

Original Article

Effect of vitamin C supplementation on concentrations of vitamins C and E in fasting plasma

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Vitamin C may 'spare' vitamin E, but this has not to date been confirmed as occurring *in vivo*. The aim of this study was to test the effect of dietary supplementation with vitamin C on total and lipid standardised vitamin E concentrations in fasting plasma, the hypothesis being that increased vitamin C intake leads to improved vitamin E levels. In this single-blinded study, 12 apparently healthy adults (seven men, five women) took 1 g/day vitamin C for 28 days, with a 28-day placebo-controlled run-in cycle and a 28-day placebo-controlled washout cycle. Concentrations of ascorbic acid, total vitamin E (as total tocopherols) and lipid standardised vitamin E (Vit E_{LS}, expressed as μmol vitamin E/ mmol total cholesterol plus triglycerides) were measured in fasting plasma after each cycle. Results showed that vitamin C supplementation led to significant increases in ascorbic acid, total vitamin E and Vit E_{LS}. These findings indicate that, by a combination of a vitamin E 'sparing' effect — perhaps via improved redox recycling of vitamin E *in vivo* — and a lipid lowering effect, increased intake of vitamin C could increase plasma vitamin E levels, and possibly vitamin E status. Further study of possible *in vivo* interrelationships between vitamins C and E, and the role of vitamin C in lipid metabolism, is needed.

Key words: anti-oxidant status, oxidative stress, ascorbic acid, tocopherol, vitamin C, vitamin E, vitamin supplementation.

Introduction

There is accumulating epidemiological evidence that increased intakes of the antioxidant vitamins C and E may help lower the risk of diseases associated with increased oxidative stress.^{1,2} Plasma concentrations of these vitamins are maintained by dietary intake, although not completely defined by it.^{3–5} Vitamin E (mainly α -tocopherol) is the major lipid soluble anti-oxidant *in vivo*; vitamin C (ascorbic acid) is found in the aqueous phase. A redox interaction between these two vitamins has been proposed, the lipid bound vitamin E being recycled from its oxidised tocopheroxyl radical form by ascorbic acid in the aqueous surroundings.^{6,7} This implies that increased vitamin C concentrations in plasma could, by improved redox recycling, appear to 'spare' vitamin E, thus augmenting defences in the lipid compartments of the body.

Some vitamin C supplementation trials have shown a trend towards sparing tissue vitamin E,^{8,9} and a study of premature infants reported an increase in plasma vitamin E levels after vitamin C supplementation.¹⁰ Results to date are inconclusive, however, and further study regarding a possible vitamin C/E interrelationship is needed. Increased intake of ascorbic acid may also lower plasma lipids but results of clinical trials have been variable and conflicting to date.^{11–13} Nevertheless, the combination of such putative effects would mean that dietary supplementation with vitamin C could lead to a significant enhancement of not only the aqueous systems of the body but also, by increasing the vitamin E to lipid ratio, an improvement in the antioxidant status of lipid systems.

In this study, the effect of vitamin C supplementation was investigated with the following questions in mind: (i) is supplementation with vitamin C associated with an increase in the concentration of total vitamin E and/or lipid standardised vitamin E in fasting plasma?; and (ii) is supplementation with vitamin C associated with a decrease in the concentration of total cholesterol and triglycerides in fasting plasma?

Methods

Subjects

A total of 12 apparently healthy, non-smoking, free-living (i.e. under no dietary or lifestyle restrictions) volunteers (seven men and five women aged between 42 and 58 years) were recruited for this single blinded supplementation study, with entry staggered over 6 weeks. Ethical approval for this study was obtained from the Ethics Sub-Committee of the Hong Kong Polytechnic University, and all procedures involving human subjects complied with the Declaration of Helsinki (1989).

Supplementation procedure

Over a period of 28 days, 1 g/day Vitamin C, as one dose, was taken. The supplementation dosage was chosen on the

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basis of previous work which reported an optimal plasma response, in terms of an increase in plasma ascorbic acid concentration, with a single oral dose of between 0.5 and 1.0 g.¹⁴ There was a 28-day placebo-controlled run-in cycle immediately before and a 28-day placebo-controlled washout cycle immediately after the vitamin supplementation cycle; placebo and active vitamin preparations (purchased from Larkhall Green Farm, London, United Kingdom) were visually identical, and volunteers were blinded as to which preparation was given. Blood samples were taken, at the end of each 28-day cycle, from the antecubital vein with the subject being in a sitting position. Collection was after 9 am following an overnight fast, with no placebo or vitamin preparation taken on these days until after blood sampling.

