

Nonalcoholic Red Wine Extract and Quercetin Inhibit LDL Oxidation without Affecting Plasma Antioxidant Vitamin and Carotenoid Concentrations

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Background: Antioxidant enrichment of LDL can increase its resistance to oxidation and hence reduce its atherogenicity. The objective of the present study was to investigate whether *in vivo* supplementation with nonalcoholic red wine extract and quercetin can increase the oxidative resistance of LDL, and also whether the supplementation has any effect on other antioxidative micronutrients present in the blood.

Methods: Twenty-one male subjects were supplemented with a placebo drink for 2 weeks and randomized into two groups. One group (n = 11) received the red wine extract (1 g/day, equivalent to 375 mL of red wine) and the other group (n = 10) quercetin (30 mg/day) for 2 weeks, followed by a 5-week washout period.

Results: In the red wine extract-supplemented group, *ex vivo* copper-initiated oxidation of LDL (lag phase, mean \pm SD) was 40 ± 11 min at the baseline, and increased significantly to 47 ± 6 min [$P < 0.05$ compared with placebo (38 ± 4 min) and the washout values (40 ± 5 min)]. In the quercetin-supplemented group, the lag phase was 44 ± 11 and 40 ± 5 min for the baseline and placebo, respectively, and increased significantly to 51 ± 7 min [$P < 0.05$ compared with placebo and washout (41 ± 9 min)] after supplementation. Plasma lipids (triglycerides, total cholesterol, LDL- and HDL-cholesterol) did not change during the study period. Supple-

mentation with red wine extract or quercetin had no effect on plasma vitamin C and E, retinol, and carotenoid concentrations.

Conclusions: Alcohol-free red wine extract and one of its components, quercetin, can inhibit LDL oxidation after *in vivo* supplementation; such "inhibition" is unrelated to changes in antioxidant vitamin and carotenoid concentrations.

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The oxidation of LDL is believed to play an important role in the pathogenesis of atherosclerosis (1) and hence the risk of cardiovascular disease. Cardio-protective effects of red wine have been suggested by several researchers, and these are attributed both to HDL-cholesterol-enhancing effects of the alcohol component (2, 3) and to antioxidant functions of its flavonoid constituents (4, 5). However, human studies that investigated the antioxidant protection of LDL after red wine consumption have produced conflicting reports, with two groups reporting positive effects (6, 7) and a third group showing no effect (8). The discrepancies in the results may be related to the methodologies used in the various studies (9) and/or variations in the flavonoid content of the wines (10, 11). The main phenolic components of red wine are anthocyanins, catechins, resveratrol, phenolic acids, and the flavonols quercetin and myricetin (9, 12). The concentrations of these components in the red wine can vary widely (13–15). Although most of the phenolic components of red wine have shown antioxidant properties *in vitro* (16–18), the antioxidant potential of individual components *in vivo* is not known.

In the present study, we investigated the effect of nonalcoholic red wine extract and one of its constituents, quercetin, on the oxidative resistance of LDL. Interaction of dietary antioxidants in blood or during absorption

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from the gut can potentially increase or reduce the effect of an antioxidant supplement. We therefore also measured the effect of supplementation on plasma vitamins A, C, and E, and carotenoid concentrations.

Materials and Methods

SUBJECTS

Twenty-two nonsmoking male volunteers (average age, 46 years; range, 33–65 years) were recruited from the University of Ulster staff. Eight subjects were regular alcohol drinkers (≥ 10 units/week), six were occasional drinkers (≤ 5 units/week), and eight were nondrinkers. All participants were asked to complete a lifestyle questionnaire. A screening blood sample showed that all subjects had values within the reference intervals for full blood count (white blood cells, platelets, and red blood cells), hemoglobin, hematocrit, and liver enzymes (alkaline phosphatase, aspartate aminotransferase, alanine transferase, and γ -glutamyltransferase) except for one whose alanine transferase concentration was slightly outside the reference interval (70 U/L). A total of 21 subjects completed the study. One subject withdrew (during the placebo period) for personal reasons.

Ethical approval was obtained from the University of Ulster, Ethical Committee, and the subjects gave written informed consent. Fifteen subjects were not on any medication or supplements. Those on medication or supplements were allowed to continue taking them at the same doses.

