ABSTRACT To determine whether the postulated sparing effect of vitamin E by ascorbic acid (AA) is important for human nutrition, we studied vitamin E status in 20 healthy pre-menopausal women (age 20–43 y) with high or low vitamin C intakes for 6 wk in a live-in metabolic unit. The experimental diet contained no fruits and vegetables and provided 5 mg/d of AA (Recommended Dietary Allowance = 60 mg/d), 3 mg/d of or-tocopherol (RDA = 10 mg/d) and 42 g/d of tocopherol-stripped safflower oil to increase the vitamin E requirement. Half of the subjects received a daily AA supplement of 495 mg (high AA group). A biochemical ascorbate deficiency was attained in the low AA group as indicated by plasma AA concentrations that reached the lower limit of normal by study d 15. Oral doses (20 mg) of hexadeuterated RRR α-tocopherol acetate (d6-αT) were given daily to all subjects on d 15–21. Measures of vitamin E status included d6-αT and unlabeled α-tocopherol concentrations in plasma, platelets, buccal cells and adipose. The levels of unlabeled α-tocopherol decreased over time in plasma and platelets and were unchanged for buccal cells and adipose, but were not significantly affected by AA intake. Likewise, the rise and fall of plasma and platelet d6-αT, and measures of lipid peroxidation, were not affected by AA intake. Although vitamin C nutriture did not significantly affect vitamin E status within the 6-wk time period of this experiment, further study of this question is warranted, because some of the present results indicate a trend toward sparing of tissue tocopherol by high AA intake. J. Nutr. 126:2268–2277, 1996.

INDEXING KEY WORDS:

• vitamin C • vitamin E • vitamin interaction
• antioxidant • humans

Although the antioxidant vitamins C and E operate in aqueous and lipid compartments, respectively, in vivo, there is considerable evidence of synergistic interaction between the vitamins in vitro. Niki (1984) has summarized some of the research showing that vitamin C can reduce the tocopheroxyl (vitamin E) radical to regenerate the active form of vitamin E (Fig. 1) in both homogeneous solutions and in model liposomal membrane systems. More recently, ascorbic acid was shown by 13C nuclear magnetic resonance monitoring to reduce the tocopheroxyl radical and tocopherolquinone back to tocopherol (Wijesundara and Berger 1994). Bowry et al. (1995) found that next to ubiquinol, ascorbate was one of the most effective co-antioxidants that prevented tocopheroxyl radical–mediated peroxidation of isolated LDL.

In animal studies, rats given supplemental vitamin C had higher plasma vitamin E concentrations than those ingest the same diet without additional vitamin C (Chen et al. 1980). Guinea pigs fed high levels of vitamin C had increased vitamin E concentrations in lung tissue at each of four different levels of vitamin E intake (Bendich et al. 1984), and guinea pigs fed vitamin C–deficient diets had decreased levels of vitamin E in lungs, liver and adrenal tissues (Kanazawa et al. 1981). In two human studies involving healthy elderly
subjects, supplementation with vitamins C and E together had a synergistic effect in lowering serum thiobarbituric acid–reactive substances (TBARS)5 (Wanta-
nowicz et al. 1984), and elderly persons in Boston taking supplemental vitamin C had higher plasma vitamin E concentrations, even after accounting for other factors such as dietary vitamin E intake (Jacob et al. 1988). Recent reports suggest that the two vitamins operate synergistically in vascular tissue to inhibit lipid peroxidation [Mezzetti et al. 1995] and improve response to vascular tissue injury [Nunes et al. 1995] and that endogenous ascorbate regenerates retinal vitamin E oxidized by UV irradiation (Stoyanovsky et al. 1995).

Although results of most in vivo studies support the hypothesis that vitamin C can regenerate or “spare” vitamin E, some studies report an antagonistic relationship between the two vitamins or report no significant interaction. In rats, excess vitamin C intake lowered tissue antioxidant potential during marginal vitamin E intake, thus suggesting an increased vitamin E requirement during high vitamin C intakes [Chen 1981]. Burton et al. (1990) studied the net rates of uptake and loss of vitamin E by feeding deuterated tocopherol as a marker of vitamin E turnover in guinea pigs receiving two levels of dietary vitamin E and three levels of vitamin C. No significant effect of vitamin C intakes on vitamin E concentrations in blood or tissues was found, whether animals were receiving ample or deficient amounts of vitamin E. However, in a further study, decreased levels of vitamin E were found in the testes, adrenal gland and lung of guinea pigs that were severely deficient in vitamin C [G. W. Burton, National Research Council Canada, personal communication].

