Relation of Serum Ascorbic Acid to Serum Vitamin B\textsubscript{12}, Serum Ferritin, and Kidney Stones in US Adults

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Background: Concern has been raised that high levels of ascorbic acid consumption may lead to potential adverse effects, such as vitamin B\textsubscript{12} deficiency, iron overload, and kidney stones.

Objective: To examine the relation of serum ascorbic acid level, which reflects intake, to serum vitamin B\textsubscript{12} level, serum ferritin level, and kidney stones.

Methods: We analyzed data collected on a random sample of the US population enrolled in the Second National Health and Nutrition Examination Survey, 1976-1980. We analyzed data using linear and logistic regression models. Serum ascorbic acid, serum vitamin B\textsubscript{12}, hemoglobin, red blood cell mean corpuscular volume (MCV), and serum ferritin levels were measured using standardized protocols. History of kidney stones was determined by self-report.

Results: After multivariate adjustment, serum ascorbic acid level was associated with higher serum vitamin B\textsubscript{12} levels among women in regression models that assumed a linear relationship; each 57-µmol/L (1.0-mg/dL) increase in serum ascorbic acid level (range, 6-153 µmol/L [0.1 to 2.7 mg/dL]) was independently associated with a serum vitamin B\textsubscript{12} level increase of 60 pmol/L (81 pg/mL) (\textit{P} < .001). Among men, serum ascorbic acid level was marginally associated with higher serum vitamin B\textsubscript{12} levels: each 57-µmol/L (1.0-mg/dL) increase in serum ascorbic acid level was associated with a serum vitamin B\textsubscript{12} level increase of 27 pmol/L (36 pg/mL) (\textit{P} = .10). In addition, serum ascorbic acid level was not associated with correlates of vitamin B\textsubscript{12} deficiency, such as higher MCV levels, macrocytosis (MCV >100), or lower hemoglobin concentrations. Serum ascorbic acid level was not independently associated with serum ferritin levels. However, among women only, serum ascorbic acid levels were associated in a nonlinear fashion with prevalence of elevated serum ferritin levels (\textit{P} = .02). We found no association between serum ascorbic acid level and prevalence of kidney stones in women or men (both \textit{P} > .05).

Conclusions: Serum ascorbic acid levels were not associated with decreased serum vitamin B\textsubscript{12} levels (or indicators of vitamin B\textsubscript{12} deficiency), prevalence of kidney stones, serum ferritin levels, or—among men—prevalence of elevated serum ferritin levels. Serum ascorbic acid levels were associated with prevalence of elevated serum ferritin levels among women. Although the clinical relevance of these findings is uncertain, it seems prudent to suggest that women with a genetic susceptibility to iron overload should consider moderating their intake of ascorbic acid.

Arch Intern Med. 1999;159:619-624

Many Americans consume ascorbic acid supplements daily.\textsuperscript{1} Although high levels of ascorbic acid ingestion have been judged safe in healthy persons,\textsuperscript{2-4} concern has been raised regarding the relation of such ingestion to vitamin B\textsubscript{12} deficiency, iron overload, and kidney stones.\textsuperscript{5-8} Most studies that have examined these relations have analyzed data from small numbers of subjects\textsuperscript{7,9-15} or from laboratory experiments.\textsuperscript{5,16-18} The use of ascorbic acid supplements may have important public health implications, particularly if adverse health effects result as a consequence.

To examine the relation of blood ascorbic acid level, which reflects usual dietary intake, to serum vitamin B\textsubscript{12} level, serum ferritin level, and kidney stones, we analyzed data from participants of the Second National Health and Nutrition Examination Survey (NHANES II), a study that surveyed a large random sample of the American population.

RESULTS

Baseline characteristics of women and men are presented in Table 1. Men had higher dietary iron intakes and serum ferritin levels compared with women but roughly comparable serum vitamin B\textsubscript{12} levels. Approximately 3% of women and 5% of men reported a history of kidney stones. Prevalence of high serum ferritin levels was approximately 5% among women and 8% among men. A varying number of subjects in our sample gave a history of using vitamin C supplements, and, as ex-
METHODS

SUBJECTS

NHANES II was a national probability survey of more than 20,000 Americans conducted between 1976 and 1980. Participants, whose age ranged from 3 to 74 years, were interviewed and examined by study personnel at 2 visits. We analyzed data from adult participants between the ages of 20 and 74 years. Data were available from 10,735 participants who answered questions regarding a history of kidney stones. Serum vitamin B₁₂ levels and serum ferritin levels were available for 1798 participants and 3407 participants, respectively.

