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LEVELS OF ANTIOXIDANT NUTRIENTS IN PLASMA AND LOW DENSITY LIPOPROTEINS: A HUMAN VOLUNTEER SUPPLEMENTATION STUDY

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A human supplementation study was undertaken in order to investigate the correlation between the intake of individual daily dosages of vitamin E (300 mg), vitamin C (250 mg), or β-carotene (15 mg) of eight week duration and their uptake *in vivo* in plasma and LDL. The effects of a combined supplement of vitamin E, vitamin C and β-carotene (Redoxon protector-75 mg, 150 mg, 15 mg respectively) were also investigated. The results show that on supplementation with the individual antioxidants the increases in plasma α-tocopherol:cholesterol levels lie in the 1.5–2 fold range and the β-carotene:cholesterol ratios give a mean 3.5 fold enhancement. The combined supplement containing the same level of β-carotene as the single dosage achieved comparative levels of uptake in plasma. The level of plasma vitamin C appears to be maximal at about 100μM regardless of the pre-supplementation level.

INTRODUCTION

The role of diet in the maintenance of health and prevention of the major chronic diseases which afflict industrialised societies is a matter of frequent debate[1]. This is especially true of cardiovascular disease where recently the protective effects of dietary antioxidant nutrients have been postulated. Studies have pointed to the importance of the antioxidant nutrients vitamin E, vitamin C and β-carotene in maintaining health, in contributing to the decreased incidence of disease and also perhaps protecting against the recurrence of pathological events[2-5]. The World Health Organisation cross-cultural epidemiological survey across 16 European countries showed an inverse correlation between plasma α-tocopherol levels and mortality from ischaemic heart disease[2,6]. The Basel prospective study described the association of poor plasma status of β-carotene and vitamin C with higher mortality from ischaemic heart disease and stroke.[7]

The Harvard Health Professionals study, based on self-supplementation of approximately 40,000 males and 80,000 females (all free of coronary heart disease and diabetes), revealed that it may be possible to begin to define levels of intake of vitamin E associated with low subsequent incidence of coronary heart disease. The results showed that those supplementing with at least 100 IU of vitamin E per day for a

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minimum of two years had, respectively, a 37% and 41% reduction in risk, for males and females[8,9]. The data did not prove a causal relationship but provided evidence of an association between a high intake of vitamin E and a lower risk of coronary heart disease in USA populations. β-Carotene was not associated with lower risk among those who never smoked but inversely related to risk among current smokers. Interim data from a sub-group of a double-blind, randomised, placebo-controlled trial involving administration of β-carotene supplements (50 mg on alternate days) to physicians with angina pectoris and/or coronary revascularisation decreased the incidence of subsequent cardiac events by 44%[10].

One of the key mechanisms relating antioxidant status in the blood to decreased risk of coronary heart disease relates to the postulate that the oxidation of low density lipoprotein (LDL) in the artery wall is an early event in atherosclerosis, for which evidence is mounting[11]. The aims of the present work were to undertake a human supplementation study in order to investigate the possible correlation between the individual dosages of vitamin E, vitamin C or β-carotene and their uptake *in vivo* in plasma and LDL. The effects of a combined supplement of vitamin E, vitamin C and β-carotene were also investigated.

MATERIALS AND METHODS

Human study design

Ethical approval was obtained from the Lewisham and North Southwark Committee on Ethical Practice. Supplements and placebo were provided by Roche, Geneva. The study was a double-blind, randomised, placebo-controlled trial, with an enrolment sample size of 50 (29 male and 21 female), 10 with placebo (soft gelatine capsules containing arachis oil) and four groups of ten with a different supplement, 300 mg vitamin E (DL-α-tocopherol acetate) or 250 mg vitamin C or 15 mg β-carotene or a combined supplement (Redoxon protector) of 15 mg β-carotene with 75 mg vitamin E and 150 mg vitamin C. The subject selection criteria were healthy subjects with no known disease, aged between 20 and 50 years, criteria for exclusion included smokers, oral contraceptive takers and aspirin users. One volunteer (female) withdrew during the course of the study. The trial dosage and duration was one oral capsule for 56 days preceded by a two week study, prior to supplementation, of baseline antioxidant levels at two time points. Volunteers were instructed to take the supplement with breakfast after an overnight fast. Blood samples were drawn on day one (the designation for the first day of the study) for basal plasma antioxidant levels. On day 15 (after two weeks), blood samples were taken for reassessment of basal antioxidant levels, for lipid levels and total plasma antioxidant activity and the subjects commenced supplementation. On day 71 subjects returned for the final blood sample after 56 days supplementation, and antioxidant and lipid levels were measured. The main parameters to be assessed were antioxidant nutrient intake compared to uptake in plasma and LDL. Compliance was assessed by counting the number of pills returned on the last visit. In all groups compliance was equally high and was at least 98%.

