The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/24669

Please be advised that this information was generated on 2019-07-31 and may be subject to change.
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 μmol·L⁻¹·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 concentrations (P < 0.01). During supplementation with vitamin E for 2 mo or β-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), α1-acid glycoprotein (r = −0.45, P = 0.002), interleukin 6 (r = −0.46, P = 0.0006), and neutrophil elastase/α1-proteinase inhibitor complexes (r = −0.34, P = 0.02). In patients with vitamin C concentrations < 40 μmol/L, all indexes of inflammation were relatively high, whereas those with concentrations > 80 μmol/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/α1-proteinase inhibitor complexes, Shwachman score, tumor necrosis factor α

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 α (TNF-α) 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-κ B (NF-κB), which is critical for the inducible expression of genes involved in inflammatory [interleukin 1 (IL-1), IL-6, and TNF-α] and acute phase responses (4). TNF-α itself stimulates neutrophils (5) and activates NF-κB (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-
VITAMIN C IN CYSTIC FIBROSIS

1859

genger of superoxide radical, hydroxyl radical, and the my-
celoperoxidase-derived oxidant hypochlorous acid (10), all of
which are released by activated neutrophils into the extracel-
lar environment. Here, ascorbic acid could prevent the ox-
idative inactivation of α1-proteinase inhibitor (α1-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 supplemen-
tes to be either higher than (33.7–850.5 µmol/L; median
94.6 µmol/L) (23) or similar to (23.5–94.2 µmol/L; median
60.1 µmol/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 man-
agement, CF patients frequently take multivitamin preparations
with low-dose vitamin C. Multivitamin preparations were rec-
ommended 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 in-
flammation-amplifying cycle of activation of alveolar macro-
phages and neutrophils, release of ROS and proinflammatory
cytokines, and inactivation of the major antiprotease α1-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 antioxi-
dants, respectively. To answer the question of whether low
plasma vitamin C concentrations are associated with increased
lipid peroxidation, plasma malondialdehyde (MDA) concentra-
tions were measured. To investigate possible interactions be-
tween vitamin C status and lung inflammation, relations be-
tween plasma vitamin C concentrations and cytokines (TNF-α
and IL-6), an acute phase reactant (α1-acid glycoprotein, α1-
AGP), and other indexes of inflammation [WBCs, bands, and
neutrophil elastase (NE)/α1-PI complexes] were studied.

SUBJECTS AND METHODS

Patients

A total of 122 patients (64 males and 58 females) with a a
median age of 9.9 y (0.4–37.9 y) and Shwachman scores (27)
of 81 ± 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 sta-
tus, 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 − Shwachman score, had to be used)

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>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</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total (n = 122)</th>
<th>Swiss (n = 62)</th>
<th>Austrian (n = 60)</th>
<th>Control subjects (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>12.1 ± 8.3</td>
<td>11.9 ± 8.1</td>
<td>12.2 ± 8.9</td>
<td>27.4 ± 6.8</td>
</tr>
<tr>
<td>Shwachman score</td>
<td>80.6 ± 17.2</td>
<td>79.8 ± 15.9</td>
<td>81.0 ± 18.5</td>
<td>NA</td>
</tr>
<tr>
<td>Plasma variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C (µmol/L)</td>
<td>0.63 ± 0.18</td>
<td>0.63 ± 0.18</td>
<td>0.64 ± 0.19</td>
<td>0.69 ± 0.16</td>
</tr>
<tr>
<td>α-Tocopherol (µmol/L)</td>
<td>26.9 ± 9.2</td>
<td>30.1 ± 11.6</td>
<td>23.6 ± 7.7</td>
<td>28.0 ± 5.6</td>
</tr>
<tr>
<td>β-Carotene (µmol/L)</td>
<td>0.35 ± 0.60</td>
<td>0.56 ± 0.80</td>
<td>0.13 ± 0.16</td>
<td>0.92 ± 0.47</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>0.69 ± 0.26</td>
<td>0.72 ± 0.31</td>
<td>0.66 ± 0.21</td>
<td>0.72 ± 0.28</td>
</tr>
</tbody>
</table>

* x ± SD; NA, not applicable.

