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# Plasma vitamin C concentrations in patients with cystic fibrosis: evidence of associations with lung inflammation<sup>1-4</sup>

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See corresponding editorial on page 1891.

**ABSTRACT** Vitamin C status and possible associations with the disease process in cystic fibrosis (CF) patients were investigated. Plasma vitamin C concentrations in patients from two different mid-European populations (Swiss,  $n = 62$ ; Austrian,  $n = 60$ ) taking no or low-dose vitamin C from multivitamin supplements did not differ from each other or from control subjects ( $n = 34$ ). Vitamin C concentrations decreased with age ( $5.05 \mu\text{mol} \cdot \text{L}^{-1} \cdot \text{y}^{-1}$ ). When followed up for 12 mo, patients had the highest plasma vitamin C concentrations in February and the lowest in May and August ( $P < 0.01$ ); the decrease in vitamin C was accompanied by increases in plasma malondialdehyde ( $P < 0.001$ ) and tumor necrosis factor  $\alpha$  concentrations ( $P < 0.01$ ). During supplementation with vitamin E for 2 mo or  $\beta$ -carotene for 12 mo vitamin C concentrations did not change. They correlated inversely with white blood cell count ( $r = -0.36$ ,  $P = 0.008$ ), bands ( $r = -0.36$ ,  $P = 0.02$ ),  $\alpha_1$ -acid glycoprotein ( $r = -0.45$ ,  $P = 0.002$ ), interleukin 6 ( $r = -0.46$ ,  $P = 0.0006$ ), and neutrophil elastase/ $\alpha_1$ -proteinase inhibitor complexes ( $r = -0.34$ ,  $P = 0.02$ ). In patients with vitamin C concentrations  $< 40 \mu\text{mol/L}$ , all indexes of inflammation were relatively high, whereas those with concentrations  $> 80 \mu\text{mol/L}$  (upper quartile of control subjects) showed clearly lower values. These results are consistent with the hypothesis that by scavenging oxygen free radicals vitamin C interacts with an inflammation-amplifying cycle of activation of alveolar macrophages and neutrophils, release of proinflammatory cytokines and oxygen free radicals, and inactivation of antiproteases. *Am J Clin Nutr* 1997;65:1858-66.

**KEY WORDS** Cystic fibrosis, vitamin C, inflammation, free radicals, cytokines, malondialdehyde, neutrophil elastase/ $\alpha_1$ -proteinase inhibitor complexes, Shwachman score, tumor necrosis factor  $\alpha$

## INTRODUCTION

Cystic fibrosis (CF) lung disease is characterized by chronic, neutrophil-dominated inflammation (1) and unopposed neutrophil elastase activity that contributes to progressive, irreversible tissue destruction (2). Activated neutrophils and alveolar macrophages are a major source of endogenous reactive oxygen species (ROS), which have been implicated in lung inflammation (3). A variety of stimuli such as bacteria, immune complexes, leukotrienes, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ )

enhance the respiratory burst of neutrophils and their degranulation, and consequently, the generation of superoxide and hydrogen peroxide (3). ROS mediate signal transduction, including the activation of the transcription factor nuclear factor- $\kappa$  B (NF- $\kappa$ B), which is critical for the inducible expression of genes involved in inflammatory [interleukin 1 (IL-1), IL-6, and TNF- $\alpha$ ] and acute phase responses (4). TNF- $\alpha$  itself stimulates neutrophils (5) and activates NF- $\kappa$ B (6). In vitro experiments showed that neutrophil elastase stimulates the secretion of IL-6 by a CF airway epithelial cell line (7). ROS have been shown to impair alveolar macrophage function and disturb membrane integrity (8). Together, all of the above constitute a vicious cycle that cannot be interrupted efficiently by the therapeutic options currently available.

Vitamin C, a potent water-soluble antioxidant (9-11) present at high concentrations in epithelial lining fluid (ELF) (12), is considered to play a major role in the extracellular defense system of the lung (13). It is accumulated by alveolar macrophages and leukocytes (14, 15), the concentration gradient between leukocytes and plasma being up to 50-fold (15, 16). Because intracellular ascorbic acid is found almost exclusively in the cytosol, it has been proposed to have a protective effect by reducing ROS that enter the cytosol from the phagolysosome (15). On the other hand, ascorbic acid may be secreted into the extracellular microenvironment to protect the cell membrane against oxidation and thus preserve cellular integrity and chemotactic function (15). Ascorbic acid is a potent scav-

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enger of superoxide radical, hydroxyl radical, and the myeloperoxidase-derived oxidant hypochlorous acid (10), all of which are released by activated neutrophils into the extracellular environment. Here, ascorbic acid could prevent the oxidative inactivation of  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -PI) (17). Upon phagocytosis, the ascorbic acid content of neutrophils decreases (18).

Vitamin C concentrations in plasma are low in critically ill patients admitted to the intensive care unit (19), in patients with rheumatoid arthritis (18, 20) and bronchial asthma (21), and in smokers (22); each is a condition that involves the activation of white blood cells (WBCs) and ROS generation.

In contrast with the statuses of vitamin E and other fat-soluble vitamins that are substantially impaired in CF patients as a result of fat malabsorption, little attention has been paid to vitamin C. Data available so far show plasma ascorbic acid concentrations in CF patients from England not taking supplements to be either higher than (33.7–850.5  $\mu\text{mol/L}$ ; median 94.6  $\mu\text{mol/L}$ ) (23) or similar to (23.5–94.2  $\mu\text{mol/L}$ ; median 60.1  $\mu\text{mol/L}$ ) those of healthy subjects (24), and in patients from North America, total vitamin C (sum of ascorbic and dehydroascorbic acids) concentrations were below normal in 25% (25). Elevated plasma ascorbic acid concentrations in CF patients were considered to impair the total radical-trapping potential (23), suggesting that vitamin C supplements might even be harmful. However, as part of routine therapeutic management, CF patients frequently take multivitamin preparations with low-dose vitamin C. Multivitamin preparations were recommended by the North American CF Consensus Committee in 1992 (26), but recommendations for the vitamin C content of these supplements were not given.

