# Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients[1–3]

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**ABSTRACT** Plasma concentrations of the antioxidant vitamin ascorbic acid were measured by high-performance liquid chromatography in critically ill patients in whom the excessive generation of reactive oxygen species could compromise antioxidant defense mechanisms. Median concentrations of both total vitamin C (ascorbic acid and dehydroascorbic acid) and ascorbic acid in these patients were $< 25\%$ ($P < 0.001$) of the values found in healthy control subjects and in subjects in two other disease groups (diabetes, gastritis) in which reactive oxygen species are reported to be increased. The low values could not be explained by age, sex, intake, or treatment differences, but were associated with the severity of the illness and were not prevented by the use of parenteral nutrition containing ascorbic acid. In addition, the vitamin was less stable in blood samples taken from critically ill patients than in similar samples from subjects in the other groups. The findings indicate that antioxidant defenses could be considerably compromised in these very sick patients. If this reduces the patient's capacity to scavenge reactive species, then the potential of these species to damage DNA and lipid membranes could be increased and compromise recovery. *Am J Clin Nutr* 1996;63: 760–5.

**KEY WORDS** Ascorbic acid, dehydroascorbic acid, critical care, reactive species, antioxidants

## INTRODUCTION

The excessive generation of reactive oxygen species has been reported to occur as part of the metabolic process that accompanies both acute and chronic disease. Because these species can damage DNA, protein, and unsaturated fat, it has been suggested that reactive oxygen species are important in encouraging the development and progression of several conditions (1, 2), especially when physiologic processes that protect the body against excessive activity of reactive oxygen species are compromised.

There is potential in critically ill patients for a massive increase in the generation of reactive oxygen species (3). If this increase exceeds the capacity of the antioxidant defense, these patients may be susceptible to further tissue damage, which could lead to complications such as adult respiratory distress syndrome (3). Micronutrients, such as vitamins A, C, and E, and carotenoids, play an important role in this defense by acting as antioxidants and scavenging reactive species (4, 5). Vitamin C is a major reducing agent in aqueous environments and acts in synergism with fat-soluble antioxidants such as vitamin E and carotenoids that protect cell membranes (6–8). We therefore investigated plasma concentrations of ascorbic acid and total vitamin C (ascorbic acid and dehydroascorbic acid) in patients who are critically ill in an intensive care unit (ICU), and attempted to assess factors that could have led to the decrease in concentrations of the vitamin C components found in these patients.

## PATIENTS AND METHODS

Patients in an ICU were selected during two periods in 1993 on the basis solely of informed consent and the ability to provide a blood sample. They were in need of intensive treatment for a variety of reasons, including accidental injury, recovery from surgery, sepsis, and major-organ failure. The only common feature of the patients was that they needed the critical care provided by the ICU to sustain life and were all therefore critically ill. Details including condition, age, length of stay in the ICU, and the use of either total parenteral nutrition or transfusions were noted. The study was approved by the Research Ethics Committee of the United Leeds Teaching Hospitals Trust.

Blood samples were collected in the morning into tubes treated with lithium heparin (10 mL) and plain tubes (5 mL) for the preparation of serum, and were processed within 40 min of collection. For the estimation of ascorbic acid, blood samples were centrifuged at room temperature at $1500 \times g$ for 5 min and plasma was transferred into two volumes of 20 g metaphosphoric acid (MPA)/L. For the estimation of total vitamin C, dithiothreitol (DTT) was added to the plasma at a concentration of 18 g/L before the addition of MPA and the MPA extract was heated for 2 h at 45 °C before HPLC analysis to convert all the dehydroascorbic acid to ascorbic acid. Some

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plasma samples were extracted with 2 volumes methanol:10 mmol EDTA/L (9:1 by vol) to give a final concentration of methanol in the sample of 60%, as an alternative to MPA (9). DTT was also added to these samples to measure total vitamin C but the extract was not heated before the assay because at this pH DTT reduces dehydroascorbic acid almost immediately at room temperature (10). Plain samples were allowed to clot and serum was removed for the estimation of C-reactive protein to assess the severity of the acute-phase response. All samples were subsequently stored at −70 °C.

