

Vitamins C and E in adolescents and young adults with HIV infection¹⁻³

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ABSTRACT

Background: Oxidative stress during HIV infection may impair immune function, cause more rapid disease progression, and increase requirements for dietary antioxidants such as vitamins C and E.

Objectives: The study had 2 principal objectives. The first was to ascertain whether HIV infection and immune activation were associated with lower plasma concentrations of ascorbate, urate, and α - and γ -tocopherols and with total antioxidant status (TAS). The second objective was to ascertain whether these antioxidants were associated with protection against oxidative damage.

Design: This was a cross-sectional study involving 241 HIV-positive and 115 HIV-negative subjects aged 14–23 y. Subjects were primarily female (76%) and African American (70%), and 21% were Hispanic.

Results: Plasma ascorbate was significantly lower, but γ -tocopherol and TAS were significantly higher in subjects with HIV infection when the analysis was adjusted for dietary intake and sex. Plasma α -tocopherol did not differ significantly by HIV status. Plasma γ -tocopherol also was higher in subjects with oxidative damage than in those without such damage. More than 90% of subjects had adequate plasma concentrations for both ascorbate and α -tocopherol, although α -tocopherol concentrations were lower than expected on the basis of third National Health and Nutrition Examination Survey data.

Conclusions: Low plasma ascorbate concentrations in HIV-positive subjects suggest that vitamin C requirements are significantly higher in those with HIV infection. Plasma tocopherol concentrations were not depressed by HIV infection and may be maintained by compensatory mechanisms such as the activity of α -tocopherol transfer protein. *Am J Clin Nutr* 2006;83:870–9.

KEY WORDS Antioxidants, α -tocopherol, γ -tocopherol, ascorbate, oxidative damage

INTRODUCTION

Vitamins C and E are antioxidant nutrients that protect cells and tissues against damage by reactive oxygen and nitrogen species. The production of such species increases during episodes of infectious disease, when the immune system is activated to eliminate pathogenic organisms. Chronic infections, such as HIV infection, place a long-term strain on antioxidant defenses, which may increase dietary antioxidant requirements. The intakes of vitamins C and E recommended for healthy subjects may thus be less than adequate to deal with the increased oxidative stress of HIV infection. This stress can damage cells and tissues

of the immune system and lead to increased severity of disease (1). Infections may also affect the absorption, tissue distribution, and excretion of nutrients (2). It is thus not surprising that both a low intake of vitamin C (3) and low plasma concentrations of vitamin E (4) have been associated with a greater risk of progression to AIDS in HIV-infected US subjects.

Studies of HIV infection and nutritional status in the United States typically have not included adolescents or young adults. However, because adolescents and young adults are at high risk of HIV infection in the United States and other countries, we chose to examine the association of antioxidant nutritional status with HIV infection in this age group. To do so, we studied subjects from the Reaching for Excellence in Adolescent Health (REACH) Study, which recruited subjects aged 14–18 y from 13 US cities. Most subjects were female and African American, and \approx 20% were Hispanic (5). The demographic characteristics of this population are unique among major HIV studies in the United States, and thus the current study provides a novel opportunity to examine the relation of nutritional status to HIV infection. The dietary intake patterns and concentrations of immune activation and oxidative damage markers in these subjects have been described previously (5–7).

In the current observational study, we examined the relation of vitamin C and E intakes, HIV status, and immune activation to biochemical indicators of antioxidant status, including plasma

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α -tocopherol, γ -tocopherol, ascorbate, and urate concentrations and total antioxidant status (TAS). In turn, we assessed the association of these variables with indicators of oxidative damage (ie, plasma malondialdehyde and protein carbonyl concentrations). Our goals were to assess the antioxidant status of these subjects, ascertain whether HIV infection was associated with lower plasma concentrations of these key antioxidant nutrients, identify markers of immune activation that may be associated with low plasma concentrations of these nutrients (and, by inference, with greater utilization), and ascertain whether plasma concentrations of these nutrients were associated with protection against oxidative damage (ie, lower concentrations of plasma oxidative damage markers).

SUBJECTS AND METHODS

Study population

The REACH Study was a prospective, observational study of HIV infection in adolescents conducted at 15 US clinical sites (8, 9). A standardized protocol was developed through the Adolescent Medicine HIV/AIDS Research Network. Between March 1996 and November 1999, 325 adolescents aged 12–18 y who had acquired HIV infection through sexual activity or intravenous drug use were recruited. In addition, 171 HIV-negative adolescents were recruited from the same sites by using selection criteria to make the HIV-negative and HIV-positive groups comparable with regard to risk-behavior profiles and demographic characteristics (including age, sex, race, and ethnicity). This report describes a supplemental study conducted during one study visit between January and October 2000. One site did not participate in the current study because of logistical difficulties. Of the 436 participants who were active in the 14 REACH Study network sites, 391 (264 HIV-positive and 127 HIV-negative) agreed to participate in this substudy. The 14 clinical sites were located in Miami, Fort Lauderdale, New Orleans, Birmingham, Los Angeles, Memphis, Washington (DC), Baltimore, Philadelphia, Newark, New York City (3 sites), and Chicago.

All participants provided written informed consent. The study was approved by human subjects research review boards at the University of California, Davis; Iowa State University; the University of Alabama at Birmingham; and each clinic site.

Data collected by the REACH Study

In the REACH Study, data were collected with face-to-face interviews, interactive computer interviews, medical record abstractions, and physical and laboratory examinations. HIV-positive subjects were seen every 3 mo, and HIV-negative subjects were seen every 6 mo. Participants were gowned and weighed at each visit by using digital scales that were accurate to 0.1 kg. Height was measured by using calibrated stadiometers that were installed at each study site. Body mass index (BMI; in kg/m^2) was calculated for each participant.

Laboratory tests were performed at local clinic sites according to the REACH Study protocol described elsewhere (8–11). Activated CD8+ T cells were measured as described previously by using CD38 and HLA-DR as markers of activation (12). Absolute CD4+ T cell counts for HIV-positive participants were stratified on the basis of Centers for Disease Control and Prevention criteria for HIV/AIDS classification: > 499, 200–499, and < 200 cells/ mm^3 . The quantitative HIV-1 RNA viral load in

plasma was measured in a centralized laboratory on frozen specimens by using either nucleic acid sequence–based amplification or NucliSens assays (Organon Teknika, Durham, NC) as described elsewhere (13). Antiretroviral therapy was coded as a dichotomous variable (receiving or not receiving therapy), and descriptive data on the use of and compliance with antiretroviral therapy were reported previously (6).