Samples

Fasting, non-haemolysed ethylene diamine tetra-acetic acid (EDTA) plasma samples were used. Blood was kept chilled and in the dark until separation of plasma, which was within 1 hour of sampling. Plasma ascorbic acid concentrations were measured within 30 min of separation. Since ascorbic acid is known to interfere with peroxidase linked assays¹⁵ of the type usually used to measure cholesterol and triglycerides, plasma for cholesterol and triglycerides measurements, as well as for vitamin E, was stored at -70°C until assayed (within 6 months, with no intermediate thawing/refreezing). Ascorbic acid is rapidly destroyed in EDTA plasma, even at very low temperatures, and no ascorbic acid was detectable in EDTA plasma stored under these conditions (Benzie, unpublished results, 1996).

Methods of measurement

Ascorbic acid was measured using a specific enzyme-assisted spectrophotometric method known as EFTSA, on a Cobas Fara centrifugal analyser (Roche Diagnostic Systems, Basle, Switzerland); within- and between-run coefficients of variation (CV) were $< 5\%$ at 25, 50, 100 and 440 $\mu\text{mol/L}$, $n = 10$ in each case.¹⁶ Vitamin E was measured as total tocopherols by a fluorimetric micromethod using a Kontron SFM 25 spectrofluorimeter (Kontron, Zurich, Switzerland); in-run CV at 24 and 44 $\mu\text{mol/L}$ were $\leq 2.0\%$, $n = 10$ in each case; between-run CV was 7.1% (mean 33 $\mu\text{mol/L}$, $n = 13$).¹⁷ This method was validated against a high performance liquid chromatography (HPLC) method for α -tocopherol. Lipid standardized vitamin E (Vit E_{LS}) was expressed as μmol of vitamin E per mmol of total cholesterol plus triglycerides;¹⁸ total cholesterol (TC) and triglycerides (Tg) concentrations in plasma were measured using commercially available enzymatic methods (Roche Diagnostic Systems) on a Cobas Fara centrifugal analyser; within- and between-run CVs were $< 4\%$.

Analysis of results

The paired Wilcoxon rank sum test was used for statistical analysis; two-tailed P values are shown.

Results

Supplementation with vitamin C led to significant changes in both vitamin C and E levels in fasting plasma. For ascorbic acid, the mean (SE) before and after supplementation was 50.7 (3.2) and 69.9 (4.8) $\mu\text{mol/L}$, respectively, while $P = 0.0068$. For total vitamin E, the mean (SE) before and after supplementation was 32.7 (2.5) and 36.9 (2.4) $\mu\text{mol/L}$, respectively, while $P = 0.042$.

Post-supplementation levels of TC and Tg were lower, although the decrease in TC was not statistically significant ($P = 0.064$); mean (SE) TC plus Tg before and after supplementation was 7.44 (0.42) and 6.74 (0.40) mmol/L, respectively, while $P = 0.038$. The combination of this decrease in plasma lipids and the modest increase in plasma total vitamin E seen resulted in a 26% mean increase in Vit E_{LS} concentrations; mean (SE) Vit E_{LS} before and after supplementation was 4.36 (0.16) and 5.49 (0.20) $\mu\text{mol}/\text{mmol}$, respectively, while $P = 0.0004$.

After the 28-day washout cycle, plasma ascorbic acid and total vitamin E concentrations had returned to near pre-supplementation levels. Consolidated results are presented in Table 1.

