al.*, 1990); thus, antioxidant intake would be expected to retard the development of atherosclerosis. Cellular antioxidant enzymatic defences exist, such as superoxide dismutase (SOD, EC 1.15.1.1), which dismutates the superoxide anion to hydrogen peroxide and oxygen, and catalase (CAT, EC 1.11.1.6) and glutathione peroxidase (GSH-Px, EC 1.11.1.9), which detoxify hydrogen peroxide (Marklund *et al.*,

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1982). Elsewhere, the protective role of glutathione (GSH) as an antioxidant and detoxifying agent has been demonstrated (Lash *et al.*, 1986); it is an ubiquitous compound which is rapidly synthesized in many tissues (Manohar & Balasubramanian, 1986).

It is well known that plant polyphenols act as free radical scavengers *in vitro* (Constantino *et al.*, 1992); tannins occur naturally in plant foods such as tea infusions, wines and fruit juices (Hertog *et al.*, 1993) and exhibit antioxidant effects (Afanas'ev *et al.*, 1989; Hertog *et al.*, 1993; Miyara *et al.*, 1993; Torel *et al.*, 1986). Tea catechins and (−)-epigallocatechin 3-O-gallate (Matsuzaki & Hara, 1985; Uchida *et al.*, 1991), procyanidins B-1 and B-3 from azuki beans (Ariga & Hamano, 1990) and procyanidolic oligomers from grape seeds (Masquelier, 1988; Meunier *et al.*, 1989) are known to have remarkable antioxidant activities in aqueous systems, which suggests that they may act *in vivo* as radical trappers. The elevated levels of free radicals during a hypercholesterolaemic pathological state with vitamin E deficiency could be due either to their overproduction and/or to the decrease in the activity of their metabolizing enzymes (SOD, CAT, GSH-Px). In previous work (Tebib *et al.*, 1994*a*,*b*) we have shown the protective effect of grape seed tannins against plasma cholesterol and LDL-cholesterol. Then, we hypothesized that tannins, through their antioxidant properties, would exert a beneficial effect against oxidant stress.

We therefore investigated the effects of grape seed tannins, either in a monomeric or in a polymeric form, on lipid peroxidation, total glutathione content and on the activity of CAT, GSH-Px, SOD in the blood and aortic tissue, and also in heart, intestinal mucosa, muscle, kidney and liver of rats which were fed a high cholesterol–vitamin E-deficient diet.

MATERIALS AND METHODS

Animals and diets

Male Sprague Dawley rats ($n=24$) weighing about 145 g were divided randomly into four dietary groups of six. All groups received an experimental semi-synthetic diet (Table 1). Group 1 was fed a high cholesterol–sufficient vitamin E diet (192 mg/kg). Group 2 was fed a high cholesterol–vitamin E-deficient diet (22 mg/kg). Groups 3 and 4 received the diet fed to Group 2, to which was added either monomeric or polymeric grape seed tannins, respectively. The animals were maintained by pair feeding over a 10 week experimental period which was chosen to adequately deplete plasma and tissue stores of α-tocopherol in the rats receiving the diet deficient in vitamin E (Bieri, 1972). The tannin concentration of the diets was established by extrapolating average tannin consumption (500 mg/day) for a 70 kg human to the equivalent for a 200 g rat. Thus, the tannin concentration of the diets supplying either monomers or polymers was set at 0.071 mg/g diet and can be related to the daily amount of tannins consumed from 0.5 litre red wine (~250 mg tannins) and food. The fatty acid composition of the diets is shown in Table 2.

Preparation of procyanidin extracts

Grape seed tannins were purchased from DRT (Les Dérivés Résiniques and Terpéniques, Dax, France); monomers and polymers were extracted and separated as previously described (Tebib *et al.*, 1994*a*,*b*), using 70% acetone solubilization and repeated ethyl acetate extractions.