DESIGN OF THE STUDY

All subjects were supplemented with a placebo drink (blackcurrant-flavored, containing sugar, citric acid, sodium citrate, aspartame, and synthetic flavor; Cambridge Manufacturing) for the first 2 weeks. The placebo drink was given as a powder in sachets, and subjects were asked to reconstitute it with water according to their individual tastes. After the placebo period, subjects were randomized into two groups. One group received the placebo drink plus one capsule containing 1 g of red wine extract (Nutrivine™; The Howard Foundation, Cambridge, UK) daily, and the second group received the placebo drink containing 30 mg of quercetin (quercetin aglycone; Sigma) daily for 2 weeks. The supplementation period was considered sufficient because previous studies have shown an antioxidant effect after supplementation with red wine for 2 weeks (6, 9). A placebo group running in parallel with two treatment groups was considered unnecessary because both within-subject and seasonal variations in the LDL lag phase have been reported to be small in un-supplemented subjects (19, 20). Furthermore, in a pilot study, we found that the LDL lag phase was not changed when measured weekly over 4 weeks in subjects not receiving any treatment (21).

Subjects were instructed to take the supplements with food. Except for their alcohol intake, they were asked to maintain a typical but consistent dietary pattern during

the course of the study. Preliminary data from the pilot study showed that the irregular consumption of alcohol, especially red wine, can affect the LDL lag phase. Subjects were therefore advised to refrain from drinking red and white wine during the study period and to limit their alcohol intake to not more than 1 unit/day. They were given food diaries to specifically record the consumption of the following foods: fruits (berries, apples, cherries, and blackcurrants), vegetables (onions, leeks, broccoli, lettuce, cabbage, tomatoes, and beans), chocolates, beverages (fruit juices, milk, tea, coffee, and cocoa) and alcohol intake. They were asked to quantify fruits and vegetables by number or portion and average size (e.g., two small apples or one large serving of beans, onions in a portion of beef stew, and so forth); beverages by glass, carton, cup, or mug (e.g., one medium glass of pure orange juice or one one-half pint carton of milk); and alcohol by bar (i.e., serving) measures (e.g., one-half pint of lager or one gin). The dietary records enabled a qualitative assessment of the amount of flavonoid-rich foods consumed during the study.

All supplements were supplied by The Howard Foundation, Cambridge and packed by Cambridge Manufacturing. The preparation of red wine extract was the same as described previously (9). The flavonoid composition of the wine extract was determined by ETS Laboratories; it contained, per gram of powder, flavonols (5 mg, including 3.5 mg of quercetin), proanthocyanidins (202 mg), phenolic acids (2 mg), monomeric catechins (18 mg), resveratrol (3.3 mg), and anthocyanins (65 mg). This dose per day was equivalent to ~ 375 mL of red wine. The quercetin-supplemented group received 30 mg of quercetin/day. The supplement amount was chosen for two reasons. Epidemiological data have shown that an intake of >20 mg of quercetin/day is protective against the risk of cardiovascular disease (22, 23). Dietary quercetin is a mixture of glycosides and aglycone forms. The glycoside form from onions is known to be absorbed to a greater extent (50% more) than the aglycone (24). Because we were using the aglycone form (only one available commercially), we hypothesized that a dose >20 mg should be used. The red wine extract used in the present study contained 1.7 mg of glycoside and 1.8 mg of aglycone form of the quercetin. A 30-mg dose of aglycone quercetin therefore provided a sixfold higher dose than the wine extract (see *Discussion*).

BLOOD SAMPLING

Fasting blood samples (10-h overnight fast) were obtained from the subjects in recumbent position using Vacutainer Tubes containing either lithium heparinate or EDTA (1.5 g/L), or no anticoagulant. The blood was collected at four time points: baseline (week 0); after the placebo supplement (week 2); after treatment with either quercetin or red wine extract (week 4); and after a 5-week washout period (week 9). A washout period of 5 weeks was used to ensure

that all effects of the treatment were removed and that the baseline values were re-established.

The EDTA blood was used for the full blood count measurements. Blood from plain tubes was used to prepare serum for the liver function tests and for the measurement of a plasma lipid profile (triglycerides, total cholesterol, and LDL- and HDL-cholesterol) done at the Causeway Health and Social Services Trust Laboratory, Coleraine, Northern Ireland. Lithium heparinate blood was used for the isolation of LDL and for analysis of plasma vitamins A, C, and E and carotenoids. The plasma was prepared after centrifugation at 1000g for 10 min at 10 °C, aspirated into a universal centrifuge tube, and centrifuged for an additional 45 min at 1000g to remove the remaining cell debris. Plasma was separated within 1 h after collection of the blood.

After centrifugation, one part of plasma (0.5 mL) was immediately stabilized with 2 parts of *meta*-phosphoric acid (Prolabo), mixed, and snap frozen in liquid nitrogen before storage at -80 °C. Plasma aliquots of 0.5 mL were frozen at -80 °C for the analysis of fat-soluble vitamins and carotenoids at a later stage. Plasma vitamin E and carotenoid analysis was completed within 6 months, and plasma vitamin C within 10 months of the completion of the study.