Interactions of biological antioxidant compounds are likely to occur in vivo, because in vitro studies have shown that they can operate in redox cycles that are often coupled, or the action of one simply “spares” the other (Jacob 1995). Research on antioxidant micronutri-

5 Abbreviations used: AA, ascorbic acid; ARS, Agricultural Research Service; δδ-α-T, unlabeled α-tocopherol; δδ-α-T, hexadeuterated RRR-α-tocopherol acetate; GC-MS, gas chromatography-mass spectrometry; HBSS, Hanks’ balanced salt solution; MDA, malondialdehyde; RDA, Recommended Dietary Allowance; SOD, superoxide dismutase; TBARS, thiobarbituric acid–reactive substances; WHNRC, Western Human Nutrition Research Center.

FIGURE 1 Postulated regeneration of α-tocopheroxyl radical by ascorbic acid. LOO• is a lipid hydroperoxide radical.

VITAMIN C-VITAMIN E INTERACTION

MATERIALS AND METHODS

Protocol and subjects. Twenty-one healthy, non-
smoking, female volunteer subjects, ages 20–43 y, were admitted to the metabolic unit of the Western Human Nutrition Research Center (WHNRC), USDA Agricultural Research Service (ARS), after medical and psychological screening. All subjects were within 90 to 130% of ideal body weight (baseline mean ± SEM of 58.9 ± 2.1 kg. The study protocol and informed consent were approved by the Institutional Review Committee of the Letterman Army Medical Center, Department of the Army, Presidio of San Francisco, CA, and by the Human Studies Review Committee of the ARS, USDA. Signed informed consent was obtained from each volunteer subject after reading of the protocol and discussion of the study’s purpose, procedures, risks and benefits. For the duration of the 42-d study, the subjects lived in and ate all meals in the WHNRC metabolic unit and were chaperoned at all times when outside the unit.

Experimental design and diet. To ensure that all subjects began the study in adequate vitamin C status, all subjects were given 500-mg ascorbic acid tablets [Walgreens, Deerfield, IL] at least 1 wk before the start of the study and instructed to take one each day. The subjects were admitted to the Metabolic Unit in two separate groups and went through the same 6-wk experimental regimen, one group after the other. Group A, consisting of 11 subjects, was studied from January 13 to February 23, 1993. Group B (10 subjects) was studied from March 3 to April 13, 1993. Data from one Group A subject who left the study early on d 32 are not included in this report, so that data analysis for this report was performed on a total of 20 subjects, 10 from...
TABLE 1

Experimental design

<table>
<thead>
<tr>
<th>Weeks</th>
<th>High AA (n = 10)</th>
<th>Low AA (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>500 mg AA/d</td>
<td>5 mg AA/d</td>
</tr>
<tr>
<td>3</td>
<td>500 mg AA/d</td>
<td>5 mg AA/d</td>
</tr>
<tr>
<td></td>
<td>+ 20 mg d6αT/d</td>
<td>+ 20 mg d6αT/d</td>
</tr>
<tr>
<td>4-6</td>
<td>500 mg AA/d</td>
<td>5 mg AA/d</td>
</tr>
</tbody>
</table>

1 The 20 subjects were studied in two groups of 10 each that received the same regimen (as shown above) in successive 6-wk periods. All took 500 mg/d AA supplements for ≈1 wk before moving in.

2 The high ascorbic acid (AA) group received a total of 500 mg/d: 5 mg/d from diet and 495 mg/d from ascorbic acid supplemented into noncaloric citrus-flavored soda (165 mg/100 mL) consumed at each of three meals.

3 d6αT = hexadeuterated-RRR-α-tocopherol acetate: all subjects received 20 mg/d with the dinner meal from d 15 to 21.

Each group. The experimental design is summarized in Table 1.