Table 1. Composition of the diets fed to rats[a]

Component (g/kg diet)	Control + vitamin E (Group 1)	Control − vitamin E (Group 2)	Experimental (Groups 3 and 4)
Casein[b] (14.45% N)	217	217	217
Cornstarch	433	433	433
Sucrose	50	50	50
Vegetable oil[c]	20	20	20
Lard	150	150	150
Mineral mix[d]	110	110	110
Vitamin mix[e]	10	—	—
Vitamin E-free vitamin mix[f]	—	10	10
Added cholesterol	10	10	10
Total vitamin E (mg/kg diet)	192	22	22

[a]Tannin content of experimental diets was 71 mg monomers/kg diet (Group 3) and 71 mg polymers/kg diet (Group 4).
[b]N×6.38.
[c]Maize oil:soybean oil (1:1).
[d]Mineral mix (g/kg mix): $CaHPO_4$, 430; KCl, 100; NaCl, 10; $MgCl_2$, 50; $MgSO_4.7H_2O$, 50; Fe_2O_3, 30; $MnSO_4.H_2O$, 2.5; $ZnSO_4.H_2O$, 2; $CuSO_4.5H_2O$, 0.5; $CoSO_4$, 0.004; KI, 0.008; cellulose Q.S.P. 1000 g.
[e]Vitamin mix (mg/kg mix): retinol, 600; cholecalciferol, 300; all-*rac*-α-tocopheryl acetate, 17000; menadione, 200; thiamin, 2000; riboflavin, 1500; nicotinic acid, 10000; pantothenic acid, 7000; pyridoxine, 1000; inositol, 15000; biotin, 30; folic acid, 500; cyanocobalamin, 0.5; ascorbic acid, 80000; para amino benzoic acid, 5000; choline, 136000; cellulose Q.S.P. 1000 g.
[f]In the vitamin mix, all-*rac*-α-tocopheryl acetate was replaced by an equal amount of cellulose.

Experimental protocols and tissue preparation

Rats were anaesthetized by an intraperitoneal injection of pentobarbital. Blood was withdrawn by cardiac puncture with heparin-moistened syringes. The aorta was dissected between diaphragm and bifurcation into iliac arteries and was rinsed in saline; the connective tissue was discarded and samples were blotted dry and stored in liquid nitrogen. Heart, kidney, liver, gastocnemius and intestinal mucosa were also collected. The subsequent steps were carried out at 4°C. Tissues were homogenized in 10 mM Tris–HCl, 150 mM KCl, 1 mM EDTA, 1 mM dithioerythritol (DTET), 0.25 mM phenylmethylsulphonyl fluoride (pH 7.4) and centrifuged at 10000*g* for 15 min; the pellet was discarded and aliquots of supernatant were stored in liquid nitrogen for later estimation of protein content, antioxidant defences and free radical damage. All samples were assayed within 20 days after collection.

Table 2. Fatty acid composition of the diets

Fatty acids	g/kg diet
14:0	2.3
16:0	40.0
16:1 (*n*−7)	3.9
18:0	23.3
18:1 (*n*−9)	70.2
18:2 (*n*−6)	24.3
18:3 (*n*−3)	1.8
20:0	0.3
20:1 (*n*−9)	1.4
22:2 (*n*−6)	0.6
Total saturated	65.9
Total monounsaturated	75.5
Total polyunsaturated	26.7

Analytical procedures

Plasma was assayed for total cholesterol (TC), HDL-cholesterol (HDLC) and LDL-cholesterol (LDLC) with Boehringer enzyme kits (Boehringer–Mannheim,

Table 3. Protein content, total glutathione concentration and lipid peroxidation level in blood, plasma and tissues from rats fed a high cholesterol–vitamin E-sufficient diet and rats fed a high cholesterol–low vitamin E diet either with or without grape seed tannins[a]

