American Journal of Epidemiology

Vol. 147, No. 6
Printed in U.S.A.

Agreement among Indicators of Vitamin C Status

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Agreement among three indicators of vitamin C status—serum ascorbate level, a 24-hour recall, and the frequency of fruit and vegetable consumption—was examined using data from the Second National Health and Nutrition Examination Survey conducted between 1976 and 1980. Agreement between pairs of these indicators was good when assessed at the group level but inconsistent at the individual level. These indicators, when classified as continuous measures, had moderately good agreement (r = 0.45–0.54), whereas agreement was poor when classified as quartiles (kappa = 0.17–0.23). Agreement between clinically based categories of serum ascorbate and total intake levels was poorer than expected (kappa = 0.25) as was agreement between low or deficient levels of both of these indicators (kappa = 0.3). Disagreement between low or deficient serum and intake levels was greater in participants who were younger, African American compared with white and other races, less educated, current smokers, nonsupplement users, and examined in the winter compared with in the summer or fall. These findings suggest that the indicators cannot be used interchangeably to assess vitamin C status because they distinguish between different aspects of status, intake level versus serum level, an indicator of available pool. Moreover, depending upon how these indicators are used in statistical analyses, they may classify individuals differently. *Am J Epidemiol* 1998;147:587–96.

ascorbic acid; biological markers; diet; nutrition surveys; nutritional status

Epidemiologic studies suggest that vitamin C may play a role in the prevention of coronary heart disease (1–4) and cancer (5–11), the two leading causes of death in the US population (12). Nevertheless, the findings from such studies are inconsistent and, consequently, the role that vitamin C plays in the etiology of chronic diseases is controversial. One explanation for these inconsistent findings is that these studies have used various methods to assess vitamin C status. The most common indicators include serum ascorbate levels and dietary vitamin C intakes, as estimated by 24-hour recalls, food records, or food frequency questionnaires. Additionally, the frequency of fruit and vegetable consumption has been used as an indirect estimate of vitamin C intake.

These methods may not be comparable because they measure different aspects of vitamin C status, that is, body pool versus intake levels. The serum ascorbate level, which estimates body pool, increases linearly with increasing ascorbic acid intake but plateaus at levels between 1.2 and 1.8 mg/dl (13, 14). Furthermore, the relation between serum and intake levels may be modified by other factors, such as smoking, disease state, and medications (3, 14–20). Thus, the serum ascorbate level may not be a good indicator of vitamin C intake across the full range of intakes. However, a low serum ascorbate level is purportedly a good indicator of a chronically low level of vitamin C intake (21, 22).

The objective of this study was to assess the agreement between the serum ascorbate level and two dietary measures of vitamin C—a single, 24-hour recall and the frequency of intake of vitamin C-rich fruits and vegetables. Because these indicators have been classified as both continuous and categorical measures in studies examining the relation between vitamin C and chronic diseases, we examined the agreement between them using several approaches to classification.

Received for publication January 17, 1997, and accepted for publication September 20, 1997.

Abbreviations: CI, confidence interval; NHANES II, Second National Health and Nutrition Examination Survey; RDA, Recommended Dietary Allowance.

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We also assessed whether age, race, education level, smoking status, oral contraceptive use, body mass index, history of diabetes, use of supplements, and seasonality predicted the disagreement between serum and intake levels. The data used in this study were collected on a large, national sample of adults living in the United States.

MATERIALS AND METHODS

The study sample was composed of participants in the Second National Health and Nutrition Examination Survey (NHANES II), conducted by the National Center for Health Statistics between 1976 and 1980. The study design and methods are reported in more detail elsewhere (23). Briefly, NHANES II had a multistage, stratified design that produced a representative sample of the civilian, noninstitutionalized US population at that time. The current study was restricted to 9,252 adults who were 30–74 years of age at the time of their examination. Seven percent of these adults were excluded because of missing serum ascorbate data; however, the distributions of demographic characteristics, education level, and several coronary heart disease risk factors were similar for the individuals with missing data compared with the remainder of the sample (data not shown). Another 2 percent were excluded because of missing data for other vitamin C indicators or covariates. After exclusions, 3,998 men and 4,458 women remained in the analytical sample.