All subjects consumed the same ascorbic acid–deficient diet throughout the study. The experimental variable was the amount of ascorbic acid supplemented into the diet (ascorbic acid fine powder USP, Roche Vitamins and Fine Chemicals, Belvidere, NJ), the high ascorbate group getting ascorbic acid supplements totaling 495 mg/d and the low ascorbate group getting no supplemental ascorbate, as shown in Table 1. The subjects were assigned to the high or low ascorbate groups by normalizing the two groups for age and body weight. Menu items are shown in Table 3. Safflower oil stripped of tocopherols and tocotrienols was added to each dinner (14 g) and evening snack (28 g) to increase the requirement for vitamin E. As calculated from food composition tables (USDA 1989), the diet provided 44% of energy from carbohydrate, 39% from fat (polyunsaturated/saturated fatty acid ratio of 1.59) and 17% from protein. The following dietary supplements were consumed with meals to ensure that the subjects received at least two-thirds of the Recommended Dietary Allowance (RDA) [NRC 1989]: iron (65 mg as ferrous sulfate, Nature’s Blend, Porterville, CA) and folic acid [800 µg, Bronson Pharmaceuticals, La Canada, CA] twice weekly, and calcium-magnesium [375 and 187 mg, Bronson Pharmaceuticals] every other day. As a marker of tocopherol turnover, capsules containing 20 mg of hexadeuterated RRR-α-tocopherol acetate (Eastman Chemical, Kingsport, TN) were consumed with the dinner meal during the third week (study d 15–21). Subjects were allowed to drink water ad libitum, instant decaffeinated coffee or tea up to 720 mL [three cups]/d, diet soda up to 710 mL [two 12 ounce cans]/d, and to use limited amounts of black pepper, salt and noncaloric sweetener. Individual energy intakes were estimated initially by the formula: 655 + [9.6 times kg-weight] + [1.8 times cm-height] times 1.6; intakes ranged from 7.95 to 10.05 MJ. Body weights were taken daily and 0.4-MJ adjustments in energy intakes were made when subjects deviated beyond ±3% of the d 1–3 average weight. Energy intakes were adjusted by changing the amounts of each food item proportionately. Subjects were escorted on two walks per day of approximately 3 km each and were allowed optional exercise on a treadmill or stationary bicycle up to 90 min a week.

As calculated from food composition tables (USDA 1989), the experimental diet provided an average of 5 mg/d of ascorbic acid (RDA = 60 mg/d), and 3 mg/d of α-tocopherol (RDA = 10 mg/d). Replicates of the four daily diets were homogenized in a blender with 400 mL of cold 200 g/L metaphosphoric acid-0.54 mmol/L Na2EDTA solution and 250 mL of cold 0.54 mmol/L Na2EDTA to preserve ascorbate and the supernate taken for immediate determination of ascorbic acid.

TABLE 2

Vitamin C and E concentrations in healthy women with high or low vitamin C intakes

<table>
<thead>
<tr>
<th>Ascorbate intake</th>
<th>Plasma</th>
<th>Leukocyte</th>
<th>Platelet</th>
<th>Buccal cell</th>
<th>Adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y</td>
<td>μmol/L</td>
<td>nmol/10^8 cells</td>
<td>μmol/mmol lipid</td>
<td>pmol/10^9 cells</td>
</tr>
<tr>
<td>500 mg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>30.2 ± 8.1</td>
<td>59.8 ± 3.7</td>
<td>86.9 ± 2.8</td>
<td>145 ± 5</td>
<td>5.60 ± 0.35</td>
</tr>
<tr>
<td>Final</td>
<td>—</td>
<td>59.3 ± 3.7</td>
<td>59.8 ± 12.0</td>
<td>169 ± 4b</td>
<td>4.50 ± 0.23b</td>
</tr>
<tr>
<td>5 mg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>29.8 ± 7.8</td>
<td>57.9 ± 2.1</td>
<td>75.0 ± 4.5</td>
<td>160 ± 7</td>
<td>4.81 ± 0.36</td>
</tr>
<tr>
<td>Final</td>
<td>—</td>
<td>57.2 ± 2.1</td>
<td>6.8 ± 1.5</td>
<td>95 ± 5b</td>
<td>4.00 ± 0.21b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM at the beginning [d 2] and end [d 41] of the study; n = 10 subjects in each ascorbate intake group. Superscript letter a indicates mean for low ascorbate group is significantly different from corresponding high ascorbate group mean (P < 0.05). Superscript letter b indicates final values within a group are different from initial values (P < 0.05).