Tissue	Group 1 +vitamin E	Group 2 −vitamin E	Group 3 −vitamin E +Monomers	Group 4 −vitamin E +Polymers
Blood				
Total glutathione (nmol/ml)	129.8 ± 5.6^{a}	37.9 ± 4.4^{b}	35.4 ± 4.5^{b}	135.7 ± 7.7^{a}
Plasma				
Total cholesterol (mg/100 ml)	106.8 ± 2.3^{b}	136.7 ± 9.6^{a}	130.0 ± 5.8^{a}	99.6 ± 5.8^{b}
HDLC (mg/100 ml)	13.3 ± 2.1^{ab}	10.0 ± 1.8^{bc}	7.1 ± 0.9^{c}	16.5 ± 2.4^{a}
LDLC (mg/100 ml)	68.3 ± 2.8^{b}	78.3 ± 2.4^{a}	76.6 ± 2.6^{a}	56.6 ± 4.0^{c}
Protein (mg/ml)	68 ± 5^{a}	66 ± 6^{a}	67 ± 5^{a}	69 ± 6^{a}
MDA (nmol/mg protein)	49 ± 5^{b}	123 ± 18^{a}	115 ± 11^{a}	41 ± 5^{b}
MDA (μmol/l)	3.27 ± 0.57^{b}	6.61 ± 1.7^{a}	6.54 ± 1.33^{a}	2.73 ± 0.28^{b}
Aorta				
Protein (mg/g wet weight)	31.3 ± 1.5^{a}	30.6 ± 1.8^{a}	29.9 ± 1.7^{a}	30.5 ± 1.4^{a}
Glutathione (nmol/mg protein)	1.43 ± 0.08^{a}	0.36 ± 0.01^{b}	0.41 ± 0.06^{b}	1.27 ± 0.07^{a}
MDA (μmol/mg protein)	25.56 ± 1.22^{b}	41.71 ± 3.11^{a}	44.32 ± 3.52^{a}	25.52 ± 1.45^{b}
Heart				
Protein (mg/g wet weight)	41.6 ± 1.3^{a}	40.6 ± 1.7^{a}	42.0 ± 1.3^{a}	41.7 ± 1.5^{a}
Glutathione (nmol/mg protein)	1.22 ± 0.08^{a}	0.36 ± 0.03^{b}	0.46 ± 0.05^{b}	1.09 ± 0.03^{a}
MDA (μmol/mg protein)	30.40 ± 1.55^{b}	53.40 ± 2.37^{a}	50.54 ± 2.98^{a}	28.93 ± 1.05^{b}
Gastrocnemius				
Protein (mg/g wet weight)	65.6 ± 3.7^{a}	68.9 ± 3.0^{a}	69.9 ± 1.7^{a}	67.2 ± 2.1^{a}
Glutathione (nmol/mg protein)	0.40 ± 0.04^{a}	0.08 ± 0.01^{b}	0.09 ± 0.01^{b}	0.38 ± 0.03^{a}
MDA (μmol/mg protein)	29.18 ± 1.56^{b}	34.20 ± 1.68^{a}	34.00 ± 1.65^{a}	28.86 ± 1.43^{b}
Intestinal mucosa				
Protein (mg/g wet weight)	53.5 ± 2.1^{a}	47.5 ± 3.1^{a}	51.2 ± 2.9^{a}	52.4 ± 3.4^{a}
Glutathione (nmol/mg protein)	120.37 ± 8.41^{a}	32.63 ± 5.26^{b}	34.96 ± 4.49^{b}	107.63 ± 4.58^{a}
MDA (μmol/mg protein)	12.68 ± 0.74^{b}	31.65 ± 3.28^{a}	35.24 ± 1.38^{a}	6.68 ± 0.40^{c}
Kidney				
Protein (mg/g wet weight)	32.6 ± 0.9^{a}	33.0 ± 1.5^{a}	32.8 ± 0.9^{a}	32.5 ± 1.2^{a}
Glutathione (nmol/mg protein)	0.49 ± 0.04^{a}	0.15 ± 0.03^{b}	0.18 ± 0.04^{b}	0.49 ± 0.05^{a}
MDA (μmol/mg protein)	7.70 ± 0.40^{b}	21.82 ± 0.85^{a}	20.15 ± 0.65^{a}	9.36 ± 0.70^{b}
Liver				
Protein (mg/g wet weight)	51.9 ± 2.1^{a}	54.0 ± 2.6^{a}	53.0 ± 2.8^{a}	51.9 ± 2.1^{a}
Glutathione (nmol/mg protein)	156.06 ± 8.47^{a}	57.22 ± 6.30^{b}	73.58 ± 6.79^{b}	159.92 ± 6.93^{a}
MDA (μmol/mg protein)	17.05 ± 1.00^{b}	33.43 ± 1.60^{a}	37.35 ± 2.64^{a}	5.87 ± 0.12^{c}

[a]Values are means ± SEM of six observations per group; values in a row with different superscript letters are significantly different ($P < 0.05$).