MEASUREMENTS

NHANES II questionnaire data included self-reported age, race, sex, menopausal status, years of education completed, level of leisure time physical activity, history of smoking, level of alcohol intake, gout, and use of diuretic medications, hormones, and nutritional supplements. Nutrition data were collected using a food frequency questionnaire and 24-hour diet recall. We calculated body mass index (a measure of weight in kilograms divided by the square of height in meters) from weight and height data recorded during the physical examination. The questionnaires, dietary methods, and examination procedures used in NHANES II have been described elsewhere in detail.

Serum ascorbic acid levels were measured at the Centers for Disease Control and Prevention by the dinitrophenylhydrazine method using a standardized protocol. Because there were a small number of extreme serum ascorbic acid values of questionable validity (ranging as high as 1028 μmol/L [18.1 mg/dL]), we excluded participants with ascorbic acid values of questionable validity (ranging as high as 1028 μmol/L [18.1 mg/dL]), we excluded participants with ascorbic acid levels in the top 0.5% of the sample (n = 54). Ascorbic acid levels were measured at the University of Kansas Medical Center for participants using the radioassay method described by Gunter et al. Serum ferritin levels were measured at the University of Kansas Medical Center, Kansas City. For both serum vitamin B₁₂ and serum ferritin assays, a subsample of persons was selected as follows: all persons whose NHANES II numbers ended in 8 and persons with 1 or more abnormal hematologic values (red blood cell count, hemoglobin, hematocrit, red blood cell mean corpuscular volume [MCV], white blood cell count). An additional ferritin subsample of 100 participants was also randomly chosen at the end of the survey for ferritin analysis. A 2-site immunoradiometric assay was performed and samples with high serum ferritin levels (>200 μg/L) and low serum ferritin levels (<20 μg/L) were reasayed. To identify participants with elevated serum ferritin levels, we used the age- and sex-specific cut points published by the NHANES II Expert Scientific Working Group on iron status of the US population. These cut points were more than 200 μg/L in men and more than 150 μg/L in women aged 20 to 44 years; more than 300 μg/L in men and 200 μg/L in women aged 45 to 64 years; and more than 400 μg/L in men and more than 300 μg/L in women aged 65 to 74 years.

A history of kidney stones was established by a positive response to either of the following 2 questions: “Have you ever had kidney stones?” and “Did a doctor ever tell you that you had kidney stones or stones in the ureter?”

STATISTICAL METHODS

We examined the distribution of ascorbic acid concentrations and other variables of interest using sample weights. We used simple and multiple linear regression models to examine the relation of serum ascorbic acid level to serum vitamin B₁₂ level, hemoglobin concentration, MCV, and serum ferritin level. For the dichotomous outcomes, ie, presence of elevated serum ferritin levels and kidney stones, we used simple and multiple logistic regression models. Level of education, age, and serum ferritin level were adjusted for multiple variables in the multivariate models.

Analyses were performed using Stata software that included survey commands for the analysis of complex survey data. We calculated linear regression coefficients and 95% confidence intervals (CIs) to estimate differences in serum vitamin B₁₂ level, hemoglobin concentration, MCV, and serum ferritin level associated with each 57-μmol/L (1.0-mg/dL) increase in serum ascorbic acid level. Odds ratios and their 95% CIs were calculated using logistic regression to estimate the relative prevalence of elevated serum ferritin levels and kidney stones. Hosmer-Lemeshow goodness-of-fit tests modified for weighted data were performed using a SAS macro program. Two-tailed P values of .05 were considered to be statistically significant, unadjusted for multiple comparisons.

Figures examining the relation of serum ascorbic acid level to serum vitamin B₁₂ level and hemoglobin concentration were calculated using linear and quadratic coefficients derived from linear regression models. Predicted levels of the outcome variables were calculated within each 6-μmol/L (0.1-mg/dL) serum ascorbic acid level and the lowest smoothing procedure was applied to produce the plots. Figures that examined the association between serum ascorbic acid level and prevalence of elevated serum ferritin level were used to plot the adjusted prevalence of elevated serum ferritin level as a function of serum ascorbic acid level. Logistic coefficients were used to compute individual predicted probability of elevated serum ferritin level. Next, we computed the mean of these probabilities within each 6-μmol/L (0.1-mg/dL) serum ascorbic acid level and then applied the lowest smoothing procedure to produce the plots.