Variables collected for the current study

In the current study, nonfasting blood was collected at a regularly scheduled REACH Study visit. Site-to-site variation within biochemical variables was minimized by providing all sites with the same blood-collection and -processing tubes from a central source and by processing and analyzing all samples collected for the current study in batches at a central laboratory. Plasma C-reactive protein (CRP), ceruloplasmin, neopterin, malondialdehyde, and protein carbonyls were measured as described (6).

The Block Food-Frequency Questionnaire [(Block FFQ) version 98.2; Block Dietary Data Systems, Berkeley, CA] was used to estimate usual dietary intake patterns and the use of vitamin supplements over the preceding year as described elsewhere (7). The Block FFQ was administered in an interview format by trained clinic staff a mean (\pm SD) $\leq 1.4 \pm 0.4$ d after the clinic visit when blood was drawn. Serving-size pictures and plates and cups were used to help estimate portion sizes. A registered dietitian reviewed the questionnaires, and, before data entry, interviewers were contacted about missing information, unusual responses, or discrepancies. Nutrient intake was measured by using SYSTAT dietary analysis software for the Block Dietary Data Systems (version 10.0; SPSS Inc, Chicago, IL).

Ascorbate and urate were measured in plasma by using HPLC with electrochemical detection, as described elsewhere (14). Plasma TAS was measured on a Hitachi 902 Autoanalyzer (Roche Molecular Systems, Alameda, CA) by using reagents from Randox (San Diego, CA). Plasma α - and γ -tocopherol concentrations were measured with isocratic reverse-phase HPLC by using a C18 column and a photodiode array detector, essentially as described elsewhere (15).

Statistical analysis

Software

All analyses were performed by using SIGMASTAT for WINDOWS (versions 2.03 and 3.01; Jandel Scientific, San Rafael, CA). Unless otherwise indicated, data are presented as means \pm SEs, and $P < 0.05$ was considered to indicate statistical significance.

Transformations

A total of 356 subjects had complete data for all 5 principal antioxidant variables: ascorbate, urate, TAS, α -tocopherol, and γ -tocopherol (Table 1). Data from these subjects were used in the current analysis. Some variables required transformation to achieve normality and constant variance. For urate, square root and \log_{10} transformations were required for bivariate and multiple regression analysis, respectively. \log_{10} transformation was required for bivariate analysis of TAS. The tocopherol values, the

TABLE 1
Demographic characteristics of the study population¹

Variable	HIV-negative subjects (n = 115)	HIV-positive subjects (n = 241)	P
Female [n (%)]	93 (81)	177 (73)	0.16
African American [n (%)]	72 (63)	176 ² (73)	0.053
Hispanic [n (%)]	28 (24)	47 ² (20)	0.37
Age (y) ³	19.3 ± 0.14 ⁴	20.1 ± 0.10	< 0.001
BMI (kg/m ²) ⁵			
Females ⁶	27.8 (22.0/33.6) ⁷	27.7 (23.4/33.1)	
Males ⁸	23.1 (21.5/27.7)	22.2 (19.9/24.1)	

¹ Categorical data were compared by using a chi-square test.

² Total n = 240.

³ Compared by using Student's *t* test.

⁴ $\bar{x} \pm SE$ (all such values).

⁵ BMI data differed by sex ($P < 0.001$, 2 way ANOVA on ranks comparing sex and HIV status; HIV status, $P = 0.383$; sex \times HIV status interaction, $P = 0.133$).

⁶ Total n = 93 for HIV-negative subjects and 176 for HIV-positive subjects.

⁷ Median; 25th/75th percentiles in parentheses (all such values).

⁸ Total n = 22 for HIV-negative subjects and 64 for HIV-positive subjects.

ratio of α -tocopherol to γ -tocopherol, and protein carbonyl values were not normalized by several transformations, and bivariate analysis was done by using nonparametric methods. Rank-order transformation of these variables achieved normality and constant variance for regression analysis, with the exception of γ -tocopherol values for HIV-positive subjects ($P = 0.018$ for the normality test). However, this lack of normality of the γ -tocopherol values of HIV-positive subjects was overlooked because the elimination of subjects to normalize the untransformed γ -tocopherol distribution resulted in the identification of the same independent variable in stepwise regression analysis as did the use of rank order. Bivariate analysis of protein carbonyl data was performed by using nonparametric methods. Log_{10} transformation was used for all analyses of malondialdehyde data.

Bivariate analysis

Bivariate analysis was performed by using Student's *t* tests and 1-way and 2-way analyses of variance. The rank-sum test or 1-way analysis of variance on ranks was used for group comparison of variables without normal distributions. Proportions were compared by using the chi-square or Fisher's exact test. For these analyses, subjects were grouped by HIV status, stage of HIV disease as indicated by CD4⁺ T lymphocyte count (<200, 200–499, and $\geq 500 \text{ mm}^{-3}$), use of antiretroviral therapy, and sex.

Regression analysis

Multiple linear regression analysis was used initially to determine whether HIV status was a significant predictor of the antioxidant variables by using HIV status, sex, nutrient intake from diet, nutrient intake from supplements (for ascorbate, α -tocopherol, γ -tocopherol, and α -tocopherol: γ -tocopherol), and study site as the independent variables (**Table 2**). Tocopherol intake from supplements was presumed to be exclusively α -tocopherol and was used in all tocopherol regression models.

TABLE 2
Multiple regression analysis to ascertain whether HIV infection has a significant association with plasma antioxidant concentrations and oxidative damage measures¹

Plasma antioxidant and oxidative damage measures	Regression coefficient for HIV status ²	SE coefficient	P
Ascorbate	−0.118	0.052	0.025
Rank α -tocopherol	0.028	0.053	0.597
Rank γ -tocopherol	0.113	0.052	0.031
Rank γ -tocopherol α -tocopherol	0.092	0.052	0.081
Log_{10} urate	−0.014	0.050	0.774
Total antioxidant status	0.121	0.048	0.013
Log_{10} malondialdehyde	0.062	0.049	0.210
Rank protein carbonyls	0.031	0.051	0.547

¹ n = 355 for ascorbate and tocopherol data and 356 for all other variables. Multiple regression analysis used HIV status, sex, and study site variables in all analyses. Intakes of vitamin C from food and supplements were included as separate variables for the ascorbate equation, and intakes of vitamin E from food (α -tocopherol) and supplements were included for the α -tocopherol equation. Intakes of vitamin E from food (total tocopherol) and supplements were used in the equations for γ -tocopherol and γ -tocopherol: α -tocopherol. The overall standardized coefficients for HIV, SEs for each coefficient, and *P* values are shown.

