Ascorbic Acid, Vitamin A, Folic Acid, and Amino Acids in Blood of Patients With Hemophilia

By Lettie Toy, Eleanor A. Young, and John B. Longenecker

Blood levels of ascorbic acid, vitamin A, folic acid, and amino acids were studied in patients at the South Texas Comprehensive Hemophilia Center, San Antonio, TX. The mean plasma ascorbic acid level in hemophilic patients was significantly lower than controls (p < 0.0001). This was observed despite a dietary ascorbic acid intake in excess of 66% of the Recommended Dietary Allowances (RDA). However, those subjects receiving specific factor replacement therapy at home and consuming at least 66% RDA of ascorbic acid maintained a mean plasma ascorbic acid level not significantly less than controls. Hemophilic subjects not on home therapy, on the other hand, had a mean plasma ascorbic acid level significantly below that of controls while receiving optimal dietary ascorbic acid. With prompt adequate medical care of bleeding episodes and with optimal nutrition, the demand for ascorbic acid needed for tissue repair in hemophilic patients may be lessened. Hemophilic subjects had mean serum vitamin A, mean serum folate, and mean red cell folate levels that were not significantly different from controls. Significantly higher mean plasma arginine and lower, but not significantly lower, mean plasma ornithine levels were found in hemophilic subjects, suggesting altered arginase activity.

DIETARY AND ANTHROPOMETRIC analyses were reported in our recent study of patients at the South Texas Comprehensive Hemophilia Center (STCHC).1 We now report selected biochemical parameters reflecting the nutritional status of these patients. It is not known whether hemophilia affects the utilization or storage of specific vitamins or whether it alters the level of specific amino acids in affected individuals. The present study compared the blood levels of ascorbic acid, vitamin A, folic acid, and amino acids of hemophilic subjects with those of controls.

MATERIALS AND METHODS

Experimental subjects, ranging in age from 4 to 62 yr, were white outpatients with hemophilia A or hemophilia B presently being treated at the South Texas Comprehensive Hemophilia Center (STCHC), Santa Rosa Medical Center, San Antonio. Control white subjects, ranging in age from 13 to 57 yr, included male siblings and fathers of experimental subjects and unrelated normal healthy males not affected with a genetic bleeding disorder. Prior approval to perform this study was obtained from the Santa Rosa Medical Center Institutional Review Board, the University of Texas Health Science Center Institutional Review Board for Human Research, and the Division of Graduate Nutrition of the University of Texas at Austin. The purpose of the study, procedure for blood collection, and risks involved in the study were explained to each volunteer or his legal guardian and informed consent was obtained.

After an overnight fast, blood samples were taken for analyses of plasma ascorbic acid and amino acids, red cell folate, as well as serum vitamin A and folate. The total plasma ascorbic acid levels (oxidized and reduced forms) were measured following the method of Tietz2 using 2,4-dinitrophenylhydrazine. Serum vitamin A analysis was performed using trifluoroacetic acid.3 Serum folic acid was determined using the method described by Herbert,4 and the method of Hoffbrand et al.3 was used to determine red cell folate levels. Vitamin determinations were performed in duplicate samples.

Plasma amino acid determinations were performed on the LKB 4400 Amino Acid Analyser (LKB Biochrom Ltd., Cambridge, England). Details of the complete procedure are described in the LKB Protein Chemistry Notes #12.

Dietary data were collected using the 24-hr recall method for all subjects with hemophilia on the day the blood was drawn. Dietary histories were collected by the nutritionist of the STCHC as a part of the annual comprehensive evaluation, as previously described.5 Both dietary data and blood were obtained during routine annual evaluation of all subjects with hemophilia. Dietary data of pediatric subjects were obtained from their parents/legal guardian. No dietary data were collected from control subjects. USDA Handbook 4566 was used to calculate dietary vitamin A and ascorbic acid of the subjects. Data from Perloff and Buttram7 and Hoppner et al.8 were used to determine total folacin in the diets of the subjects. The dietary intake of the subjects was compared to the 1980 Recommended Dietary Allowances (RDA)9 and expressed as a percent of the RDA.

Hemophilic patients were subdivided according to severity of the disease. Severe hemophiliacs have less than 1%-2% factor activity level; moderate cases range from 2% to 5% and mild cases have 6%-30%.10 The presence of hemophilic arthropathy was documented in the medical record of each subject following orthopedic examination. Any subject who had limitation of motion in any joint or synovitis was classified as having joint disease.