We hypothesized that by efficiently scavenging ROS in the lung of CF patients, ascorbic acid could interact with an inflammation-amplifying cycle of activation of alveolar macrophages and neutrophils, release of ROS and proinflammatory cytokines, and inactivation of the major antiprotease  $\alpha_1$ -PI. The purpose of this observational study was to gain further insight

into the vitamin C status of CF patients and its possible associations with the disease process by 1) evaluating CF patients from two different mid-European populations (Swiss and Austrian), because plasma vitamin C concentrations are likely to reflect, at least in part, the dietary intake of fruit and vegetables rich in vitamin C, which is known to vary among populations; and 2) analyzing changes over time in plasma vitamin C concentrations both in the absence and presence of changes in supplementation with other antioxidants to detect possible seasonal influences and interactions between antioxidants, respectively. To answer the question of whether low plasma vitamin C concentrations are associated with increased lipid peroxidation, plasma malondialdehyde (MDA) concentrations were measured. To investigate possible interactions between vitamin C status and lung inflammation, relations between plasma vitamin C concentrations and cytokines (TNF- $\alpha$  and IL-6), an acute phase reactant ( $\alpha_1$ -acid glycoprotein,  $\alpha_1$ -AGP), and other indexes of inflammation [WBCs, bands, and neutrophil elastase (NE)/ $\alpha_1$ -PI complexes] were studied.

## SUBJECTS AND METHODS

### Patients

A total of 122 patients (64 males and 58 females) with a median age of 9.9 y (0.4–37.9 y) and Shwachman scores (27) of  $81 \pm 17$  were investigated. They comprised two cohorts of CF patients. Group A included 58 patients from Switzerland and 4 patients from Germany; this group is further referred to as the Swiss group ( $n = 62$ ). Group B comprised 60 patients from Austria. Demographic data for the two patient groups are shown in Table 1. Disease severity was assessed by using the Shwachman score (5–25 points are given for each of four categories: general activity, physical findings, nutritional status, and chest X-ray findings, with a perfect score being 100 points). Shwachman scores (to obtain normality the square root of the mirror scale,  $100 - \text{Shwachman score}$ , had to be used)

**TABLE 1**  
Comparison of Swiss and Austrian cystic fibrosis (CF) patients taking 0–150 mg vitamin C and CF patients and healthy control subjects taking no supplements<sup>1</sup>

	CF patients			Control subjects ( $n = 31$ )
	Total ( $n = 122$ )	Swiss <sup>2,3</sup> ( $n = 62$ )	Austrian <sup>4</sup> ( $n = 60$ )	
Age (y)	12.1 $\pm$ 8.3	11.9 $\pm$ 8.11	12.2 $\pm$ 8.9	27.4 $\pm$ 6.8
Shwachman score	80.6 $\pm$ 17.2	79.8 $\pm$ 15.9	81.6 $\pm$ 18.5	NA
Plasma variables				
Vitamin C ( $\mu\text{mol/L}$ )	63.6 $\pm$ 18.8	63.3 $\pm$ 18.3	64.0 $\pm$ 19.5	69.5 $\pm$ 16.6
$\alpha$ -Tocopherol ( $\mu\text{mol/L}$ )	26.9 $\pm$ 9.2	30.1 $\pm$ 11.6 <sup>5</sup>	23.6 $\pm$ 7.7	28.0 $\pm$ 5.6
$\beta$ -Carotene ( $\mu\text{mol/L}$ )	0.35 $\pm$ 0.60 <sup>6</sup>	0.56 $\pm$ 0.80 <sup>7,8</sup>	0.13 $\pm$ 0.16	0.92 $\pm$ 0.47
Malondialdehyde ( $\mu\text{mol/L}$ )	0.69 $\pm$ 0.26	0.72 $\pm$ 0.31	0.66 $\pm$ 0.21	0.72 $\pm$ 0.28

<sup>1</sup>  $\bar{x} \pm \text{SD}$ . NA, not applicable.

<sup>2</sup> Includes four patients from Germany.

<sup>3</sup> All supplemented with vitamin B; 21 also supplemented with  $\beta$ -carotene.

<sup>4</sup> All supplemented with vitamin E.

<sup>5</sup>  $\text{Log}_{10}$  value significantly different from Austrian group,  $P < 0.0001$  ( $t$  test).

<sup>6</sup>  $\text{Log}_{10}$  value significantly different from control subjects,  $P < 0.0001$  ( $t$  test).

<sup>7</sup>  $\text{Log}_{10}$  value significantly different from Austrian group,  $P < 0.0001$  ( $t$  test).

<sup>8</sup> In patients taking  $\beta$ -carotene supplements ( $n = 21$ ) concentration was  $1.29 \pm 0.86 \mu\text{mol/L}$  compared with  $0.14 \pm 0.14 \mu\text{mol/L}$  in those not taking  $\beta$ -carotene.

correlated inversely with  $\sqrt{\text{age}}$  ( $r = -0.48$ ,  $P < 0.0001$ ), as expected, because of disease progression with age. Because of continuous nutritional support, the majority of patients had good nutritional status: median Z scores for upper arm circumference for the Swiss group were  $-1.26$  ( $-3.94$ – $2.88$ ) and for body weight were  $-0.80$  ( $-3.13$ – $3.73$ ), compared with Swiss reference values established by Prader et al (28). There were no major differences in the overall therapeutic regimen for CF patients among the participating CF centers, nor did patients differ with respect to age and disease severity (Table 1). About one-third (21 of 62) of the Swiss group had taken  $\beta$ -carotene supplements ( $0.5 \text{ mg all-trans-}\beta\text{-carotene} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) long-term before this investigation in addition to vitamin E. These patients were enrolled only in the overall comparison of vitamin C status between Swiss and Austrian patients, not in the study of correlations between vitamin C status and indexes of inflammation because  $\beta$ -carotene status has been shown to affect at least one of the indexes of inflammation assessed in this study (29).

Subgroups of the patients included in this observational study had been enrolled previously in either a vitamin E (36 patients from group A, ie, 32 Swiss and 4 German patients, and 13 patients from group B, ie, Austrian patients, further referred to as the Swiss/Austrian group) or  $\beta$ -carotene (54 patients from group B, ie, Austrian patients only, further referred to as the Austrian group) supplementation trial, with 7 Austrian patients enrolled in both. These studies were conducted 2 y apart, allowing for investigation of vitamin C status in the same season (summer) and for all patients enrolled in the  $\beta$ -carotene trial to be in a steady state for vitamin E status. Forty-seven patients enrolled in the vitamin E supplementation study had a second determination of plasma vitamin C concentrations after they had taken 268 mg *RRR*- $\alpha$ -tocopherol (400 IU)/d for 2 mo, and 28 patients enrolled in the  $\beta$ -carotene supplementation trial after they had taken  $0.5 \text{ mg all-trans-}\beta\text{-carotene} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 12 mo. Possible seasonal changes were evaluated every 3 mo in 26 patients receiving vitamin E supplements long-term and followed up for 12 mo in the absence of changes in the therapeutic regimen (placebo group of the  $\beta$ -carotene supplementation trial). Details of the vitamin E study were reported (30) and a report is in preparation for the  $\beta$ -carotene supplementation trial.