² A negative coefficient indicates that HIV infection is associated with lower plasma measures, whereas a positive coefficient indicates that HIV infection is associated with higher plasma measures.

Total tocopherol intake from foods was used for regression analysis of plasma γ -tocopherol and α -tocopherol: γ -tocopherol, whereas α -tocopherol intake from foods was used for analysis of plasma α -tocopherol.

Backwards stepwise linear regression analysis was used to identify significant predictors of the plasma antioxidant measures (ascorbate, urate, TAS, α -tocopherol, γ -tocopherol, and α -tocopherol: γ -tocopherol) from among the following independent variables (**Table 3**): demographic variables—age, sex, BMI, race, ethnicity, and smoking; intake variables—intake of vitamins C and E from food and supplements; HIV variables—HIV status, CD4⁺ T cell count, plasma virus load, and use of antiretroviral therapy; and immune activation variables—activated CD8⁺ T cells, neutrophil count, plasma neopterin, plasma CRP, and plasma ceruloplasmin. This analysis was performed on all subjects, HIV-negative subjects and HIV-positive subjects independently, with variables for HIV status, sex, and nutrient intake (for the vitamin C and E models) from food and supplements forced into the model. Variables included in the final model (Table 3) were significant ($P < 0.05$) in 1 of the 3 groupings of subjects. If a variable was not significant in the model with all subjects, it was included only if the coefficients differed at $P < 0.10$ between the HIV-positive and HIV-negative subjects, as assessed by Student's *t* test. Because significant variations in the markers of oxidative damage were seen between the different clinical study sites (6), dummy variables were included in all models to represent the different study sites, but coefficients were not reported. Analysis of α -tocopherol: γ -tocopherol identified essentially the same independent variables that were identified when γ -tocopherol and α -tocopherol concentrations were analyzed separately, and the results of the former combined analysis are not presented here.



TABLE 3
Multiple linear regression analysis to predict plasma antioxidant concentrations¹

Independent variables	All subjects			HIV-negative subjects			HIV-positive subjects			P_{HIV}^2
	Coefficient	SE	<i>P</i>	Coefficient	SE	<i>P</i>	Coefficient	SE	<i>P</i>	
Ascorbic acid ($\mu\text{mol/L}$) ³										
HIV	-0.119	0.052	0.022							
Female	-0.075	0.056	0.181							
Dietary vitamin C	0.075	0.053	0.160							
Supplemental vitamin C	-0.011	0.052	0.836							
Log ₁₀ CRP	-0.129	0.053	0.015							
Rank α -tocopherol ⁴										
HIV	0.095	0.056	0.092	— ⁵	—	—	—	—	—	—
Female	0.069	0.060	0.251	0.031	0.117		0.102	0.071		0.436
Dietary α -tocopherol	-0.028	0.053	0.604	-0.089	0.104		0.016	0.062		0.520
Supplemental α -tocopherol	0.033	0.052	0.526	0.263	0.100	0.010	-0.005	0.061	0.933	0.024
BMI	-0.228	0.055	<0.001	-0.189	0.109		-0.250	0.065		0.106
Log ₁₀ neutrophils	0.222	0.058	<0.001	0.122	0.106	0.252	0.244	0.064	<0.001	0.071
Log ₁₀ ceruloplasmin	0.175	0.058	0.003	0.104	0.125	0.407	0.234	0.068	<0.001	0.063
Rank γ -tocopherol ⁶										
HIV	0.107	0.050	0.033							
Female	0.074	0.055	0.177							
Dietary total tocopherol	-0.070	0.052	0.179							
Supplemental α -tocopherol	-0.121	0.050	0.017							
BMI	0.287	0.053	<0.001							
Log ₁₀ total antioxidant status (mmol/L) ⁷										
HIV	0.139	0.045	0.002	—	—	—	—	—	—	—
Female	-0.140	0.053	0.009	-0.213	0.098		-0.122	0.067		0.397
Log ₁₀ ceruloplasmin	-0.206	0.050	<0.001	-0.050	0.098	0.612	-0.245	0.062	0.001	0.052
Log ₁₀ urate	0.302	0.049	<0.001	0.391	0.088		0.256	0.062		0.190
Log ₁₀ α -tocopherol	0.098	0.045	0.032	0.218	0.082		0.061	0.059		0.502

¹ CRP, C-reactive protein. Independent variables were selected by backward stepwise multiple regression analysis as described in Subjects and Methods. The regression model for all subjects is shown for all dependent variables. When ≥ 1 variable in the model differed at $P < 0.10$ between HIV-positive and HIV-negative subjects by comparing coefficients between these groups by using Student's *t* test, the models for HIV-positive and HIV-negative subjects are also shown with *P* values for the coefficients that differed at $P < 0.10$.

² Comparing individual coefficients by Student's *t* test between HIV-positive and HIV-negative subjects. Variables for HIV status, sex, and nutrient intake from diet or supplements were forced into the equations.

³ $n = 351$. $R^2 = 0.124$.

⁴ $n = 349$, 115, and 234 for all subjects, HIV-negative subjects, and HIV-positive subjects, respectively. $R^2 = 0.171$, 0.185, and 0.266 for the same groups, respectively.

⁵ Variable not appropriately included.

⁶ $n = 355$. $R^2 = 0.183$.

⁷ $n = 354$, 115, and 239 for all subjects, HIV-negative subjects, and HIV-positive subjects, respectively. $R^2 = 0.349$, 0.452, and 0.321 for the same groups, respectively.

All of the variables mentioned above (except vitamin intake) were also used as described in backward stepwise regression analysis to predict oxidative damage (plasma protein carbonyl, data not shown; plasma malondialdehyde, **Table 4**). Equations predicting oxidative damage by using these demographic, HIV, and immune variables have been reported elsewhere (6).

RESULTS

Subject characteristics

Subjects from the REACH Study were predominately female and African American. Twenty-one percent reported their ethnicity as Hispanic, and BMI for females was greater than that for males (Table 1). The prevalence of obesity was high in the REACH Study subjects, as was reported previously in greater detail (5, 6).