PREPARATION OF LDL

The LDL was isolated from the fresh lithium heparinate blood by density ultracentrifugation using a modified method of Chung et al. (25). Briefly, the density of plasma was adjusted by adding 0.32 g KBr/mL of plasma. The density-adjusted plasma (3.5 mL) was layered below EDTA containing a 1 g/L KBr solution (density = 1.006; pH 7.4) in Optiseal polyallomer tubes (Beckman) and centrifuged for 2.5 h under reduced pressure in a Beckman XL-70 centrifuge at 30 000g rpm at 7 °C using a NVT65 rotor. The LDL fraction was removed by puncturing the tube wall with a hypodermic needle attached to a syringe. A fraction of LDL (0.5 mL) was dialyzed immediately, and the remaining LDL was stored under nitrogen at 4 °C. Gel electrophoresis of LDL using Paragon lipoprotein gels (Beckman) showed that the LDL was free from contamination with plasma proteins and HDL.

DIALYSIS AND OXIDATION OF LDL

LDL samples (0.5 mL) were transferred to Visking dialysis tubes [size 1; diameter, 6.3 mm (8/32 inches); Medicell International Limited] and dialyzed in deoxygenated phosphate-buffered saline (0.01 mol/L Na₂HPO₄, 0.16 mol/L NaCl, and 0.3 mmol/L chloramphenicol, pH 7.4) both in the absence and presence of 10 μmol/L EDTA (sodium salt). Duplicate samples were dialyzed in buffers containing either 10 μmol/L or 2 μmol/L EDTA at 4 °C for the first hour. After 1 h, those samples from the buffer containing 2 μmol/L EDTA were transferred to a buffer containing no EDTA. Samples in both flasks were dialyzed an additional 15 h at 4 °C under nitrogen.

After dialysis, the cholesterol content of the dialyzed LDL was determined using a CHOD-PAP kit from Boehringer. The kinetics of the LDL oxidation were followed by continuously monitoring the formation of conjugated dienes at 234 nm after the addition of copper as described by Puhl et al. (26). The concentrations of LDL and copper in the final reaction mixture of EDTA-free PBS buffer were 0.25 g/L total LDL (equivalent to 0.1 μmol/L LDL) and 11.7 μmol/L copper. A control, LDL prepared from pooled plasma stored in sucrose (final concentration, 6 g/L) at -80 °C, was used with every oxidation. The interassay imprecision of the lag phase (the resistance of LDL to oxidation) measurements was calculated from the lag phase measurements of control LDL and gave a CV of 4%. The intraassay CV for eight measurements on pooled dialyzed LDL was <3%.

APOLIPOPROTEIN B ANALYSIS

The apolipoprotein B (apoB) content of LDL was measured on fresh samples using Sigma diagnostic kits. The analysis was done on a COBAS Fara centrifugal analyzer by measuring the absorbance at 340 nm. The concentration of apoB was determined from a calibration curve obtained using multilevel calibrators. Two control serum samples provided with the kit were used with each run. For the first 4 weeks of the study, the mean apoB concentration was 566 ± 3.3 mg/L (CV = 0.58%) for control 1 and 1060 ± 13.0 mg/L (CV = 1.2%) for control 2. A new apoB kit was used after the washout period; with this kit, the values obtained for controls 1 and 2 were 656 and 987 mg/L, respectively. The overall CV of the assay using different kits was ~5%.

VITAMIN C (ASCORBIC ACID) ANALYSIS

The ascorbic acid concentrations in *meta*-phosphoric acid extracts of plasma were determined on stored samples by the method described by Heiliger (27). Briefly, samples were thawed on a roller-mix, mixed well by inversion, and centrifuged on a microcentrifuge at 7000g for 5 min. Clear extract (20 μL) was separated on a 100 × 4.6 mm cartridge column (Phase-Sep) containing 3-μm ODS-2 Spherisorb particles. The sample was pumped at 1.0 mL/min through the column, with a pH 5.5 mobile phase containing 0.1 mol/L sodium acetate, 1 mmol/L octylamine (Fluka Chemicals), 200 mg/L disodium EDTA, and 100 mg of DL-homocysteine. An output to a chromatographic data handling system (Maxima 820, Waters; Millipore) allowed manual peak integration. The concentration of ascorbic acid was quantified based on a response factor calculated by direct injection of 40 μmol/L ascorbic acid calibrator in 100 g/L *meta*-phosphoric acid. Pooled plasma extract and 40 μmol/L ascorbic acid extract stored at -80 °C were run with every batch as control samples. The interassay CV was 5%, and the intraassay CV was 3%.