Patients were compared with 34 healthy Swiss adults, median age 25.7 y (19.0–43.8 y), who volunteered as control subjects; it was not considered ethically acceptable to enroll healthy children. Patients received either no or 30–150 mg (median 40 mg) vitamin C from multivitamin supplements daily (different preparations from different companies); these doses were not changed during the observation periods. Control subjects consumed an average diet and did not take supplements. All investigations were approved by the Ethics Committees of either the Department of Pediatrics, University of Zurich, Switzerland, or the Faculty of Medicine, University of Innsbruck, Austria, and informed consent was obtained from the patients or their parents and from the control subjects.

### Analytic methods

Blood was drawn after an overnight fast into tubes containing either potassium EDTA or lithium heparin, protected from light with aluminum foil where indicated, and centrifuged immediately at  $2000 \times g$  for 8 min at room temperature;

plasma was separated and the different aliquots were processed as follows. For plasma total vitamin C determinations, 0.5 mL plasma with heparin was mixed with 4.5 mL 5% (wt:vol) metaphosphoric acid within a maximum of 30 min after blood was drawn and kept at  $-80^\circ\text{C}$  until analyzed in the laboratory of the Vitamin Research Department of Hoffmann-La Roche (Basel, Switzerland); a fluorimetric method with iodine oxidation followed by condensation with 1,2-phenylenediamine was used (31). Plasma samples with EDTA for MDA determination were also stored at  $-80^\circ\text{C}$  until analyzed; the HPLC MDA-thiobarbituric acid method used was based on the method of Wong et al (32) as described previously, with a mean ( $\pm$  SD) of  $0.61 \pm 0.22 \mu\text{mol/L}$  used as the reference value for healthy subjects (33). Aliquots for the determination of the fat-soluble antioxidants  $\alpha$ -tocopherol and  $\beta$ -carotene were stored at  $-20^\circ\text{C}$  for a maximum of 4 d before analysis; the HPLC method of Hess et al (34) was used. The other samples were either processed immediately or stored at  $-80^\circ\text{C}$  until analyzed. Plasma concentrations of NE/ $\alpha_1$ -PI complexes were determined by enzyme-linked immunosorbent assay (ELISA) with a test kit from Merck (Darmstadt, Germany), as published recently (29), with  $45.6 \pm 18.7 \mu\text{g/L}$  used as the reference value for healthy subjects. TNF- $\alpha$  (normal  $< 20 \mu\text{g/L}$ ) and IL-6 concentrations (normal  $< 3 \text{ ng/L}$ ) were determined by ELISA with test kits from Medgenix Diagnostics (Fleurus, Belgium), and  $\alpha_1$ -AGP by nephelometry using the QM300 AAG antibody from Sanofi Diagnostics Pasteur (Chaska, MN) (normal  $< 1.2 \text{ g/L}$ ). The normal percentage of white blood cells classified as bands in our laboratory was  $< 15\%$ .

### Statistical analyses

To obtain approximately normal distributions, some variables had to be either log-transformed or square root-transformed. In all cases the transformation that resulted in the best approximation of a Gaussian distribution was applied. Pearson correlations and multiple-regression analysis were used to study relations between plasma ascorbic acid concentrations and different variables. To correct for the influence of age, partial correlations were calculated. Repeated-measures analysis of variance (ANOVA) was applied to analyze seasonal changes in different variables. STATGRAPHICS Plus for Windows, version 1 (STSC, Rockville, MD) and GRAPHPAD INSTAT, version 2 (GraphPad Software, San Diego) were used for statistical procedures.  $P < 0.05$  was considered significant. Values are expressed as means  $\pm$  SDs unless otherwise indicated.

## RESULTS

### CF patients compared with healthy subjects

Plasma total vitamin C concentrations in the whole patient group did not differ significantly from those in healthy control subjects (Table 1). Plasma  $\beta$ -carotene concentrations (log-transformed) were significantly lower in patients than in the control subjects ( $P < 0.0001$ ), whereas  $\alpha$ -tocopherol and MDA concentrations (both log-transformed) did not differ. Plasma vitamin C concentrations in the Swiss and Austrian groups were similar. Plasma  $\alpha$ -tocopherol and  $\beta$ -carotene concentrations were higher in the Swiss group ( $P < 0.0001$ ) but there were no differences for MDA concentrations.

### Plasma vitamin C concentrations and age, Shwachman score, and overall nutritional status

Multiple-regression analysis, including plasma vitamin C concentration as the dependent variable and age (square root-transformed) and Shwachman score ( $\sqrt{100 - \text{Shwachman score}}$ ) as the independent variables, showed lower vitamin C concentrations in older patients ( $P = 0.001$ ) (Figure 1), but no dependency on Shwachman scores (data not shown). No relations between plasma vitamin C concentrations and sex and population (Austrian or Swiss) were found. In the Swiss group ( $n = 62$ ), data on upper arm circumference (Z scores), considered a reliable index of nutritional status in CF, were available and showed a positive correlation with vitamin C concentrations ( $r = 0.32$ ,  $P = 0.04$ ).

### Correlations between plasma vitamin C concentrations and indexes of lung inflammation

Because different indexes were investigated in the two patient groups, further correlations were studied only in either one of the two groups. Data from the Swiss/Austrian group enrolled in a vitamin E supplementation trial were used from the second examination after patients had received 400 IU *RRR*- $\alpha$ -tocopherol/d for 2 mo, and the Austrian group had their vitamin E status corrected long-term before entry into the  $\beta$ -carotene supplementation trial; thus, both groups had normal vitamin E status because of efficient vitamin E supplementation. Both the second investigation of the Swiss/Austrian group and the first of the Austrian group were conducted in summer. In the Swiss/Austrian group, three indexes of inflammation showed significant inverse correlations with plasma vitamin C concentrations: bands as a percentage of WBC count ( $r = -0.36$ ,  $P = 0.02$ ),  $\alpha_1$ -AGP ( $r = -0.45$ ,  $P = 0.002$ ), and NE/ $\alpha_1$ -PI complex concentrations ( $r = -0.34$ ,  $P = 0.02$ ) (Figure 2). In the Austrian group, WBC count ( $r = -0.36$ ,  $P = 0.008$ ) and plasma IL-6 concentrations ( $r = -0.46$ ,  $P = 0.0006$ ) correlated inversely with plasma vitamin C concentrations (Figure 3), whereas TNF- $\alpha$  did not (data not shown). Because plasma vitamin C concentrations showed age-dependency, as did disease severity, partial correlations of vitamin C with these indexes were calculated, with the influence of age controlled for (Table 2). The influence of age was more pronounced in the Swiss/Austrian group (on bands,  $\alpha_1$ -AGP,

