Serum vitamin C concentrations and diabetes: findings from the third National Health and Nutrition Examination Survey, 1988–1994[1,2]

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ABSTRACT

Background: Previous studies suggested that diabetes mellitus may lower serum vitamin C concentrations, but most of these studies used clinic-based populations with established diabetes of varying duration and did not adjust for important covariates.

Objective: Using a population-based sample and adjusting for important covariates, we asked whether serum vitamin C concentrations in persons with newly diagnosed diabetes differed from those in persons without diabetes.

Design: Data were obtained from the third National Health and Nutrition Examination Survey (1988–1994). Serum vitamin C was assayed by using reversed-phase HPLC with multiwavelength detection. Diabetes status (n = 237 persons with diabetes; n = 1803 persons without diabetes) was determined by oral-glucose-tolerance testing of the sample aged 40–74 y.

Results: After adjustment for age and sex, mean serum vitamin C concentrations were significantly lower in persons with newly diagnosed diabetes than in those without diabetes. After adjustment for dietary intake of vitamin C and other important covariates, however, mean concentrations did not differ according to diabetes status.

Conclusion: When assessing serum vitamin C concentrations by diabetes status in the future, researchers should measure and account for all factors that influence serum vitamin C concentrations. *Am J Clin Nutr* 1999;70:49–52.

KEY WORDS Ascorbic acid, blood chemical analysis, diabetes mellitus, diet surveys, epidemiology, population, NHANES III, vitamin C, oral-glucose-tolerance test, third National Health and Nutrition Examination Survey

INTRODUCTION

Previous research suggests that persons with diabetes mellitus have lower circulating vitamin C concentrations than those without this disorder (1). If this is indeed true, this deficit in persons with diabetes may be one factor contributing to their increased risk of infection, damage to connective tissue, and oxidative tissue damage (2). Several explanations for reduced serum vitamin C concentrations in persons with diabetes might be considered: *1*) renal reabsorption of vitamin C may be reduced by hyperglycemia, *2*) blood glucose may compete with vitamin C for uptake into certain cells and tissues, *3*) cellular regulation of vitamin C may be impaired, and *4*) increased oxidative stress may deplete antioxidant reserves (1). Alternatively, lower vitamin C concentrations observed in persons with diabetes might be explained by methodologic limitations in previous research. Most studies have not fully considered factors known to be associated with lower plasma vitamin C concentrations, such as low dietary intake of vitamin C, tobacco use, and poor health status (1, 2). Studies that have compared vitamin C concentrations of persons with diabetes attending outpatient clinics with those of likely healthier persons are especially troubling because lower observed concentrations may simply reflect illness. For example, people with recent myocardial infarction or gastritis have been shown to have lower plasma vitamin C concentrations than their healthier counterparts (3, 4).

To better assess whether persons with diabetes have low serum vitamin C concentrations, we used a population-based survey of US citizens to compare those newly diagnosed with diabetes with those without diabetes. We accounted for many important cofactors, including dietary intake of vitamin C, physical activity, and number of cigarettes smoked during the 5 d preceding examination.

METHODS

We used data from phase 1 and phase 2 (1988–1994) of the third National Health and Nutrition Examination Survey (NHANES III), a stratified multistage probability study generalizable to the noninstitutionalized civilian population of the United States (5). Ethical approval was obtained and written, informed consent was received from all subjects, as described previously (5). Of 39 695 persons selected to participate in NHANES III, 33 994 (86%) were interviewed in their homes. Of these, 30 818 (78%) received clinical examinations.

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Serum vitamin C was assayed at the NHANES laboratory at the Centers for Disease Control and Prevention by using reversed-phase HPLC with multiwavelength detection. Detailed laboratory procedures for this assay were published elsewhere (6). To improve the normality of the serum vitamin C distribution, we log-transformed the serum vitamin C concentrations.