Vitamin C measures

Blood was drawn from participants, stabilized with metaphosphoric acid, and shipped on dry ice to the Centers for Disease Control and Prevention for further processing. Plasma was analyzed for total serum vitamin C using the 2,4-dinitrophenylhydrazine method (24, 25). Approximately one half of the sample was randomly selected to fast 10–16 hours before their examination, although only 39 percent reported actually fasting 8 or more hours.

A 24-hour dietary recall was administered using three-dimensional models to record the amount of food consumed (23). Dietary intakes were recalled from weekdays only because of examination schedules. Vitamin C intake was calculated by the National Center for Health Statistics from the recalled foods and portion size estimates using food composition data from the US Department of Agriculture and food manufacturers (23).

Participants were asked about the kind of vitamin supplements taken during the week prior to their examination. If they took supplements every day or almost every day, participants were classified as regular users. All other users were classified as irregular users. Because serum ascorbate levels of irregular users did not differ from those of nonusers of supplements, we combined these groups. Data for participants taking more than one supplement were recoded so that the content of all supplements was recorded instead of just the first listed as previously released by the National Center for Health Statistics (26). Supplements were categorized according to their vitamin content and as either multivitamins or specialized products containing vitamin C. The latter classification was used to estimate the amount of vitamin C consumed in supplements because the amount was not consistently collected. Multivitamins usually contain the US Recommended Dietary Allowance (RDA) for each vitamin (27), and most contain vitamin C (28). Specialized products, containing three or fewer nutrients (27), usually contain more than the US RDA for each nutrient. For these reasons, it was assumed, in calculating total vitamin C intakes, that multivitamins contained 60 mg of vitamin C, the US RDA, and that specialized vitamin C products contained 150 mg, 250 percent of the US RDA.

A food frequency questionnaire was also administered that contained one item concerning intake of vitamin C-rich fruits and vegetables during the 3 months prior to the interview. The list included the following: avocado, cantaloupe, grapefruit, lemon, lime, orange, strawberries, brussels sprouts, broccoli, cauliflower, green peppers, tomato, and greens. The frequency of intake was converted to number of times per week.

Predictors of disagreement

The following age categories were used in all analyses: 30–44, 45–54, 55–64, and 65–74 years. In preliminary analyses, levels of the vitamin C indicators among African Americans differed from those of whites and other races. Because there were few participants of other races and their vitamin C status indicators did not differ from those of whites, these two groups were combined. Individuals were grouped according to their highest level of education attained: 0–8, 9–11, 12, or greater than 12 years. Participants were classified as never, past, or current smokers and, if current smokers, as smoking less than, equal to, or greater than one pack per day. Body mass index (weight (kg)/height $(m)^2$) was calculated using height and weight measured during the examination. Women were asked separately about oral contraceptive use at the time of and during the 6 months prior to their examination, and the responses were combined. Participants were classified as having a history of diabetes if they reported that they were taking insulin or had

been told by a doctor that they had diabetes. As part of the food frequency instrument, subjects were asked the frequency of consumption of beer, wine, and other alcoholic beverages. The responses to these questions were summed to calculate total alcohol consumption per day.

Statistical methods

Quartiles of vitamin C measures were calculated separately for women and men because of sex differences in vitamin C indicators. Serum and total intake levels were also classified into clinically based categories. The following definitions were used for serum ascorbate level (17): deficient (<0.2 mg/dl), low (0.2–0.39 mg/dl), normal (0.4–0.99 mg/dl), and saturated (≥1.0 mg/dl). Sex-specific definitions for total vitamin C intakes were as follows for men and women, respectively (17): deficient (<20, <15 mg/day), low (20–29, 15–24 mg/day), normal (30–149, 25–79 mg/day), and saturated (>149, >79 mg/day). The same classification was used to categorize serum and total intake levels as low and deficient or normal and saturated by combining the lowest and highest categories, respectively.