2 Value is only for the last of the two groups studied, n = 5.
All ascorbic acid determinations were performed by ion pairing HPLC separation on a reverse-phase column, electrochemical detection, and quantification against an isoascorbic acid internal standard (Kutnink et al. 1987). The average ascorbic acid content of the four daily diets by HPLC analysis was 1.9 mg [the daily values were 1.5, 2.1, 1.9 and 1.9 mg]. The ascorbic acid supplementation of the high ascorbate group with 495 mg/d was accomplished by preparation of an ascorbic acid-supplemented non-caloric citrus-flavored soda solution containing 3.30 g ascorbic acid dissolved in 2 L of decarbonated (“flat”) soda (165 mg/100 mL), and providing 100 mL of this solution at each of three meals for a total supplement of 495 mg/d. The solution was kept refrigerated throughout the day. The low ascorbate group received the decarbonated soda without any added ascorbic acid.

**Specimen collections and analytical methods.** Fifteen times during the study, blood was taken by venipuncture (at 0700–0800 h after 12 h of overnight fasting) for various biochemical determinations. Plasma (EDTA anticoagulant) was taken weekly for determination of ascorbic acid. Ascorbic acid in plasma was initially determined by a simple acid phosphotungstate colorimetric method (Kyaw 1978). This method, however, gave results that seemed to be biased high: many fasting values were 85 to 113 μmol/L in the high ascorbate group, and most values in the low ascorbate group remained above 45 μmol/L throughout (the lower limit of normal is 23 μmol/L). Because of this concern, the more specific HPLC method was used for determining plasma ascorbic acid beginning with the last blood draw of the first group studied (Group A) and for all of the second group B. Only the HPLC results are used in this report. Plasma for the HPLC determination of ascorbic acid was stabilized by addition of an equal volume of a 100 g/L metaphosphoric acid and 0.54 mmol/L Na₂EDTA solution containing a known quantity of 0.114 mmol/L isoascorbic acid internal standard. After centrifugation, the protein-free supernatant was frozen at -70°C until thawed for ascorbic acid analysis by the HPLC method of Kutnink et al. (1987).

Plasma was taken 15 times for tocopherol measurements. EDTA-anticoagulated plasma was frozen at -70°C until thawed for determination of tocopherols. The labeled hexadeuterated RRR-α-tocopherol acetate (d6-αT) and unlabeled α-tocopherol (d0-αT) were determined by a gas chromatography-mass spectrometry (GC-MS) method involving extraction of tocopherols into heptane, purification by HPLC, and conversion to trimethylsilyl ether derivatives (Burton et al. 1988, Ingold et al. 1987). Malondialdehyde (MDA) was determined by a procedure modified from Chirico (1994). Plasma MDA results are expressed as micromolar MDA equivalents based on tetraethoxypropane as calibrator. Erythrocyte superoxide dismutase (SOD, EC 1.15.1.1) was determined by a method based on reduction of cytochrome c (Wheeler et al. 1990). Erythrocyte glutathione peroxidase (EC 1.11.1.9) was determined by an automated continuous-
flow colorimetric method using dichloroindophenol as substrate (Hawkes and Craig 1990).