Participants aged 40–74 y who were not pregnant, not taking insulin, and who did not have certain medical conditions were eligible for an oral-glucose-tolerance test (OGTT). Those who agreed to undergo an OGTT were randomly assigned to a morning, afternoon, or evening session, but only those assigned to the morning session were asked to fast for ≥10 h, which is the recommendation of the World Health Organization (WHO; 7). Because we used the WHO criteria to classify persons by diabetes status, we restricted our analyses to those participants attending the morning session (n = 3882). We excluded all persons who said they had previously diagnosed diabetes or who indicated that they did not know whether they had diabetes (n = 462). Of the 3420 persons remaining, we excluded the 259 persons who did not fast for ≥10 h and the 265 persons who did not have their blood drawn at 2 h ± ≥16 min. Of the 2896 persons still remaining, we excluded those who received an examination in their home (n = 86) because we wanted to focus on healthy, ambulatory persons. We also eliminated participants who did not answer the complete set of questions used in our analyses (n = 288). Finally, we excluded persons who had impaired glucose tolerance (IGT)—a fasting plasma glucose concentration (FPG) of <7.8 mmol/L (140 mg/dL) with a 2-h value of 7.8–11.0 mmol/L (140–199 mg/dL) (n = 482). Thus, our final sample consisted of 2040 men and women. Persons with an FPG of ≥7.8 mmol/L or <7.8 mmol/L with a 2-h value of ≥11.1 mmol/L (200 mg/dL) were considered to have newly diagnosed diabetes (n = 237). Persons with an FPG of <7.8 mmol/L with a 2-h value <7.8 mmol/L were considered to have normal glucose tolerance (n = 1803).

We examined serum vitamin C concentrations by diabetes status while controlling for sociodemographic, behavioral, anthropometric, and dietary variables, which included age (y), sex, race (white or other), education (y), body mass index (in kg/m^2), number of cigarettes smoked during the past 5 d, alcohol consumed in the previous 24 h (g), length of fast before clinic attendance (h), and physical activity (vigorously active, moderately active, lightly active, or sedentary).

Being vigorously active was defined as participating ≥3 times/wk in an activity with a metabolic equivalent (MET) level of ≥6 for participants who were aged ≥60 y and 7 for participants who were <60 y. The term "moderately active" was defined as participating ≥5 times/wk in physical activities, ≤2 of which were defined as vigorous. "Lightly active" was defined as participation that was not "vigorously active" or "moderately active." "Sedentary" was defined as engaging in no leisure-time physical activity.

We also adjusted for total vitamin C consumed in the previous 24 h, which was estimated by summing the amount from food and from supplements assumed to have been taken in the previous 24 h. For supplements, we relied on a series of questions about the vitamins or minerals the participant had taken in the previous month. We used the answers to these to estimate the average daily amount of supplemental vitamin C (the participant was not asked about specific supplements during the 24-h recall). For example, if total supplemental vitamin C for 1 mo was estimated at 4800 mg, we calculated the average daily intake as 160 mg. We added this amount to the amount of vitamin C from food only if the respondent responded positively to the question, "Have you taken any vitamins or minerals during the past 24 h?" For food, we relied on a single 24-h recall that was administered at the clinic examination; results were coded by using the US Department of Agriculture survey nutrient database (8).

TABLE 1

Characteristics of study persons aged 40–74 y who attended the morning clinic examination, by diabetes status: NHANES III[1]

Characteristic	Diabetes (n = 237)	No diabetes (n = 1803)
Serum vitamin C (μmol/L)		
Arithmetic mean	37.5 ± 2.2[2]	42.4 ± 1.1[3]
Logarithmic mean	03.3 ± 0.1	03.5 ± 0.0
Mean vitamin C from food (mg)[4]	103.5 ± 9.7	104.1 ± 6.1
Consumed supplement[4]		
No	74.7 ± 3.9	73.9 ± 2.0
Yes	25.3 ± 3.9	26.1 ± 2.0
Mean vitamin C from supplement (mg)[5]	159.5 ± 41.7	264.7 ± 28.6[3]
Mean total intake of vitamin C (mg)[4]	143.8 ± 16.5	173.1 ± 10.8
Mean age (y)	60.3 ± 1.0	52.8 ± 0.5[6]
Sex (%)		
Male	52.6 ± 5.1	48.1 ± 1.3
Female	47.4 ± 5.1	51.9 ± 1.3
Race (%)		
White	86.8 ± 3.0	88.4 ± 1.2
Other	13.2 ± 3.0	11.6 ± 1.2
Mean education (y)	11.6 ± 0.4	12.7 ± 0.1[6]
Mean BMI (kg/m^2)	30.4 ± 0.6	26.8 ± 0.2[6]
Mean cigarettes smoked during past 5 d	27.2 ± 5.9	27.5 ± 1.7
Mean alcohol consumed (g)[4]	7.7 ± 2.2	8.2 ± 0.8
Physical activity (%)		
Vigorously active	4.5 ± 1.9	7.2 ± 1.0
Moderately active	35.6 ± 5.0	36.1 ± 2.0
Lightly active	46.1 ± 4.7	44.2 ± 2.0
Sedentary	13.8 ± 3.5	12.5 ± 1.0
Mean time fasted (h)	14.1 ± 0.2	13.4 ± 0.0[7]

[1] NHANES III, third National Health and Nutrition Examination Survey.
[2] $\bar{x}$ ± SE.
[3] Significantly different from those with newly diagnosed diabetes, $P < 0.05$.
[4] In past 24 h.
[5] Among those taking vitamin C supplements.
[6,7] Significantly different from those with newly diagnosed diabetes: [6] $P < 0.0001$, [7] $P < 0.01$.