Subjects were given a dietary diary, designed by Roche, Welwyn, to complete daily for the duration of the study to monitor nutritional status. The diet sheets consisted of recorded dietary intake of salad, carrots, green vegetables, fresh fruit, fruit juice, fish, red wine, red meat, natural milk and butter. Information was also recorded of intake of medication and occurrence of illnesses during the study.

Venous blood was collected into EDTA for the lipid profiles and into 3.8% trisodium citrate (1 volume to 9 volumes of blood) for antioxidant measurements. Plasma was obtained by centrifugation of the blood at 1200 g for 15 min at 20°C. Plasma samples (for α-tocopherol, β-carotene and total antioxidant activity determination) were immediately frozen at −70°C until assayed. For vitamin C determinations, plasma samples (1 vol) were deproteinised with 10% trichloroacetic acid (4 vol) and stored at −70°C until assayed.

ANALYSIS OF ANTIOXIDANT LEVELS

Measurement of α-tocopherol and β-carotene

Plasma concentrations of α-tocopherol and β-carotene were determined by normal-phase high performance liquid chromatography[12]. Plasma (1 volume), containing δ-tocopherol as an internal standard, was extracted with 4 volumes of hexane following the addition of 2 volumes of methanol. After centrifugation of the sample at 1500 g for 15 min, α-tocopherol and β-carotene were measured by normal phase HPLC (Novapak silica column-4μM, 3.9 × 156 mm). The mobile phase consisted of 92% hexane and 8% methyl tert-butyl ether pumped at a flow rate of 1 ml/min. Detection and quantification of α-tocopherol and β-carotene were performed fluorimetrically (excitation 295 nm and 450 nm, emission 340 nm and 470 nm respectively). LDL samples (0.125 mg/ml) were extracted with 1 ml of hexane following the addition of 1 ml of methanol and antioxidant determinations similarly undertaken.

Measurement of vitamin C

Plasma total ascorbate was measured fluorimetrically[13]. Briefly, ascorbic acid was oxidised to dehydroascorbic acid with iodine, followed by condensation with ortho-phenylenediamine (OPD) to form a fluorescent quinoxaline which was measured at an excitation wavelength of 348 nm, emission at 423 nm.

Total antioxidant assay

Plasma total antioxidant activity was measured by the Trolox Equivalent Antioxidant Activity (TEAC) method[14,15]. The assay involves the interaction of the phenothiazine compound 2,2′-azinobis-(3-ethyl benzo-thiazoline-6-sulphonic acid) (ABTS) with activated myoglobin to produce the radical cation ($ABTS^{\cdot+}$) with the characteristic absorption maxima at 645, 734 and 815 nm. In the presence of hydrogen-donating antioxidants the formation of the radical cation is suppressed. The suppression of the absorbance at 734 nm is directly related to the antioxidant capacity (or activity) of the plasma sample being investigated, using dilutions of Trolox (Hoffman-La Roche) as an antioxidant standard. The following (final) reagent concentrations were used: 2.5 μM metmyoglobin, 150 μM ABTS, 0.84% plasma sample and the reaction was started by adding the hydrogen peroxide (final concentration 375 μM). Absorbances were read with a Cobas Bio centrifugal analyser 3.25 mins after the addition of hydrogen peroxide and a dose-response curve for Trolox derived using a logit/log 4 curve fitting procedure[16] where

$$R = R_0 + K_c \frac{1}{1 + \exp[-(a + b \cdot \ln C)]}$$

R = rate of absorbance change of unknown sample
R_O = predicted rate for standard of zero concentration
C = concentration of unknown sample
K_c = the predicted difference between the rate of a standard with infinite concentration and R_O
a,b= parameters defining the non-linear elements of each mathematical function.