Antioxidant status

Vitamin C

Plasma ascorbate was significantly ($P = 0.005$) greater in males ($45 \pm 2.0 \mu\text{mol/L}$) than in females ($39 \pm 2.0 \mu\text{mol/L}$) and marginally ($P = 0.064$) lower in HIV-positive ($40 \pm 1.2 \mu\text{mol/L}$) than in HIV-negative ($44 \pm 1.9 \mu\text{mol/L}$) subjects (**Figure 1**). HIV status was a significant negative predictor of plasma ascorbate when multiple regression analysis was used to adjust for sex, vitamin C intake, and study site (Table 2). Plasma ascorbate in HIV-positive subjects did not differ by stage of HIV disease (data not shown; $P = 0.90$). When divided into quartiles based on age- and sex-specific data from the third National Health and Nutrition Examination Survey (NHANES III; 16), a significantly ($P = 0.002$) lower percentage of female subjects than expected was in the highest quartile, but the opposite trend was seen in males, of

TABLE 4

Multiple linear regression to predict log₁₀ plasma malondialdehyde concentration (μmol/L)¹

Independent variables	All subjects ²			HIV-negative subjects ³			HIV-positive subjects ⁴			P _{HIV} ⁵
	Coefficient	SE	P	Coefficient	SE	P	Coefficient	SE	P	
HIV	0.036	0.054	0.506	— ⁶	—	—	—	—	—	—
Female	-0.141	0.057	0.014	-0.041	0.101	—	-0.148	0.068	—	0.415
Antiretroviral therapy	—	—	—	—	—	—	0.168	0.064	0.009	—
Log ₁₀ neutrophils	-0.008	0.056	0.892	-0.246	0.092	0.009	0.049	0.064	0.450	0.006
Log ₁₀ ceruloplasmin	-0.101	0.056	0.070	0.052	0.108	0.634	-0.205	0.068	0.003	0.033
Log ₁₀ α-tocopherol	0.143	0.050	0.005	0.179	0.089	—	0.148	0.065	—	0.686
Log ₁₀ γ-tocopherol	0.145	0.050	0.004	0.106	0.088	—	0.175	0.060	—	0.365

¹ Independent variables were selected by backward stepwise multiple regression analysis as described in Subjects and Methods. Variables for sex and HIV status were forced into the equations. *P* values for each coefficient are shown for the model for all subjects and for the other 2 models when coefficients differed at *P* < 0.10 between HIV-positive and HIV-negative subjects.

² *n* = 350. *R*² = 0.228.

³ *n* = 115. *R*² = 0.354.

⁴ *n* = 235. *R*² = 0.264.

⁵ Comparing individual coefficients by Student's *t* test between HIV-positive and HIV-negative subjects.

⁶ Variable not appropriately included.

whom a significantly (*P* = 0.043) greater percentage than expected was in the highest quartile (Figure 2). The overall prevalence of low and deficient plasma ascorbate (<17 μmol/L; 17) was 8.7% (31/356). The prevalence was marginally higher in females than in males [10.4% (28/270) and 3.5% (3/86); *P* = 0.080]. No difference was seen by HIV status (*P* = 0.31). When stepwise multiple regression analysis was used to identify significant predictors of plasma ascorbate, only plasma CRP was identified as a significant, negative predictor (Table 3).

Urate

Plasma urate was significantly (*P* < 0.001) greater in males (359 ± 11 μmol/L) than in females (277 ± 5.4 μmol/L), as expected (18), and it did not differ significantly (*P* = 0.79) between HIV-positive (316 ± 5.9 μmol/L) and HIV-negative (319 ± 10 μmol/L) subjects (Figure 1 and Table 2). Plasma urate in HIV-positive subjects did not differ by stage of disease (data not shown; *P* = 0.40). The overall mean values for REACH Study subjects (296 μmol/L) is similar to the mean value reported from NHANES I for subjects < 45 y old (316 μmol/L; 18).

Vitamin E

α-Tocopherol. Plasma α-tocopherol concentrations did not differ significantly (*P* = 0.19) between females (median: 18.3 μmol/L; 25th/75th percentiles: 15.4/22.1 μmol/L; *n* = 270) and males (17.2 μmol/L; 14.4/20.8 μmol/L; *n* = 86) or between HIV-positive (18.0 μmol/L; 15.2/21.8 μmol/L; *n* = 241; *P* = 0.80) and HIV-negative (18.4 μmol/L; 15.2/22.0 μmol/L; *n* = 115) subjects (Figure 1 and Table 2). Nor did concentrations in HIV-positive subjects differ significantly by stage of disease (data not shown; *P* = 0.79). When divided into quartiles based on age- and sex-specific national reference data (NHANES III), a greater percentage of subjects than expected was in the lowest quartile (Figure 2). The distribution for females and males analyzed together was significantly (*P* ≤ 0.001) different from that expected according to the national standard. The distributions of male and female REACH Study subjects did not differ significantly (*P* = 0.79). With the use of a cutoff of 12 μmol/L (18), the prevalence of vitamin E deficiency was 7.0%

(25/356). The prevalence in males (11.6%, 10/86) was twice that in females (5.6%, 15/270), but the difference was not significant (*P* = 0.094). No significant difference was seen by HIV status (*P* = 0.79).

Multiple regression analysis found that BMI was negatively associated with plasma α-tocopherol (Table 3). In addition, the use of vitamin E supplements was positively associated with plasma α-tocopherol in HIV-negative but not HIV-positive subjects (Table 3). This association was also seen in bivariate regression analysis for HIV-positive subjects, although, without adjustment for other variables, the association did not differ significantly from that seen in HIV-negative subjects (Figure 3). Two markers of immune activation, the acute phase protein ceruloplasmin and the neutrophil count, also were positively associated with α-tocopherol in HIV-positive but not HIV-negative subjects (Table 3).

γ-Tocopherol and α-tocopherol:γ-tocopherol. Plasma γ-tocopherol was significantly (*P* = 0.003) higher in females (median: 4.35; 25th/75th percentiles: 3.05/5.68 μmol/L; *n* = 270) than in males (3.45; 2.28/4.96 μmol/L; *n* = 86), but it did not differ significantly between HIV-positive (4.18; 2.88/5.68 μmol/L; *n* = 241, *P* = 0.19) and HIV-negative (3.88; 2.59/5.42 μmol/L; *n* = 115) subjects (Figure 1). Multiple regression analysis after adjustment for sex, study site, and vitamin E intake did find a significant positive association of HIV status with plasma γ-tocopherol concentration (Table 2). Plasma γ-tocopherol concentrations did not differ significantly (*P* = 0.14) by stage of disease in HIV-positive subjects.

Mean plasma α-tocopherol:α-tocopherol was marginally (*P* = 0.056) higher in females (median: 0.231; 25th/75th percentiles: 0.159/0.318 μmol/L; *n* = 270) than in males (0.196; 0.126/0.299 μmol/L; *n* = 86), but it did not differ significantly between HIV-positive (0.229; 0.157/0.314 μmol/L; *n* = 241, *P* = 0.28) and HIV-negative (0.213; 0.148/0.318 μmol/L; *n* = 115) subjects (Figure 1). Adjustment for sex and study site showed a positive association of HIV status with α-tocopherol:γ-tocopherol, but the difference was not statistically significant (Table 2). The ratio did not differ by stage of disease among HIV-positive subjects (*P* = 0.18).



