PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 363–367 (1982)

Effects of Vitamin C Loading on Serum Constituents in Man[1] (41358)

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Abstract. Loading doses of vitamin C in volunteer subjects produce a variety of changes in serum components. When the grouped means of baseline studies are compared with the results of 12 weeks of vitamin C loading (3.0 g/day), there are significant differences. Serum ascorbic acid, lactate dehydrogenase, and albumin all show increases; sodium, uric acid, alanine aminotransferase, and globulins are significantly decreased when evaluated by Student's *t* test. Although biweekly variations in the lipid parameters of serum cholesterol, HDL cholesterol, triglyceride, lecithin:cholesterol acyltransferase activity, and bile acids were found, there were no significant differences in the grouped means for baseline and experimental periods. There was a significant increase in leukocyte ascorbic acid, but no other change in cell distribution or number. It is suggested that these types of studies might also be interpreted in terms of R. J. Williams' "biochemical individuality" as well as more conventional statistical studies.

The biochemical effects of vitamin C in man have been widely investigated, and some controversy exists regarding the effects on serum lipids and other biochemical constituents. Bates (1) reported a positive correlation between vitamin C status and the level of plasma HDL cholesterol in a group of elderly men. Ginter (2) showed that the conversion of cholesterol to bile acids is slowed in guinea pigs with latent vitamin C deficiency. Kallner (3) studied serum bile acids during the vitamin C supplementation in man and found little change. Spittle (4) observed that vitamin C supplementation affected serum cholesterol to various degrees in different age groups and proposed that ascorbic acid caused increased metabolism of cholesterol and a mobilization of cholesterol deposits in some subjects. Ginter (5) showed that the higher the initial cholesterolemia, the greater the hypocholesterolemic effect after prolonged administration of ascorbate. The interference of aspirin with vitamin C metabolism was investigated by Loh *et al.* (6), who showed that aspirin prevented uptake of ascorbic acid by leukocytes and this change was associated with a high plasma ascorbic acid and an increase in urinary excretion of ascorbic acid.

Stein *et al.* (7) reported ascorbic acid induced uricosuria which is inhibited by pyrazinamide and low-dose acetylsalicylic acid. Krumdieck and Butterworth (8) reviewed ascorbate–cholesterol–lecithin interactions. Van Steirteghem *et al.* (9) studied multiple biochemical parameters before and after administration of vitamin C to 10 healthy adults. The present study was undertaken to define further the biochemical parameters affected by loading doses of ascorbic acid in man in his usual dietary environment.

Methods and Materials. The 26 subjects (22 male and 4 female) were well-motivated, intelligent volunteers, whose ages ranged from 25 to 76 years. They recorded their usual diet for 2 weeks to establish a baseline vitamin C intake. During this period three blood samples were obtained from each subject after a 12-hr fast. These samples served to define baseline values for each individual, and each person thus was his own control. A single 24-hr urine collection was also obtained during this period. Each subject was then instructed to follow the dietary pattern he previously established, and to supplement his diet with

[1] Supported in part by the Wallace Genetic Foundation, South Salem, N.Y., and the Baptist Hospitals Foundation, Birmingham, Ala.

0037-9727/82/030363-05$01.00/0

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1 g of sodium ascorbate three times daily with meals. While it is recognized that this is not dietary control, it does relate in a practical way to a long-term protocol likely to be followed by most individuals. Blood samples were collected at 2-week intervals after a 12-hr fast. A 24-hr urine collection was made during the sixth week of vitamin C supplementation.