Platelets were taken 10 times for tocopherol measurements. Platelet-rich plasma was isolated from whole blood (EDTA anticoagulant) by centrifugation at 190 times g for 10 min at 5°C. A 40-µL aliquot of the platelet-rich plasma was taken and the platelet count obtained using the prediluted mode of a Serono System 9000 Differential automated cell counter (Serono Baker Diagnostics, Allentown, PA). Platelets were then isolated as a pellet after centrifugation of 2.0 mL of the platelet-rich plasma at 950 times g for 10 min at 5°C. The platelets were washed once with chilled Hanks' balanced salt solution (HBSS) and recentrifuged, and the final pellet was gently broken up with 1.00 mL of chilled HBSS. The solution was then frozen at −70°C until thawed for tocopherol extraction and analyses by GC-MS. For tocopherol extraction, 50–100 mg of sodium ascorbate and 0.5 nmol of vitamin E internal standard (25 µL of 0.02 mmol/L d9-αT) were added to 0.8 mL of the thawed platelet-HBSS solution and vortexed for 10 s. Sequential additions of 0.5 mL of 0.5 mol/L SDS (10 s vortex), 2 mL of absolute ethanol (10 s vortex), and 2 mL of heptane (1 min vortex) were performed. The mixture was centrifuged for 3 min at 1700 g, and the top layer was taken with a Pasteur pipette and placed into an HPLC vial that was capped and stored at −20°C until HPLC purification.

For isolation of mononuclear leukocytes, 5 mL of whole blood (EDTA anticoagulant) was added to 5-mL LeucoPREP® cell separation tubes (Becton Dickinson Labware, Lincoln Park, NJ), and the tubes were centrifuged at room temperature for 19 min at 1730 times g. The tubes were then chilled, the plasma layer removed, and the mononuclear leukocyte band above the gel removed and placed in a centrifuge tube with 10 mL of chilled PBS. This solution of cells was gently mixed and centrifuged at 100 times g for 15 min at 5°C. The cell pellet was gently washed again with a second 10-mL portion of chilled PBS. A 1.00-mL portion of chilled PBS was then added to the cell pellet, the solution mixed gently, and a leukocyte count obtained using the whole blood mode of the automated cell counter. The solution was then centrifuged at 400 times g for 15 min at 5°C, and 500 µL of an ascorbic acid preservative solution containing 31 g/L metaphosphoric acid, 0.54 mmol/L Na2EDTA and 14.2 µmol/L isosorbidic acid was added to the isolated pellet. The solution was then vortexed to disrupt the pellet and frozen at −70°C until thawing for ascorbic acid determination by HPLC. Values for mononuclear leukocyte ascorbic acid were not obtained for the last 3 wk of the first group studied and the first 2 wk of the second group due to sample processing problems.

Buccal cells were collected four times with a soft bristle toothbrush. Before the collection, the subjects rinsed their mouths with distilled water. The inner surface of the cheek was gently brushed 20 strokes on one side of the mouth. The toothbrush was rinsed in ~20 mL of chilled saline solution and the procedure repeated on the other side. The subjects then rinsed their mouths vigorously with 20 mL of isotonic saline, which was then added to the brush rinsings. The saline rinsings were centrifuged at 380 times g for 15 min at 5°C. The supernate was discarded and 1.00 mL of saline added. A 20-µL portion was taken for protein determination by a modified Lowry method (Peterson 1977), and the remainder was frozen at −70°C until thawed for extraction and analysis of tocopherols by GC-MS. Extraction of tocopherols was accomplished using 1 mL of the buccal cell saline suspension and the same procedures described earlier for extraction of tocopherols from platelets.

Adipose samples of 0.3 to 94 mg were taken twice by gluteal liposuction, near the beginning and end of the study. Adipose samples from the liposuction syringe were expressed directly onto a nylon-screen of a vacuum-filter apparatus. The samples were rinsed with isotonic saline and blood clots removed with tweezers. Samples were allowed to air dry for 10–15 min and then transferred to a pre-weighed tube to obtain sample weight. The samples were frozen at −70°C until thawing for extraction and analysis of tocopherols by GC-MS. To extract tocopherols, the weighed adipose tissue sample was minced in a screw-top centrifuge tube and 1 mL of distilled water was added. Vitamin E internal standard (5 nmol as 25 µL of 0.2 mmol/L d9-αT) and 2 mL of ethanol containing 10 g/L sodium ascorbate were added, and the solution was vortex-mixed for 10 s. After addition of 0.3 mL of saturated KOH, the tube was vortexed for 10 s, capped tightly and heated at 70°C for 30 min (for small samples of 25 mg or less, the sample was not minced, the amount of internal standard was reduced to 1 nmol, and the heating time was reduced to 10 min). The tube was then cooled in ice, 1 mL of water and 3 mL of heptane were added, and the tube was vortexed for 1 min. The mixture was centrifuged for 3 min at 1700 times g, and the top layer was removed with a Pasteur pipette and placed into an HPLC vial that was capped and stored at −20°C until HPLC purification step.