Table 1. Changes in plasma lipids and apoB from subjects during the study period.

	Red wine extract group ^a (n = 11)				Quercetin group ^a (n = 10)			
	Baseline	Placebo	Treatment	After 5-week washout	Baseline	Placebo	Treatment	After 5-week washout
Plasma triglycerides, mmol/L	1.39 ± 0.42	1.33 ± 0.60	1.56 ± 0.59	1.32 ± 0.51	1.19 ± 0.69	1.19 ± 0.79	1.18 ± 0.70	1.39 ± 0.69
Total cholesterol, mmol/L	5.41 ± 0.58	5.45 ± 0.64	5.41 ± 0.74	5.39 ± 0.71	5.56 ± 0.98	5.60 ± 0.96	5.70 ± 0.98	5.61 ± 0.90
LDL-cholesterol, mmol/L	3.63 ± 0.57	3.69 ± 0.54	3.56 ± 0.65	3.60 ± 0.64	3.66 ± 1.04	3.65 ± 0.92	3.88 ± 1.06	3.69 ± 0.97
HDL-cholesterol, mmol/L	1.15 ± 0.24	1.15 ± 0.24	1.14 ± 0.27	1.19 ± 0.33	1.36 ± 0.32	1.41 ± 0.31	1.29 ± 0.30	1.28 ± 0.29
Total:HDL cholesterol ratio	4.86 ± 0.89	4.89 ± 1.03	4.92 ± 1.03	4.72 ± 0.91	4.28 ± 1.15	4.15 ± 1.09	4.62 ± 1.22	4.56 ± 1.08
LDL:HDL cholesterol ratio	3.28 ± 0.75	3.15 ± 0.71	3.23 ± 0.76	3.32 ± 0.78	2.83 ± 1.00	2.72 ± 0.85	3.16 ± 1.09	3.02 ± 0.94
LDL apoB, μmol/L	0.108 ± 0.02 ^b	0.094 ± 0.02 ^{b,c}	0.095 ± 0.02 ^c	0.095 ± 0.02 ^c	0.105 ± 0.03	0.098 ± 0.02	0.096 ± 0.02	0.096 ± 0.02
LDL-cholesterol:apoB ratio, mmol:μmol	41 ± 2	45 ± 8	43 ± 5	41 ± 9	40 ± 5	41 ± 3	46 ± 7	46 ± 19
LDL lag phase, min	40 ± 1.1 ^{b,c}	38 ± 4 ^b	47 ± 6 ^c	40 ± 5 ^b	44 ± 11 ^{d,e}	40 ± 5 ^d	51 ± 7 ^e	41 ± 9 ^d

^a The results are shown as the mean ± SD.

^{b-e} Within each group, results not sharing a common superscript (b and c for red wine extract, d and e for quercetin) are significantly different at $P < 0.05$ (Wilcoxon paired-rank test).

VITAMIN E AND CAROTENOID ANALYSIS

The plasma and LDL concentrations of vitamin E (α - and γ -tocopherol), retinol, and carotenoids were determined as described previously (28). Briefly, 0.1 mL of aqueous sodium dodecyl sulfate and 0.2 mL of an ethanolic solution of internal standard (40 mg/L tocopherol acetate) were added to 0.1 mL of sample; 1 mL of heptane containing 0.5 g/L butylated hydroxytoluene was added to the resulting mixture, and the solution was mixed vigorously for 3 min on a vortex-type mixer. The samples were centrifuged at 800g for 10 min at 10 °C. The heptane layer (0.7 mL) was removed, evaporated to dryness under a stream of nitrogen at 37 °C, and reconstituted in 0.1 mL of reconstitution mobile phase (mobile phase plus 90 mg/L butylated hydroxytoluene). A 50- μ L sample was separated on a 3- μ m Spherisorb ODS-2 column (10 cm \times 4 mm) with mobile phase (acetonitrile-methanol-dichloromethane, 500:500:128 by volume, plus 0.01 g/L butylated hydroxytoluene) pumped at 1 mL/min. The tocopherols, retinol, and carotenoids were detected simultaneously at 292, 325, and 450 nm, respectively. The data were collected and integrated using Maxima software (Waters). The carotenoid, retinol, and tocopherol concentrations were calculated using authentic standards as described previously (28). Pooled control plasma was run with every batch of samples to calculate interassay imprecision. The interassay imprecision (CV) was <5% for the tocopherols and retinol and <10% for carotenoids.

STATISTICAL ANALYSIS

Statistical analysis was performed by using Statistical Package for Social Sciences, Ver. 6 (SPSS Inc). The data were skewed both before and after transformation to logarithms. Therefore, the nonparametric Wilcoxon paired-rank test was used for statistical analysis. $P < 0.05$ was considered significant.

