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) \((P = .001)\). 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) \((P = .10)\). 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 \((P = .02)\). We found no association between serum ascorbic acid level and prevalence of kidney stones in women or men (both \(P > .05)\).

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.

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Many Americans consume ascorbic acid supplements daily.\textsuperscript{1} Although high levels of ascorbic acid ingestion have been judged safe in healthy persons,\textsuperscript{2,3} 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{3,16-18} 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.

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 1,798 participants and 3,407 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 levels in the top 0.5% of the sample (n = 54). Ascorbic acid levels for the remaining 99.5% of the participants were measured at the Cen- ters 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 levels in the top 0.5% of the sample (n = 54). Ascorbic acid levels for the remaining 99.5% of the participants ranged from 6 to 153 µmol/L (0.1-2.7 mg/dL).

Serum vitamin B₁₂ levels were measured in a subgroup of participants using the radioassay method described by Gunter et al and Laut 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 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.

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

### 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</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>
</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.

†N = 5785 and 5187 for women and men, respectively.

‡N = 1795 and 1612 for women and men, respectively.

§N = 5785 and 5187 for women and men, respectively.

¶N = 5782 and 5213 for women and men, respectively.

### Table 2. Relation of Serum Ascorbic Acid Level (per 57 µmol/L [1 mg/dL]) to Serum Vitamin B12 (pmol/L)*

<table>
<thead>
<tr>
<th>Vitamin B12 (pmol/L)</th>
<th>Women Slope (95% CI)</th>
<th>Men Slope (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>489</td>
<td>44 (13 to 75)†</td>
<td>890 27 (−6 to 60)</td>
</tr>
<tr>
<td>600</td>
<td>42 (0.8)</td>
<td>890 27 (−6 to 60)</td>
</tr>
</tbody>
</table>

*Multivariate models adjusted for age, race, body mass index, smoking, education level, alcohol intake, level of physical activity, and vitamin B supplement use. CI indicates confidence interval.

Appendix 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.

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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 B$_{12}$ 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 B$_{12}$ 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 B$_{12}$ LEVELS**

Using the same vitamin B$_{12}$ radioassay used in NHANES II, Herbert and Jacob$^7$ reported that the addition of ascorbic acid to homogenized test meals resulted in the destruction of vitamin B$_{12}$. These findings, however, could not be replicated.$^8$ Herbert and colleagues$^9$ later reported that 4 of 18 patients with spinal cord injury who received 2 g/d of ascorbic acid for periods up to several years were vitamin B$_{12}$ 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 B$_{12}$ levels.$^3$ Examination of the relation of high intakes of ascorbic acid in 10 patients with spinal cord injury$^9$ and among 40 children with myelomeningoceles$^3$ failed to reveal an association between ascorbic acid and vitamin B$_{12}$ deficiency. To our knowledge, there have been no large population-based studies examining the relation of ascorbic acid to vitamin B$_{12}$ status. We found no evidence that serum ascorbic acid levels are associated inversely with serum vitamin B$_{12}$ levels. On the contrary, it appeared that higher ascorbic acid serum levels were, on average, associated with higher serum vitamin B$_{12}$ 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.$^3$ Ascorbic acid enhances the absorption of nonheme iron$^3$ 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.$^6$ In assessing the prevalence of elevated serum ferritin levels based on age- and sex-specific cut points,$^2$ we found no association between ascorbic acid and prevalence of elevated serum ferritin levels among men but observed a nonlinear relation among women. To our knowledge, the relation between ascorbic acid and elevated serum ferritin levels has not been...
Men

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. 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. 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.

KIDNEY STONES

Most kidney stones are composed of calcium oxalate or uric acid. Although not observed uniformly, some studies have reported that urinary oxalate and uric acid excretion are increased by the ingestion or administration of large amounts of ascorbic acid. Studies that examined the relation of ascorbic acid to oxalate and uric acid excretion have generally examined small numbers of subjects. 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 others 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.

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. 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 radioassay used by NHANES II measures both biologically active and inactive vitamin B12 analogs. 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 radioimmunoassay, when compared with the microbiologic vitamin B12 assay, suggests that the comparative rank order of serum values is likely to be similar across assays.30 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,31 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.24

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REFERENCES