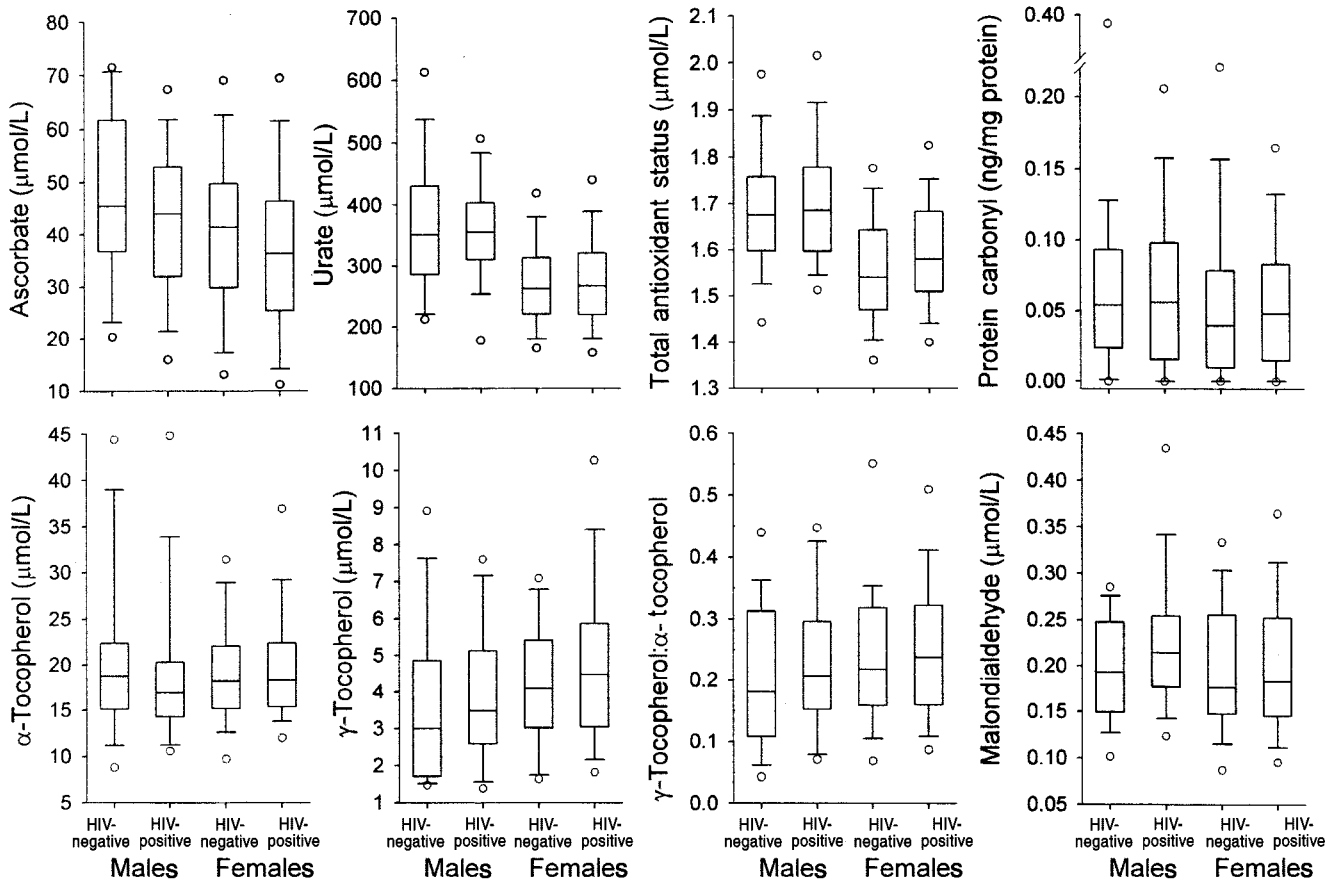


FIGURE 1. Distribution of plasma antioxidant (ascorbate, urate, total antioxidant status, α - and γ -tocopherol, and the ratio of α -tocopherol to γ -tocopherol) and oxidative damage (protein carbonyl and malondialdehyde) measures in the Reaching for Excellence in Adolescent Health (REACH) Study cohort. Box plots show median (horizontal line in center of each box), 25th and 75th percentiles (bottom and top of each box), 10th and 90th percentiles (bottom and top error bars), and the 5th and 95th percentiles (○). Data are shown separately for HIV-positive males ($n = 64$) and females ($n = 177$) and for HIV-negative males ($n = 22$) and females ($n = 93$). Ascorbate, urate, total antioxidant status, and malondialdehyde were compared by using 2-way ANOVA (for sex and HIV status). Values for ascorbate, urate, and total antioxidant status differed significantly by sex ($P < 0.05$). No significant differences were seen for HIV status, although the difference for ascorbate was nearly significant ($P = 0.064$). No sex \times HIV status interactions were seen. The tocopherol values and protein carbonyl values were not normally distributed and were compared by using the Mann-Whitney rank-sum test. The difference between the sexes in γ -tocopherol was significantly ($P = 0.003$) different when all subjects were analyzed together, and that for α -tocopherol: γ -tocopherol was nearly significant ($P = 0.056$).

Multiple regression analysis found that intake of vitamin E from supplements was negatively associated with plasma γ -tocopherol concentrations (Table 3). In agreement with this observation, plasma γ -tocopherol was significantly ($P = 0.004$) lower in subjects who were using vitamin E supplements (median: 3.60; 25th/75th percentiles: 2.28/5.26 $\mu\text{mol/L}$, $n = 121$) than in subjects who were not using supplements (4.36; 3.11/5.80 $\mu\text{mol/L}$, $n = 234$). In contrast to the negative association that was seen between BMI and plasma α -tocopherol, BMI had a positive association with γ -tocopherol (Table 3).

Total antioxidant status

Plasma TAS values were significantly ($P < 0.001$) higher in males (1.70 ± 0.017 mmol/L) than in females (1.58 ± 0.0089 mmol/L). Values in HIV-positive subjects (1.65 ± 0.010 mmol/L) did not differ significantly ($P = 0.10$) from those in HIV-negative subjects (1.62 ± 0.016 mmol/L; Figure 1). However, multiple regression analysis after adjustment for sex and study site found a significant, positive association of HIV status with TAS (Table 2). TAS values for HIV-positive

subjects did not differ by stage of disease ($P = 0.49$). Plasma antioxidants were included in the stepwise regression analysis to predict TAS values because they contribute directly to the total antioxidant capacity of plasma. As expected, urate and α -tocopherol showed positive associations with TAS (Table 3). Ceruloplasmin, on the other hand, showed a negative association with TAS in HIV-positive but not HIV-negative subjects (Table 3).