Results

EFFECT OF SUPPLEMENTATION ON THE LAG PHASE

A qualitative examination of food diaries completed during the placebo and supplementation periods indicated that dietary habits with respect to flavonoid-rich foods did not change during the study period. However, the LDL lag phase was significantly increased in both the red wine extract- and quercetin-supplemented groups compared with the placebo ($P < 0.01$, Wilcoxon paired-rank test; Table 1) and washout ($P < 0.02$) time points.

Because there was no difference in the lag phase measured on dialyzed LDL prepared in non-EDTA- and EDTA (10 μ mol/L)-containing buffers, an average of two measurements was taken. Results shown in Table 1 are subject means using the average of two lag phase measurements. The Mann-Whitney test showed that the change in the lag phase observed after supplementation

Table 2. Changes in blood biochemistry and liver enzymes of subjects over the study period.

	Red wine extract group ^a				Quercetin group ^a			
	Baseline	Placebo	Treatment	After washout	Baseline	Placebo	Treatment	After washout
RBC, ^b × 10 ¹² /L	5.2 ± 0.38 ^{c,d}	5.1 ± 0.38 ^c	5.2 ± 0.39 ^d	5.2 ± 0.27 ^{c,d}	5.15 ± 0.27 ^e	4.99 ± 0.28 ^f	5.16 ± 0.37 ^e	5.07 ± 0.35 ^e
WBC, × 10 ⁹ /L	6.6 ± 1.05	6.4 ± 1.22	6.9 ± 1.01	6.4 ± 0.97	6.53 ± 2.12	6.15 ± 1.78	6.64 ± 1.99	6.58 ± 1.93
Platelets, × 10 ⁹ /L	247 ± 39	238 ± 39	245 ± 39	237 ± 29	259 ± 60	246 ± 56	267 ± 69	248 ± 65
ALP, U/L	170 ± 41	172 ± 37	170 ± 39	174 ± 39	135 ± 27	138 ± 31	141 ± 34	134 ± 22
ALT, U/L	31 ± 18	33 ± 18	31 ± 16	27 ± 13	20 ± 5	22 ± 7	20 ± 4	21 ± 5
AST, U/L	23 ± 8	25 ± 8	25 ± 8	23 ± 8	24 ± 6	27 ± 14	26 ± 9	27 ± 5
GGT, U/L	31 ± 19	32 ± 25	30 ± 21	29 ± 18	24 ± 10 ^e	26 ± 9 ^f	26 ± 10 ^f	27 ± 11 ^{e,f}

^a Results are the mean ± SD.

^b RBC, red blood cells; WBC, white blood cells; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate amino-transferase; GGT, γ -glutamyl transferase.

^{c-f} Significance of difference was measured using Wilcoxon paired-rank test. Data not sharing the same superscript (c and d for extract, e and f for quercetin) were significantly different at $P < 0.05$.

was not significantly different between the red wine extract and quercetin groups.

Lag phase measurements at baseline, after placebo treatment, and after the 5-week washout period were not different.

CHANGES IN PLASMA LIPIDS AND LDL apoB CONCENTRATIONS DURING THE STUDY

Table 1 shows that both red wine extract and quercetin treatment had no effect on plasma triglycerides and cholesterol (total, LDL, and HDL). There was no change in the total:HDL and LDL:HDL cholesterol ratios. The LDL apoB was decreased compared with baseline in the red wine extract-supplemented group and remained low throughout the study, including after the washout period. The LDL-cholesterol:apoB ratio was not changed in either group.

The results in Table 2 show the hematological and hepatic biochemistry in the volunteers. The results remained constant throughout the study except the small changes in red blood cell count and serum γ -glutamyl-transferase concentrations.

EFFECT OF SUPPLEMENTATION ON PLASMA MICRONUTRIENTS

Plasma ascorbic acid, tocopherol (α - and γ -), and retinol concentrations before and after supplementation are shown in Table 3. There was no significant change in any of the variables between time points. Likewise, there were no significant changes in the plasma and LDL (data not shown) carotenoids after red wine extract and quercetin supplementation (Figs. 1 and 2). Both groups showed a trend in reduction of plasma retinol, tocopherol, and hydroxy-carotenoid concentrations following the placebo period, and this decrease was more apparent in alcohol drinkers. To investigate whether alcohol drinking habits had a significant effect on plasma micronutrients, a few extra subjects (two drinkers, four occasional drinkers, and two nondrinkers) were asked to take placebo supplements for 2 weeks, and alcohol drinkers were asked to limit their alcohol intake to not more than 1 unit/day. The results are shown in Table 4. After the placebo period, there were significant decreases in plasma retinol ($P = 0.03$, Wilcoxon rank test,) and lutein ($P = 0.01$) concentrations in the regular drinkers ($n = 10$). In occasional