The analytical methods are those in use in the Baptist Medical Center laboratories. Cholesterol, triglyceride, glucose, and uric acid were determined enzymatically with the Technicon 12/60 using reagents supplied by Boehringer-Mannheim Corporation. Vitamin C was assayed in serum and leukocytes by the method of Denson and Bowers (10). Inorganic phosphorus, calcium, aspartate aminotransferase, lactate dehydrogenase, creatine phosphokinase, total protein, alkaline phosphatase, bilirubin, sodium, potassium, chloride, bicarbonate, creatinine, and urea nitrogen were all determined using standard Technicon methodology. Bile acids were determined by a radioimmunoassay technique originally described by Spenney (11). Thyroxine was determined by a double antibody RIA technique. HDL cholesterol was determined by the Mn^{2+}-heparin technique described in the manual of the Lipid Research Clinics (12). Protein fractions were determined by electrophoresis on cellulose acetate followed by densitometry. Lecithin:cholesterol acyltransferase (LCAT) activity was measured by the Soloff (13) modification of the method described by Stokke and Norum (14). The data are reported as mean values ± SEM and were evaluated by Student's *t* test for significance of the differences between paired means.

Results. Statistically significant differences ($P < 0.01-0.001$) were found in the grouped data for the following constituents: ascorbic acid in serum and leukocytes; uric acid, sodium and chloride; aspartate aminotransferase, lactate dehydrogenase, albumin and globulin in serum (Table I). Constituents which did not change significantly ($P > 0.05$) were the following: cholesterol, HDL cholesterol, triglyceride, bile acids, LCAT, glucose, rbc, wbc, hemoglobin, hematocrit, urea nitrogen, creatinine, creatine phosphokinase, alkaline phosphatase, bilirubin, inorganic phosphate, calcium, bicarbonate, potassium, thyroxine, amylase, total protein and α-1, α-2, and β globulins.

TABLE I. SERUM CONSTITUENT LEVELS AFTER ASCORBIC ACID LOADING

Serum constituent	Baseline	After ascorbic acid loading
Serum ascorbic acid[a]	1.2 ± 0.10	2.1 ± 0.12
Leucocyte ascorbic acid[b]	28 ± 1.2	37 ± 1.2
Lactate dehydrogenase[c]	149 ± 6.67	167 ± 8.24
Aspartate aminotransferase[c]	36 ± 2.8	28 ± 1.8
Uric acid[a]	6.2 ± 0.24	5.7 ± 0.22
Sodium[d]	141 ± 0.451	139 ± 0.353
Chloride[d]	105 ± 0.392	103 ± 0.588
Albumin[e]	3.9 ± 0.045	4.0 ± 0.047
Globulin[e]	1.24 ± 0.053	1.14 ± 0.057

Note. Values are means ± SE for 26 subjects.
[a] mg/dl.
[b] $\mu g/10^8$ cells.
[c] U/liter.
[d] meq/liter.
[e] g/dl.

The possibility of ascorbic acid interference with analytical procedures was evaluated as follows. A quality control serum (Monitrol I) was prepared using solutions of 2.5 and 5.0 mg/dl of ascorbic acid as diluents. These control sera were analyzed along with the control and experimental samples. These concentrations of ascorbic acid were chosen because they bracketed the range of ascorbic acid values found in serum samples of the subjects following loading doses of ascorbic acid. All the Monitrol samples were from a single lot number. These samples were prepared and run with the experimental samples until 48 replicate sets of data were obtained.

The statistical data concerning ascorbate interferences are shown in Table II. The only significant difference ($P > 0.01$) was a slight inhibition of the enzymic cholesterol analysis as originally observed by Allain *et al.* (15). All other constituents which changed after ascorbic acid loading were unaffected by 2.5–5.0 mg/dl of ascorbic acid in these *in vitro* studies.

Discussion. *Serum and leukocyte ascorbic acid values.* The serum ascorbic acid baseline in 26 subjects was 1.2 ± 0.10

TABLE II. THE EFFECTS OF ASCORBIC ACID *IN VITRO* ON SERUM CONSTITUENTS AFFECTED BY 3.0 g/DAY *IN VIVO*

Constituent[a]	Control baseline[b]	Ascorbate supplement: Control + 2.5 mg/dl	Ascorbate supplement: Control + 5.0 mg/dl	*P*
Sodium	144 ± 0.144	145	144	NS[c]
Potassium	5.9 ± 0.010	5.9	5.9	NS
Aspartate aminotransferase	32.3 ± 0.289	31.4	31.0	NS
Lactate dehydrogenase	248 ± 0.91	247	247	NS
Uric acid	6.8 ± 0.023	6.9	6.8	NS
Albumin	3.45 ± 0.023	3.42	3.40	NS
Globulin	0.67 ± 0.016	0.68	0.70	NS
Cholesterol	132 ± 0.88	128	125	0.01
HDL cholesterol	45.9 ± 0.253	45.3(NS)	42.8	0.01