We examined whether serum ascorbic acid level was associated with serum vitamin B₁₂ level in several models (Table 2). In univariate, age-adjusted, and multivariate models, serum ascorbic acid level was associated with higher serum levels of vitamin B₁₂ in women; in the multivariate model, each 57-μmol/L (1.0-mg/dL) increase in serum ascorbic acid level was independently associated with
a serum vitamin B12 level increase of 60 pmol/L (81 pg/ml) in women (P < .001). Among men, each 57-µmol/L (1.0-mg/dL) increase in serum ascorbic acid level was marginally associated with a serum vitamin B12 level increase of 27 pmol/L (36 pg/mL) (P = .10). Multivariate models controlled for the effects of a number of variables, including age, race, smoking, alcohol consumption, and use of vitamin B supplements. Because we also identified significant curvilinear relations between serum ascorbic acid and serum vitamin B12 levels (P = .09 in men and .04 in women) and because the proportion of variance explained by both the linear and nonlinear models was almost identical, we also present these relations using models containing linear and quadratic terms (Figure 1).

Less than 1% of participants had low serum vitamin B12 levels (< 221 pmol/L [300 pg/ml]) consistent with vitamin B12 deficiency. Serum ascorbic acid level was associated with a decreased prevalence of low serum vitamin B12 levels in women (P = .001) but was not associated with prevalence of low serum vitamin B12 levels in men (P = .38). Because the assay used in NHANES II cannot distinguish between active and inactive analogs of vitamin B12, we also examined whether serum ascorbic acid levels were associated with indicators of vitamin B12 deficiency, such as hemoglobin concentration, red blood cell MCV, and macrocytosis (MCV > 100). Among participants in whom measurements of serum vitamin B12 were performed, we found no association between serum ascorbic acid level and hemoglobin concentrations in men (P = .34) but found a significant curvilinear relation among women (P ≤ .001) (Figure 2). We also examined whether serum ascorbic acid level was a correlate of red blood cell MCV and prevalence of macrocytosis. Serum ascorbic acid level was not associated with either MCV or macrocytosis in women or men (all P > .08). Furthermore, self-reported ascorbic acid supplement use was not independently associated with serum vitamin B12 levels in either women (P = .92) or men (P = .11).

We also assessed the relation between serum ascorbic acid and ferritin levels (Table 3). In univariate models, serum ascorbic acid level was significantly associated with serum ferritin level among women (P < .01) but not among men (P = .99). The association between ascorbic acid and iron status among women was attenuated after multivariate adjustment (P = .05) and further attenuated after controlling for the additional effects of menopausal status and hormone use (P = .14). Serum ascorbic acid level was not associated with elevated serum ferritin levels in men (P = .59) (Table 4) but was independently associated in a nonlinear fashion with elevated serum ferritin levels in women (P = .02) (Figure 3). Women with the highest serum ascorbic acid levels were associated with indicators of vitamin B12 deficiency, such as hemoglobin concentration, red blood cell MCV, and macrocytosis (MCV > 100).

Table 1. Characteristics of the Study Subjects (5785 Women and 5214 Men)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women, Mean (SD)</th>
<th>Men, Mean (SD)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ascorbic acid level, µmol/L (mg/dL)†</td>
<td>62 (28)</td>
<td>51 (28)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum B12 level, pmol/L, [µg/mL]†</td>
<td>489 (181)</td>
<td>483 (146)</td>
<td>.46</td>
</tr>
<tr>
<td>Serum ferritin level, µg/L‡</td>
<td>60 (65)</td>
<td>119 (99)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>42.5 (15.6)</td>
<td>42.1 (15.3)</td>
<td>.19</td>
</tr>
<tr>
<td>Dietary iron intake, mg/d</td>
<td>10.7 (5.7)</td>
<td>16.2 (8.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Dietary calcium intake, mg/d</td>
<td>600 (442)</td>
<td>899 (659)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.1 (5.6)</td>
<td>25.5 (4.0)</td>
<td>.01</td>
</tr>
<tr>
<td>White, %</td>
<td>87</td>
<td>86</td>
<td>.37</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>34</td>
<td>42</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Level of alcohol consumption, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥6 drinks/wk</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>1 to 5 drinks/wk</td>
<td>51</td>
<td>56</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>&lt;1 drink/wk</td>
<td>42</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Level of education, %</td>
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</tr>
<tr>
<td>High school or greater</td>
<td>68</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Some high school</td>
<td>18</td>
<td>16</td>
<td>.03</td>
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<tr>
<td>No high school</td>
<td>14</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Level of leisure time</td>
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<td></td>
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</tr>
<tr>
<td>physical activity, %</td>
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</tr>
<tr>
<td>Frequent</td>
<td>31</td>
<td>39</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Seldom</td>
<td>56</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>13</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>History of kidney stones, %§</td>
<td>3</td>
<td>5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diuretic therapy, %¶</td>
<td>13</td>
<td>6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>History of gout, %¶</td>
<td>2</td>
<td>4</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*P values for continuous variables and categorical variables were obtained by linear regression and logistic regression, respectively, taking into account sample weights and survey design effects. All numbers reflect sample weights.