Two freeze-dried pooled plasma samples were used for precision monitoring; intra-assay imprecision was in the range 0.5–1.5% and inter-assay imprecision was 2.5%.

Lipid analyses

Total plasma cholesterol and triacylglycerols were measured according to the methods of Warnick[17]. High density lipoprotein cholesterol was measured directly by precipitation with phosphotungstate[18] and the low-density lipoprotein (LDL) cholesterol calculated by the Friedewald formula[19].

Isolation of low density lipoprotein

The plasma low density lipoproteins were prepared using a modified density gradient ultracentrifugation method[20]. The density of the plasma was adjusted to 1.3 g/ml by addition of sodium bromide. Ultracentrifuge tubes containing 20 ml 0.9% sodium chloride solution (density 1.006 g/ml) were underlaid with aliquots of 10 ml plasma (density 1.3 g/ml) and centrifuged at 149 000 g for two hours at 16°C in a Beckman L70 ultracentrifuge using a Type 70 T1 rotor. The low density lipoprotein-containing fraction was recovered and 20 ml aliquots were placed into ultracentrifuge tubes containing 6 ml sodium bromide solution (density 1.154 g/ml, with 100 μM EDTA). The tubes were then made up to volume using sodium bromide solution (density 1.063 g/ml with 100 μM EDTA) and centrifuged for a further 14 hours, 149 000 g at 16°C. The supernatant containing the low density lipoprotein fraction was recovered and dialysed for eight hours against degassed 10 mM phosphate-buffered saline (PBS) containing 10 μM EDTA (pH 7.4) at 4°C. Following dialysis the low density lipoprotein solution was filtered using a 0.2 μm filter (Millipore). The concentration of protein was estimated[21] and LDL (125 ug/ml) stored in duplicate aliquots at −70°C under nitrogen.

Statistics

Results are expressed as mean ± standard deviation. For each individual, the baseline value prior to treatment i.e. the mean of the first (day 1) and second (day 15) visits (or just the second visit where relevant parameters were not assessed at visit 1) was subtracted from the post-treatment value (day 71) and the mean changes in the different groups were compared by one-way analysis of variance (ANOVA). Statistical differences in changes in the plasma antioxidants after supplementation with those of the placebo group were sought by Student's unpaired t-test.

TABLE 1
Baseline characteristics of the volunteers

Supplementation Group	No of Subjects	Sex Ratio (M/F)	Age (yr)	BMI (kg/m^2)
Placebo	10	7/3	30.8 ± 8.1	25.1 ± 4.5
Vitamin E	10	6/4	30.2 ± 8.8	22.4 ± 2.8
Vitamin C	10	6/4	27.5 ± 7.3	22.9 ± 5.2
β-carotene	9	5/4	37.2 ± 9.6	23.9 ± 2.0
Vitamin E + Vitamin C + β-carotene	10	5/5	27.4 ± 6.4	24.6 ± 4.2

RESULTS

The base-line characteristics of the volunteers, mean ages and sex ratios are shown in Table 1. There is relatively little difference in the body mass indices among the groups. The lipid profiles pre-and post-supplementation (Table 2) show that the mean levels of cholesterol, LDL, HDL and triglycerides for each individual group are not significantly different from the mean levels of the 50 volunteers and closely match the reported means of the standard reference intervals[22].

The ingestion of the supplements, α-tocopherol (300 mg), β-carotene (15 mg) or vitamin C (250 mg) (Table 3) resulted in a 1.7 fold increase of plasma α-tocopherol and α-tocopherol: cholesterol ratio, a 3.5 fold increase in β-carotene: cholesterol ratio, and a mean 1.3-fold increase in vitamin C after 56 days. The plasma level of vitamin C seems to be closely dependent on the initial level (Fig 1) in that volunteers with pre-supplementation plasma levels of the order of 80–95 μM showed minimal enhancement, while those with initial levels well below this increased to varying extents not exceeding a mean value of 80 μM. There were no significant changes in the plasma antioxidant levels of the placebo group, the levels pre- and post-supplementation being similar. Furthermore, supplementation with 300 mg of α-tocopherol for the eight weeks of the study had no effect on β-carotene levels of these subjects nor did supplementation with 15 mg β-carotene influence plasma α-tocopherol levels.