Complete 24-h urine collections were taken throughout the study, except for the last day. The daily urine collection jugs were kept refrigerated and 6-d pools prepared by adding together 10% of each daily urine for 6 d and mixing well. Aliquots of the daily and pooled urines were taken for determination of creatinine by a modified Jaffe method (Kroll et al. 1986). Aliquots of the pooled urine samples were frozen for later determination of free and amino acid–conjugated MDA by the size-exclusion HPLC method of Lee et al. (1992) and of urinary TBARS (Lee et al. 1992).

Statistics. Data were analyzed using SAS for Personal Computers, Version 6.08 (SAS Institute Inc., Cary, NC), SigmaStat 1.00 statistical software and Sigmaplot graphing software 1.02 (Jandel, San Rafael, CA).
and InStat 2.02 statistical software (GraphPad Software, San Diego, CA). Repeated measures ANOVA was used to assess the effect of time and ascorbate intake on concentrations of vitamin E and lipid peroxidation measures. Regression analysis was used to fit and analyze curves for the appearance and disappearance of d6-αT in plasma, platelets and buccal cells. Two sample t tests were used to assess differences between ascorbate groups at single time points and between d6-αT curve parameters, slopes and area under the curve. Paired t tests were used to determine differences between two time points within a group. The Mann-Whitney and Wilcoxon Signed Rank nonparametric tests were used where data were not normally distributed (Glantz 1992). The 0.05 level of significance was used for all statistical tests. Values are presented as means ± SEM.

RESULTS

Initial and final values for age, body weight, ascorbic acid and unlabeled α-tocopherol (d0-αT) in the high and low ascorbate groups are summarized in Table 2. Weekly values for plasma and mononuclear leukocyte ascorbate are shown in Figure 2 (for the first group studied, Group A, only the last day’s plasma ascorbate value is shown). Plasma ascorbate was significantly lower in the low ascorbate intake group than in the high ascorbate group at all time points, and leukocyte ascorbate was significantly lower from d 28 on, by t test (P < 0.05).

Unlabeled plasma α-tocopherol and total α-tocopherol (d0 + d6-αT) concentrations per millimole of lipid (cholesterol + triglycerides), shown in Figure 3, were lower in the low ascorbate group compared with the high ascorbate group throughout, but this difference was not statistically significant except at d 36 [plasma cholesterol values were also lower in the low ascorbate intake group, but the differences were not significant and did not change over time]. The final d 42 values of d0-αT and total-αT within each ascorbate intake group are significantly lower than initial d 2 values by paired t test, P < 0.05 [Table 2 and Fig. 3], with the decreases being 3% greater for the high ascorbate intake group.

The rise and fall of hexadeuterated α-tocopherol (d6-αT) in plasma and platelets are shown in Figure 4. There was no significant difference in the plasma or platelet d6-αT curve parameters by ascorbate intake group for comparisons of individual time points, areas under the curve, positive (rise) or negative (disappearance) slopes, when plasma d6-αT data was calculated per millimole of lipid, per liter of plasma, or when plasma and platelet d6-αT was calculated per unit of d0-αT or total-αT, i.e., d6-αT/(d0 + d6)-αT. Platelet d0-αT was not significantly different by ascorbate intake group. Platelet d0-αT decreased significantly over time