Total vitamin C and ascorbic acid were measured in the supernates of the MPA and methanol samples, with and without DTT, respectively, by HPLC as described previously (11), but with a mobile phase consisting of 0.2 mol sodium acetate/L:acetonitrile (95:5 by vol), pH 4.3, and containing 10 mmol octylamine/L. The concentration of dehydroascorbic acid in the samples was estimated by the difference between the concentrations of total vitamin C and ascorbic acid. The technique has a between-batch CV of 8.2% for total vitamin C, 7.8% for ascorbic acid, and 34% for dehydroascorbic acid with the use of a low plasma control with a total vitamin C concentration (21 μmol/L) within the range of the ICU patient values. Plasma total vitamin C measured by this method showed good agreement when assessed externally with a fluorometric technique (correlation coefficient between the two techniques for 92 samples, $r = 0.951$; mean values (μmol/L): 41.6, HPLC; 43.0, fluorometry) (12). Recovery of added ascorbic acid as ascorbic acid and total vitamin C was 85% and 103%, respectively. Treatment of plasma samples with ascorbate oxidase spatulas at pH 5.5 led to zero values for ascorbic acid in all samples tested, indicating the specificity of the technique. Subsequent treatment of these samples with DTT, followed by MPA extraction and heating, gave a mean recovery of 83% for dehydroascorbic acid as total vitamin C.

### Statistical analysis

For comparison with the results in critically ill patients, samples were collected at the same time from healthy control subjects (laboratory and hospital staff) and from patients in two other disease groups (diabetes and gastritis) in which increased generation of reactive species has been suggested (13–15). Statistical analysis was by paired *t* test for comparisons of different treatments on the same sample and by Wilcoxon's rank test for comparisons between patient groups in which distributions were nonparametric. The association between vitamin C concentrations and the concentration of C-reactive protein was assessed by Pearson product-moment correlation analysis.

## RESULTS

### Concentrations in the different groups

Total vitamin C and ascorbic acid concentrations in the plasma of ICU patients were considerably lower than those in any other group and there were no overlaps in the interquartile ranges (**Table 1**). All these differences between the ICU patients and the other groups were significant ($P < 0.001$, Wilcoxon's rank test). In ICU patients, age and sex, reported to have slight effects on plasma ascorbic acid concentrations (16), could not account for the differences between the groups (Table 1).

Dehydroascorbic acid values were very low in all groups, but mean concentrations were significantly greater than zero, except in the control subjects ($P < 0.001$, $P < 0.05$, and $P < 0.01$ for subjects in the ICU, gastritis, and diabetes groups, respectively), indicating the presence of traces of dehydroascorbic acid in disease. However, in contrast with the other vitamin C indexes, dehydroascorbic acid was not significantly different in ICU patients, although the proportion of total vitamin C as dehydroascorbic acid was greater. Some subjects had a negative value for dehydroascorbic acid (Table 1), but this simply reflected a combination of the imprecision of both the ascorbic acid and total vitamin C measurements used to calculate this component and the fact that mean concentrations of dehydroascorbic acid in these samples were close to zero.

When the concentration of ascorbic acid in the plasma from 27 ICU patients (initial values 2.6–32.3 μmol/L) was increased by 57–228 μmol/L by adding commercial ascorbate to the sample, percentage recovery ($\bar{x} \pm$ SD) of this added vitamin C was good: total vitamin C, 88 ± 9.8%; ascorbic acid, 92 ± 8.5%, indicating that inaccuracy was not the cause of the low ascorbate concentrations in the ICU patients. Furthermore, an alternative method of sample preparation, the use of methanol-EDTA, did not yield measurements of higher concentrations of ascorbic acid or total vitamin C in these patients (**Table 2**).

### Cause of low concentrations in ICU patients

Examination of subgroups within the ICU population indicated that the elderly tended to have the lowest ascorbic acid and total vitamin C concentrations, although even at the age extremes the differences did not reach conventional levels of significance (**Table 3**). Blood transfusions after surgery did not affect ascorbic acid concentrations compared with concentrations in similar patients who received no transfusions.