The influence of additional supplemental antioxidant nutrients on the uptake of β-carotene in the plasma was monitored by comparing the β-carotene supplementation group with the group supplemented with the same level of β-carotene but in a combined supplement which also contained 75 mg α-tocopherol and 150 mg vitamin C (Table 4). The increase in plasma β-carotene with the combined supplement was comparable to that observed with the individual supplement and not significantly different [$p = 0.31$]. It is of interest to note the mean 1.3 fold increase in plasma α-tocopherol level after supplementation with the combined nutrients, which included 75 mg α-tocopherol, with the 1.7-fold increase in α-tocopherol from the individual supplemental dosage four times higher at 300 mg α-tocopherol (Table 3).

The increases in α-tocopherol and β-carotene concentrations in LDL isolated from the plasma of the study volunteers were also assessed. The level of β-carotene in LDL after supplementation (Table 5) with β-carotene alone showed an increase from $0.85 \pm 0.46 \rightarrow 3.89 \pm 1.32$ μM, with no change in the α-tocopherol concentrations. However, in the α-tocopherol-supplemented group, the increase (mean 1.4 fold) in

TABLE 2
Lipid profiles pre- and post-supplementation

	Cholesterol (mM)		LDL (mM)		HDL (mM)		Triglycerides (mM)	
	Prior	Post	Prior	Post	Prior	Post	Prior	Post
Placebo	4.68 ± 0.84 [10]	4.60 ± 0.80 [10]	2.40 ± 0.53 [3]	2.84 ± 0.79 [10]	1.01 ± 0.3 [3]	1.22 ± 0.48 [10]	1.44 ± 0.99 [10]	1.18 ± 0.55 [10]
Vitamin E 300 mg	4.97 ± 1.09 [10]	4.73 ± 0.87 [10]	3.44 ± 0.88 [7]	3.06 ± 0.96 [10]	1.44 ± 0.42 [7]	1.23 ± 0.37 [10]	0.84 ± 0.15 [10]	0.92 ± 0.28 [10]
Vitamin C 250 mg	5.23 ± 0.97 [10]	5.30 ± 1.00 [10]	2.82 ± 0.65 [6]	3.17 ± 0.72 [10]	1.58 ± 0.41 [6]	1.50 ± 0.79 [10]	1.31 ± 1.00 [10]	1.33 ± 0.79 [10]
β-Carotene 15 mg	4.83 ± 0.65 [9]	4.87 ± 0.55 [8]	2.88 ± 0.24 [7]	3.14 ± 0.52 [8]	1.28 ± 0.21 [7]	1.12 ± 0.22 [8]	1.01 ± 0.41 [9]	1.29 ± 0.96 [8]
Combined supplement*	5.04 ± 1.32 [10]	4.98 ± 1.27 [10]	2.97 ± 1.75 [3]	3.19 ± 0.34 [10]	1.39 ± 0.34 [3]	1.30 ± 0.37 [10]	1.06 ± 0.75 [10]	1.08 ± 0.89 [10]
Mean for all volunteers	4.95 ± 0.98 [49]	4.90 ± 0.13 [48]	3.00 ± 0.8 [26]	3.07 ± 0.8 [48]	1.37 ± 0.4 [26]	1.28 ± 0.5 [48]	1.10 ± 0.7 [49]	1.15 ± 0.7 [48]

* 75 mg vitamin E/15 mg β-Carotene/150 mg vitamin C
Prior corresponds to Day 15 (see Methods).

