Assessment of Human Vitamin C Status\textsuperscript{1,2}

**ROBERT A. JACOB**

Western Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture, Presidio of San Francisco, CA 94129

**ABSTRACT** Since no reliable functional markers of human vitamin C status have been demonstrated, determination of vitamin C levels in blood plasma and/or leukocytes remains the current choice for individual and population assessments. Newer analytical techniques, especially high-performance liquid chromatography, allow determination of reduced (ascorbic acid), oxidized (dehydroascorbic acid), or total amounts of vitamin C in biological specimens or foods. Plasma levels of vitamin C forms are easily determined but may not reflect tissue content as well as leukocyte levels. The vitamin C content of leukocyte cell types varies severalfold and, unlike plasma, leukocytes may contain an appreciable fraction of dehydroascorbic acid. The effects of sex, age, cigarette smoking, drugs, and physiological factors on vitamin C levels are better known for plasma than leukocytes. To realize the potential of leukocytes as measures of vitamin C status, continued work is needed in standardizing the methodology and interpretive guidelines and simplifying the technique for blood processing. The search for specific functional markers of vitamin C deficiency should continue. Candidate markers may involve pathways of carnitine or collagen metabolism, immunocompetence, or antioxidant defense. *J. Nutr.* 120:1480–1485, 1990.

**INDEXING KEY WORDS:** vitamin C • vitamin C status • vitamin C methods • ascorbic acid • nutritional assessment

Populations that suffered scurvy symptoms are recorded back to ancient civilizations. The devastating effects of scurvy on sailors during long ocean voyages prompted the classical British Navy studies, which led to rations of citrus juices in 1795 as a cure. The isolation, characterization, and synthesis of ascorbic acid in the early 1930's marked the beginning of the end for scurvy as a major nutrition-deficiency disease.

Today, although isolated pockets and individual cases of scurvy exist, the following questions are at the forefront: 1) What is the appropriate range of human vitamin C requirement beyond that required to prevent scurvy (5–10 mg/d)\textsuperscript{1,2}, and 2) What tools or measures can we use to determine the requirement and the vitamin C body status? A great deal of experimental data suggests a requirement for vitamin C beyond antiscorbutic action, particularly for antioxidant defense (1, 2), immune function, and a variety of biochemistries requiring reducing equivalents. Measures for assessing vitamin C status must, therefore, be sensitive to preclinical deficiency states, i.e., "marginal status," and should define stages of body compartment (pool) levels, as has been demonstrated for iron. Data on how vitamin C assessment measures reflect total body pool and vitamin C intake over time need to be provided. Clarifying the role of vitamin C intake in health maintenance of populations requires the use of methods that are simple to perform in large numbers and that define relatively common states due to marginally low and high vitamin C intakes. Criteria that relate blood vitamin C measures to marginal status need to be established.

**METHODOLOGY OPTIONS**

Current options for assessing vitamin C status are limited when compared with many other micronutri-
ents. No assessment (indeed concept) of vitamin C storage (such as serum ferritin for the iron pool) is available. Reduced serum or urinary ascorbate levels after oral vitamin C loads can reliably index a depleted ascorbate body pool, however, these saturation tests are not practical for survey use. Despite the vitamin’s requirement in a variety of biochemical reactions, no reliable function-based method has been demonstrated. Currently the measurements of plasma and leukocyte vitamin C levels are the most practical and reliable tests for assessing human vitamin C status. Work is needed to establish interpretive guidelines, simplify techniques, and standardize methodology in order for these “static” markers to be more useful. Concurrently, research on specific functional markers of vitamin C deficiency should continue. Functional measures have the advantage of relating directly to health or body function and avoid the problems of interpreting results across demographic variables such as age and sex.

**PLASMA VERSUS LEUKOCYTE ASCORBIC ACID**

Plasma ascorbic acid (AA) levels generally have been shown to correlate with dietary vitamin C intake [3–6] and with leukocyte vitamin C levels [3, 6, 7] in both epidemiologic and experimental studies.

Bates et al. [3] assessed vitamin C status of 23 healthy elderly persons living in the north of England over 18 mo. Plasma vitamin C correlated strongly with buffy-coat vitamin C both cross-sectionally between subjects and longitudinally within subjects. Plasma and buffy-coat vitamin C were strongly correlated with vitamin C intake both between and within subjects. The strongest correlations were for biochemical values related to the most recent 7-d dietary intake assessment. In this study, plasma vitamin C was judged nearly as good an index of long-term vitamin C status as buffy-coat levels [3].