Table 3. Effect of red wine extract and quercetin supplementation on plasma micronutrient concentrations of the subjects over the study period.^a

	Ascorbate, $\mu\text{mol/L}$	γ -Tocopherol, $\mu\text{mol/L}$	Retinol, $\mu\text{mol/L}$	α -Tocopherol, $\mu\text{mol/L}$	α -Toc:chol ^b
Red wine extract group	n = 8	n = 11	n = 11	n = 11	n = 11
Baseline	55 ± 13	2.49 ± 0.81	2.59 ± 0.61	37 ± 12	6.6 ± 2.1
Placebo	55 ± 10	2.17 ± 0.98	2.11 ± 0.45	33 ± 6	5.9 ± 0.8
Supplementation	52 ± 14	2.19 ± 0.55	2.23 ± 0.26	32 ± 7	5.9 ± 1.1
After 5-week washout	57 ± 19	2.56 ± 1.02	2.29 ± 0.25	31 ± 4	5.8 ± 0.8
Quercetin group	n = 8	n = 10	n = 10	n = 10	n = 10
Baseline	48 ± 15	2.28 ± 1.01	2.55 ± 0.46	41 ± 21	7.6 ± 4.4
Placebo	45 ± 15	2.34 ± 0.87	2.56 ± 0.36	36 ± 11	6.3 ± 1.1
Supplementation	47 ± 23	2.02 ± 1.16	2.70 ± 0.91	33 ± 11	5.8 ± 1.8
After 5-week washout	48 ± 24	2.25 ± 0.88	2.82 ± 0.52	35 ± 10	6.2 ± 1.3

^a Results are expressed as the mean ± SD. There was no significant change in plasma micronutrients over the study period (Wilcoxon paired-rank test).

^b α -Toc:chol, α -tocopherol:cholesterol ratio ($\mu\text{mol}/\text{mmol}$).

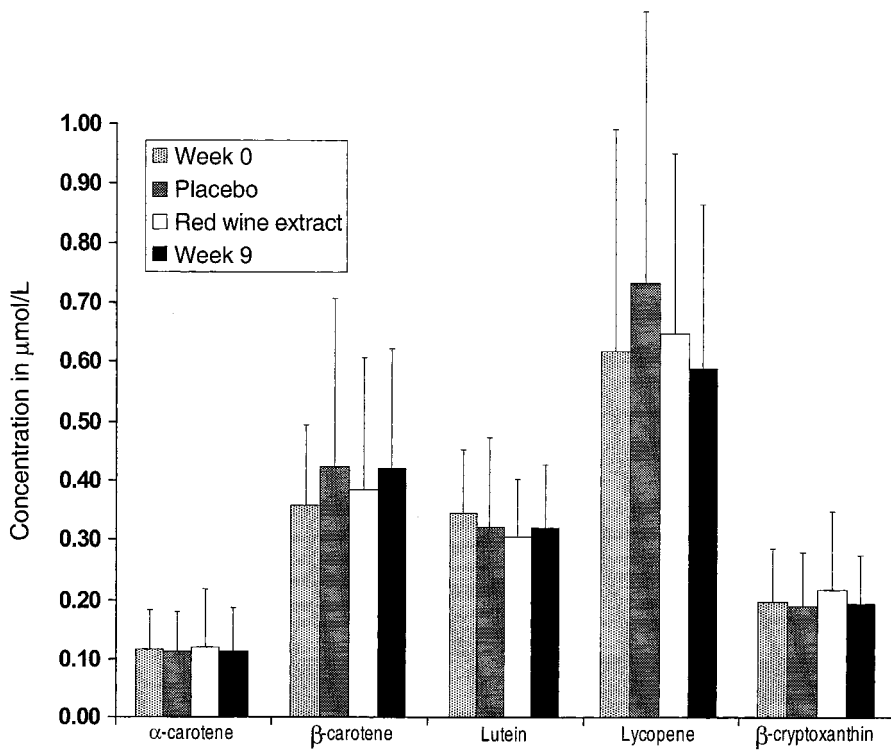


Fig. 1. Changes in plasma carotenoids in the red wine extract-supplemented group (n = 11).

There were no significant changes in plasma carotenoids at any time point (not significant, Wilcoxon rank test). Bars, SD.

drinkers (n = 10), only retinol (P = 0.02) was significantly lower; there was no change in plasma micronutrients in nondrinkers (n = 9).