$\times \pm$ S.D.
Number of subjects in brackets

TABLE 3
Effects of antioxidant nutrient supplementation on plasma levels

	Supplement			
	Vitamin E 300 mg [n = 10]	Vitamin C 250 mg [n = 10]	β-carotene 15 mg [n = 9]	Placebo [n = 10]
Vitamin E (μM)				
Prior	20.2 ± 4.7	23.9 ± 6.4	19.6 ± 4.4	23.1 ± 4.2
Post	34.9 ± 10.4	22.5 ± 5.2	19.2 ± 5.1	22.6 ± 2.9
	P<0.001	NS	NS	NS
Vitamin E/ Cholesterol (mmoles/mole)				
Prior	4.25 ± 1.37	4.76 ± 1.09	3.84 ± 0.42	4.99 ± 1.46
Post	7.60 ± 2.81	4.28 ± 0.82	3.84 ± 0.86	4.98 ± 0.85
	P = 0.001	NS	NS	NS
Vitamin C (μM)				
Prior	54.1 ± 19.6	63.6 ± 18.9	58.2 ± 13.0	59.6 ± 19.6
Post	48.8 ± 19.8	81.8 ± 13.2	54.0 ± 16.4	52.7 ± 21.2
	NS	P = 0.001	NS	NS
β-carotene (μM)				
Prior	1.17 ± 0.58	1.14 ± 0.59	0.87 ± 0.33	0.87 ± 0.47
Post	0.95 ± 0.55	0.99 ± 0.42	3.07 ± 1.05	0.77 ± 0.31
	NS	NS	P<0.001	NS
β-carotene/ Cholesterol (mmoles/mole)				
Prior	0.22 ± 0.07	0.23 ± 0.11	0.18 ± 0.08	0.19 ± 0.10
Post	0.21 ± 0.14	0.19 ± 0.09	0.61 ± 0.25	0.17 ± 0.07
	NS	NS	P<0.001	NS

Results expressed as mean ± standard deviation. For the statistics, the mean changes between the antioxidant levels of the supplemented groups and the pre-supplementation groups are compared with the changes for the placebo group. (See Material and Methods)

The p value is derived from the statistical differences on comparison of the changes in the antioxidants after supplementation with those of the placebo group. For each individual the baseline value prior to supplementation was subtracted from the post-treatment value and the mean changes in the different groups were compared by one-way analysis of variance (ANOVA)

α-tocopherol was of marginal significance when compared to the placebo group, p = 0.115:

vitamin E group: $[\alpha\text{-tocopherol}]_{post}$-$[\alpha\text{-tocopherol}]_{pre}$ = 5.24 ± 3.52;

placebo group: $[\alpha\text{-tocopherol}]_{post}$-$[\alpha\text{-tocopherol}]_{pre}$ = 2.66 ± 2.35.

The combined supplement, which contained the same amount of β-carotene as the individual supplement, augmented LDL β-carotene levels from 1.01 ± 0.58 → 2.37 ± 1.08μM but with an approximately 1.2 fold enhancement of the α-tocopherol level. Other workers have shown that in order to attain a doubling of LDL α-tocopherol level, a high supplementation dosage of 1,600 mg/day for eight weeks was required[23]. However, a high dosage of β-carotene of 60 mg/day for 13 weeks enhanced the levels of that nutrient in LDL nearly 20 fold[24].