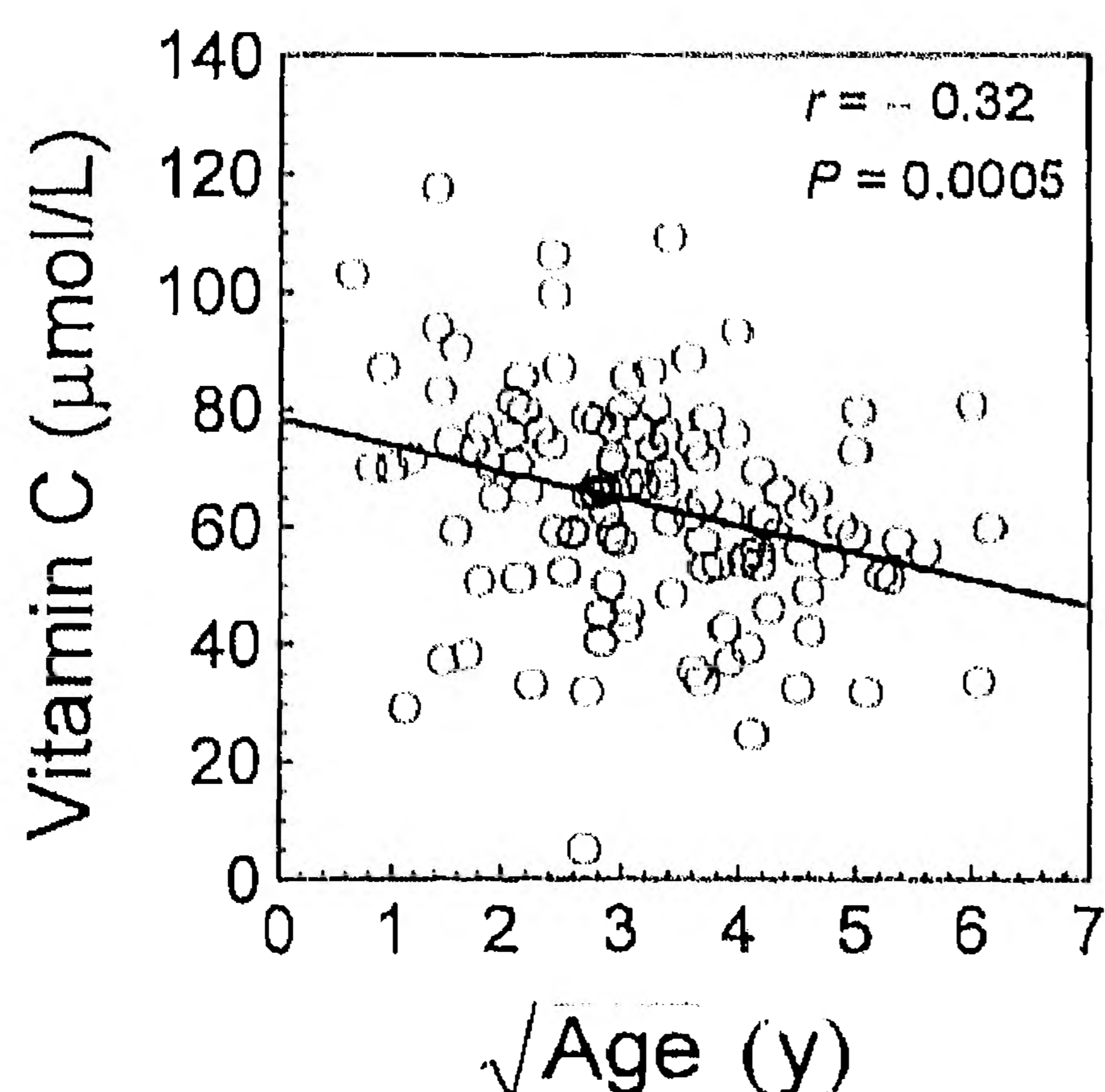


FIGURE 1. Regression of plasma vitamin C concentrations on  $\sqrt{\text{age}}$  for 122 cystic fibrosis patients. Vitamin C concentrations decreased significantly with age ( $-5.05 \mu\text{mol} \cdot \text{L}^{-1} \cdot \text{y}^{-1}$ ).