FIGURE 2 Weekly plasma (panel A) and mononuclear leukocyte (panel B) ascorbic acid values (means ± SEM) for healthy women receiving either 500 or 5 mg/d of ascorbic acid. The Low Norm reference lines represent the lower limits of normal: 23 μmol/L for plasma ascorbate and 120 nmol/10^8 cells for mononuclear leukocyte ascorbate. Panel A: Circle symbols are for the second group studied (n = 5 each), and triangle symbols are for the last day of the first group studied (n = 5 each). All mean values for the low ascorbate intake group are significantly different from the corresponding values for the high ascorbate intake group, by t test (P < 0.05). Seven-day period of supplementation with 20 mg/d of hexadeuterated α-tocopherol acetate is shown by arrows. Panel B: Values are for five subjects except for d 15 and 22 where n = 10. *Mean values are significantly different from those of the high ascorbate intake group (P < 0.05 by t test).
FIGURE 3 Plasma d0 [panel A] and d0 + d6 [panel B] α-tocopherol concentrations per mmol lipid (cholesterol + triglycerides) for healthy women receiving either 500 or 5 mg/d of ascorbic acid. Values are means ± SEM for 10 subjects each. Final d 42 values within each ascorbate intake group are significantly lower than initial d 2 values by paired t test (p < 0.05). *Mean value is significantly greater than for low ascorbate group (p < 0.05 by t test). Seven-day period of supplementation with 20 mg/d of hexadeuterated α-tocopherol acetate is shown by arrows.

FIGURE 4 The rise and fall of hexadeuterated α-tocopherol [d6-αT] in plasma [panel A] and platelets [panel B] of healthy women receiving either 500 or 5 mg/d of ascorbic acid. Values are means ± SEM for 10 subjects each. Subjects were given capsules containing 20 mg of hexadeuterated RRR-α-tocopherol acetate with the dinner meal during the period indicated by the arrows (study d 15–21). Mean values are not significantly different by ascorbate intake.
FIGURE 5 Incorporation of hexadeuterated α-tocopherol (d₆-αT) into buccal cells of healthy women receiving either 500 or 5 mg/d of ascorbic acid. Values are means ± SEM for 10 and nine subjects in the high and low ascorbate groups, respectively. Subjects were given capsules containing 20 mg of hexadeuterated RRR-α-tocopherol acetate with the dinner meal during the period indicated by the arrows (study d 15–21). *Mean value for low ascorbate group is significantly greater than for high ascorbate group at d 22 (P < 0.003, by t test).

± SEM, n = 10 subjects) for urinary TBARS were 366 ± 60 and 220 ± 33 for the high ascorbate group and 293 ± 42 and 149 ± 34 for the low ascorbate group. Erythrocyte SOD activity (per 10⁹ cells) was not different by ascorbate intake, but also decreased significantly over time. Erythrocyte glutathione peroxidase activity (per gram of protein) was not different between ascorbate intake groups, but increased over time in the high ascorbate group and remained the same throughout for the low ascorbate group.

DISCUSSION

Subjects receiving the ascorbic acid supplement maintained an adequate to high ascorbate status throughout, whereas subjects receiving no supplement attained a state of moderate ascorbate deficiency, i.e., low body levels of the vitamin with no physical signs of scurvy. In the low ascorbate group, plasma ascorbate concentrations indicating deficiency were attained at about d 15, whereas tissue ascorbate deficiency as represented by the ascorbate levels of mononuclear leukocytes (primarily lymphocytes) was attained by d 28. Lymphocytes command a relatively high metabolic priority for available ascorbate, and low values represent a state of significant tissue ascorbate deficiency [Jacob et al. 1992].

The decreases in plasma and platelet tocopherol (d₀-αT) over the course of the study (Table 2 and Fig. 3) are likely due to 1) the low tocopherol content of the experimental diet (3 mg/d), 2) the increased vitamin E requirement due to supplementation of the diet with 42 g/d of stripped safflower oil, or 3) the effect of supplementation with 20 mg/d of d₆-αT during the third week. The latter effect is supported by noting that the fall of plasma d₀-αT occurs primarily during the period that the deuterated tocopherol doses were given [Fig. 3A]. This is consistent with results of previous tocopherol supplementation studies, such as that of Acuff et al. (1994), who found that unlabeled plasma tocopherol concentrations decreased significantly after six healthy adults were given 150 mg/d of deuterated tocopherols for 11 d. The decreases in plasma d₀-αT over the course of the present study are moderate, however, as the ±2 SD range at the beginning (10–36 μmol/L) and end (11–27 μmol/L) of the study compare well with the range reported by Lehmann et al. (1988) for 20 healthy women and men (13–35 μmol/L).