Data analyses were performed using the Statistical Package for the Social Sciences (SPSS).11,12 The mean, standard error of the mean, t test, and one-way analysis of variance with pairwise multiple comparison of means were computed using SPSS. One-way analysis of variance with pairwise multiple comparison of means was used for

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the statistical analysis of the ascorbic acid, vitamin A, folic acid, and arginine data where comparisons within groups were made. Student’s t test was used for all other amino acid data where no comparisons within groups were made. Correlation coefficient by multiple regression was calculated using standard formulas.13

RESULTS

Plasma Ascorbic Acid

The median dietary ascorbic acid intake of subjects with hemophilia who were not taking vitamin C supplement was 188% of the RDA. There was poor correlation between the dietary intake of ascorbic acid, as measured by dietary recall, and plasma ascorbic acid levels \((r = -0.0994)\).

The mean and standard error of the mean of plasma ascorbic acid levels found in controls and hemophilic subjects are given in Table 1. The plasma ascorbic acid levels of 29 subjects with hemophilia was determined; 27 subjects were not taking vitamin C supplement and 2 subjects were taking vitamin C supplement at the time of blood collection. The plasma ascorbic acid levels of the 2 subjects taking vitamin C supplement were not included in the statistical analysis. The mean plasma ascorbic acid level of 40 controls was significantly higher than the mean plasma ascorbic acid of 27 hemophilic subjects \((p < 0.0001)\). Subjects with moderate hemophilia had a mean plasma ascorbic acid level significantly lower than controls \((p < 0.01)\). There was no significant difference in mean plasma ascorbic acid level between those subjects with severe and moderate hemophilia.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Plasma Ascorbic Acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1.21 ± 0.07* (40)</td>
</tr>
<tr>
<td>Hemophilic subjects</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.77 ± 0.08 (27)</td>
</tr>
<tr>
<td>Severe</td>
<td>0.79 ± 0.12† (9)</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.68 ± 0.11 (16)</td>
</tr>
<tr>
<td>Mild</td>
<td>0.85 ± 0.36 (2)</td>
</tr>
<tr>
<td>With joint disease</td>
<td>0.77 ± 0.12 (14)</td>
</tr>
<tr>
<td>Without joint disease</td>
<td>0.68 ± 0.12 (13)</td>
</tr>
<tr>
<td>Vitamin C intake</td>
<td></td>
</tr>
<tr>
<td>&lt;50% RDA</td>
<td>0.53 ± 0.09 (6)</td>
</tr>
<tr>
<td>&lt;66% RDA</td>
<td>0.59 ± 0.14 (8)‡</td>
</tr>
<tr>
<td>&gt;66% RDA</td>
<td>0.82 ± 0.11 (18)‡</td>
</tr>
<tr>
<td>&gt;100% RDA</td>
<td>0.85 ± 0.11 (15)</td>
</tr>
<tr>
<td>On home therapy</td>
<td>0.85 ± 0.12 (15)</td>
</tr>
<tr>
<td>Not on home therapy</td>
<td>0.57 ± 0.09 (12)</td>
</tr>
<tr>
<td>On home therapy, vitamin C intake &gt;66% RDA</td>
<td>0.99 ± 0.13† (11)</td>
</tr>
<tr>
<td>Not on home therapy, vitamin C intake &gt;66% RDA</td>
<td>0.52 ± 0.06 (6)</td>
</tr>
</tbody>
</table>

*Mean ± SEM; numbers in parentheses indicate number of subjects.
†Not significantly different from controls.
‡Dietary data not available from one subject.

Both groups of hemophilic subjects with joint disease and without joint disease had plasma ascorbic acid levels significantly lower than normal controls \((p < 0.01)\). There was no significant difference in mean plasma ascorbic acid between hemophilic subjects with joint disease and hemophilic subjects without joint disease. There was poor correlation between the frequency of bleeding episodes experienced by a patient with hemophilia during a 1-yr period prior to the time of blood collection and the plasma ascorbic acid level \((r = 0.187)\). However, as noted in Table 1, plasma ascorbic acid levels tend to increase with a greater intake of dietary ascorbate. Hemophilic subjects who consume greater than 66% RDA of ascorbic acid and would otherwise be considered to be receiving adequate dietary ascorbate have a mean plasma ascorbic acid level significantly lower than controls \((p < 0.05)\).