**Figure 1** shows the response of plasma and leukocyte [mixed cell population] vitamin C to experimental changes in dietary vitamin C intake from 5 to 605 mg/d in healthy men over 14 wk [6]. Both parameters accurately reflected the changes in vitamin C intake when measured several weeks after the intake changed. A more recent report of experimental vitamin C depletion and repletion in healthy adult women also showed a parallel response of plasma and leukocyte AA to changes in AA intake [8]. The data are generally consistent with previous studies that suggest plasma vitamin C levels are more responsive to recent dietary intake, whereas leukocyte levels more closely reflect cellular stores and total body pool [7]. Thus, the data in Fig. 1 show that after the final 1-wk period of vitamin C repletion (study wk 13–14), plasma ascorbate increased fourfold, to well above baseline, while leukocyte ascorbate increased only to 50% of baseline, with most values remaining below normal. The repletion dynamics of various body compartments or pools are different when the vitamin intake during repletion is limited, however. In such cases the tissues or body pools with the highest metabolic priority will replete preferentially.

Blanchard et al. [9] recently determined plasma, mononuclear [MN] leukocyte, and polymorphonuclear [PMN] leukocyte vitamin C levels in eight young [20–29 yr] and eight elderly [65–71 yr] healthy women after they consumed their free-living diet, a vitamin C deficient diet (<10 mg/d) for 5 wk, and a vitamin C supplement [500 mg/d] for 3 wk [9]. The mean values of plasma and leukocyte vitamin C changed in parallel with one another and with the vitamin intake. However, the vitamin C levels in plasma did not correlate with leukocyte levels at each time point, and the individual changes in plasma levels between depleted and supplemented states did not correlate with the changes in the leukocyte levels. Thus, while the average plasma levels reflected changes in the mean MN and PMN vitamin C levels, plasma measurements were not reliable for predicting individual cellular levels at a fixed ascorbate intake or changes in cellular levels after vitamin intake changed.

**METHODOLOGY**

Analytical methods for determining vitamin C in biological samples and foods have been reviewed recently [7, 10, 11]. Most notable has been the variety of techniques reported in the past decade that allow specific determination of reduced, oxidized, or total vitamin C forms. The term “vitamin C” is the generic term for all compounds exhibiting qualitatively the biological activity of ascorbic acid. The reduced form of the vitamin is referred to as AA and the oxidized form as dehydroascorbic acid (DHAA). The sum of AA + DHAA represents the total naturally occurring biologically active vitamin C.

Many of the newer techniques utilize reductants (dithiothreitol, homocysteine) or oxidants (ascorbate oxidase) to convert all vitamin C in the specimen to one form, followed by specific determination of that form, usually by high-performance liquid chromatography (HPLC). This provides a value for the total vitamin C, both forms can be determined, however, if the specimen is analyzed similarly without the exogenous reductant or oxidant added. For example, AA is determined specifically by electrochemical detection after HPLC separation [7], and DHAA can be determined by detection of its fluorescent derivative with O-phenylenediamine [12]. Ascorbic acid and DHAA have been determined simultaneously in a variety of
samples by post-column derivatization with dimethyl-O-phenylenediamine (13) and by use of dual ultraviolet (UV) detectors (10).

**INTERPRETIVE GUIDELINES**

The ability to determine oxidized, reduced, or total vitamin C raises the question of specific reference ranges for these measures. For interpreting plasma values, this is apparently not a problem, since >95% of plasma vitamin C exists as ascorbic acid, and studies comparing total plasma vitamin C (AA + DHAA) with levels of AA have shown good agreement over a variety of conditions (9, 14). In general, plasma or serum AA values < 11 μmol/L (<0.20 mg/100 mL) are considered to represent frank vitamin C deficiency (biochemical and/or clinical symptoms). Plasma AA values between 11 and 23 μmol/L (0.2–0.4 mg/100 mL) represent marginal status, i.e., moderate risk of developing clinical signs of vitamin C deficiency due to low vitamin intakes or depleted body pool (6). Some investigators have used 28 μmol/L (0.5 mg/100 mL) as the lower limit of normal for plasma AA (6, 9). Sauberlich et al. (8) have recently shown—in adult females supplemented (600 mg/d) with erythorbic (isoascorbic) acid, a common food additive—that a major fraction of the apparent plasma AA concentration determined by non-HPLC methods was due to erythorbic acid. The authors caution that erroneously high plasma AA values may result when non-HPLC analysis methods are used to evaluate subjects whose diet contains significant amounts (>100 mg/meal) of erythorbic acid. Erythorbic acid has little antiscorbutic vitamin C activity but possesses antioxidant properties similar to AA.