Because the liver can readily mobilize α-tocopherol to the plasma pool [Traber et al. 1994], it is not surprising that plasma and platelet d₀-αT levels were unaffected by vitamin C intake. There was also no significant effect of ascorbic acid nutriment on the rise and fall of d₆-αT in plasma and platelets or on markers of in vivo lipid peroxidation. These results suggest that ascorbate nutriment in the adequate to deficient range had no significant effect on tocopherol concentrations over the time periods of this experiment; however, the trend toward lower plasma MDA values in the high ascorbate intake group [Fig. 6] may indicate increased

FIGURE 6 Plasma malondialdehyde equivalents for healthy women receiving either 500 or 5 mg/d of ascorbic acid. Values are means ± SEM for 10 subjects each. Subjects were given capsules containing 20 mg of hexadeuterated RRR-α-tocopherol acetate with the dinner meal during study d 15–21, indicated by the arrows. *Mean values are significantly different from baseline (P < 0.05). Mean values are not significantly different by ascorbate intake.
protection against lipid peroxidation by the high ascorbate intake. The initial rise in plasma MDA for both groups may be due to consumption of the tocopherol-stripped safflower oil in the experimental diet.

The d0-αT levels in buccal cells and adipose did not decrease over time as did the levels in plasma and platelets. Hence, tissue tocopherol content was not significantly decreased over the course of the study. The lack of significant differences in lipid peroxidation indices between high and low ascorbate groups is somewhat surprising in view of previous work such as that of Chakraborty et al. (1994), which showed that ascorbate protected guinea pig tissues against lipid peroxidation. This may be because the subjects in the present study received 20 mg/d of d6-αT during the third week and maintained their tissue tocopherol status throughout. A significantly greater portion of the d6-αT dose was incorporated into the buccal cells (Fig. 5) and adipose of the low compared with the high ascorbate group. This suggests an effect of ascorbate nutriture, but may also be due to the lower initial tocopherol concentrations of the low ascorbate group (Table 2), which may predispose to relatively greater absorption and retention of incoming tocopherol.

Two results, although not statistically significant because of small sample number and high variance, do suggest a trend toward sparing of tissue tocopherol by the high ascorbate intake relative to the low: 1) the lower buccal cell d6-αT disappearance rates for the high compared with the low ascorbate intake groups, and 2) the observation that total and d0-αT adipose concentrations tended to increase over the course of the study in the high ascorbate group and decrease in the low ascorbate group, even though the low ascorbate group started with lower overall tocopherol status and incorporated more of the new labeled tocopherol. The latter trend is consistent with the trend toward higher plasma MDA in the high compared with the high ascorbate intake group.

Although a significant effect of ascorbate nutriture on tocopherol status was not seen in this study, the results were somewhat mixed, and do not conclusively prove that this interaction does not take place in vivo. Previous research showed that other endogenous antioxidants may reduce oxidized tocopherol; chief among these is the lipid-soluble antioxidant ubiquinol. Bowry et al. (1995) found that the pro-oxidant activity of α-tocopherol towards isolated LDL devoid of ubiquinol was effectively inhibited by a number of co-antioxidants, including ascorbate, bilirubin and various phenolic antioxidants. In other studies, thiols (glutathione, dihydrolipoic acid and metallothionein) and NADH-cytochrome b5 reductase activity were shown to be capable of recycling oxidized vitamin E and related phenoxy radicals (Constantinescu et al. 1993, Kagan et al. 1994). Kohar et al. (1995) found that several biologic systems, including FAD/NADH, are able to reduce α-tocopherol quinone to α-tocopherol and that such reactions are likely to occur in vivo. Hence, it seems that the in vivo capability to regenerate reduced tocopherols from their oxidized forms is diverse and not likely dependent on a single biological reductant. In addition to the factors discussed above, it is possible that the period of ascorbate deficiency in the present study was not long enough to affect tocopherol metabolism and status to a significant degree.

In conclusion, the results of this study showed that a wide range of vitamin C intake had no major effect on vitamin E concentrations over the 6-wk period. Because some of these results suggest a trend toward sparing of tissue tocopherol by a higher vitamin C intake, however, this research question should be further pursued, especially with cellular, tissue and in vivo studies and with the use of more sensitive measurements such as vitamin E metabolites.

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LITERATURE CITED