Because of the complex survey design of NHANES III, we used SUDAAN (9) to conduct a weighted regression analysis of diabetes status on the logarithm of serum vitamin C concentrations while adjusting for the variables listed previously. This regression analysis produced a test of the difference of mean serum vitamin C concentration between those persons with newly diagnosed diabetes and those without diabetes. Adjusted mean vitamin C concentrations were produced by using SAS (10).

To test some biological mechanisms that may influence serum vitamin C concentrations, we independently examined the relations of 4 variables with serum vitamin C concentrations after adjusting for all of the factors described above. These 4 variables were urinary frequency (an indicator of failure to reabsorb vitamin C) and concentrations of fasting plasma glucose, fasting insulin, and C-reactive protein (a measure of inflammation).

TABLE 2
Associations for categorical variables with serum vitamin C concentrations for persons aged 40–74 y who attended the morning clinic examination: NHANES III[1]

	Serum vitamin C	Log serum vitamin C	Age- and sex-adjusted log mean	Fully adjusted log mean[2]
	μmol/L	*μmol/L*	*μmol/L*	*μmol/L*
Diabetes				
None	42.41 ± 0.96[3]	3.46 ± 0.03	3.47[4]	3.33
Newly diagnosed	37.49 ± 2.17	3.26 ± 0.10	3.21	3.21
Sex				
Male	37.21 ± 1.01	3.29 ± 0.04	3.28[5]	3.11[5]
Female	47.24 ± 1.21	3.61 ± 0.04	3.62	3.44
Race				
White	43.08 ± 1.08	3.47 ± 0.04	3.46	3.28
Other	37.23 ± 1.34	3.33 ± 0.06	3.38	3.28
Physical activity				
Vigorously active	52.80 ± 2.36	3.85 ± 0.08	3.87[6]	3.48[7]
Moderately active	46.84 ± 1.55	3.60 ± 0.05	3.60	3.33
Lightly active	38.91 ± 1.27	3.35 ± 0.04	3.34	3.20
Sedentary	37.13 ± 1.89	3.24 ± 0.07	3.12	3.10

[1] NHANES III, third National Health and Nutrition Examination Survey.
[2] Adjusted for all variables given in Tables 2 and 3.
[3] $\bar{x}$ ± SE.
[4,5] Significantly different from mean in the row below: [4] $P < 0.01$, [5] $P < 0.0001$.
[6,7] Significant linear trend: [6] $P < 0.0001$, [7] $P < 0.01$.

RESULTS

On average, persons with newly diagnosed diabetes were older, less educated, and had higher body mass indexes than persons without diabetes (**Table 1**). The 2 groups did not differ significantly in the amounts of vitamin C consumed from food, but the diabetic group consumed significantly less vitamin C from supplements than did the group without diabetes. No significant differences were found between the groups for cigarette smoking, alcohol consumption, or physical activity.

After adjustment for differences in age and sex, persons with diabetes had significantly lower serum vitamin C concentrations than did persons without diabetes ($P = 0.01$) (**Table 2**). After adjustment for all the covariates shown in Table 2 and **Table 3**, however, there was no significant difference between the 2 groups ($P = 0.10$). Higher serum vitamin C concentrations were found in women, those who engaged in moderate or vigorous activity, those with a higher dietary intake of vitamin C, older persons, those who were more educated, and those who smoked fewer cigarettes.

In examinations of the association of each of the 4 biological variables with serum vitamin C concentrations after adjustment for all the variables in Tables 2 and 3 except diabetes status, we found that both serum C-reactive protein ($P = 0.0001$) and urinary frequency ($P = 0.002$) were inversely associated (data not shown). On the other hand, neither glucose nor insulin concentration was associated with vitamin C concentration. When we again assessed possible associations between diabetes and serum vitamin C concentrations by adjusting for either serum C-reactive protein or urinary frequency plus all the variables in Tables 2 and 3 except diabetes status, we found that the association between diabetes status and serum vitamin C concentrations was weakened further ($P = 0.19$ when adjusted for C-reactive protein; $P = 0.15$ when adjusted for urinary frequency).