Discussion

Results of this small study suggest that supplementation with vitamin C is associated with a significant increase in fasting plasma concentrations of vitamin E. The observed increase in Vit E_{LS}, which averaged 26%, was related to a combination of decreased lipids and increased total vitamin E concentrations. It is possible that there was a direct ascorbic acid-induced 'sparing' of vitamin E *in vivo* owing to enhanced aqueous anti-oxidant defence. Alternatively, or in addition, there may have been improved redox recycling of the tocopheroxyl radical in plasma lipoproteins owing to the increased ascorbic acid concentration in the immediate aqueous surroundings.¹⁹

The apparently prolonged effect on lipid concentrations indicates that there may be a role for ascorbic acid at both the biosynthetic and catabolic stages of lipid metabolism, as previously suggested.^{11,20} This may involve vitamin C-dependent increases in lecithin cholesterol acyltransferase and conversion of cholesterol to bile acids, inducing a response at these two stages of cholesterol metabolism. This could help explain why shorter periods of supplementation did not appear to lower lipid levels in smokers.¹³ It has also been reported that vitamin C modulates insulin action, and this could help explain the decrease in plasma Tg levels observed in the current study.²¹ It must be noted, however, that intra-

Table 1. Mean (SE) concentrations of vitamin C and E and lipids in fasting plasma after 28-day supplementation cycles

	Vitamin C $\mu\text{mol/L}$	Vitamin E $\mu\text{mol/L}$	Vit E _{LS} $\mu\text{mol}/\text{mmol}$	TC mmol/L	Tg mmol/L	TC + Tg mmol/L
After run-in cycle (placebo)	50.7 (3.2)	32.7 (2.5)	4.36 (0.16)	5.9 (0.21)	1.65 (0.22)	7.44 (0.42)
After 1g/day vitamin C	69.9 (4.8)**	36.9 (2.4)*	5.49 (0.20)**	5.4 (0.29)	1.32 (0.15)*	6.74 (0.40)*
After wash-out cycle (placebo)	47.0 (3.9)**	31.5 (2.6)**	4.69 (0.25)**	5.3 (0.23)	1.32 (0.17)	6.67 (0.38)

Significantly different from the corresponding value at the end of the previous cycle * $P < 0.05$, ** $P < 0.01$.

individual (biological) variation in lipids, and particularly in triglycerides, can be very large.²²

While it is unlikely that the direction of biological variation would have been the same for all 12 subjects, the decrease in Tg could have been a chance finding, and the results of this current study must be interpreted with caution owing to the small sample size. In addition, the apparently prolonged effects on lipid levels suggest that a 28-day intervention and washout period may not be long enough to reveal slow or prolonged effects. Alternatively, it is possible that some other dietary factor(s) may have accounted for the change. A follow-up study with a larger sample size and cross-over design would be useful to address and help resolve these issues.

Vitamin E is the major lipid soluble anti-oxidant *in vivo*, and increased Vit E_{LS} signifies an absolute increase in the anti-oxidant status of lipoproteins.^{4,5,18} Low density lipoprotein (LDL), the most cholesterol-rich lipoprotein, can be oxidised into a much more atherogenic form.^{23,24} Vitamin C scavenges reactive oxygen species (ROS) capable of initiating peroxidation of polyunsaturated fatty acids in LDL.^{25,26} Vitamin E breaks chains of peroxidation initiated by ROS generated within the lipoprotein particle, or ROS which have made it through the aqueous-phase anti-oxidant defences unscathed.^{4,27-30} There is a clear biochemical rationale, therefore, linking increased ascorbic acid levels with decreased oxidative stress, and this rationale supports the strong and varied epidemiological and experimental evidence for the health benefits of vitamin C-rich diets.^{1,2,31-35}

Results of previous studies exploring vitamin C supplementation effects on vitamin E levels and on biomarkers of lipid peroxidation have been conflicting.^{6,13,25,28,31,34} Data presented here support the putative *in vivo* interaction between vitamin C and vitamin E, and indicate that supplementation with ascorbic acid could lead to a decrease in oxidative damage to lipid- as well as to aqueous-components of the body owing to an increase in the vitamin E to lipid ratio.^{7,8,19} Further study is needed into possible vitamin C-supplementation effects in relation to vitamin E status and to lipid oxidation.

In conclusion, the results of this study indicate that an increased intake of vitamin C may increase plasma vitamin E levels, and possibly vitamin E status. Vitamin C supplementation may, therefore, effectively enhance anti-oxidant status of both the aqueous and lipid compartments of the body.

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