Hemophilic subjects not on home therapy have a mean plasma ascorbic acid level significantly lower than controls \((p < 0.001)\), whereas those subjects on home therapy have a mean plasma ascorbic acid level not significantly different from controls (Table 1). The median dietary ascorbic acid intake of hemophilic subjects not on home therapy was 85% RDA. The mean plasma ascorbic acid level was numerically higher for hemophilic subjects on home therapy than for those not on home therapy, but the difference was not statistically significant. Among hemophilic subjects whose dietary intake was greater than 66% RDA, only those subjects who receive home therapy maintained a mean plasma ascorbic acid level that was not significantly different from controls. Hemophilic subjects not on home therapy and who had a dietary ascorbic acid intake greater than 66% RDA had a mean plasma ascorbic acid level significantly lower than controls \((p < 0.05)\). These same hemophilic subjects also had a mean plasma ascorbic acid level similar to the mean plasma ascorbic acid level of hemophilic subjects whose dietary ascorbic acid intake was less than 66% RDA. One-way analysis of variance indicated that there was no significant difference in mean plasma ascorbic acid levels of subjects on home therapy with intakes >66% RDA for ascorbic acid and mean plasma ascorbic acid levels of hemophilic subjects not on home therapy with intakes of vitamin C greater than 66% RDA.

The mean plasma ascorbic acid level of the two subjects with hemophilia who were taking vitamin C supplement was 1.35 ± 0.07 mg/dl, and the median dietary ascorbic acid intake, excluding vitamin supplement, was 440% RDA. One subject could not recall the specific amount of vitamin C supplement he ingested daily. Both subjects in the vitamin-supplemented
group were diagnosed with severe hemophilia, had degenerative joint disease, and were on home therapy. No control subjects were taking vitamin C supplements at the time of blood collection. No subjects in the entire study population had a plasma ascorbic acid level less than 0.20 mg/dl.

**Serum Vitamin A**

The mean and standard error of the mean for the serum vitamin A levels in hemophilic subjects and controls are given in Table 2. The 2 subjects with mild hemophilia had a mean serum vitamin A level of 56.41 ± 18.23 μg/dl. There was no significant difference in mean serum vitamin A levels between controls and hemophilic subjects. There was no correlation between the dietary vitamin A intake, as measured by dietary recall, and the mean serum vitamin A levels among hemophilic subjects (r = 0.066). Ten percent of all hemophilic and control subjects had serum vitamin A levels less than 20 μg/dl.

**Serum and Red Cell Folate**

The mean serum folate and mean red cell folate activity levels for controls and subjects with hemophilia are presented in Table 2. There was no significant difference in the means of serum folate or red cell folate levels between controls and subjects with hemophilia. There was no correlation between the dietary intake of total folacin, as measured by dietary recall, and serum folate (r = -0.214) or red cell folate levels (r = -0.045). Forty-eight percent of the hemophilic subjects reported a dietary intake of total folacin below 50% RDA, but these subjects had a mean serum and red cell folate level of 11.21 ng/ml and 233 ng/ml, respectively. Twenty-eight percent of the hemophilic subjects and 15% of controls had red cell folate levels below 140 ng/ml.

**Plasma Amino Acids**

Eighteen plasma amino acid levels were studied in persons with hemophilia and controls: threonine, serine, glutamine, proline, glyoxine, alanine, citrulline, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, lysine, histidine, and arginine. With the exception of arginine, there were no significant differences in mean plasma amino acid levels of persons with hemophilia and controls. The mean arginine level for all subjects with hemophilia was significantly higher than the mean arginine level for controls (p < 0.0002) (Table 3). Subjects diagnosed with severe or moderate hemophilia had a mean arginine level that was significantly different (p < 0.001 and p < 0.05, respectively) from controls (Table 3).

The amino acids involved in the urea cycle include arginine, ornithine, and citrulline. No significant differences were found in the mean ornithine or citrulline levels of persons with hemophilia compared to controls. However, with respect to ornithine, significance was approached (p < 0.1), and this suggests that a statistical significance would probably have been found with a larger number of subjects (Table 3).