Meylan, France). Free radical damage was estimated by specifically measuring malondialdehyde (MDA) using the LPO-586 assay kit purchased from Bioxytech (Bioxytech S.A., Bonneuil sur Marne, France). Briefly, samples (200 μl) were added to 650 μl of 8.5 mM chromogenic reactive (*N*-methyl phenyl indole); the reaction was started by adding 150 μl of 37% aqueous HCl. The mixture was incubated for 40 min at 45°C. The amount of MDA in the supernatant was determined at 586 nm using tetramethoxypropane as the standard. The activity of SOD was assayed by the method of Paoletti *et al.* (1986) and Paoletti & Mocali (1990). One unit of SOD activity was defined as the amount of protein which produced 50% inhibition of the rate of NADH oxidation observed in the control. Catalase activity was measured according to Lück (1965). Glutathione peroxidase activity was assessed by the method of Paglia and Valentine (1967) modified by Lawrence and Burk (1976) using cumene hydroperoxide as the substrate. For analysis of glutathione, 100 mg samples of tissue were homogenized in 15 vols perchloric acid (2 g/litre). After centrifugation, the supernatant fractions (200 μl) were assayed for total glutathione according to the procedure of Anderson (1985) where GSH content was determined by its reaction with 5-5′-dithiobis-(2-nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid at 412 nm. Whole blood total glutathione was analysed according to Kelly (1993). Protein determinations were performed according to Smith *et al.* (1985) using bovine serum albumin as standard. The fatty acid composition of the diets was analysed by gas chromatography after conversion of fatty acids to methyl esters.

Statistical analysis

Data are shown as the mean ± SEM of six observations per group. The statistical significance of differences between treatments was established by ANOVA using a Stat View 512+ microcomputer program (Brain Power, Calabasas, CA, USA) incorporating a calculation of least significant difference from the pooled estimate of variance. Differences were considered significant when $P < 0.05$.

RESULTS

Plasma cholesterol analyses are presented in Table 3. In the presence of sufficient dietary vitamin E (Group 1) and in that of polymeric tannins (Group 4), plasma total cholesterol was significantly reduced compared to other groups. Feeding polymers led to the highest HDLC concentration (not significant relative to Group 1).

Table 4. Antioxidant enzyme activities in plasma and tissues from rats fed a high cholesterol–vitamin E sufficient diet and rats fed high cholesterol–low vitamin E diets containing or not containing grape seed tannins[a]

Tissue	Group 1 +vitamin E	Group 2 −vitamin E	Group 3 −vitamin E +monomers	Group 4 −vitamin E +polymers
Plasma				
GSH-Px (U/mg protein)	0.260 ± 0.013^a	0.083 ± 0.008^b	0.050 ± 0.008^b	0.239 ± 0.026^a
Aorta				
Catalase (U/mg protein)	0.28 ± 0.02^a	0.13 ± 0.01^b	0.15 ± 0.01^b	0.26 ± 0.01^a
GSH-Px (U/mg protein)	0.300 ± 0.009^a	0.095 ± 0.012^b	0.089 ± 0.005^b	0.236 ± 0.015^a
SOD (U/mg protein)	2.03 ± 0.13^a	0.99 ± 0.08^b	0.85 ± 0.15^b	1.80 ± 0.10^a
Heart				
Catalase (U/mg protein)	13.3 ± 0.88^a	1.71 ± 0.16^b	1.62 ± 0.20^b	12.10 ± 0.72^a
GSH-Px (U/mg protein)	0.247 ± 0.020^a	0.028 ± 0.003^b	0.032 ± 0.002^b	0.242 ± 0.014^a
SOD (U/mg protein)	2.14 ± 0.15^a	0.72 ± 0.06^b	0.69 ± 0.08^b	2.06 ± 0.14^a
Gastocnemius				
Catalase (U/mg protein)	8.64 ± 0.55^a	0.71 ± 0.02^b	0.86 ± 0.10^b	8.51 ± 0.39^a
GSH-Px (U/mg protein)	0.111 ± 0.003^a	0.013 ± 0.002^b	0.015 ± 0.002^b	0.098 ± 0.004^a
SOD (U/mg protein)	0.88 ± 0.03^a	0.37 ± 0.06^b	0.40 ± 0.04^b	1.03 ± 0.09^a
Intestinal mucosa				
Catalase (U/mg protein)	0.38 ± 0.03^a	0.18 ± 0.01^b	0.17 ± 0.02^b	0.32 ± 0.03^a
GSH-Px (U/mg protein)	0.178 ± 0.019^a	0.086 ± 0.004^b	0.046 ± 0.006^c	0.165 ± 0.019^a
SOD (U/mg protein)	1.19 ± 0.09^a	0.59 ± 0.05^b	0.22 ± 0.02^c	1.46 ± 0.19^a
Kidney				
Catalase (U/mg protein)	10.54 ± 0.51^a	0.70 ± 0.08^c	0.80 ± 0.06^c	8.12 ± 0.3^b
GSH-Px (U/mg protein)	0.093 ± 0.012^a	0.038 ± 0.004^b	0.046 ± 0.006^b	0.105 ± 0.012^a
SOD (U/mg protein)	1.30 ± 0.09^a	0.56 ± 0.08^b	0.67 ± 0.10^b	1.22 ± 0.19^a
Liver				
Catalase (U/mg protein)	13.9 ± 0.62^a	0.84 ± 0.02^c	0.54 ± 0.01^c	$9,82 \pm 0.19^b$
GSH-Px (U/mg protein)	0.224 ± 0.008^a	0.031 ± 0.002^c	0.033 ± 0.001^c	0.186 ± 0.008^b
SOD (U/mg protein)	1.75 ± 0.13^a	0.20 ± 0.01^b	0.11 ± 0.01^b	1.70 ± 0.07^a