Poor diet could potentially be a major cause of low plasma concentrations, but intake is difficult to assess in critically ill patients. It can be measured in the ICU but most patients had

**TABLE 1**
Concentration of plasma vitamin C metabolites in patients in the intensive care unit (ICU) compared with other patient groups[1]

| Study group | Age | Total vitamin C | Ascorbic acid | Dehydroascorbic acid |
|---|---|---|---|---|
| | *y* | *μmol/L* | *μmol/L* | *μmol/L* |
| ICU (*n* = 37M, 25F) | 60 | 11.0 (8–22)[2] | 9.0 (5–15)[2] | 1.4 (−0.8 to 2.9) |
| Healthy (*n* = 17M, 17F) | 29 | 61.8 (55–72) | 61.4 (56–68) | 2.3 (−2.9 to 5.8) |
| Gastritis (*n* = 21M) | 49 | 47.4 (28–57) | 45.7 (29–57) | 2.3 (0.6–2.9) |
| Diabetes (*n* = 15M, 9F) | 66 | 44.9 (27–60) | 41.5 (22–57) | 2.8 (0.0–6.3) |

[1] Median; interquartile range in parentheses.
[2] Significantly different from concentrations in all other groups, $P < 0.001$ (Wilcoxon's rank test).

**TABLE 2**
Comparison of measurements of vitamin C metabolites found in the plasma of healthy subjects and in patients in the intensive care unit (ICU) through use of two different extraction techniques[1]

| | Total vitamin C | | Ascorbic acid | | Dehydroascorbic acid | |
|---|---|---|---|---|---|---|
| Study group | Meth | MA | Meth | MA | Meth | MA |
| ICU ($n$ = 9) | 12.0 ± 8.6 | 14.8 ± 9.2 | 10.4 ± 8.5 | 12.9 ± 9.9 | 1.6 ± 2.0 | 1.9 ± 3.8 |
| Healthy ($n$ = 12) | 59.6 ± 8.2 | 58.2 ± 7.0 | 56.5 ± 6.0 | 60.4 ± 6.7[2] | 3.1 ± 7.0 | −2.2 ± 0.8[2] |

[1] $\bar{x}$ ± SD. Meth, methanol-EDTA extraction; MA, metaphosphoric acid extraction.
[2] Significantly different from methanol-EDTA value, $P < 0.05$ (paired $t$ test).

only been in the ward a short time before a blood sample was taken (median time: 2 d). Detailed metabolic studies in humans show that, even with zero intake, it takes ≈20 d for plasma concentrations to fall to the values seen here, with a 7% fall expected in 2 d (17, 18). Intake in the weeks before admission would therefore provide a better measure but it is impossible to obtain accurate information in patients whose conscious state is often impaired. We were, however, able to classify the ICU patients into three groups in which vitamin C intake differed considerably. The highest intakes (200 mg/d for an average of 13 d before sampling) were in five patients receiving total parenteral nutrition, but their plasma concentrations of ascorbate were not significantly different from those in the rest of the ICU population (Table 3). Eleven patients were admitted after accidental injury and it is assumed that their intakes before admission would have been similar to the UK average [a mean of 64 mg/d (19)]. The remaining 46 ICU patients investigated would, on average, have had a poorer recent intake because of ongoing major disease that had led to their current need for critical care because of sepsis, organ failure, or major surgery. However, no significant difference in vitamin C status was noted between those with accidental injury and those in this group [total vitamin C and ascorbic acid (median values) were 12.3 and 11.3 μmol/L in the accidental injury group compared with 10.3 and 8.5 μmol/L in the major disease group, respectively]. Blood was sampled on average 2 d after admission in both groups. It is evident from the similar plasma vitamin C concentrations in all three groups that recent vitamin C intake was not a major determinant of the low plasma concentrations found in ICU patients.

**TABLE 3**
Effect of patient variables on plasma total vitamin C and ascorbic acid concentrations in the intensive care unit (ICU) patients[1]

| Variable | Total vitamin C | Ascorbic acid |
|---|---|---|
| | μmol/L | |
| Age | | |
| <30 y ($n$ = 9) | 19.8 (13.9) | 18.7 (13.1) |
| >75 y ($n$ = 8) | 13.7 (13.5) | 8.3 (8.6) |
| Blood transfusion[2] | | |
| None ($n$ = 10) | 12.1 (10.8) | 9.9 (8.4) |
| Present ($n$ = 7) | 15.2 (10.8) | 12.2 (9.3) |
| TPN | | |
| None ($n$ = 57) | 14.5 (10.8) | 12.4 (8.9) |
| Present ($n$ = 5) | 16.9 (10.3) | 14.4 (9.3) |
| Days in ICU[3] | | |
| ≤2 d ($n$ = 13) | 20.6 (21.8) | 17.8 (15.0) |
| ≥8 d ($n$ = 15) | 12.2 (10.0)[4] | 9.7 (8.7)[4] |
| Days after surgery[5] | | |
| 2 d ($n$ = 8) | 14.9 (11.2) | 10.1 (9.3) |
| 14 d ($n$ = 8) | 14.3 (10.9) | 11.2 (9.5) |

[1] $\bar{x}$; median in parentheses.
[2] Postoperative patients only.
[3] Total number of days patient spent in the ICU.
[4] Significantly different from ≤2 d, $P < 0.02$ (Wilcoxon's rank test).
[5] Same subjects 2 d after major surgery when critically ill in ICU and during recovery, 14 d after surgery and no longer in ICU.