Oxidative damage

We (6) previously showed that the use of antiretroviral therapy and the markers of immune activation (see Table 4) are associated with oxidative damage in the REACH Study subjects. In the current study, we analyzed the association of ascorbate, urate, TAS, α -tocopherol, and γ -tocopherol with the markers plasma malondialdehyde and plasma protein carbonyls. We anticipated that protection against oxidative damage by one of these antioxidants would result in a negative association between the antioxidant and the oxidative damage variables. However, both α - and γ -tocopherol had a positive

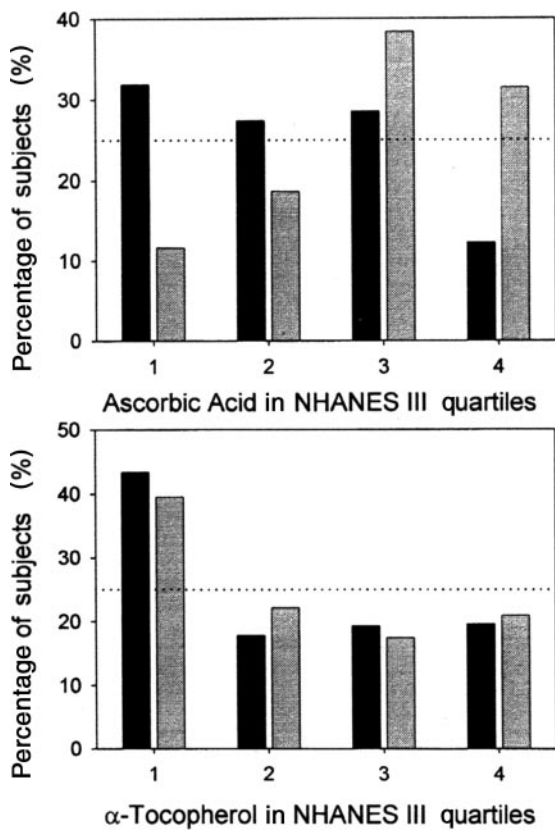


FIGURE 2. Distribution of plasma ascorbic acid and α -tocopherol concentrations for male ($n = 86$; □) and female ($n = 270$; ■) Reaching for Excellence in Adolescent Health (REACH) Study subjects based on quartiles derived from age- and sex-appropriate data from the third National Health and Nutrition Examination Survey (NHANES III) US population sample (17). The expectation for each quartile (25% of subjects) on the basis of these standards is shown by the horizontal dotted line. $n = 10, 16, 33,$ and 27 males and $86, 74, 77,$ and 33 females in the ascorbic acid distribution. $n = 34, 19, 15,$ and 18 males and $117, 48, 52,$ and 53 females in the α -tocopherol distribution. For ascorbic acid, both distributions differed from the expectation of 25% of subjects in each quartile ($P < 0.05$), and the distributions differed from one another ($P < 0.05$). For α -tocopherol, the distributions for the 2 sexes together and for females only differed from the expectation ($P < 0.05$); the distributions for female and male did not differ significantly.

association with malondialdehyde (Table 4). No significant association was found between antioxidant variables and plasma protein carbonyls.

In a previous report (6), we used both of these oxidative damage variables to categorize subjects as with or without oxidative damage (by using the 90th percentile for each variable as a threshold to identify those with oxidative damage). Using these categories, we compared plasma tocopherol values in those with and those without oxidative damage and found that the median γ -tocopherol concentration was 25% higher ($P < 0.001$) in those with oxidative damage than in those without (Figure 4). Similarly, median γ -tocopherol: α -tocopherol was 20% greater ($P = 0.003$) in subjects with oxidative damage than in those without oxidative damage. A significant difference was not seen for α -tocopherol independently.

DISCUSSION

Vitamin C

Plasma ascorbate was lower in subjects with HIV infection, which suggests that such subjects have greater vitamin C requirements than do persons without HIV infection. Although oxidized ascorbate can be recycled, increased oxidative stress is presumed to increase ascorbate depletion (19). Thus, plasma ascorbate may be decreased by the chronic immune activation of HIV infection even if dietary intake is at a level judged to be adequate for healthy persons, as was true for the REACH Study subjects (7). However, plasma ascorbate was lower in HIV-positive than in HIV-negative REACH Study subjects. Previous studies have made similar observations (20–23) but did not control for diet. Thus our results suggest that vitamin C utilization is increased by HIV infection. Higher intakes during HIV infection should help prevent oxidative damage and maintain normal immune function.

Allard et al (24) found that vitamin C and E supplements decreased oxidative damage and tended to decrease disease severity in HIV-positive Canadian adults. High intakes of vitamin C also were associated with a lower risk of progression to AIDS in an observational study of US men (3). Trials in Africa of multinutrient supplements containing vitamins C and E found a lower risk of death due to HIV infection in Tanzanian women (25) and a small increase in CD4 T lymphocyte counts in Kenyan women (26), although an increase in vaginal HIV shedding was also seen in the latter study. Whereas the results of these intervention trials cannot be attributed only to the antioxidant components of the supplements, the studies do show that multinutrient supplements designed to address underlying deficiencies in antioxidant nutrients can diminish the severity of HIV disease. Risks may also result from such supplements, as seen by the increased virus shedding in the Kenyan study.

Vitamin E

α -Tocopherol

Almost 40% of REACH Study subjects have vitamin E intakes below the estimated average requirement (7). It thus appears that low dietary intake of vitamin E is a principal cause of the relatively poor vitamin E status of the REACH Study subjects. Assessment of dietary intake by using an FFQ has limitations. For example, subjects with HIV infection may change their intakes during the recall period covered by the questionnaire, and thus the questionnaire would be more likely to reflect current intakes than the intakes at the beginning of the study period. Such an example raises questions of validity and reverse causality. Nevertheless, the agreement of these dietary data with plasma α -tocopherol concentrations supports the validity of their use in the current study.