Because of the nonnormal distributions of serum and dietary indicators, medians and interquartile ranges were used to describe these measures. The Kruskal-Wallis test was used to assess agreement at the group level. Kappa statistics and Spearman's correlation coefficients were used to assess agreement at the individual level. Predictors of the disagreement between low serum and intake levels were determined using logistic regression. To assess the effect of the complex sample design, we repeated the logistic regression analyses using appropriate software (29) and weights, which account for oversampling, nonresponse, and poststratification (23). Results of the weighted analyses were consistent with those derived from the unweighted analyses. For simplicity, only the estimates from the unweighted analyses are presented. All estimates were calculated using the Statistical Analysis System (30).

RESULTS

Table 1 shows the distributions of each vitamin C status indicator by selected characteristics. Median vitamin C serum and dietary intake levels tended to be higher at older ages in women but not in men and were higher with higher education levels in men but less so in women. Serum levels tended to be lower for African Americans of both sexes compared with whites and other races; however, dietary intakes were lower only

TABLE 1. Median serum vitamin C levels, dietary vitamin C intake, and weekly frequency of fruit and vegetable intake by selected variables among participants in the Second National Health and Nutrition Examination Survey, 1976–1980*

	Men (n = 3,998)				Women (n = 4,458)			
	%	Serum (mg/dl)	Dietary intake (mg/day)	FV† intake (times/week)	%	Serum (mg/dl)	Dietary intake (mg/day)	FV intake (times/week)
Age, years								
30–44	28	0.9 (0.5–1.2)‡	65 (30–136)	3 (1–7)	29	1.0 (0.6–1.3)	51 (22–110)	4 (1–7)
45–54	16	0.9 (0.4–1.3)	72 (30–138)	4 (1–7)	16	1.1 (0.7–1.4)	71 (16–134)	5 (2–7)
55–64	28	0.9 (0.4–1.3)	79 (32–144)	4 (1–7)	27	1.3 (0.9–1.6)	85 (34–141)	7 (2–7)
65–74	28	1.0 (0.5–1.4)	76 (33–138)	3 (1–7)	29	1.3 (0.9–1.6)	90 (36–145)	7 (2–7)
Race								
Black	10	0.6 (1.0–0.3)	52 (17–124)	3 (0.5–7)	10	0.9 (0.5–1.2)	72 (29–134)	5 (2–7)
White or other	90	1.0 (1.3–0.5)	75 (33–139)	3 (1–7)	90	1.2 (0.8–1.5)	75 (29–137)	5 (2–7)
Education, years								
0–8	28	0.7 (0.3–1.1)	54 (21–114)	2 (0.5–7)	24	1.1 (0.6–1.4)	64 (22–121)	4 (1–7)
9–11	18	0.8 (0.4–1.2)	63 (29–128)	3 (1–7)	21	1.1 (0.6–1.4)	55 (25–118)	4 (1–7)
12	29	1.0 (0.5–1.3)	81 (39–141)	4 (1–7)	35	1.2 (0.8–1.5)	79 (31–141)	6 (3–7)
≥13	25	1.1 (0.7–1.4)	96 (44–160)	6 (2–7)	20	1.3 (1.0–1.6)	94 (41–157)	7 (3–7)
Vitamin C supplement use								
None	82	0.8 (0.4–1.2)	67 (30–131)	3 (1–7)	78	1.1 (0.5–1.4)	67 (27–128)	5 (1–7)
Multivitamin	11	1.3 (1.1–1.4)	101 (43–166)	6 (2–7)	14	1.4 (1.2–1.7)	94 (40–159)	7 (2–7)
Specialized	6	1.5 (1.2–1.8)	101 (47–161)	5 (1–7)	9	1.5 (1.4–1.9)	90 (35–146)	7 (2–7)

* Unweighted data.
† FV, vitamin C-rich fruit and vegetable intake estimated from a 3-month qualitative food frequency.
‡ Numbers in parentheses, interquartile range.

among African-American men compared with other races. Serum levels also tended to be higher for multivitamin users and even higher for users of specialized products containing vitamin C when compared with nonusers of supplements. Dietary vitamin C intakes among users of supplements were generally higher than those of nonusers.