Length of stay in the ICU was related to ascorbate concentration. Those in the ward for ≥ 8 d had significantly lower plasma concentrations of total vitamin C and ascorbate than did patients whose stay was shorter (Table 3). One difference between these two groups, who were of similar average age (51.7 and 53.8 y for those with a short or long stay, respectively), was the fact that blood samples were taken closer to admission in the short-stay group (median time since admission was 1 d in the short-stay group compared with 6 d in the long-stay group). This could have allowed time for plasma ascorbate to fall to lower concentrations in patients in the long-stay group. However, in six subjects who became long-stay patients, but from whom, similar to the short-stay patients, blood samples were taken within 2 d of admission, ascorbate values were both much lower than those in the short-stay group and not significantly different from the mean in the long-stay group: mean (median in parentheses) total vitamin C was 13.1 (10.1) μmol/L and mean ascorbic acid was 9.0 (9.4) μmol/L (compare these values with those in Table 3). This indicates that the length of time that the patient is in the ICU, rather than the closeness of sampling to admission, is the important variable. Associations with length of stay could therefore be a reflection of disease severity, complications, or both.

Selecting 14 patients from the ICU group who had sepsis, who subsequently died, or both to represent those who were the most critically ill resulted again in ascorbate measurements that were lower than those in the group as a whole (median total vitamin C: 9.5 μmol/L, and median ascorbic acid: 7.9 μmol/L), but no satisfactory "less-sick" group could be identified from the remaining ICU patients for comparison. Plasma C-reactive protein concentration is a marker of the acute-phase response and the severity of illness. Vitamin C concentrations showed a moderate inverse association with this protein ($r = -0.543$) in patients who had been in the ICU for > 1 d (to allow time for C-reactive protein concentrations to reflect the severity of disease). This suggests that the severity of the acute-phase response was in part responsible for the lowering of the ascorbate concentrations.

Overall, the findings indicate that the inverse relation of ascorbate to length of stay is a crude reflection of an association with disease severity and magnitude of the acute-phase response. However, partial recovery (14 d) does not lead to

improvement of ascorbate status because plasma concentrations measured after patients had left the ICU for other wards were similar to those recorded in the ICU (Table 3).

The acute-phase effect on vitamin C could be caused by rapid oxidation, utilization, or redistribution of the vitamin. There is some evidence for oxidation. Ascorbic acid in ICU patients' blood that had been kept at room temperature before storage was more unstable than that in samples from the other patient groups (**Figure 1**). Although the absolute decrease in total vitamin C and ascorbic acid in the ICU patients was less than in the other groups, the percentage decrease over 4 h (34%) was significantly greater (compared with 19% and 22% in the control and diabetic groups, respectively, $P < 0.05$, Wilcoxon's rank test). This decay was more striking when ascorbic acid was added to blood from ICU patients (spiked sample) in an amount similar to that seen in the control group (Figure 1). Now not only was the percentage change much larger in the ICU group (45.3%), but the absolute fall was also significantly larger ($P < 0.001$, ICU compared with control group, Wilcoxon's rank test). Although ascorbic acid concentrations fell considerably in blood kept in this way, dehydroascorbic acid concentrations did not accumulate. This loss of ascorbic acid and total vitamin C did not persist during normal storage of the sample. In 12 ICU samples that were initially precipitated with MPA and stored at −70 °C for 5 wk, the mean values for total vitamin C and ascorbic acid were 21.6 and 19.7 μmol/L before storage and 22.7 and 20.1 μmol/L after storage.