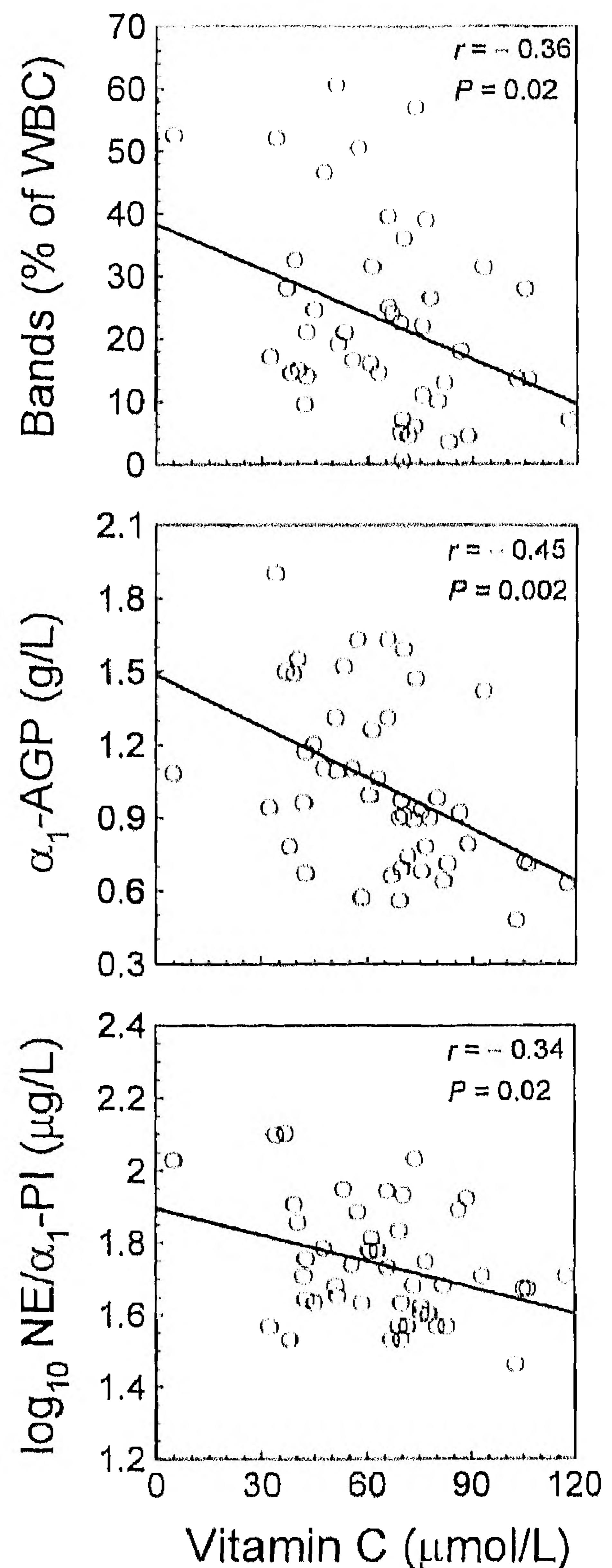
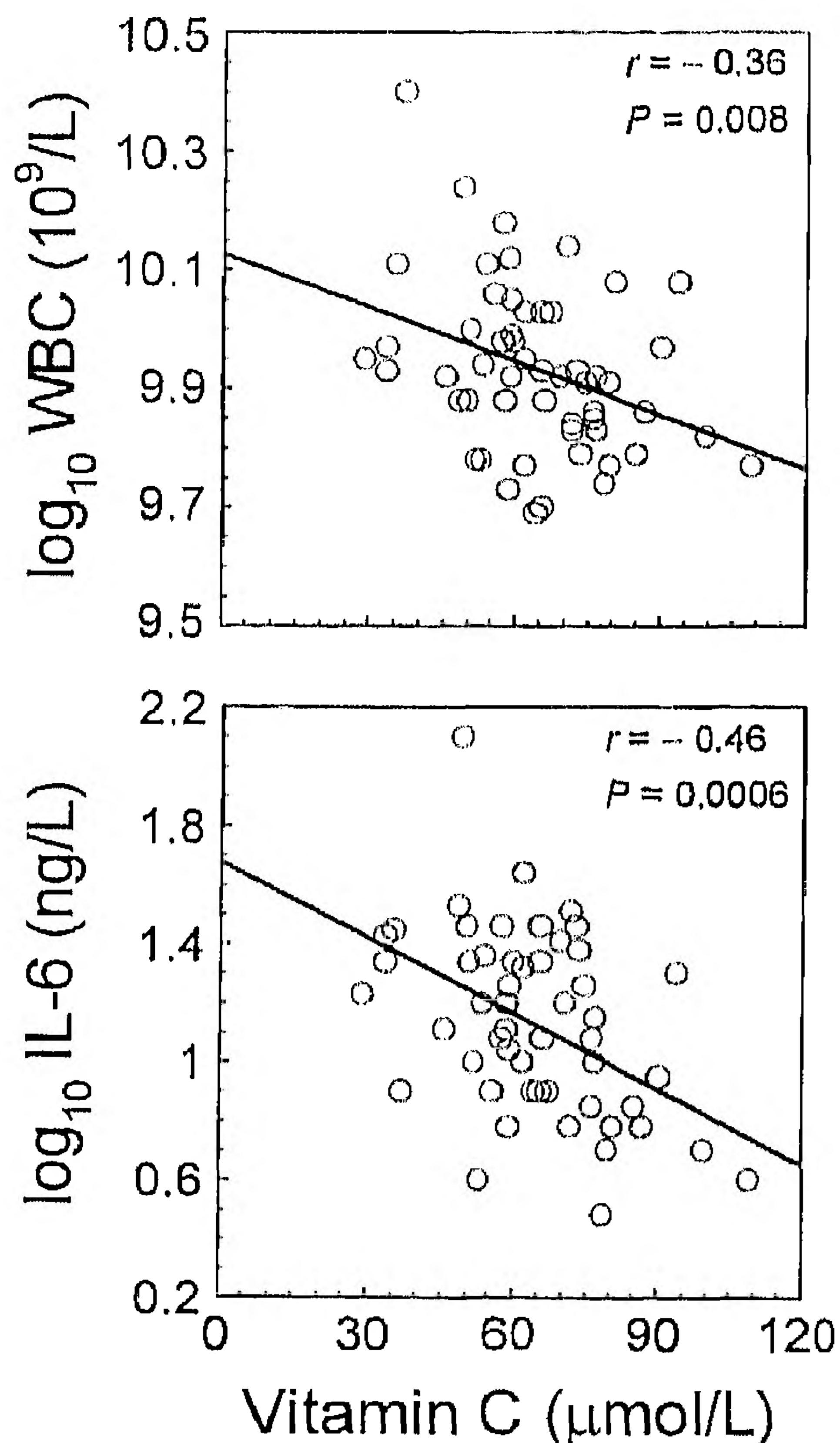


FIGURE 2. Inverse correlations between plasma vitamin C concentrations and indexes of inflammation in cystic fibrosis (CF) patients taking vitamin E but not  $\beta$ -carotene (second evaluation of the vitamin E supplementation trial): the percentage of bands contributing to the total white blood cell (WBC) count, plasma  $\alpha_1$ -acid glycoprotein ( $\alpha_1$ -AGP) concentrations, and plasma neutrophil elastase/ $\alpha_1$ -proteinase inhibitor (NE/ $\alpha_1$ -PI) complex concentrations (log-transformed) in the Swiss/Austrian group ( $n = 47$ ). Normal band counts are  $< 15\%$ , normal  $\alpha_1$ -AGP concentrations are  $< 1.2 \text{ g/L}$ , and normal NE/ $\alpha_1$ -PI concentrations in healthy subjects are  $45.6 \mu\text{g/L}$  ( $\log_{10} = 1.66$ ).

and NE/ $\alpha_1$ -PI complex concentrations) compared with the Austrian group (on WBC count and IL-6 concentrations).

### Changes in plasma vitamin C concentrations during vitamin E and $\beta$ -carotene supplementation

During supplementation with 400 IU *RRR*- $\alpha$ -tocopherol/d for 2 mo (starting in spring) plasma vitamin C concentrations increased slightly but not significantly (Table 3). There were no changes in vitamin C concentrations in patients taking  $0.5 \text{ mg all-trans-}\beta\text{-carotene} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 12 mo.



**FIGURE 3.** Inverse correlations between plasma vitamin C concentrations and indexes of inflammation studied in the Austrian group taking vitamin E but not  $\beta$ -carotene ( $n = 51$ ) (baseline evaluation of the  $\beta$ -carotene trial; complete data were missing for three patients): total white blood cell count (WBC) (data from one patient missing) and plasma interleukin 6 (IL-6) concentrations (both log-transformed). Note that normal IL-6 concentrations are  $< 3$  ng/L ( $\log_{10} = 0.48$ ).