Discussion

The day-to-day variation of the LDL lag phase has been reported to be low in unsupplemented volunteers (19–21). In the present study, each subject acted as his own control. Three measurements were done at time points

when subjects were not receiving any treatment, i.e., baseline, placebo, and after a 5-week washout period, and there were no significant differences in the lag phase at these three time points (Table 1). Several in vitro studies have shown an antioxidant effect of red wine and fractionated phenolic compounds on LDL oxidation (5, 11, 29, 30). The results of the present study show that supplementation of human subjects with alcohol-free red wine extract can inhibit oxidation of LDL ex vivo. Our

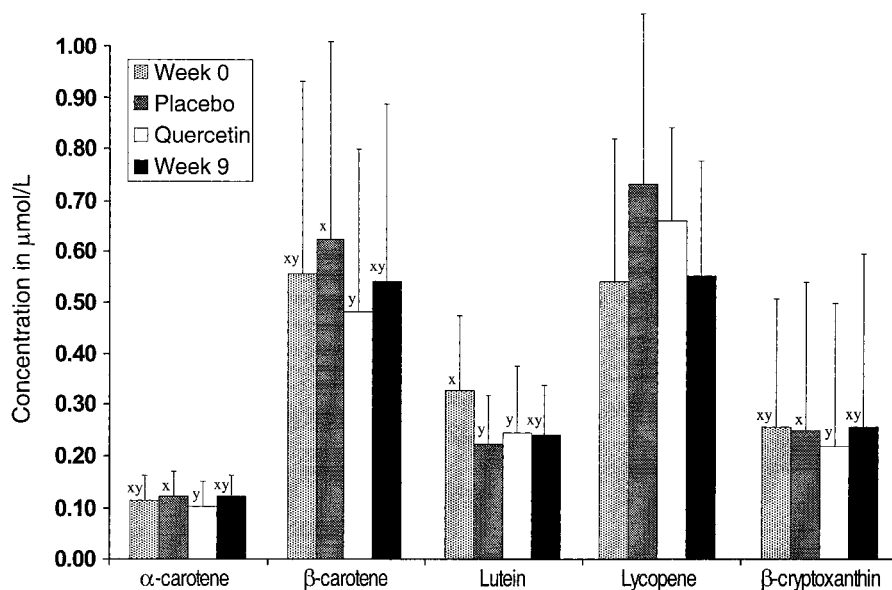


Fig. 2. Changes in plasma carotenoids in quercetin-supplemented group (n = 10).

For each carotenoid, columns not sharing the same letter are significantly different at P < 0.05 (Wilcoxon rank test). Bars, SD.

Table 4. Changes in fat-soluble micronutrients in drinkers and nondrinkers following the 2-week placebo treatment.^a

	Drinkers ^b (n = 10)			Occasional drinkers ^b (n = 10)			Nondrinkers ^b (n = 9)		
	Baseline	Placebo (2 weeks)	P ^c	Baseline	Placebo (2 weeks)	P ^c	Baseline	Placebo (2 weeks)	P ^c
α -Tocopherol, $\mu\text{mol/L}$	39 \pm 12	32 \pm 7	0.06	37 \pm 17	33 \pm 9	NS ^d	36 \pm 10	36 \pm 10	NS
γ -Tocopherol, $\mu\text{mol/L}$	2.4 \pm 0.9	2.3 \pm 0.7	NS	3.4 \pm 2.0	2.4 \pm 1.2	NS	2.3 \pm 1.0	2.2 \pm 1.1	NS
Retinol, $\mu\text{mol/L}$	2.9 \pm 0.9	2.3 \pm 0.4	0.03	2.7 \pm 0.9	2.2 \pm 0.6	0.02	2.4 \pm 0.4	2.3 \pm 0.5	NS
α -Carotene, $\mu\text{mol/L}$	0.11 \pm 0.06	0.10 \pm 0.06	NS	0.07 \pm 0.03	0.07 \pm 0.02	NS	0.15 \pm 0.06	0.15 \pm 0.07	NS
β -Carotene, $\mu\text{mol/L}$	0.38 \pm 0.24	0.39 \pm 0.19	NS	0.36 \pm 0.13	0.35 \pm 0.11	NS	0.59 \pm 0.34	0.58 \pm 0.39	NS
Lutein, $\mu\text{mol/L}$	0.35 \pm 0.20	0.27 \pm 0.19	0.01	0.32 \pm 0.15	0.27 \pm 0.11	NS	0.33 \pm 0.17	0.34 \pm 0.11	NS
Lycopene, $\mu\text{mol/L}$	0.61 \pm 0.25	0.63 \pm 0.27	NS	0.58 \pm 0.37	0.67 \pm 0.41	NS	0.56 \pm 0.44	0.63 \pm 0.49	NS
β -Cryptoxanthin, $\mu\text{mol/L}$	0.19 \pm 0.10	0.17 \pm 0.09	NS	0.17 \pm 0.15	0.13 \pm 0.09	NS	0.38 \pm 0.32	0.33 \pm 0.28	NS

^a Subjects were asked to restrict their alcohol intake to <1 unit/day. The effect of alcohol withdrawal on plasma micronutrient concentrations was measured.