1 Includes four patients from Germany.
2 All supplemented with vitamin E; 21 also supplemented with β-carotene.
3 All supplemented with vitamin E.
4 Log10 value significantly different from Austrian group, P < 0.0001 (t test).
5 Log10 value significantly different from control subjects, P < 0.0001 (t test).
6 Log10 value significantly different from Austrian group, P < 0.0001 (t test).
7 In patients taking β-carotene supplements (n = 21) concentration was 1.29 ± 0.86 µmol/L compared with 0.14 ± 0.14 µmol/L in those not taking β-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 cir-
cumference 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 mg all-trans-$\beta$-carotene $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$) long-
term before this investigation in addition to vitamin E. These
patients were enrolled only in the overall comparison of vita-
m 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 mg all-trans-$\beta$-carotene $\cdot$ kg$^{-1}$ $\cdot$ 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 supple-
mentation trial). Details of the vitamin E study were reported
(30) and a report is in preparation for the $\beta$-carotene supple-
mentation trial.

Patients were compared with 34 healthy Swiss adults, me-
dian 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. Con-
trol subjects consumed an average diet and did not take sup-
plements. All investigations were approved by the Ethics Com-
nittees 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 contain-
ing 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 $-80^\circ$C until analyzed in the laboratory
of the Vitamin Research Department of Hoffmann-La Roche
(Basel, Switzerland); a fluorimetric method with iodine oxida-
tion followed by condensation with 1,2-phenylenediamine
was used (31). Plasma samples with EDTA for MDA determi-
nation were also stored at $-80^\circ$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 (± SD)
of 0.61 ± 0.22 mmol/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
$-20^\circ$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 $-80^\circ$C until anal-
alyzed. 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 ± 18.7 mg/L used as the reference
value for healthy subjects. TNF-$\alpha$ (normal < 20 mg/L) and
IL-6 concentrations (normal < 3 mg/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 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 vari-
ables had to be either log-transformed or square root-trans-
formed. 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 analy-
sis of variance (ANOVA) was applied to analyze seasonal
changes in different variables. STATGRAPHICS Plus for Win-
dows, 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 ± 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 concen-
trations 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 \(\text{RRR-}\alpha\)-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, 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 \(\text{RRR-}\alpha\)-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 mg \(\text{all-trans-}\beta\)-carotene • kg\(^{-1}\) • d\(^{-1}\) for 12 mo.

**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 g/L, and normal NE/\(\alpha_1\)-PI concentrations in healthy subjects are ~45.6 \(\mu\text{g} /\text{L}\) (log\(_{10}\) ~1.66).
Subjects, was 14.3% (1.4-42.1%). Concentrations over a whole year, calculated for the 26 individual spring and summer, the mean CV in plasma vitamin C concentrations were also highest in winter and lowest in considered equal. Thus, on an individual basis, plasma vitamin C status only, plasma vitamin C concentrations were significantly higher in February than in August and May. Differ from each other, Plasma vitamin C concentrations were increased from the first investigation in August to the second in November, achieved a plateau up to February and then dropped 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-α 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-α 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-α concentrations showed a distinct pattern that was inverse for MDA and TNF-α 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 µmol/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 β-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 α1-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.
The same holds true for the correlation supplement7
Changes in plasma concentrations of vitamin C, 
TAKLR3
establish a possible cause and effect relation between lower
vitamin C concentrations and Shwaehman scores are needed to
course, including irreversible lung damage that may have oc­
Swiss/Austrian group (n = 47)
ß-Carotene supplementation
Vitamin E supplementation
Austrian group (n = 51)
Interleukin 6 (log10)
White blood cell count (log10)
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, Shwach­
man 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 de­
mands 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 en­
tirely 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, depend­
ing 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 α-tocopherol and β-car­
otene concentrations did not change. Interestingly, plasma
MDA concentrations showed inverse behavior, with the high­
est 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-α concentrations were also highest in
May and returned to baseline values. These simultaneous sea­
sonal 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, re­
sulting in increased lipid peroxidation. The simultaneous in­
crease in TNF-α concentrations may reflect increased produc­
tion 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 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 β-carotene1

<table>
<thead>
<tr>
<th>Vitamin C concentration</th>
<th>Pearson correlation</th>
<th>Partial correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Swiss/Austrian group (n = 47)2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bands (% of white blood cells)</td>
<td>-0.36</td>
<td>0.02</td>
</tr>
<tr>
<td>α1-Acid glycoprotein</td>
<td>-0.45</td>
<td>0.002</td>
</tr>
<tr>
<td>Neutrophil elastase/α1-proteinase inhibitor (log10)</td>
<td>-0.34</td>
<td>0.02</td>
</tr>
<tr>
<td>Austrian group (n = 51)3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin 6 (log10)</td>
<td>-0.46</td>
<td>0.0006</td>
</tr>
<tr>
<td>White blood cell count (log10)</td>
<td>-0.36</td>
<td>0.008</td>
</tr>
</tbody>
</table>

1 Partial correlations were applied to control for age.
2 After 2 mo of 268 mg (400 IU) RRR-α-tocopherol/d. Group includes four patients from Germany.
3 After long-term vitamin E supplementation (100–400 IU/d).
4 n = 50.