Plasma α -tocopherol was not associated with HIV infection in the REACH Study subjects. This lack of association suggests that dietary intakes of vitamin E, although low overall, was adequate to maintain plasma α -tocopherol despite the oxidative stress of HIV infection. In support of this view, we previously reported that vitamin E intakes in REACH Study subjects were higher in HIV-positive than in HIV-negative males (7). However, only 24% of REACH Study subjects in the current analysis were male. Thus, higher dietary intake of vitamin E in those with HIV infection is not the principal reason for the maintenance of plasma α -tocopherol concentrations. A more important factor may be



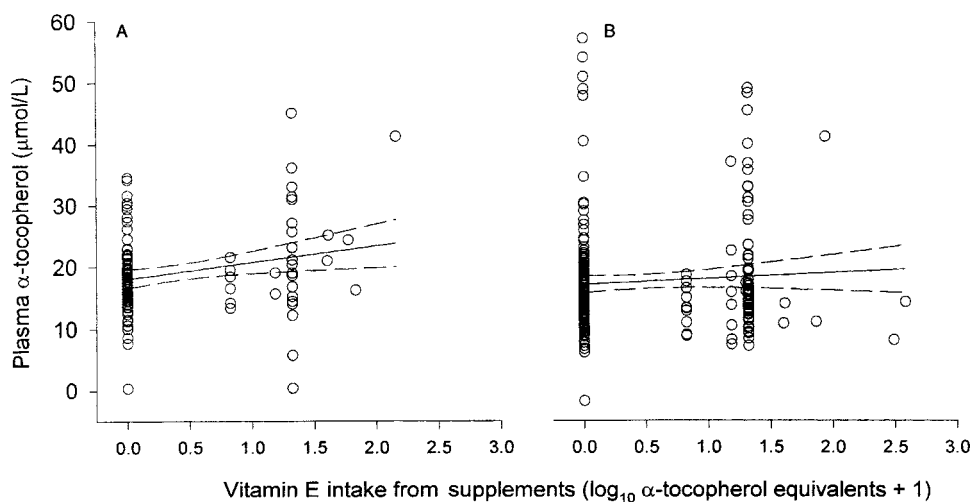


FIGURE 3. Association of vitamin E intake from supplements with plasma α -tocopherol in HIV-positive (B) and HIV-negative (A) subjects (\circ). The slope (\pm SE) of the regression line (solid line) for HIV-negative subjects was 2.593 ± 1.037 ($n = 115$; $P = 0.014$), and the slope for HIV-positive subjects was 0.845 ± 0.818 ($n = 240$; $P = 0.30$). The slopes for these bivariate regressions did not differ significantly from one another ($P = 0.21$), but they did differ significantly in multiple regression analysis ($P = 0.027$; see Table 3). The dashed lines represent 95% CIs for the regression line. Considering all subjects together, the following doses of α -tocopherol supplementation were taken daily: 0 ($n = 234$), 5.7 ($n = 16$), 14 ($n = 10$), 19 ($n = 2$), 20 ($n = 82$), 39 ($n = 2$), 40 ($n = 2$), 58 ($n = 1$), 67 ($n = 1$), 73 ($n = 1$), 87 ($n = 1$), 144 ($n = 1$), 308 ($n = 1$), and 383 ($n = 1$) mg.

the activity of α -tocopherol transfer protein (ATTP), which regulates the transport of α -tocopherol from the liver into the plasma (27). It is possible that ATTP activity maintained plasma α -tocopherol concentrations in these HIV-positive subjects despite the increased oxidative stress resulting from inflammation caused by HIV infection.

Results of the current study are consistent with the hypothesis that HIV infection increases vitamin E requirements. For example, the positive association of vitamin E supplement use with plasma α -tocopherol that was seen in HIV-negative REACH Study subjects was not seen in HIV-positive subjects, which suggests greater need for or less absorption of vitamin E during

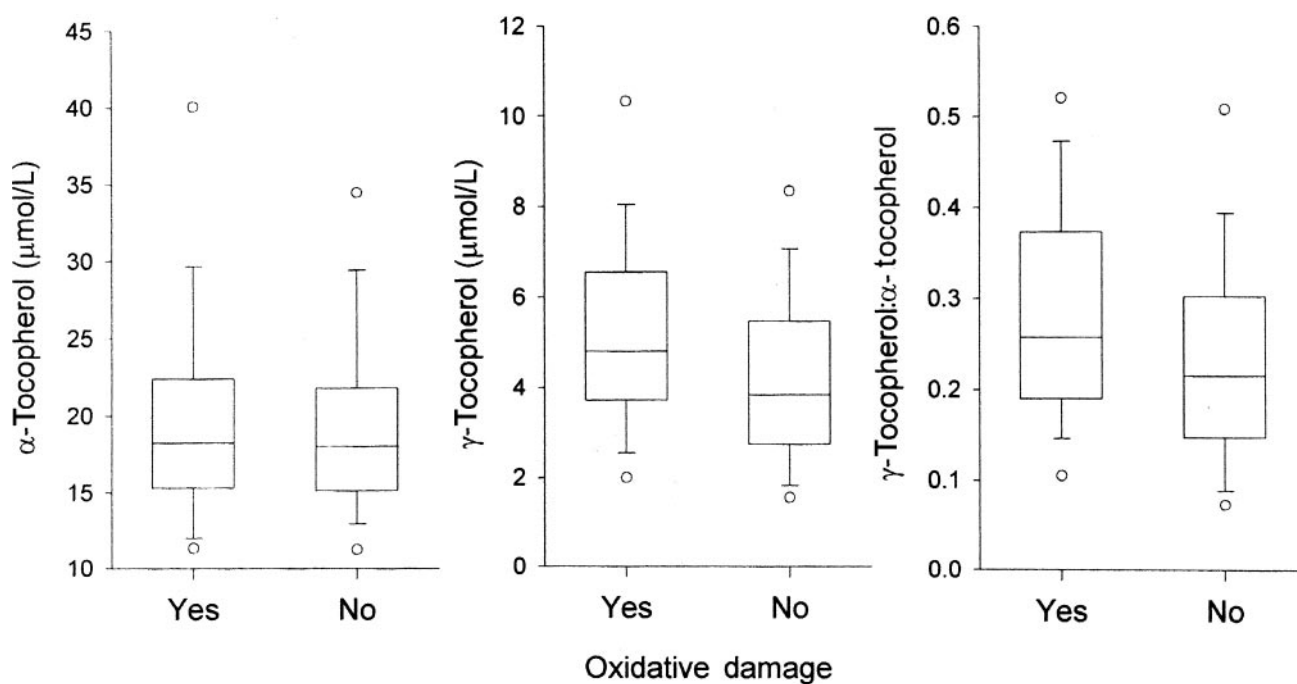


FIGURE 4. Distribution of plasma α -tocopherol, γ -tocopherol, and the ratio of α -tocopherol to γ -tocopherol in all subjects categorized by oxidative damage. Subjects with oxidative damage had a plasma oxidative damage measure (malondialdehyde or protein carbonyl) greater than the 90th percentile for this group of subjects (6). Box plots show the median (horizontal line in the center of each box), 25th and 75th percentiles (bottom and top of each box), 10th and 90th percentiles (bottom and top error bars), and the 5th and 95th percentiles (\circ). Data are shown separately for subjects with [(Yes) $n = 64$] and without [(No) $n = 292$] oxidative damage. Differences between those with and without oxidative damage were compared by using the Mann-Whitney rank-sum test. Differences were significant for γ -tocopherol ($P < 0.001$) and α -tocopherol: γ -tocopherol ($P = 0.003$) but not for α -tocopherol ($P = 0.60$).