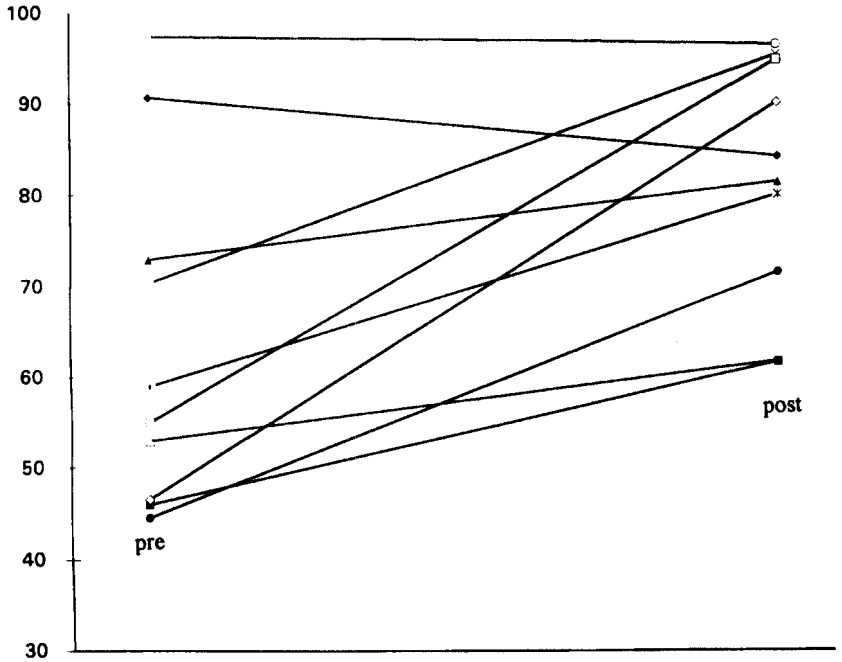

FIGURE 1 The levels of plasma vitamin C (μM) before and after supplementation (250 mg) for each volunteer in the vitamin C – supplementation group.

DISCUSSION

In the investigations reported here at the specified levels of supplementation, each individual antioxidant led to a significant increase in plasma concentrations of the respective antioxidant and in antioxidant/cholesterol ratios in the case of lipid-soluble antioxidants. The increase in plasma α-tocopherol levels and the α-tocopherol: cholesterol ratios on supplementation lie in the 1.5–2 fold range. The attainment of plasma levels of β-carotene of 3.07 ± 1.05 μM, after eight weeks supplementation, compared with pre-supplementation levels of 0.87 ± 0.33 μM (a 3.5- fold enhancement) did not affect α-tocopherol or vitamin C levels in the plasma. Combined supplementation of 15 mg β-carotene with α-tocopherol and vitamin C did not significantly attenuate plasma β-carotene uptake compared with the single supplement of β-carotene. This observation is consistent with previous reports (reviewed in reference[25]).

No significant change was observed in the mean plasma total antioxidant activity (Table 6) of any group before and after supplementation. The mean value for the plasma total antioxidant activity of a normal healthy population is 1.46 ± 0.14 mM (n = 312), the reference interval being 1.32–1.60 mM[14]. When considering that the maximal mean increment of the plasma antioxidant concentration in any group is 0.063 mM, the observation is not unexpected. This finding is consistent with the results of others[26] who observed no significant modification of total plasma antioxidant activity as measured by the TRAP assay[27] after supplementation with 180 mg β-carotene for two weeks.

TABLE 4
Comparative effects of β-carotene supplementation alone compared with supplementation in combination with vitamins E and C on plasma levels

	β-carotene (μM)		β-car/chol		Vitamin E (μM)		Vit E/chol		Vitamin C (μM)	
	Prior	Post	Prior	Post	Prior	Post	Prior	Post	Prior	Post
Sole supplement β-carotene (15 mg)	0.87 ± 0.33	3.07 ± 1.05	0.18 ± 0.08	0.61 ± 0.25	19.6 ± 4.4	19.2 ± 5.1	3.84 ± 0.42	3.84 ± 0.86	58.2 ± 13.0	54.0 ± 16.4
Combined supplement β-carotene 15 mg vitamin E 75 mg vitamin C 150 mg	1.01 ± 0.43	2.75 ± 1.4	0.19 ± 0.08	0.53 ± 0.22	22.6 ± 6.84	29.7 ± 11.6	4.60 ± 0.56	5.81 ± 1.09	59.9 ± 9.7	74.3 ± 20.8

Results expressed as mean ± standard deviation

TABLE 5
Levels of β-carotene and vitamin E in LDL post- versus pre-supplementation