Note. Results are means ± SE for 48 replicate analyses.
[a] Units same as Table I.
[b] Monitrol (Dade, Amer. Sci. Products).
[c] Not significant.

mg/dl. After the ascorbic acid loading period, the values increased to a mean of 2.1 ± 0.12 mg/dl. Only three subjects had values greater than 3.0 mg/dl during the loading period. Leukocyte ascorbate during the baseline periods was found to have a mean value of 28.0 ± 1.2 $\mu g/10^8$ cells while following loading with ascorbate, the mean was 37.0 ± 1.2 $\mu g/10^8$ cells.

These data compare favorably with Loh and Wilson (16) and in general reflect the findings reviewed by Irwin and Hutchins (17). Loh and Wilson studied the relationship between leukocyte and plasma ascorbic acid concentrations. They found the mean plasma ascorbic acid to be 0.87 ± .082 mg/dl in 14 normal adult males in the age group of 33–49 years. The leukocyte values were 30.4 ± 2.3 $\mu g/10^8$ cells. A linear correlation between leukocyte and plasma ascorbate levels was demonstrated. Kallner *et al.* (18) suggested that the body ascorbic acid pool is saturated at about 1500 mg and cannot be increased by additional intake of ascorbic acid. They found plasma concentrations of 1.9 mg/dl after one week of daily doses of 2000 mg of ascorbate.

Uric acid. Decreases in serum levels of uric acid occurred in 22 of 26 subjects and the other 4 did not change.

The uricosuric effect of ascorbate is well known. Berger *et al.* (19) studied the clearance of ascorbic acid and uric acid in man. The clearance of ascorbic acid in five normal men ranged from 28.6 to 85.4 mg/min after loading doses of ascorbate. Endogenous clearance in the same subjects ranged from 2.4 to 21 mg/min. Berger suggested that ascorbate competes with urate for renal tubular reabsorptive transport, probably in the proximal tubule. Stein *et al.* (7) also found uricosuria with diminished serum urate levels. These investigators also noted that the uricosuria was inhibited by a low dose of acetylsalicylate. We had previously reported (20) similar effects of acetylsalicylate in man. When acetylsalicylate and ascorbic acid are administered together, Loh *et al.* (6) reported that the uptake of ascorbate into leukocytes is reduced, and this change is associated with a high plasma ascorbic acid and an increase in the urinary excretion of ascorbic acid.

Sodium and chloride. Despite a small daily sodium load of approximately 15 meq/liter from sodium ascorbate, the serum sodium and chloride values showed decreases following ascorbate loading ranging from 1 to 6 meq/liter in 19 of 26 subjects. The mean serum sodium during the ascorbate loading period decreased to 139.2 meq/liter from the baseline mean of 140.8. Statistical analysis of the data by grouped means shows the differences in the means to be significant at the 0.001 level. This effect of ascorbate loading has not been previously reported.

While there is no direct explanation for the serum sodium decrease, it could be inferred from the work of Selkurt and Houck (21). They reported in 1943 that hypertonic sodium and potassium chloride solutions impaired the reabsorptive activity of the tubules for ascorbic acid so that the reabsorptive T_m for ascorbic acid was depressed. The reciprocal relationship should follow. This finding is supported by comparison of sodium data from 24-hr urine collections done at the midpoint of the baseline and vitamin C studies. These data show that 13 of 16 subjects had increases in 24-hr sodium excretion ranging from 3.6 to 77% following ascorbic acid.