Group-level comparisons

Median serum ascorbate levels increased in a continuous, graded fashion with increasing quintile of dietary intake and intake of vitamin C-rich fruits and vegetables among nonusers of supplements (table 2). In contrast, serum levels were not related to dietary intake among persons who took either multivitamins or specialized products containing vitamin C. The median dietary intake of vitamin C per day was progressively higher at higher levels of fruit and vegetable intake among all subjects (table 3).

Continuous measures

Agreement between continuous measures of vitamin C indicators, as assessed by Spearman's correlation coefficients, was generally high. It was highest for serum levels and total intake ($r = 0.54$, $p = 0.0001$), slightly lower for the two dietary measures ($r = 0.47$, $p = 0.0001$), and lowest for serum levels compared with intake of vitamin C-rich fruits and vegetables ($r = 0.40$, $p = 0.0001$). When analyses were restricted to persons who did not take supplements, the correlation coefficients were similar for serum levels with both total intake ($r = 0.46$, $p = 0.0001$) and the frequency of fruit and vegetable intake ($r = 0.45$, $p = 0.0001$). As expected, there was less association between the frequency of fruit and vegetable intake and serum levels among multivitamin supplement users ($r = 0.17$, $p = 0.0001$) and even less among persons taking specialized vitamin C products ($r = 0.04$, $p = 0.04$). Similarly, the association was low between total

TABLE 2. Median serum ascorbate levels (mg/dl) by quintile of dietary intake and weekly frequency of fruit and vegetable intake by use of multivitamin and vitamin C supplements among participants in the Second National Health and Nutrition Examination Survey, 1976–1980†

	Median serum ascorbate level (mg/dl) by the following supplement use		
	None (n = 6,743)	Multivitamin (n = 1,062)	Vitamin C (n = 651)
Dietary intake, mg/day‡			
Men			
<26	0.4 (0.3–0.8)*,§	1.2 (0.9–1.4)**	1.4 (1.1–1.6)
26–53	0.6 (0.3–0.9)	1.2 (1.0–1.4)	1.4 (1.2–1.6)
54–96	0.8 (0.5–1.1)	1.3 (1.0–1.5)	1.6 (1.3–1.9)
97–156	1.0 (0.6–1.3)	1.2 (1.1–1.4)	1.5 (1.3–1.8)
>156	1.2 (0.9–1.4)	1.3 (1.2–1.5)	1.5 (1.2–1.8)
Women			
<23	0.6 (0.4–1.0)*	1.2 (1.0–1.5)*	1.6 (1.3–2.0)
23–51	0.9 (0.5–1.2)	1.3 (1.1–1.6)	1.5 (1.3–1.7)
52–95	1.1 (0.7–1.3)	1.4 (1.2–1.6)	1.6 (1.4–1.9)
96–151	1.3 (1.0–1.5)	1.4 (1.2–1.7)	1.7 (1.3–2.0)
>151	1.4 (1.1–1.6)	1.5 (1.3–1.8)	1.6 (1.4–1.9)
Fruit and vegetable intake, times/week¶			
Men			
<0.5	0.4 (0.2–0.7)*	1.1 (0.9–1.7)	1.6 (1.2–1.8)
0.5–1.9	0.5 (0.3–0.9)	1.2 (0.9–1.4)	1.5 (1.2–1.7)
2.0–4.9	0.7 (0.4–1.0)	1.2 (1.0–1.5)	1.4 (1.2–1.6)
5.0–6.9	0.9 (0.5–1.1)	1.3 (1.1–1.6)	1.4 (1.2–1.7)
>6.9	1.1 (0.8–1.3)	1.3 (1.1–1.4)	1.5 (1.3–1.9)
Women			
<1.0	0.6 (0.4–1.0)*	1.2 (0.9–1.5)*	1.6 (1.3–1.9
1.0–2.9	0.8 (0.5–1.2)	1.3 (1.2–1.7)	1.5 (1.2–1.7)
3.0–6.9	1.0 (0.6–1.3)	1.3 (1.1–1.6)	1.6 (1.4–2.0)
>6.9	1.3 (1.0–1.5)	1.5 (1.2–1.7)	1.6 (1.4–1.9)

* Quintiles differ from one another ($p < 0.001$) as assessed by a Kruskal-Wallis test; ** quintiles differ from one another ($p < 0.01$) as assessed by a Kruskal-Wallis test.
† Unweighted data.
‡ Estimated from a 24-hour dietary recall.
§ Numbers in parentheses, interquartile range.
¶ Vitamin C-rich fruit and vegetable intake estimated from a 3-month qualitative food frequency.