The interpretation of leukocyte vitamin C concentrations, unlike plasma, is complicated by the different concentrations of vitamin C in various leukocyte cell fractions and by the possible presence of a portion of total leukocyte vitamin C as the oxidized form, DHAA. MN cells are generally found to contain up to two- or threefold higher concentrations of vitamin C than PMN cells (Table 1) (7, 9, 12, 15–18). The leukocytes contain considerably higher amounts of vitamin C than platelets on a per-cell (but not per-volume) basis, and all three cell types concentrate 2 to 80
times as much vitamin C on a per-volume concentration basis as plasma [15, 16]. Numerous clinical and physiological factors have been cited that increase the inherent variability among cell populations and their vitamin C content [14]. Hence, use of the heterogeneous “buffy-coat” mixture of blood cells for assessing cellular vitamin C status has been criticized as likely to provide inconsistent and possibly misleading results [14, 15].

In leukocytes, especially in phagocytic cells, a dynamic relationship exists between the oxidized and reduced forms of the vitamin [17]. Reported levels of DHAA in human leukocytes range from zero to nearly half of the total cellular vitamin C. VanderJagt et al. [12] found that the fraction of total vitamin C as DHAA in MN leukocytes of 17 healthy elderly men and women ranged from 23 to 48%. The range of mean values for leukocyte DHAA as percent of total vitamin C from five reports summarized by Schaus et al. [17] was 13–42%. The choice of analytical method is of greater concern for leukocyte assays than for plasma, since determination of the reduced form of the vitamin in leukocytes may provide only a fraction (albeit a major fraction) of the total biochemically available vitamin C. More work is needed to determine the extent to which reported DHAA levels in leukocytes represent true in vivo levels or methodological artifacts.

As seen in Table 1, considerable variation exists in reported levels of leukocyte vitamin C. Adding to the difficulty of comparing reported values are differences in units, including vitamin C levels calculated per number of cells, per volume of cells, and per milligram cellular protein. The various reported units are not easily or reliably interconverted.

Although AA levels in whole blood, erythrocytes, and urine have shown some value in assessing vitamin C status, plasma or leukocyte AA levels are preferred [6, 7, 8, 11]. The additional complexity and tedium involved in analyzing an isolated leukocyte cell fraction, as opposed to plasma or the Buffy coat, is especially important if large-scale population studies are planned. Recent technical advancements, such as commercially available tubes with density separation materials already in place, are simplifying the cell isolation task somewhat. The question remains as to what leukocyte cell fraction might be the best marker of vitamin C status. Upon studying 41 unsupplemented and 16 supplemented (2 g AA/d for 5 d) individuals, Evans et al. [15] found that vitamin C levels of the PMN cells and platelets, but not MN cells, correlated with plasma levels. In the Blanchard et al. [9] study of young and elderly women, the PMN cell fraction and plasma AA levels were more responsive to the changes in AA intake than was the MN cell fraction. This suggests that the plasma and PMN cell fraction are more sensitive indicators of AA status and that the MN cells represent a deeper metabolic pool, which is less easily depleted of vitamin C. The ability of the MN cells (lymphocytes) to concentrate vitamin C and their apparent functional importance in immunocompetence suggests that decreases in MN-cell AA levels may reflect a more serious functional deficit of tissue vitamin C. Further work is needed to establish the relative

### Table 1

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Method used</th>
<th>Total vitamin C</th>
<th>AA</th>
<th>DHAA</th>
<th>Population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN</td>
<td>DNPH</td>
<td>-207,25-81</td>
<td></td>
<td></td>
<td>Healthy females (21) and males (20), age 17-55 yr</td>
<td>Evans et al., 1982 [15]</td>
</tr>
<tr>
<td>PMN</td>
<td></td>
<td>1.4-5.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MN</td>
<td>HPLC</td>
<td>30-60</td>
<td>15-25</td>
<td></td>
<td>Healthy adults (20)</td>
<td>Lee et al., 1982 [16]</td>
</tr>
<tr>
<td>PMN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MN</td>
<td>DNPH</td>
<td>170-835</td>
<td>18-106</td>
<td></td>
<td>Six healthy adult males</td>
<td>Ikeda 1984 [18]</td>
</tr>
<tr>
<td>PMN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MN</td>
<td>HPLC</td>
<td>40-130</td>
<td>7-32</td>
<td></td>
<td>Healthy elderly females [9] and males [8]. Vitamin C intakes from 30 to 283 mg/d</td>
<td>VanderJagt et al., 1989 [12]</td>
</tr>
<tr>
<td>PMN</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td></td>
<td>1.0-1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN</td>
<td></td>
<td>5-500</td>
<td>5-560</td>
<td></td>
<td>Eight young (20-29 yr) and 8 elderly (65-71 yr) healthy women</td>
<td>Blanchard et al., 1989 [9]</td>
</tr>
</tbody>
</table>

1 Vitamin C concentrations reported in various units by the cited references were all converted to nmol/10⁸ cells. Abbreviations: MN, mononuclear; PMN, polymorphonuclear; HPLC, high-performance liquid chromatography; DNPH, dinitrophenylhydrazine.