Supplement Group	Vitamin E (nmoles/mg LDL protein)		P	β-carotene (nmoles/mg LDL protein)		P
	Pre-	Post-		Pre-	Post-	
Placebo	10.98 ± 1.85 [8]	13.64 ± 3.63 [8]		1.09 ± 1.11 [8]	0.74 ± 0.70 [8]	
Vitamin E	13.19 ± 3.15 [7]	18.4 ± 4.88 [7]	0.115	1.41 ± 1.14 [7]	1.31 ± 0.66 [7]	NS
Vitamin C	12.45 ± 1.31 [4]	15.43 ± 6.43 [4]	NS	0.95 ± 0.87 [4]	1.30 ± 0.28 [4]	
β-carotene	11.77 ± 1.8 [6]	11.83 ± 1.46 [6]	NS	0.85 ± 0.46 [6]	3.89 ± 1.32 [6]	<0.001
Vitamin E + Vitamin C + β-carotene	12.5 ± 2.00 [7]	14.96 ± 1.48 [7]	NS	1.01 ± 0.58 [7]	2.37 ± 1.08 [7]	= 0.001

Results expressed as mean ± standard deviation. For the statistics, the mean changes between the antioxidant levels of the supplemented groups and the pre-supplementation groups are compared with the changes for the placebo group. (See Material and Methods)

The p value is derived from the statistical differences on comparison of the changes in the antioxidants after supplementation with those of the placebo group. For each individual the baseline value prior to supplementation was subtracted from the post-treatment value and the mean changes in the different groups were compared by one-way analysis of variance (ANOVA)

In the case of vitamin C supplementation, measurement of the level of this vitamin in plasma reveals a maximum of around 100 μM regardless of the pre-supplementation level. This suggests that, at this concentration, the plasma is saturated with vitamin C and the level controlled by renal clearance, in confirmation of the report of Friedman *et al.*[28] In studies by Abbey *et al.*[29] (Table 7) a combined supplement including 900 mg/day vitamin C for 13 weeks [66 μM basal → 84 μM plasma vitamin C] a similar maximal level was observed. Another study with combined supplements (600 mg

TABLE 6
The effects of antioxidant nutrient supplementation on the total plasma antioxidant activity

Supplement Group	Total Plasma Antioxidant Activity		
	Pre-	Post-	P
Placebo [10]	1.39 ± 0.07	1.39 ± 0.05	NS
Vitamin E [10]	1.36 ± 0.05	1.41 ± 0.06	NS
Vitamin C [10]	1.39 ± 0.05	1.39 ± 0.03	NS
β-carotene [9]	1.38 ± 0.03	1.38 ± 0.04	NS
Combination [10]	1.37 ± 0.05	1.37 ± 0.04	NS

Results are expressed as mean ± standard deviation

TABLE 7
Summary of selected earlier supplementation studies; effects on plasma level of nutrients of healthy normal individuals

Reference	Number	Dosage	Duration (Weeks)	Mean Plasma Levels
32	25 nonsmokers	280 mg E	10	31.3 → 44.1 μM
	25 smokers	280 mg E		26.2 → 44.5 μM
	25 placebo			
33	8	440 mg E	4	17.4 → 34.8 μM
	23	880 mg E		17.4 → 34.8 μM
	18	1320 mg E		18 → 34 μM
34	7	200 IU-E	2	39 → 68 μM
35	20: 10 placebo	800 IU-E	5	23.2 → 69 μM
36	44: 15 placebo		16	
	15	800 IU-E		24.6 → 47.1 μM
	14	30 mg β-carotene		3.2 → 10.4 μM
37	8	45 IU-E	3	28.5 → 37.6 μM
38	16	400 IU-E	4	46 → 92 μM
39	12: 4 placebo		3	
	2	150 IU-E		24.5 → 35 μM
	2	225 IU-E		29 → 50 μM
	2	800 IU-E		27 → 49 μM
	2	1200 IU-E		27 → 65 μM
40	8	1200 IU-E	8	31.1 → 101 μM
41	18 nonsmokers	20 mg β-carotene	4	0.41 → 3.5 μM
	20 placebo			
	14 smokers	20 mg β-carotene		0.29 → 3.4 μM
	11 placebo			
42	45: 8 placebo		40	
	10	15 mg β-carotene		ca 0.56 → 1.68 μM
	10	30 mg β-carotene		ca 0.56 → 2.24 μM
	8	45 mg β-carotene		ca 0.56 → 2.4 μM
	9	60 mg β-carotene		ca 0.56 → 2.6 μM
43	12: 6 placebo	90 mg β-carotene	3	0.76 → 6.45 μM
30	78: 39 placebo	Combination:	20	
	(paired)	300 mg-E		23.2 → 47.4 μM
		600 mg-C		28.4 → 75.5 μM
		27 mg β-carotene		0.57 → 3.71 μM
		75μg-Se		
31	24: 12 placebo	Combination:	13	
		800 IU-E		20.3 → 81.6 μM
		1 g C		66 → 140 μM
		30 mg β-carotene		0.37 → 5.4 μM
29	45: 23 placebo	Combination:	13	
		200 mg E		29 → 45 μM
		900 mg C		66 → 84 μM
		18 mg β-carotene		0.4 → 2 μM