### Seasonal changes in plasma vitamin C concentrations

In 23 of the 26 Austrian patients who took vitamin E supplements long-term and were followed up for 12 mo without changes in vitamin E supplementation and other aspects of the therapeutic regimen, and who also had a complete set of data for the different variables, plasma vitamin C concentrations increased from the first investigation in August to the second in November, achieved a plateau up to February and then dropped between February and May ( $P < 0.01$ ) without further changes between May and August (Figure 4). Values at the beginning and end of the 12-mo study period (both in August) did not differ from each other. Plasma vitamin C concentrations were significantly higher in February than in August and May ( $P < 0.01$ ). Looking at the changes in the individual patients in vitamin C status only, plasma vitamin C concentrations were highest in February in 11 of 26 patients and lowest in August and May in 16 of 26 and 8 of 26, respectively. Because two investigations were conducted in August, the frequencies of lowest values in August (16 of 26) and May (8 of 26) can be considered equal. Thus, on an individual basis, plasma vitamin C concentrations were also highest in winter and lowest in spring and summer. The mean CV in plasma vitamin C concentrations over a whole year, calculated for the 26 individual subjects, was 14.3% (1.4–42.1%).

### Concomitant changes in lipid peroxidation and TNF- $\alpha$ concentrations

The significant increase in plasma vitamin C concentrations from August to February in 23 patients was accompanied by a small decrease in plasma MDA concentrations; the significant drop in plasma vitamin C concentrations between February and May was accompanied by a significant increase in MDA concentrations ( $P < 0.001$ , repeated-measures ANOVA; Figure 4). Both plasma vitamin C and MDA concentrations returned to baseline values after 12 mo. TNF- $\alpha$  concentrations were significantly elevated in May compared with August and February ( $P < 0.01$ ), decreased thereafter, and values at the end of the study period were comparable with those at the beginning (Figure 4). Thus, plasma vitamin C, MDA, and TNF- $\alpha$  concentrations showed a distinct pattern that was inverse for MDA and TNF- $\alpha$  compared with vitamin C concentrations. No corresponding changes in WBC counts and IL-6 concentrations were observed (data not shown).

### DISCUSSION

The data presented show that plasma total vitamin C concentrations in CF patients are widely scattered and scorbutic concentrations, ie,  $< 11$   $\mu\text{mol/L}$  (35) can be observed occasionally. The average values, however, are only slightly lower in CF patients than in healthy adult subjects when the majority of patients are taking low-dose vitamin C supplements and the control subjects are not taking supplements. Even though age-matched healthy control subjects (aged 0–38 y) would have been preferable, comparison with the control subjects of this study (aged 19–44 y) seemed to be appropriate because plasma vitamin C concentrations in a French population showed no evidence of a distinct evolutionary pattern of plasma vitamin C concentrations between the ages of 6 and 40 y (36).

There were no differences between patients from Switzerland and Austria, in contrast with elevated plasma ascorbic acid concentrations in CF patients from England that were associated with impaired total radical-scavenging potential (23). There were no signs of increased lipid peroxidation in the patients of our study, most likely because of efficient vitamin E supplementation. Whereas a small but nonsignificant increase in plasma vitamin C concentrations was observed during 2 mo of vitamin E supplementation, no changes occurred during 12 mo of  $\beta$ -carotene supplementation, suggesting that no major interactions between these antioxidants and vitamin C take place.

Given the progressive nature of CF lung disease, a relation between age and disease severity, assessed by Shwachman scores, was expected for individual patients, but it also held true for the study patients as a group. In line with this observation, different indexes of inflammation also correlated positively with age. In general, this can be explained by more advanced lung disease in the older patients because healthy subjects of a similar age range do not show an increase with age, for instance, in  $\alpha_1$ -AGP (37, 38), the variable that showed the closest correlation with age in CF patients. However, that older patients would have lower plasma vitamin C concentrations than younger ones could not be expected a priori, but is in line with an observation made in another group of CF patients (24). When age and Shwachman scores were entered

TABLE 2

Pearson and partial correlations for plasma vitamin C concentrations and indexes of inflammation in two cohorts of cystic fibrosis patients taking vitamin E but not  $\beta$ -carotene<sup>1</sup>

	Vitamin C concentration			
	Pearson correlation		Partial correlation	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Swiss/Austrian group ( <i>n</i> = 47) <sup>2</sup>				
Bands (% of white blood cells)	-0.36	0.02	-0.19	0.20
$\alpha_1$ -Acid glycoprotein	-0.45	0.002	-0.29	0.05
Neutrophil elastase/ $\alpha_1$ -proteinase inhibitor (log <sub>10</sub> )	-0.34	0.02	-0.17	0.25
Austrian group ( <i>n</i> = 51) <sup>3</sup>				
Interleukin 6 (log <sub>10</sub> )	-0.46	0.0006	-0.44	0.001
White blood cell count (log <sub>10</sub> ) <sup>4</sup>	-0.36	0.008	-0.39	0.004

<sup>1</sup> Partial correlations were applied to control for age.

<sup>2</sup> After 2 mo of 268 mg (400 IU) *RRR*- $\alpha$ -tocopherol/d. Includes four patients from Germany.

<sup>3</sup> After long-term vitamin E supplementation (100–400 IU/d).

<sup>4</sup> *n* = 50.

into multiple-regression analysis, only age was an explanatory variable for plasma vitamin C concentrations and Shwachman scores were not. This seems surprising, but can be explained by the fact that in contrast with current vitamin C status, Shwachman scores are highly influenced by the long-term disease course, including irreversible lung damage that may have occurred years ago. Data on the long-term evolution of plasma vitamin C concentrations and Shwachman scores are needed to establish a possible cause and effect relation between lower plasma vitamin C concentrations and lower Shwachman scores in older patients. The same holds true for the correlation between vitamin C status and lung function, both of which have been shown recently to correlate negatively with age in CF patients (24). Therefore, the question of whether impaired vitamin C status facilitates the progression of lung disease in CF patients long-term, or whether patients with more advanced lung disease have lower plasma vitamin C concentrations as a

result of lower vitamin C intake or increased vitamin C demands or both when they are sicker, remains open.

In critically ill patients (19) and smokers (22), low plasma and leukocyte vitamin C concentrations cannot be explained entirely by low intake of vitamin C, which raises the question of increased ascorbic acid turnover or consumption. We did not estimate the vitamin C intake in this study, because the vitamin C content of a specific food item varies considerably, depending on its source (39), storage time, and food preparation, all of which can hardly be accounted for in the evaluation of dietary records of patients consuming a free diet and not admitted to the hospital. Patients took either no or a dose of median 40 mg vitamin C daily in multivitamin supplements. Possible effects on plasma vitamin C concentrations of these low doses may have been easily hidden by differences in dietary intakes of vitamin C. Therefore, relations between supplement intake and plasma concentrations were not analyzed.