[a]Values are means ± SEM of six observations per group; values in a row with different superscript letters are significantly different ($P < 0.05$).

Plasma LDLC concentration was highest in rats fed the low vitamin E diet (Group 2) and this value was unaffected by monomers (Group 3); it was lower in animals receiving enough vitamin E (group 1) and lowest in the presence of dietary polymeric tannins (Group 4). Data on GSH and lipid peroxide concentration are given in Table 3. The low vitamin E diet and that supplying monomers, in comparison with the vitamin E-sufficient diet, decreased the amount of blood total GSH by about 70%; this was the same as the diet containing polymers. In all tissues, total GSH concentration was not significantly different in rats receiving a vitamin E sufficient diet (Group 1) or in rats receiving polymeric tannins; on the other hand, vitamin E deficiency (Group 2) led to a decreased GSH concentration and addition of monomers (Group 3) failed to restore it. In plasma and all tissues analysed, lipid peroxidation, i.e. MDA concentration (Table 3), was significantly lowered by polymers as well as by vitamin E compared with other dietary groups. Table 4 shows the effect of diets on antioxidant enzyme activities; generally, animals fed the diet supplying sufficient vitamin E (Group 1) and the diet supplying polymeric tannins (Group 4) showed significantly higher GSH-Px activities than did the other groups. Overall, CAT and SOD activities were in the same range after sufficiently feeding vitamin E or polymeric grape seed tannins, and were significantly higher than in the other groups.