†P = 908 and 890 for women and men, respectively.
‡P = 5618 and 5117 for women and men, respectively.
§P = 5750 and 1587 for women and men, respectively.
¶P = 908 and 890 for women and men, respectively.

Figure 1. Relation between serum ascorbic acid concentration and serum vitamin B12 level among 888 women (O) and 880 men (+) enrolled in the Second National Health and Nutrition Examination Survey (NHANES II), 1976-1980, based on the multivariate model noted in Table 2.
Concern has been expressed that high levels of ascorbic acid consumption may be placing many Americans at risk for adverse health outcomes, such as vitamin B₁₂ deficiency, iron overload, and kidney stones. Several studies that have examined these issues were performed in a laboratory setting or on small numbers of subjects. Among a representative sample of the adult US population surveyed between 1976 and 1980, we found no evidence that higher serum ascorbic acid levels resulting from ascorbic acid consumption adversely affected serum vitamin B₁₂ levels, prevalence of kidney stones, or, among men, prevalence of elevated serum ferritin levels. We did, however, find a nonlinear relation between serum ascorbic acid level and prevalence of elevated serum ferritin levels among women, which appeared similar to the relation between serum ascorbic acid level and hemoglobin concentration among women.

**SERUM VITAMIN B₁₂ LEVELS**

Using the same vitamin B₁₂ radioassay used in NHANES II, Herbert and Jacob reported that the addition of ascorbic acid to homogenized test meals resulted in the destruction of vitamin B₁₂. These findings, however, could not be replicated. Herbert and colleagues later reported that of 18 patients with spinal cord injury who received 2 g of ascorbic acid for periods up to several years were vitamin B₁₂ deficient. Others, however, have judged these findings to be, at least in part, the result of an artifact of the assay used to measure vitamin B₁₂ levels. Examination of the relation of high intakes of ascorbic acid in 10 patients with spinal cord injury and among 40 children with myelomeningoceles failed to reveal an association between ascorbic acid and vitamin B₁₂ deficiency. To our knowledge, there have been no large population-based studies examining the relation of ascorbic acid to vitamin B₁₂ status. We found no evidence that serum ascorbic acid levels are associated inversely with serum vitamin B₁₂ levels. On the contrary, it appeared that higher ascorbic acid serum levels were, on average, associated with higher serum vitamin B₁₂ levels among women and men. These findings could be the result of a correlated intake of these nutrients, either in the diet or in nutritional supplements.

**SERUM FERRITIN LEVELS**

Although serum ferritin levels may be elevated as a consequence of inflammatory conditions, they generally reflect total body iron stores and are considered the best measure to assess iron status in epidemiologic studies. Ascorbic acid enhances the absorption of nonheme iron and, therefore, may aid in the treatment of iron-deficiency anemia. However, ascorbic acid may be detrimental to individuals who carry the gene for hemochromatosis. Concern has been expressed that high levels of ascorbic acid consumption may be placing many Americans at risk for adverse health outcomes, such as vitamin B₁₂ deficiency, iron overload, and kidney stones.

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**Figure 2.** Relation between serum ascorbic acid concentration and hemoglobin concentration among 888 women enrolled in the Second National Health and Nutrition Examination Survey (NHANES II), 1976-1980, based on a multivariate model that included menopausal status, hormone use, and all variables noted in Table 2.

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<table>
<thead>
<tr>
<th>Table 3. Relation of Serum Ascorbic Acid Level (per 57 µmol/L [1 mg/dL]) to Serum Ferritin Level (µg/L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>Univariate model</td>
</tr>
<tr>
<td>Age-adjusted model</td>
</tr>
<tr>
<td>Multivariate model</td>
</tr>
</tbody>
</table>

* Multivariate models adjusted for age, race, body mass index, smoking, education level, alcohol intake, level of physical activity, dietary iron intake, and use of iron supplements (including pills and injections). For women, multivariate models also adjust for menopausal status and hormone use. CI indicates confidence interval.