Seasonal variations in plasma vitamin C concentrations were observed, with similar values at the beginning and the end of the 12-mo study period in August. Values were highest in winter and lowest in summer. Plasma  $\alpha$ -tocopherol and  $\beta$ -carotene concentrations did not change. Interestingly, plasma MDA concentrations showed inverse behavior, with the highest values in May when plasma vitamin C concentrations were lowest; they also returned to baseline values at the end of the 12-mo study period. TNF- $\alpha$  concentrations were also highest in May and returned to baseline values. These simultaneous seasonal changes suggest a dependency of one or more indexes on the others, but certainly are not sufficient to prove that one exists. A possible explanation is that in spring vitamin C status was not sufficient to efficiently neutralize oxidant stress, resulting in increased lipid peroxidation. The simultaneous increase in TNF- $\alpha$  concentrations may reflect increased production of this cytokine by alveolar macrophages, either as a cause or consequence of increased ROS release in the presence of lower vitamin C concentrations. Although a 1-y follow-up study of healthy subjects did not note changes in plasma ascorbic acid concentrations (40), another study in the elderly showed the highest plasma total vitamin C concentrations and lowest C-reactive protein concentrations in summer, in associ-

TABLE 3

Changes in plasma concentrations of vitamin C,  $\alpha$ -tocopherol, and  $\beta$ -carotene in cystic fibrosis patients during vitamin E and  $\beta$ -carotene supplementation<sup>1</sup>

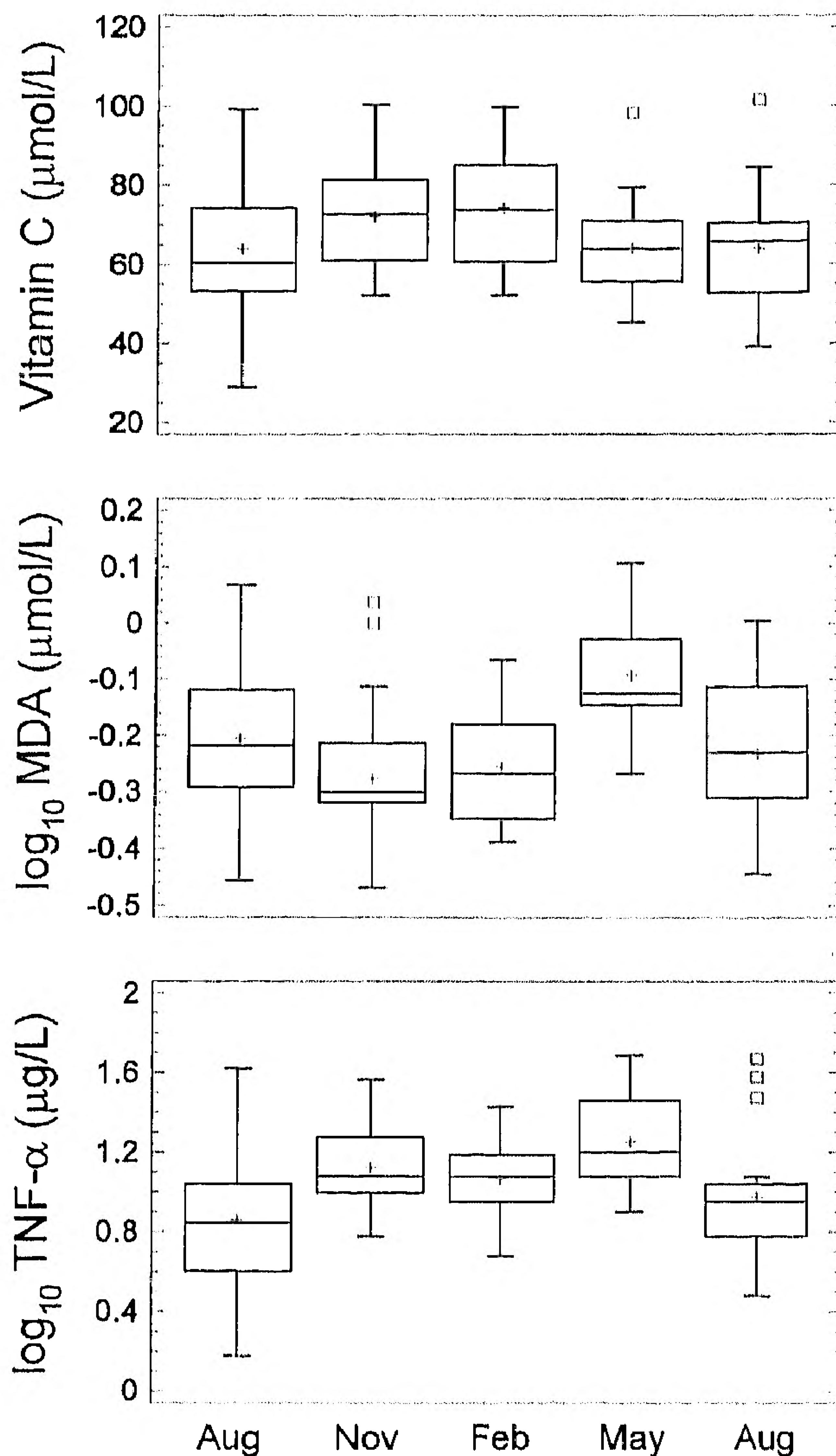
	Vitamin C	$\alpha$ -Tocopherol	$\beta$ -Carotene
	$\mu\text{mol/L}$		
Vitamin E supplementation of Swiss/Austrian group ( <i>n</i> = 47) <sup>2</sup>			
Baseline	61.2 $\pm$ 19.7	17.3 $\pm$ 12.0	0.14 $\pm$ 0.25
2 mo	66.1 $\pm$ 20.4	31.0 $\pm$ 9.18 <sup>3</sup>	0.15 $\pm$ 0.29
$\beta$ -Carotene supplementation of Austrian group ( <i>n</i> = 28) <sup>4</sup>			
Baseline	64.7 $\pm$ 17.3	21.6 $\pm$ 7.38	0.11 $\pm$ 0.16
12 mo	63.3 $\pm$ 15.9	20.8 $\pm$ 6.65	0.99 $\pm$ 0.64 <sup>3</sup>

<sup>1</sup>  $\bar{x} \pm$  SD.

<sup>2</sup> 268 mg (400 IU) *RRR*- $\alpha$ -tocopherol/d. Group includes four patients from Germany.

<sup>3</sup> Significantly different from baseline, *P* < 0.0001 (Wilcoxon matched-pairs signed-rank test).

<sup>4</sup> 0.5 mg/kg water-miscible *all-trans*- $\beta$ -carotene/d.