DISCUSSION

We have reported previously that 9 week 2% dietary polymeric grape seed tannins prevent an increase in total and LDL plasma cholesterol in high cholesterol-fed rats (Tebib *et al.*, 1994*a*). Although the differences are not as large as in the former study, we confirmed these data using a lower dose of dietary tannins and vitamin E-restricted diets. Since, in Groups 1 and 4, plasma tissue MDAs were lower than in other groups, it can be expected that plasma LDL would be less oxidized, strengthening the beneficial effect of decreased LDLC concentration. Elsewhere, Ng and Hegele (1993) suggested that HDL particles may have an antioxidant effect and the ability to reduce LDL uptake by epithelial cells; this cannot be ruled out here for Group 4, which exhibited the highest HDLC level. The measurement of MDA enabled us to assess lipid peroxidation; our work demonstrates a significant role of polymeric grape seed tannins, as important as that of vitamin E in preventing *in vivo* peroxidative tissue damage. Vitamin E deficiency with or without the presence of monomeric tannins elevated the level of tissue MDA; the addition of polymers in the vitamin E-deficient diet resulted in a decrease of MDA in all tissues analysed. These data suggest that rats fed grape seed tannins are less susceptible to peroxidative damage under the challenge of oxidative stress, such as a high cholesterol diet. The supply of polymeric tannins restored the activity of all the antioxidant enzymes which were decreased by a vitamin E deficiency. It has been shown previously (Lane *et al.*, 1979) that rats fed Torula yeast diets devoid of vitamin E exhibited low GSH-Px activity in the small intestine, colon and liver tissues and catalase activity in the liver, compared with rats fed corn–soybean diets. Elsewhere, Gomi *et al.* (1995) reported that vitamin E-deficient rats had lower hepatic GSH-Px activity than normal rats after oxygen inhalation, whereas CAT and SOD activities remained unchanged. These findings confirmed other anterior studies such as those reported by Chow (1977), Chen *et al.* (1980), Suga *et al.* (1984) and Chen and Thacker (1986). However, a comparison with our results was somewhat difficult since diets, oxidative stress and duration of the experiments were different. Total GSH concentration, which is also an important antioxidant defence (Burk, 1983), was low in Groups 2 and 3; since it is a specific substrate for GSH-Px (Stadman, 1980), this finding may explain the loss in the tissue enzyme activity. Elsewhere, superoxide anion is known to inactivate CAT (Kono & Fridovich, 1982) and GSH-Px (Blum & Fridovich, 1985) and H_2O_2 to inactivate SOD (Bray *et al.*, 1974); thus, increased amount of oxyradicals and lipid peroxidation products would lead to an oxidative stress and to an exhaustion or inhibition of defences. It can be hypothesized that the reasons for decrease in tissue antioxidant enzyme activities in Groups 2 and 3 would be that increased levels of $O_2^{\cdot-}$ inactivate CAT and GSH-Px; inactivation of CAT and GSH-Px would lead to an enhancement of H_2O_2 level which, in turn, would inactivate SOD. On the other hand, the enhancement of lipid peroxidation products content in animals fed the low vitamin E diet was not surprising. Whatever may be the mechanism involved, this is a specific effect of tannins, since other enzyme activities such as hepatic glutamate–pyruvate transaminase and intestinal alkaline phosphatase, aminopeptidase *N* and sucrase were not significantly decreased, even in the presence of 0.2% dietary grape seed tannins (Vallet *et al.*, 1994); this suggests that one is not looking at a more generalized mechanism in these experiments. To our knowledge, this general restoration of antioxidant mechanisms by polymeric grape seed tannins is the first *in vivo* report and is in accordance with findings in other *in vitro* studies (De Whalley *et al.*, 1990; Frankel *et al.*, 1993; Kanner *et al.*, 1994): inhibitory effects of vitamin E and wine phenols on LDL oxidation were compared and it has been shown that diluted wine containing 10 μmol/litre phenolics had the same antioxidant ability as 10 μmol/litre quercetin; in contrast, vitamin E was only 50–60% as potent an antioxidant as the wine or quercetin. Iron, which is required for initiation of lipid peroxidation and which can rapidly catalyse additional radical-mediated tissue injury, might be involved also, since phenolic compounds such as tannins present a strong affinity for iron ions (Garcia-Lopez *et al.*, 1990). Thus, the antiperoxidative capability of tannins could

be ascribed to concomitant activities of scavenging free radicals and chelating iron. An additional mechanism by which wine tannins exert antioxidant activity is by complexing Cu^{2+} and reducing it to Cu^{+} which, in turn, may reduce hydroperoxides (Frankel *et al.*, 1993). Polymeric grape seed tannins appeared to have a supplementing effect in hypercholesterolaemic–vitamin E-deprived rats, since tissue lipid peroxidation products content is lower in Group 4 than in Group 2. Implications for the observed changes in antioxidant mechanisms are that tissue capabilities to compensate for oxidant stress may be protected by polymeric grape seed tannins and that one consequence of lipid peroxidation, i.e. the development of hypercholesterolaemic atherosclerosis, may be affected. Finally, our results represent an important contribution in explaining how wine phenolic antioxidants may protect against cardiovascular diseases via prevention of LDL oxidation.

Nevertheless, further studies are necessary to examine why monomeric tannins do not exhibit as beneficial an effect as polymers.

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