HIV infection. Thus, HIV infection may increase vitamin E requirements.

Previous studies showed lower plasma α -tocopherol concentrations in subjects with HIV infection or AIDS than in healthy controls (28–30), but we did not see such a difference in the current study. A principal reason for this discrepancy may be that the REACH Study subjects were studied earlier in the disease than were subjects in previous studies, and thus they were relatively healthy. For example, only 13% of the REACH Study subjects have CD4 T lymphocyte counts $< 200/\mu\text{L}$ (6). This interpretation is also consistent with the report that plasma α -tocopherol concentrations decrease with time during HIV infection (31).

Multiple regression analysis found apparently paradoxical positive associations between 2 markers of immune activation, ceruloplasmin and neutrophil count, and α -tocopherol. Ceruloplasmin is an acute phase protein that also has antioxidant activity (32). The latter activity may explain its positive association with α -tocopherol if similar factors regulate both ceruloplasmin and α -tocopherol concentrations to maintain antioxidant protection. The positive association of neutrophil counts with α -tocopherol is harder to interpret but may be due to reverse causality. For example, subjects with higher plasma α -tocopherol may have less severe tissue inflammation due to better antioxidant protection. This could decrease extravasation of neutrophils and help maintain higher blood neutrophil counts.

γ -Tocopherol

Although an age- and sex-appropriate US reference population was not available for comparison, the median γ -tocopherol concentration in REACH Study subjects ($4.1 \mu\text{mol/L}$; $n = 356$) was similar to that in male US physicians aged 40–84 y who were at low risk of cardiovascular disease ($4.2 \mu\text{mol/L}$) (33) and to that of healthy, postmenopausal US women ($5.0 \mu\text{mol/L}$) (34). In contrast, the mean for REACH Study subjects ($4.5 \mu\text{mol/L}$) was 2.8-fold the mean for a sample of apparently healthy Greek men and women aged 30–82 y ($1.8 \mu\text{mol/L}$) (35). α -Tocopherol: γ -tocopherol was also higher than expected in British adolescents and young adults (36). Poor diet quality (eg, high total energy intakes and low fruit and vegetable intakes; 34) and high intakes of γ -tocopherol-rich foods (eg, vegetable oils and nuts; 37) may account for the high γ -tocopherol concentrations seen in the REACH Study subjects.

A striking finding of the current study was the elevation of plasma γ -tocopherol in subjects under physiologic stress, including HIV infection, oxidative damage, and high BMI. This association with BMI was reported previously (34, 36). It is tempting to speculate that increased oxidative stress is the common factor that results in the elevation of plasma γ -tocopherol in these situations. However, this is counterintuitive, because disposal of γ -tocopherol occurs via oxidation by liver cytochrome P450 enzymes (27). Disposal of γ -tocopherol is relatively rapid: a recent tracer study reported the half-life of plasma γ -tocopherol as 13 h, whereas that for α -tocopherol is 57 h (38). To account for the observations of the current study, one would have to speculate that the activity of these enzymes is reduced by HIV infection or oxidative stress. However, it was recently reported that liver α -tocopherol concentrations are positively correlated with cytochrome P450 (specifically, Cyp3a) activity and the γ -tocopherol oxidation product γ -carboxyethyl hydroxychroman (39). On the


basis of this association, one could speculate that, if liver α -tocopherol concentrations are reduced by oxidative stress (at the same time that plasma concentrations are maintained by ATTP), that could reduce Cyp3a activity, thus decreasing γ -tocopherol degradation and increasing plasma γ -tocopherol concentrations. ATTP activity also may explain the positive association of plasma α -tocopherol with oxidative damage seen in the current study. Thus, it would be interesting to examine tissue α -tocopherol concentrations in subjects undergoing oxidative stress.

The negative association of vitamin E intake from supplements with plasma γ -tocopherol has been reported previously (40, 41). Whereas the 2 forms of vitamin E are equivalently absorbed and transported to the liver, ATTP preferentially binds and re-secretes α -tocopherol from the liver into the plasma. Thus, higher liver concentrations of α -tocopherol may further reduce the secretion of γ -tocopherol from the liver, as well as increasing its catabolism, as discussed above.

Total antioxidant status

TAS is an aggregate measure of the ability of plasma to prevent the oxidation of a water-soluble indicator by a peroxidase (metmyoglobin) and hydrogen peroxide (42). Thus, the positive association of the plasma antioxidants urate and α -tocopherol with TAS was expected. However, ceruloplasmin, which also has antioxidant properties, was negatively associated with TAS, which may be due to the ability of ascorbate plus copper (which may be released from ceruloplasmin during the assay by oxidation) to generate hydroxyl radicals from the hydrogen peroxide that is present in the TAS assay system (19). This prooxidant activity could increase the oxidation of the indicator and thereby reduce the TAS values of subjects with elevated ceruloplasmin. However, this negative association was seen only in HIV-positive subjects, which suggests that a physiologic difference due to HIV infection, rather than an artifact of the assay method that would affect all subjects, underlies this difference.

SUMMARY

Data from the current study support the conclusion that HIV infection increases the requirements for both vitamin C and vitamin E. Plasma ascorbate concentrations were lower in subjects with HIV infection despite adequate intake and relatively high overall plasma ascorbate concentrations. On the other hand, vitamin E intake was low in many REACH Study subjects, but plasma α -tocopherol concentrations were not reduced by HIV infection, perhaps because of compensatory mechanisms such as ATTP activity that help maintain plasma concentrations and the transport of α -tocopherol from the liver to peripheral tissues. Such a compensatory mechanism may also explain the elevation of γ -tocopherol in the REACH Study subjects under conditions of greater oxidative stress, including HIV infection. 

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CBS, GSM, SDD, and CMW all participated in the design and implementation of this study. LAK trained clinic staff to perform dietary intake and



performed quality control on dietary data collection and analysis. RAJ helped design and implement the collection of samples for ascorbate analysis and supervised that analysis. None of the authors had any personal or financial conflict of interest.

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