**FIGURE 4.** Boxplots for plasma vitamin C, malondialdehyde (MDA), and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) concentrations are shown for five time points with 3-mo intervals in 23 patients (starting in August) taking vitamin E but not  $\beta$ -carotene (placebo group of the  $\beta$ -carotene trial). See Methods for details of the studies. Significant differences with repeated-measures ANOVA with Tukey-Kramer multiple comparisons test: for vitamin C in February compared with August (beginning and end) and May,  $P < 0.01$ ; for MDA ( $\log_{10}$ ) in May compared with August (beginning),  $P < 0.01$ , and May compared with November, February, and August (end),  $P < 0.001$ ; for TNF- $\alpha$  ( $\log_{10}$ ) in May compared with August (beginning),  $P < 0.001$ , and May compared with February and August (end)  $P < 0.01$ . Note that mean plasma MDA concentrations of healthy subjects are  $0.72 \mu\text{mol/L}$  ( $\log_{10} = -0.14$ ) and normal TNF- $\alpha$  concentrations are  $< 20 \mu\text{g/L}$  ( $\log_{10} = 1.30$ ). The boxplots divide the data into four areas of equal frequency: the box encloses the middle 50% of data; the median is the horizontal line and the mean value the "+"; the lower (upper) whisker is drawn from the lower (upper) quartile to the smallest (largest) data point within 1.5 interquartile ranges from the lower (upper) quartile; individual points are data points that fall within three interquartile ranges (suspect outliers); no far outliers were observed.

ation with higher vitamin C intake in summer (41). Lower vitamin C intake, either from food or supplements, or both, could be a plausible explanation for the decrease in plasma vitamin C concentrations in our study as well, but in the

absence of intake data, redistribution of vitamin C from plasma to the site of inflammation should be considered as well.

For further study, confounding seasonal influences were excluded by analyzing data obtained in the same season, ie, from the second examination of the Swiss/Austrian group and the first of the Austrian patients, both of which were conducted in summer. Low plasma vitamin C concentrations were associated with increased proinflammatory cytokine release (TNF- $\alpha$  and IL-6), enhanced hepatic acute phase response ( $\alpha_1$ -AGP), increased plasma NE/ $\alpha_1$ -PI complex concentrations, and elevated WBC and band counts. The association with IL-6 concentrations and WBC count was significant even after the age influence (which, to an unknown extent, is proposed to reflect long-term disease progression) was controlled for. These findings are in line with observations in critically ill patients with different underlying diseases showing low plasma vitamin C concentrations associated with the severity of the illness and C-reactive protein concentrations (19), in elderly subjects with higher plasma vitamin C concentrations in association with lower C-reactive protein concentrations (41), and in smokers, in whom 21% lower plasma ascorbic acid concentrations were associated with 38% higher TNF- $\alpha$  and 16% higher IL-6 concentrations than in nonsmokers (22).

Perhaps even more than smoking, bacterial infection stimulates proinflammatory cytokine production and the acute phase response and activates neutrophils to release ROS (3). Unopposed oxidants not only cause lipid peroxidation (eg, MDA formation), but enhance the production, for instance, of TNF- $\alpha$  and IL-6 (3, 4). ROS are likely to oxidatively inactivate the  $\alpha_1$ -PI in the lung of CF patients (1, 17, 29). This may result in further neutrophil activation and proteolytic lung damage (2) and thus in propagation of the disease process. Under the type of oxidant stress that is exerted by activated neutrophils in CF (42), ascorbic acid is highly effective in vitro.

When human plasma was challenged with ROS released from activated neutrophils, ascorbic acid was depleted rapidly together with other antioxidants (43). Lipid peroxidation was prevented completely as long as ascorbic acid was present (43), perhaps because it intercepts oxidants in the aqueous phase before they can attack and cause detectable damage to lipids. In vitro experiments have shown that ascorbic acid protects  $\alpha_1$ -PI against inactivation during exposure to stimulated neutrophils (17). Taken together, these data suggest that ascorbic acid may indeed play an important role in protecting lung tissue from oxidant injury. Thereby, we speculate that ascorbic acid may accumulate at the site of inflammation, with enhanced uptake by activated inflammatory cells, and, as a consequence, plasma concentrations may decrease.

In ex vivo studies the low-density lipoprotein (LDL) of patients of this study, when efficiently supplemented with vitamin E, showed normal resistance to a defined oxidative stress (30). In vitro experiments identified  $40 \mu\text{mol/L}$  as the minimum concentration of ascorbic acid that significantly inhibited oxidative modification of LDL (11). Indeed, the majority of CF patients whose LDL was tested showed plasma vitamin C concentrations  $\geq 40 \mu\text{mol/L}$ . Interestingly, in patients with plasma vitamin C concentrations  $< 40 \mu\text{mol/L}$ , all indexes of inflammation included in the current investigation were markedly increased, whereas those with concentrations  $> 80 \mu\text{mol/L}$  showed clearly lower values. These results suggest that plasma vitamin C concentrations in the upper quartile



of our healthy control subjects (82.9–112.4  $\mu\text{mol/L}$ ) may benefit CF patients. From correlation studies (44, 45) we attempted to extrapolate the vitamin C dose that may be required for CF patients to reach the goal of steady state plasma concentrations  $> 80 \mu\text{mol/L}$ . One study showed that intake of  $> 200 \text{ mg}$  vitamin C/d is associated with these target plasma concentrations in  $\approx 50\%$  of healthy individuals (44). Results from another study suggest that daily doses as high as 1 g may be required, ie, the dose that led to complete plasma saturation in healthy subjects (45). By contrast, saturation of neutrophils occurred at 100 mg/d, again, in healthy subjects (45). Vitamin C pharmacokinetics and requirements may differ substantially between healthy subjects and CF patients. Clearly, further studies are needed to establish both optimal plasma vitamin C concentrations and vitamin C intake in CF patients.

Even though correlations do not prove a cause and effect relation, the correlations between plasma vitamin C concentrations and different indexes of inflammation observed in CF patients in this study may be the first indications that vitamin C plays a role in the inflammatory disease process. In CF patients, interactions between nutritional status and infection and inflammation have been postulated, even though this is supported by indirect evidence such as higher life expectancy in patients receiving more aggressive nutritional support (46), rather than by clearly focused clinical trials. It remains to be shown in controlled intervention studies that improvement of vitamin C status ameliorates inflammation in CF patients, not only short-term, but more importantly, long-term. Although intake of up to 1 g vitamin C/d appears to be safe in healthy subjects (45, 47), vitamin C may act as a prooxidant under certain conditions (48). These include pathologic events in which iron is unloaded from its main carrier proteins, transferrin and lactoferrin. This may, in general, be the case in the low-pH microenvironment of activated phagocytes (49) and, specifically, in the *Pseudomonas aeruginosa*-infected lung of CF patients (50). Therefore, before supplementation with high-dose vitamin C in CF patients can be recommended, controlled clinical studies addressing the issue of potential adverse effects need to be conducted. ■

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