vitamin C/day for 20 weeks) [28.4 μM basal $\rightarrow$ 75.5 μM supplemented] was in accord with this observation[30]. However, other workers found that a combined supplement including 1 g/day vitamin C for 13 weeks increased the plasma levels from 66 μM (basal)$\rightarrow$ 140 μM (supplemented)[31], which may indicate that at very high levels of supplementation other factors are involved.

Table 7 summarises several recent studies concerning antioxidant levels in plasma[29-43]. In most of the published investigations tabulated, despite the range of dosages of α-tocopherol (45 IU to 1200 IU), or the duration of administration (2–16 weeks) for individual supplements, or for combined supplements containing α-tocopherol, the plasma level increased by 1.5–2 fold, the same extent as the study reported here. This was regardless of the basal pre-supplement levels, which range from 17.4 μM to 46 μM. The exceptions to this were among the high doses 800 IU for five weeks[35], 1200 IU for eight weeks[40], both of which produced 3-fold increases, and the combination study[31] including 800 IU for 13 weeks which produced a 4-fold increase.

Previous studies have suggested that a number of dietary and life-style factors moderate absorption of carotenoids in humans although the information is inconsistent. Specifically, non-smokers and lean subjects are thought to exhibit the largest responses to β-carotene supplementation; for example, individuals with large body surface areas and high adiposity are said to require higher doses of β-carotene than normal to reach a target plasma concentration[44,45]. The presence of dietary fats in the digestive tract has also been suggested to aid carotene absorption[46]. This may prove to be the single most important factor that influences carotenoid absorption.

Willett *et al.*[36] have proposed that α-tocopherol supplements (800 IU/16 weeks) cause modest overall decreases in plasma endogenous carotenoid levels, but contrariwise a supplement of 30 mg β-carotene for the same duration did not affect plasma α-tocopherol levels. However, others[39] found no such effects on β-carotene levels up to supplemental levels of 1200 IU of α-tocopherol over a period of three weeks. Earlier observations were from animal experiments[47,48], in which the reduced utilisation of β-carotene by large doses of α-tocopherol apparently depended on simultaneous administration. In our study the addition of vitamin E and vitamin C with β-carotene did not alter significantly the increased plasma β-carotene levels compared to the single β-carotene dose. However, in the case of LDL β-carotene levels after the combined supplement, compared with those attained with the single β-carotene supplement, the addition of α-tocopherol and vitamin C with the β-carotene apparently reduced the enhancement of LDL β-carotene levels, $p<0.05$:

β-carotene group: mean of $([\beta\text{-carotene}]_{post}-[\beta\text{-carotene}]_{pre}) = 3.03 \pm 1.71$;

Redoxon protector group: mean of $([\beta\text{-carotene}]_{post}-[\beta\text{-carotene}]_{pre}) = 1.36 \pm 0.88$.

Several other studies have supported the contention that intake of one fat-soluble vitamin can interfere with the utilisation of others (but this may be more significant on long-term administration). For example, studies in human subjects with β-carotene supplementation for 40 weeks resulted in lowered plasma tocopherol levels by 40%[42], following on from the observation of Bendich and Shapiro[49] that β-carotene supplementation of rat diets reduces plasma α-tocopherol levels by 50%. We have not found this to be the case in our investigations in human plasma, consistent with the finding of Reaven *et al.*[24]. From individual supplementation studies, Maxwell *et al.*[50] have drawn the tentative conclusion from studies on healthy individuals during exercise

regimes that supplementation with α-tocopherol under these conditions may protect vitamin C from oxidation.

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