TABLE 3. Median dietary vitamin C intake (mg/day) by quintile of frequency of fruit and vegetable intake among participants in the Second National Health and Nutrition Examination Survey, 1976–1980†

Fruit and vegetable intake, times/week‡	Dietary intake, mg/day§
Men	
<0.5	36 (14–63)*,¶
0.5–1.9	44 (21–79)
2.0–4.9	56 (28–105)
5.0–6.9	80 (32–139)
>6.9	133 (79–192)
Women	
<1.0	31 (14–68)*
1.0–2.9	40 (20–81)
3.0–6.9	53 (24–103)
>6.9	114 (70–175)

* Quintiles differ from one another ($p < 0.001$) as assessed by a Kruskal-Wallis test.
† Unweighted data.
‡ Vitamin C-rich fruit and vegetable intake estimated from a 3-month qualitative food frequency.
§ Estimated from a 24-hour dietary recall.
¶ Numbers in parentheses, interquartile range.

intake and serum levels among multivitamin users ($r = 0.20$, $p = 0.001$) and persons who took specialized vitamin C products ($r = 0.08$, $p = 0.04$) even though supplement use was accounted for in total intake. Correlation coefficients between the different measures did not differ markedly when stratified by season of data collection, fasting status, or smoking status (data not shown).

Quartiles

Agreement was poor when quartiles of serum level were compared with quartiles of total intake, that is, the combined intake from the dietary recall and supplements (kappa = 0.23, 95 percent confidence interval (CI) 0.21–0.24). Individuals were classified into different quartiles in 58 percent of cases and, even though most disagreement was only one quartile different, disagreement was evenly spread throughout the range of each quartile (table 4). Similar percentages of individuals were classified into a higher (32 percent) as into a lower (27 percent) quartile based on serum levels compared with intake.

Table 4 also shows the agreement between serum ascorbate levels and intake of vitamin C-rich fruits and vegetables among persons not taking supplements. Agreement (kappa = 0.17, 95 percent CI 0.15–0.18) was only slightly lower than it was for quartiles of total intake compared with serum levels, but the pattern of disagreement differed markedly. Twice as many individuals were classified into a higher (40 percent) as into a lower (23 percent) quartile based on intake of fruits and vegetables compared with serum levels. Much of this disagreement occurred among persons in the highest quartile of fruit and vegetable intake, those consuming fruits and vegetables at least once per day. Over one fifth of the total disagreements occurred among individuals in both the upper quartile of fruit and vegetable intake and the third quartile of

TABLE 4. Number of participants in the Second National Health and Nutrition Examination Survey, 1976–1980, classified jointly by serum ascorbate level and two indicators of dietary vitamin C intake among nonusers of supplements, and by two dietary indicators of vitamin C intake among all participants regardless of supplement use*

Quartile	Quartile of total intake†					Quartile of fruit and vegetable intake‡				
	1	2	3	4	Total	1	2	3	4	Total
Serum§										
1	1,015 (12)¶	579 (7)	238 (3)	87 (1)	1,919 (23)	877 (13)	498 (7)	228 (3)	269 (4)	1,872 (28)
2	635 (8)	681 (8)	453 (5)	248 (3)	2,017 (24)	440 (6)	475 (7)	300 (4)	615 (9)	1,830 (27)
3	320 (4)	534 (6)	742 (9)	652 (8)	2,248 (26)	235 (3)	330 (5)	278 (4)	895 (13)	1,738 (26)
4	142 (2)	322 (4)	680 (8)	1,128 (13)	2,272 (27)	109 (2)	186 (3)	151 (2)	857 (13)	1,303 (19)
Total	2,112 (25)	2,116 (25)	2,113 (25)	2,115 (25)	8,456 (100)	1,661 (25)	1,489 (22)	957 (14)	2,638 (39)#	6,743 (100)**
Dietary intake††										
1						849 (10)	605 (7)	296 (4)	361 (4)	2,111 (25)
2						656 (8)	593 (7)	360 (4)	504 (6)	2,113 (25)
3						304 (4)	360 (4)	307 (4)	1,150 (14)	2,121 (25)
4						162 (2)	238 (3)	214 (2)	1,497 (18)	2,111 (25)
Total						1,971 (23)	1,796 (21)	1,177 (14)	3,512 (42)	8,456 (100)