TABLE 3
Changes in plasma concentrations of vitamin C, α-tocopherol, and β-carotene in cystic fibrosis patients during vitamin E and β-carotene

<table>
<thead>
<tr>
<th>Vitamin C</th>
<th>α-Tocopherol</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmol/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vitamin E supplementation
of Swiss/Austrian group (n = 47)2
Baseline 61.2 ± 19.7 17.3 ± 12.0 0.14 ± 0.25
2 mo 66.1 ± 20.4 31.0 ± 9.18 0.15 ± 0.29

β-Carotene supplementation
of Austrian group (n = 28)4
Baseline 64.7 ± 17.3 21.6 ± 7.38 0.11 ± 0.16
12 mo 63.3 ± 15.9 20.8 ± 6.65 0.99 ± 0.64

1 ± SD.
2 268 mg (400 IU) RRR-α-tocopherol/d. Group includes four patients from Germany.
3 Significantly different from baseline, P < 0.0001 (Wilcoxon matched-pairs signed-rank test).
4 0.5 mg/kg water-miscible all-trans-β-carotene/d.
could he a plausible explanation for the decrease in plasma vitamin C concentrations in our study as well, but in the 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-α and IL-6), enhanced hepatic acute phase response (α1-AP), increased plasma NE/α1-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-α 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-α and IL-6 (3, 4). ROS are likely to oxidatively inactivate the α1-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 α1-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 μmol/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 ≥ 40 μmol/L. Interestingly, in patients with plasma vitamin C concentrations < 40 μmol/L, all indexes of inflammation included in the current investigation were markedly increased, whereas those with concentrations > 80 μmol/L showed clearly lower values. These results suggest that plasma vitamin C concentrations in the upper quartile

![FIGURE 4. Boxplots for plasma vitamin C, malondialdehyde (MDA), and tumor necrosis factor α (TNF-α) concentrations are shown for five time points with 3-mo intervals in 23 patients (starting in August) taking vitamin E but not β-carotene (placebo group of the β-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 (log10) in May compared with August (beginning), P < 0.01, and May compared with November, February, and August (end), P < 0.001; for TNF-α (log10) in May compared with August (beginning), P < 0.01, and May compared with February and August (end) P < 0.01. Note that mean plasma MDA concentrations of healthy subjects are 0.72 μmol/L (log10 = −0.14) and normal TNF-α concentrations are < 20 μg/L (log10 = 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.](image-url)
of our healthy control subjects (82.9–112.4 μmol/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 μmol/L. One study showed that intake of > 200 mg vitamin C/d is associated with these target plasma concentrations in ~50% of healthy individuals (44). Results from another study suggest that daily doses as high as 1 g may be required, i.e., 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.

We acknowledge the excellent cooperation of the CF outpatient clinics of the Departments of Pediatrics in St Gallen (Felix Sennhauser), Luzern (Johann Spitingler), Aarau (Peter Künzle), and Chur (Dieter Vischer), and of the University Hospital in Zurich (Erich Ruessi), Switzerland, as well as of the University Children’s Hospital in Freiburg, Germany (Peter Greiner) and Department of Pediatrics, Landeskrankenhaus Feldkirch, Austria (Udo Müller), who allowed us to study their patients. We thank Richard Salkeid and Willy Schlip (Vitamin Research Department of Hoffmann-La Roche, Basell, Switzerland) for the determination of plasma antioxidants, Manfred Herold (Clinical Laboratory of the Department of Internal Medicine, University of Innsbruck, Austria) for TNF-α and IL-6 determinations, and Frank Kaden and Christiane Wilhelm (Department of Pediatrics, University of Innsbruck) for help with sample and data processing. We furthermore thank the nurses, Irene Tantner, Johann Spielter, Dominika Stoeessel (University Department of Pediatrics, Zurich), and Ingeborg Wild (University Department of Pediatrics, Innsbruck) for their help in the enrollment of patients and control subjects.

REFERENCES