†P < .01.
examined in other large population-based studies. One small nonrandomized study of 17 healthy college students revealed no adverse effect from high-dose ascorbic acid ingestion (2 g/d) on serum ferritin levels and iron stores.14 The clinical significance of our findings in women is uncertain. Several reviews have concluded that, for most individuals, high levels of ascorbic acid consumption are safe.2-4 Furthermore, other analyses of NHANES data have reported improved health outcomes and lower mortality rates among individuals with high serum or intake levels of ascorbic acid.33-35

**KIDNEY STONES**

Most kidney stones are composed of calcium oxalate or uric acid. Although not observed uniformly,12,36,37 some studies have reported that urinary oxalate and uric acid excretion are increased by the ingestion or administration of large amounts of ascorbic acid.2 Studies that examined the relation of ascorbic acid to oxalate and uric acid excretion have generally examined small numbers of subjects.2 We were able to examine the relation of serum ascorbic acid level to history of kidney stones among a random sample of more than 10 000 Americans and found no evidence to indicate that high serum ascorbic acid levels increased the prevalence of kidney stones. On the contrary, among men, each 57-µmol/L (1.0-mg/dL) increase in serum ascorbic acid level was independently associated with an approximately 28% decrease in the prevalence of kidney stones (P = .06). These findings agree with the conclusions of others2 that in general, high levels of ascorbic acid ingestion do not result in increased kidney stone formation and may, in fact, be associated with a decreased prevalence of kidney stones.38

The main limitation of our study derives from the cross-sectional nature of the analyses. Thus, we cannot be certain that the outcomes did not affect ascorbic acid consumption and, in turn, serum ascorbic acid levels. For example, it is possible that persons with kidney stone disease decreased their ascorbic acid intake and consequently lowered their blood ascorbic acid level, thereby producing the ostensible protective association between ascorbic acid and kidney stones. Although we cannot exclude this possibility, it is also biologically plausible that ascorbic acid may, in fact, lower the risk of kidney stones by decreasing urinary calcium excretion.13 Because most people are unlikely to know their serum ferritin level or vitamin B12 level, it seems less probable that these associations resulted from individuals changing their ascorbic acid intake. The serum vitamin B12 radiodilution assay used by NHANES II measures both biologically active and inactive vitamin B12 analogs.39 Hence, we cannot be certain that the higher levels of serum vitamin B12 associated with serum ascorbic acid levels reflect higher levels of the biologically active form of the vitamin. However,
examination of the reliability of the serum vitamin B₁₂ radiodilution assay, when compared with the microbiologic vitamin B₁₂ assay, suggests that the comparative rank order of serum values is likely to be similar across assays. Higher serum ascorbic acid levels were not associated with lower hemoglobin concentrations, higher red blood cell MCVs, or increased prevalence of macrocytosis, which further suggests that serum ascorbic acid levels, which reflect ascorbic acid intake, are unlikely to be associated with decreased serum levels of biologically active vitamin B₁₂. Although serum ascorbic acid levels reflect intake patterns over days to weeks, we had only a single measurement of serum ascorbic acid, which may not reflect longer-term intake. Finally, because the genotype of participants is not known, we cannot comment on the relation of serum ascorbic acid levels to elevated serum ferritin levels among individuals categorized by their genetic susceptibility to iron overload.

There are 2 notable strengths to these analyses. First, NHANES II surveyed a large random sample of the US population using standardized procedures and protocols. Hence, our findings should be generalizable. Second, the measurement of serum ascorbic acid levels on such a large number of persons provides a more accurate and precise assessment of ascorbic acid status as a correlate of serum vitamin B₁₂ level, serum ferritin level, and history of kidney stones than studies using dietary and supplement estimates only.

Establishing the safety of high levels of ascorbic acid ingestion is of public health importance in view of the large percentage of Americans consuming supplements containing ascorbic acid. Our results suggest that ascorbic acid serum levels, reflecting a broad range of ascorbic acid intakes, do not adversely affect vitamin B₁₂ status, prevalence of kidney stones, or, among men, prevalence of elevated serum ferritin levels. Because women with the highest serum ascorbic acid levels had an increased prevalence of elevated serum ferritin levels, it seems prudent to recommend that women with a genetic susceptibility to iron overload moderate their consumption of ascorbic acid. Although our results should be interpreted cautiously in view of the cross-sectional nature of the study design, they do, in general, concur with the findings of other investigators.

Accepted for publication July 14, 1998.

This study was supported by grant HL53479 from the Public Health Service, Bethesda, Md, and an unrestricted research grant from Hoffmann-La Roche Inc, Parsippany, NJ. Reprints: Joel A. Simon, MD, General Internal Medicine Section (111A1), San Francisco Veterans Affairs Medical Center, 4150 Clement St, San Francisco, CA 94121 (e-mail: jasimon@itsa.ucsf.edu).

REFERENCES