**DISCUSSION**

This study indicates that plasma ascorbic acid levels are lower in persons with hemophilia than among controls. Subjects with moderate hemophilia have a mean plasma ascorbic acid level significantly lower than controls. The mean plasma ascorbic acid level of subjects with severe hemophilia was numerically lower than controls, but not significantly lower, which could be due to the small number of subjects in the group.

| Table 2. Mean Serum Vitamin A and Folate and Red Cell Folate Levels of Subjects |
|-----------------------------|-----------------|-----------------|
| Subjects | Serum Vitamin A (μg/dl) | Serum Folate (ng/ml) | Red Cell Folate (ng/ml) |
| Control | 81.04 ± 25.93* (29) | 9.22 ± 0.73* (40) | 211 ± 14* (40) |
| Hemophilic subjects | | | |
| All | 85.77 ± 9.93 (21) | 8.88 ± 1.05 (25) | 235 ± 27 (25) |
| Severe | 75.00 ± 13.28 (7) | 6.88 ± 1.42 (9) | 275 ± 48 (9) |
| Moderate | 96.77 ± 14.93 (12) | 9.80 ± 1.46 (15) | 244 ± 32 (15) |
| With joint disease | 81.61 ± 14.45 (11) | 8.21 ± 1.66 (13) | 269 ± 38 (13) |
| Without joint disease | 90.34 ± 14.05 (10) | 9.60 ± 1.28 (12) | 210 ± 37 (12) |

*Mean ± SEM; numbers in parentheses indicate number of subjects.

| Table 3. Mean Plasma Arginine and Ornithine Levels of Subjects |
|-----------------------------|-----------------|
| Subjects | Plasma Arginine (mg/dl) | Plasma Ornithine (mg/dl) |
| Controls | 2.13 ± 0.19* (24) | 4.06 ± 0.58* (24) |
| Hemophilic subjects | | |
| All | 3.79 ± 0.33† (24) | 2.64 ± 0.78† (25) |
| Severe | 4.45 ± 0.56† (10) | 3.81 ± 1.73† (11) |
| Moderate | 3.48 ± 0.45† (12) | 1.81 ± 0.23† (12) |

*Mean ± SEM; numbers in parentheses indicate number of subjects.

†Analysis of variance indicates no significant difference in hemophilic subjects compared to controls.

‡Analysis of variance indicates significantly greater values in hemophilic subjects compared to controls.
Plasma ascorbic acid levels do not appear to be a function of the severity of hemophilia, the presence of degenerative joint disease, nor the frequency of bleeding episodes. Although we found a poor correlation between plasma ascorbic acid levels and dietary ascorbic acid intake, as determined by dietary recall, hemophilic subjects in our present study who consume less than 66% RDA of ascorbic acid had a numerically lower mean plasma ascorbic acid level than hemophilic subjects who consume greater than 66% RDA of ascorbic acid. Hemophilic subjects who consume greater than 66% RDA of ascorbic acid had a mean plasma ascorbic acid level that was significantly less than controls. The Recommended Dietary Allowances (RDA) are the levels of intake of essential nutrients considered to be adequate to meet the known nutritional needs of virtually all healthy persons. Since the recommended allowance for most nutrients is increased by two standard deviations above the mean requirement, an intake of 66% of the RDA cannot be interpreted as being inadequate for an individual. However, as dietary intake falls below 50% of the RDA, the risk of nutritional deficiency increases.

Among hemophilic subjects who consume greater than 66% RDA of ascorbic acid, those subjects who receive home therapy maintain a mean plasma ascorbic acid level that was not significantly less than controls. Persons with hemophilia are often placed on a home therapy program so that when a bleeding episode occurs, the patient can promptly transfuse himself with factor replacement. The numerically lower mean plasma ascorbic acid level found in hemophilic subjects not on home therapy, but whose median intake of dietary ascorbic acid was adequate, suggests that the demand for ascorbic acid needed for tissue repair may be lessened when replacement therapy is prompt.

Ascorbic acid is a known cofactor for the hydroxylation of proline in collagen synthesis and has been found to be necessary for an intact peripheral vascular system. Kramer et al. studied the effect of ascorbic acid on collagen production and wound healing in the guinea pig. They concluded that megadose ascorbic acid therapy resulted in a higher production of hydroxyproline during the acute phase of healing, but in long-term healing, megadose ascorbic acid therapy showed no difference in hydroxyproline production.

The nutritional demands imposed by physical stress have been linked to alterations of various vitamins and minerals in humans. Psychologic stress, such as anxiety or emotional tension, can often result in spontaneous hemorrhages into muscles and joints for the patient with hemophilia. A greater physical stress may be placed on the patient with hemophilia not on home therapy as a result of the delay in receiving factor replacement after an injury. Without prompt medical treatment for bleeding episodes, a greater degree of tissue destruction may occur and, therefore, more ascorbic acid may be needed for tissue repair.