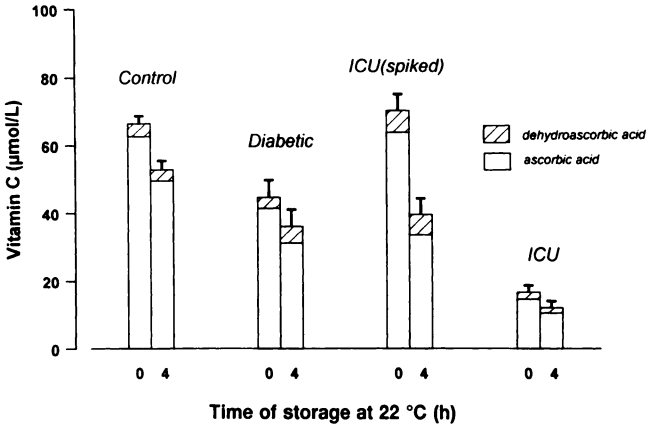


**FIGURE 1.** Stability of vitamin C metabolites in whole blood from different patient groups. Plasma for the 0-h samples was prepared for analysis immediately after a specimen was taken into tubes treated with lithium heparin, and the whole blood was then kept at 22 °C for 4 h before a further plasma sample was precipitated with metaphosphoric acid. For the "spiked" samples, ascorbic acid was added to increase its concentration in specimens from patients in the intensive care unit (ICU) by 57 μmol/L immediately after collection. The spiked samples were then treated in the same way as the unspiked samples. $\bar{x} \pm$ SE; $n = 23$ control samples, 22 diabetic samples, 22 ICU spiked samples, and 38 ICU samples. Changes (0–4 h) in total vitamin C and ascorbic acid but not in dehydroascorbic acid were significant in all study groups ($P < 0.001$, paired $t$ test). The percentage change in the ICU group and the absolute and percentage changes in the ICU spiked group were significantly greater than changes in both control and diabetic groups: $P < 0.05$ and $P < 0.001$ for the unspiked and spiked ICU samples, respectively (Wilcoxon's rank test).

## DISCUSSION

To our knowledge there are only two other studies reporting vitamin C concentrations in critically ill patients. In one, very low concentrations of ascorbic acid were described but only eight patients were examined (20). In the other, decreased ascorbate concentrations were found but they were not as low as those reported here (21). However, the lowest concentrations were associated with the most severe disease. Three further studies of plasma total vitamin C in patients who were acutely ill, but whose illness was not severe enough for them to require critical care, reported concentrations intermediate between those of the ICU patients and control subjects in this study (22–24). This confirms our proposal that the low ascorbate and total vitamin C concentrations observed in the ICU patients are probably explained in part as an effect of the disease process because of evidence that the lowest concentrations occured in those who were the most critically ill.

Comparison of ascorbate concentrations between ICU patients with different diseases or drug regimens was inappropriate because of the wide range of conditions and treatments, but the largest disease group (those recovering from surgery) had average values that were not different from the overall ICU mean (compare the values for the postsurgery groups in Table 3 with those for ICU patients in Table 1). In addition, apart from ascorbic acid itself, a wide range of drug therapies have been shown to have no effect on plasma vitamin C (25).

In cases in which malabsorption, increased utilization, or redistribution do not exert an effect on vitamin C requirements, intake is by far the major determinant of plasma ascorbate concentrations (26). As already explained, intake in the ICU is for most patients we examined not typical of recent diet, and obtaining an estimate of recent intake by accurate recall would be impossible. However, dividing the ICU patients into three groups in which average intake would have ranged from < 60 to > 200 mg/d did not lead to any significant difference in the mean plasma concentrations of the groups. Concentrations in all categories were still low, even in those receiving 200 mg ascorbate/d parenterally, compared with patients with gastritis and diabetes, disease groups for which intakes were previously estimated to be 51 and 77 mg/d, respectively (27, 28).

Taken together, these considerations strongly indicate that the acute-phase response largely accounted for the differences seen between ICU patients and those in other groups. It is not possible to determine whether this response represented redistribution of vitamin C, as has been suggested for plasma proteins (29), or increased utilization or oxidation of ascorbate. However, redistribution is unlikely to have been the only cause because one would have expected the patients receiving ascorbate as part of their total parenteral nutrition to become saturated with the vitamin if it was not utilized. It is probable that the generation of free radicals during the inflammatory response, particularly by white cells (3, 5, 20), could have accounted for the excessive oxidation of ascorbate and its subsequent loss from plasma. Rapid oxidation might be expected to lead to higher concentrations of dehydroascorbic acid, the oxidized form of the vitamin, but we did not observe this. Concentrations of dehydroascorbic acid were significantly greater than zero in all disease groups but we make no claim for the accuracy of these values because of the large imprecision of the measurement of this analyte. In these circumstances it is