DISCUSSION

Factors generally associated with lower serum vitamin C concentrations in other studies were found to be associated in our study as well—lower consumption of vitamin C, being male, and cigarette smoking (2). In addition, after simple adjustment for age and sex, our results appeared to support most previous studies in finding that persons with diabetes have lower serum vitamin C concentrations than do persons without diabetes (1). After multivariate adjustment that included total vitamin C consumption, body mass index, educational level, cigarette smoking, and other factors, however, we found that the association between diabetes and vitamin C concentrations was no longer significant. In addition, we found no associations between fasting plasma glucose or fasting serum insulin and vitamin C concentrations.

Of 23 studies reviewed previously (1), only 7 found that blood vitamin C concentrations in persons with diabetes were not significantly lower than concentrations in persons without diabetes (4, 11–16). In 2 of those 7 studies, persons with diabetes actually had higher blood concentrations than the comparison group (4, 11). In 1 of those 2 studies, it seems likely that results were influenced by the provision of extensive dietary instruction to persons with diabetes that promoted the benefits of consuming fruit and vegetables (11). In the other, persons with diabetes were compared with critically ill persons without diabetes (4). Paralleling our finding for a measure of inflammation (concentration of C-reactive protein), researchers in that study found that illness was associated with lower plasma vitamin C concentrations. In the remaining studies that did not find vitamin C concentrations to be lower in persons with diabetes, comparability of the 2 groups was uncertain because dietary intake and illness status were generally not reported. We note that none of the studies examined urinary frequency, a factor we found to be inversely associated with serum vitamin C concentrations.

TABLE 3
Associations of continuous variables with serum vitamin C concentrations for persons aged 40–74 y who attended the morning clinic examination: NHANES III[1]

	Age- and sex-adjusted β-coefficient	Fully adjusted β-coefficient[2]
Total vitamin C intake	0.0012 ± 0.0001[3]	0.0010 ± 0.0001[3]
Age	—	0.0103 ± 0.0024[4]
Education	0.0682 ± 0.0079[3]	0.0336 ± 0.0077[4]
BMI	−0.0159 ± 0.0063[5]	−0.0087 ± 0.0058
Number of cigarettes smoked	−0.0048 ± 0.0008[3]	−0.0038 ± 0.0008[3]
Alcohol	−0.0027 ± 0.0017	−0.0020 ± 0.0013
Number of hours fasted	−0.0440 ± 0.0224	−0.0204 ± 0.0203

[1] β-coefficient ± SE. NHANES III, third National Health and Nutrition Examination Survey.
[2] Adjusted for all variables in Tables 2 and 3.
[3–5] Significantly different from zero: [3] $P < 0.0001$, [4] $P < 0.001$, [5] $P < 0.05$.

Our study was an improvement over previous studies in that it compared relatively healthy persons with newly diagnosed diabetes with their nondiabetic counterparts and adjusted for several important covariates. As in all cross-sectional studies, however, serum vitamin concentrations and diabetes are both assessed at the same point in time. Thus, if a positive association had been detected, it would have been difficult to determine whether serum vitamin C concentrations predicted diabetes status or vice versa. Furthermore, our assessment of vitamin C intake was probably not entirely satisfactory. First, to assess intake of supplements we used an indirect method that assumed that use over 1 mo could be applied to a single 24-h period in which the exact type of supplement was not known. For persons who do not take the same supplements every day, this approach seems particularly suspect. Second, the 24-h recall method for estimating dietary vitamin C intakes could have produced some data that misrepresented actual experience. For example, people may overreport consumption of those foods they eat most frequently (17), and those who are sensitive to criticism or praise may overreport those foods they believe are healthiest (18). Inaccuracies in reporting could distort comparisons of mean concentrations between persons with and without diabetes, but more information would be needed before conclusions on any effects of misreporting could be drawn.

On the other hand, the NHANES III 24-h dietary recall was administered by using standardized, computerized probes; edited carefully for completeness; and verified to determine the accuracy of extreme values (5). Indeed, 95% of participants completed the 24-h dietary recall, which suggests that errors introduced in our study because of poor survey methods should have been quite small.

In summary, after we adjusted for several important covariates, we found that serum vitamin C concentrations did not differ significantly by diabetes status. Future investigations on this topic should adjust serum concentrations for dietary intake of the vitamin and other factors that are related to both serum concentrations and diabetes status. In particular, researchers may want to examine the extent to which frequent urination or degree of inflammation lowers serum vitamin C concentrations. By conducting more rigorous scientific studies, it may be possible to achieve consensus on whether diabetes causes low serum vitamin C concentrations. Until that time, persons with diabetes (and all others) can likely attain adequate serum vitamin C concentrations by not smoking cigarettes and by following the *Dietary Guidelines for Americans* (19), which suggests that most energy be obtained from grains, fruit, and vegetables.

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