2 Range calculated as mean ± 2 SD.
merits of the leukocyte cell fractions as markers of vitamin C status.

**Effects of age, sex, and smoking on vitamin C levels.** No definitive relationship between age and vitamin C status in adults has been established [the data have been briefly reviewed in recent publications [9, 11]]. In general, many reports have indicated that serum and/or leukocyte AA levels decline with age; this trend, however, has not been shown to be a consequence of senescence per se, i.e., after differences in dietary AA intakes and body mass have been taken into account. In 677 healthy Boston-area elderly persons [60–98 yr] with adequate vitamin C intakes, no relationship between age and plasma vitamin C levels was observed [5]. Blanchard et al. [9] found no significant age-related differences in the vitamin C levels of plasma, MN, or PMN cells in free-living young and elderly women receiving low, normal, and high intakes of vitamin C. Females consistently show higher levels of vitamin C in body fluids and tissues than males, and nonsmokers generally have higher levels than smokers, even when dietary AA intakes are normalized [4, 5, 11, 12]. Overall, the data suggest a higher vitamin C requirement for males versus females and smokers versus nonsmokers. The recent Tenth Edition of the U.S. Recommended Dietary Allowances (RDAs) leaves the RDA for adult men and women at 60 mg/d, but recommends that cigarette smokers consume at least 100 mg daily [19].

**FUNCTIONAL MEASURES**

Vitamin C is required for many hydroxylation reactions and acts as a reversible biological reducing agent. Roles have been defined in collagen formation, carnitine and steroid syntheses, iron absorption, immune functions; and the metabolism of folic acid, amino acids, cyclic nucleotides, cholesterol, and glucose. Despite the known role of AA in a number of biochemical reactions, no reliable functional marker of human vitamin C status has been established. The requirement for AA to hydroxylate proline and lysine residues in forming mature collagen is well known. Previous studies in humans or animal models, however, have not shown that the flux of these two amino acids (or their hydroxylated forms) in serum or urine consistently relates to vitamin C status. Recent reports of experimental vitamin C depletion in adult men have shown a decrease in gingival integrity [20] along with a significant increase in the excretion of urinary hydroxyproline [21]. The increases in urinary hydroxyproline were not sufficiently distinct to provide a reliable measure of vitamin C deficiency [21]. Markers of collagen metabolism appear to be good candidates for functional assessment tests, however, as this pathway appears to be disturbed in subclinical as well as in overt vitamin C deficiency.

In humans, vitamin C is required, together with iron, at two steps in the biochemical pathway synthesizing carnitine from methionine and lysine [22]. Muscle weakness and fatigue are symptoms of both scurvy and carnitine deficiency, and, in guinea pigs, AA deficiency lowers muscle carnitine levels. Several studies have failed to show a relationship between vitamin C deficiency and plasma carnitine. Davies et al. [23], however, found that the urinary carnitine/creatinine ratio correlated positively with leukocyte AA in elderly female hospital patients, and that healthy elderly males showed increased urinary carnitine excretion after supplementation with 200 mg/d of AA. Urinary rather than plasma carnitine may be an indicator of AA depletion, and further work on the carnitine pathway as related to AA status is warranted.

Evidence is accumulating that AA plays an important role in immunocompetence and in the antioxidant defense system. Recent reports link vitamin C status with lymphocyte blastogenesis, antibody response to viral antigens, and circulating levels of the immune modulators complement C1q and interleukin-1. New data also demonstrate the importance of AA as a plasma antioxidant [1, 2], and the in vitro measurement of the ascorbate free radical as a clinical index of oxygen radical attack has been suggested [24]. No AA-related measure of immune or antioxidant function has been shown to be sufficiently specific, however, to serve as an index of AA status. Certainly work must continue to establish a reliable functional measure of AA status. For now, measurement of plasma and/or leukocyte vitamin C levels, properly determined and interpreted, provides a reliable method for assessing human vitamin C status.

**LITERATURE CITED**