Mullen and Wilson reported that patients with rheumatoid arthritis and progressive joint disease have significantly lower plasma and leukocyte ascorbic acid levels than controls due to an increased rate of ascorbic acid utilization. Moran and Greene reported that large amounts of ascorbic acid given orally or parenterally will not result in sustained high concentration of ascorbic acid in the blood, since the maximum tubular reabsorption of ascorbic acid is easily exceeded at serum levels of 1.4 mg/dl. The subjects taking ascorbic acid supplement in this study were possibly at or near tissue saturation level. Sauberlich et al. reported that the first signs of scurvy appear when the plasma ascorbic acid levels range from 0.13 to 0.24 mg/dl. There were no subjects in our study with plasma ascorbic acid below 0.2 mg/dl.

This study shows that plasma ascorbic acid levels of persons with hemophilia can be maintained at near-normal levels through an adequate intake of ascorbic acid, along with prompt medical treatment of bleeding episodes at home. At this time, there is inadequate data to indicate that persons with hemophilia need to ingest large amounts of ascorbic acid in excess of the RDA. Until more information is known about the rate of ascorbic acid utilization in persons with hemophilia, it is recommended that persons with hemophilia consume a well balanced diet and include daily sources of ascorbic-acid-rich foods to maintain an acceptable level of plasma ascorbic acid.

Neither the severity of hemophilia nor the presence of joint disease affected the level of serum vitamin A in persons with hemophilia. The Ten-State Nutrition Survey reported that subjects with serum vitamin A less than 20 μg/dl are at high risk for developing vitamin A deficiency. The majority of hemophilic subjects in the present study did not show a high risk for developing vitamin A deficiency. Serum vitamin A provides limited information about the actual storage of the vitamin in the body, especially in the liver, since serum levels can be maintained relatively constantly until liver stores are nearly exhausted.

While the mean serum and red cell folate levels in subjects with hemophilia appear to be adequate when compared to normal controls, 28% of hemophilic subjects had red cell folate levels below 140 ng/ml, and 8% of subjects with hemophilia had serum folate levels below 3 ng/ml. Neither the severity of hemophilia nor the presence of degenerative joint disease affected folate status in persons with hemophilia. Herbert, Hoffbrand, and others suggested that serum and
red cell folate levels below 3 ng/ml or 140 ng/ml, respectively, are "deficient" levels. The red cell folate level is considered to be a more accurate and less variable quantitative index than serum folate in determining folate deficiency. 

Alter et al. studied serum folate levels of patients with rheumatoid arthritis. They reported that 49% of their study population had serum folic acid levels between 3 and 5 ng/ml, and 22% had serum folate levels less than 3 ng/ml. Although no correlation could be made between poor dietary intake of total folacin and low serum or red cell folate levels in the present study, more than 50% of the subjects with hemophilia consumed less than 66% of the RDA for total folacin. More recent data on the folate content of foods is necessary to better evaluate the dietary intake of the folic acid.

Plasma amino acid levels of persons with hemophilia have never been documented in the medical literature. The significantly higher arginine level and the lower, although not significantly lower, mean ornithine level of persons with hemophilia strongly suggest that persons with hemophilia have lower levels of arginase activity than normal controls. Meister has suggested that increased arginine may have a protective effect against toxic levels of ammonia, presumably by increasing the activity of the urea cycle. A high incidence of hepatitis in patients with hemophilia due to the increased use of factor VIII or IX blood coagulation concentrates prepared from plasma pools of multiple donors has been reported. Chronic abnormalities of liver enzymes, such as elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), have been observed in persons with hemophilia. Cascino et al. reported high levels of aromatic and sulfurated amino acids and low levels of branched-chain amino acids in patients with liver disease. At present, the arginase activity of hemophiliacs is not known. The data from this study raise further questions concerning the relationship of liver enzymes and hemophilia and a possible metabolic block in the urea cycle in patients with hemophilia.

This study, as well as our earlier report, provides important knowledge of the influence of hemophilia on nutritional status and the role of the nutritionist in the care of hemophiliacs. Further research regarding the metabolism of ascorbic acid and arginine in patients with hemophilia is needed, as well as research into other nutrients that may be altered by this chronic illness.

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