Aspartate transaminase and lactate dehydrogenase. Twenty-four of twenty-six subjects had decreases in aspartate aminotransferase serum levels relative to the control period. Similarly, 23 of 26 subjects showed an increase in lactate dehydrogenase. The *t* values indicate significance of these data at 0.001 level for both determinations. The findings are apparently not analytical artifacts as indicated by the data in Table II.

These findings contrast with those reported by Steirteghem *et al.* (9) who reported variable responses of both these enzyme activities following 3 g of ascorbic acid per day administered to 10 adults for 18 days. They did, however, show a common decrease in alanine aminotransferase in which all individuals appeared to respond similarly. Perhaps the differences in their findings and ours are related to the period required to attain a new level of activity in response to the ascorbic acid. In contrast, Knodell *et al.* (22) showed a decrease in the serum aminotransferase (GPT) in ascorbate-treated hepatitis patients, although the differences in the data for six subjects were not significant.

Albumin and globulin. There were statistically significant differences ($P < 0.01$) in the baseline means and ascorbate values for albumin and globulin. As Cerna and Ginter (24) have stated, "statistical significance does not necessarily mean clinical significance." These data are reported because many studies have implicated vitamin C in a variety of immunological roles such as affecting the common cold, post-transfusion hepatitis (22), and possible alterations in immune status (24). These data, along with the enzyme data, may hold some clue for further investigation relative to liver cell function and/or the immune system.

Cholesterol, HDL cholesterol, triglycerides, and bile acids. The negative findings in this study relative to serum lipid constituents are mentioned because of the lack of agreement in the literature regarding the effects of ascorbate on these constituents. Much data have been obtained from subjects who might be considered to be borderline deficient in vitamin C. Ginter (5) found the hypocholesterolemic effect of vitamin C related to initial cholesterol levels. He found no effect in healthy people with initial cholesterol values below 200 mg/dl, but a definite effect in hypercholesterolemic subjects with low vitamin C intake. Although there were two subjects in the present study whose baseline plasma vitamin C was 0.2 and 0.4 mg/dl, and whose cholesterol was greater than 200 mg/dl, there was no significant change in the individual or the grouped data after 12 weeks of 3.0 g/day of ascorbate. Peterson (25) has also reported no significant changes in cholesterol or triglyceride levels in hypercholesterolemic subjects receiving ascorbic acid supplements. Bates study (1) of elderly healthy people living at home showed a strong positive correlation between vitamin C status and plasma HDL cholesterol in men but not in women. Neither men nor women showed significant correlation between serum or leukocyte ascorbate and total plasma cholesterol. Van Steirteghem (9) found no significant changes in triglycerides after feeding 3 g/day of ascorbate for 18 days. They also reported no significant group changes in cholesterol levels, but different individual responses ranging from −190 to +160 mg/liter. Pederson (26) gave 10 healthy persons 1 g of ascorbic acid daily for 7 to 15 days, and the total bile acids, cholesterol, and phospholipids in bile were determined. He found no support for the hypothesis that vitamin C controls the transformation of cholesterol into bile acids.

Horning and Weiser (27) showed in guinea pigs that total bile acids were significantly reduced when the animals were deficient in vitamin C. When the animals were on an adequate intake, conversion of cholesterol to bile acids could not be further stimulated. Ginter (28) showed that animals deficient in vitamin C accumulated cholesterol in the blood serum and postulated a decreased rate of transformation in the liver of ascorbate-deficient animals.

In this study, we found no significant differences in the grouped means for cholesterol, HDL cholesterol, triglycerides, or bile acids when the data for the 6-week

control period is compared with the 12-week experimental period. However, there are individual variations that become apparent in plots of serum cholesterol values for individual subjects which agree with the findings of Spittle (4). It is also interesting to note that 17 of 26 subjects had increases in serum bile acids averaging 45% even though differences between the grouped means are not significant ($P > 0.05$). Perhaps these kinds of data should be evaluated in terms of biochemical individuality (29) as well as the more conventional approach of considering a group of individuals as a statistical clone.

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Received February 25, 1981. P.S.E.B.M. 1982, Vol. 169.