^b Mean \pm SD.

^c Wilcoxon paired-rank test.

^d NS, not significant.

results differed from two previously published reports. In one report, red wine extract (1 g/day, similar to the present study) inhibited LDL oxidation only in the samples dialyzed in the absence of EDTA in the dialysis buffer (9). The authors suggested that there was an inhibiting effect of EDTA in the copper-diene assay when polyphenols were examined. In our study, LDL oxidation was inhibited by the wine extract irrespective of the presence of EDTA in the buffer. The previous study (9) used a different dialysis technique: they used cassettes for the dialysis, and the dialysis buffer was not degassed or flushed with nitrogen. In our study, instead of cassettes, dialysis tubing was used and samples were dialyzed in a buffer flushed with nitrogen. It is possible that the different dialysis conditions may have contributed to the discrepancies between the two studies, but at present there is no other obvious explanation. In the second study, supplementation with alcohol-free red wine increased the antioxidant capacity of plasma but had no effect on LDL oxidation (31). The study protocol was similar to ours except that the polyphenol content of the wine extract was different. There were similar amounts of flavonols and anthocyanins, but the catechin and proanthocyanin concentrations were one-half of those used in our study. Both catechins and proanthocyanin have been reported to show antioxidant properties in vitro (32,33), and the differences in their concentrations may have contributed to the differences in the findings from our study with those of Carbonneau et al. (31).

Quercetin, a major flavonol in red wine, when given on its own also inhibited LDL oxidation. The red wine extract and quercetin supplements had the same effect on the lag phase. The red wine extract contained 1.7 mg of quercetin as glycoside and 1.8 mg as aglycone. It has been reported that only 24% of the aglycone is absorbed from onions (24). Provided the same is true for pure supplements, then \sim 7.2 mg of quercetin from the pure supplements and 1.2 mg from the red wine extract would have been absorbed. The lag phase was raised by 23% in the red wine extract-

supplemented group and 27% in the quercetin-supplemented group. The red wine extract, however, also contained other phenolic components, such as resveratrol, procyanidins, anthocyanins, and catechins (see *Materials and Methods* for detailed composition), all of which have been reported to show antioxidant properties (17, 18, 32–34). The results of the present study, therefore, suggest that quercetin may not be the only protective component of the red wine.

Both red wine extract and quercetin supplementation failed to have an effect on plasma lipid concentrations. This, however, could be attributable to the fact that most subjects in the present study were normolipidemic. Previous studies have shown that flavonoids, in particular quercetin, can lower lipid concentrations in hyperlipidemic rats (35). Preliminary studies in our laboratory have suggested that red wine extract can lower plasma cholesterol, particularly LDL-cholesterol, but only in hyperlipidemic subjects (>6.5 mmol/L cholesterol). However, these observations require further confirmation through carefully planned studies.

In contrast to a previous report (31), we did not find any increase in plasma or LDL (data not shown) α -tocopherol and carotenoid concentrations after quercetin or wine extract supplementation. Indeed, there was evidence of a decrease in these analytes, particularly in those who regularly drank alcohol before the study and reduced their alcohol intake to <1 unit/day. In those previously consuming alcohol, there was a significant reduction in plasma lutein and retinol concentrations. It is interesting to note that in a recent Scottish Health Survey (36), people who drank alcohol had significantly higher retinol concentrations than nondrinkers, supporting the observation that alcohol consumption may influence plasma tocopherol, lutein, and retinol. Only polar nutrients were affected by alcohol consumption. The effect of alcohol might be related to increased absorption of these nutrients through a solvating effect of alcohol. However, this would be most surprising in the case of retinol because plasma

concentrations are homeostatically controlled (37), unless alcohol affects the synthesis or transport of retinol-binding protein, thereby influencing plasma retinol concentrations indirectly.

In summary, the consumption of alcohol-free red wine can increase the antioxidant capacity of LDL but has no effect on plasma vitamin A, C, and E and carotenoid concentrations. It is, however, noteworthy that because alcohol withdrawal was found to have a significant effect on some of the plasma fat-soluble vitamins, future studies in which subjects are asked to refrain from alcohol consumption during the course of the study should take into account possible effects of alcohol withdrawal on plasma vitamin concentrations.

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