* Unweighted data.
† Quartiles of total intake, estimated from a 24-hour recall and supplement use: *men*: <37, 37–85, 86–165, ≥166 mg/day; *women*: <35, 35–91, 92–169, ≥170 mg/day.
‡ Quartiles of frequency of vitamin C-rich fruit and vegetable intake, estimated from a 3-month qualitative food frequency: *men*: <1, 1–2.9, 3–6.9, ≥7 times per week; *women*: <2, 2–4.9, 5–6.9, ≥7 times per week.
§ Quartiles of serum ascorbate level: *men*: <0.5, 0.5–0.89, 0.9–1.29, ≥1.3 mg/dl; *women*: <0.7, 0.7–1.19, 1.2–1.49, ≥1.5 mg/dl.
¶ Numbers in parentheses, percentage.
# More than 25% because 23% reported consuming a vitamin C-containing fruit or vegetable once per day.
** Nonusers of supplements only; quartiles were calculated for all persons regardless of supplement use.
†† Quartiles of dietary intake, estimated from a 24-hour recall: *men*: <32, 32–72, 73–138, ≥139 mg/day; *women*: <29, 29–74, 75–133, ≥134 mg/day.

serum levels, equivalent to the lower range of saturated serum ascorbate levels.

Agreement among quartiles of the two dietary intake measures was also poor (kappa = 0.18, 95 percent CI 0.16–0.19). Individuals were classified into different quartiles in 61 percent of cases (table 4), and more were classified into a higher quartile based on fruit and vegetable intake (39 percent) compared with dietary intake from the 24-hour recall (23 percent). As in the comparison of fruit and vegetable intake with serum level, much of this disagreement occurred for persons in the highest quartile of fruit and vegetable intake. Almost one quarter of the total disagreement occurred among individuals in both the highest quartile of fruit and vegetable intake and the third quartile of dietary intake.

Agreement among quartiles of serum levels and each dietary measure did not change when analyses were restricted to nonusers of vitamin C supplements, even though agreement was much poorer in persons taking either multivitamins or specialized vitamin C supplements (data not shown). Kappa statistics did not differ between smokers and nonsmokers or between participants who fasted at least 8 hours and those who did not fast (data not shown).

Clinically based categories

Agreement improved slightly (kappa = 0.25, 95 percent CI 0.24–0.27) between clinically based categories of serum level and total intake (table 5). Fewer individuals (48 percent) disagreed but, unlike the comparison of quartiles, the disagreement was not evenly distributed. Over one third of the disagreements occurred when serum was classified as saturated and intake as normal. Twice as many individuals were classified as having better vitamin C status on the basis of serum (32 percent) compared with intake (15 percent) levels.

Low versus normal vitamin C status

Approximately the same proportion of individuals were classified as low or deficient (18–19 percent) versus normal or saturated (81–82 percent) using either serum or total intake levels. Using this classification, serum and total intake levels agreed in 79 percent of individuals. Disagreement was equally split between higher (11 percent) and lower (10 percent) serum levels compared with total intake.

The largest contributor to disagreement between low or deficient serum and total intake levels was supplement use (table 6). Disagreement was greater in participants who were younger, especially among women, in African Americans compared with whites and other races, and in those who were less educated, current smokers, nonsupplement users, and examined in the winter compared with in the summer or fall. Alcohol intake, body mass index, history of diabetes, and fasting status did not predict disagreement between low serum and intake levels.

Among individuals where disagreement was present, a separate logistic regression was used to determine factors associated with classifying individuals with low or deficient status based on serum level but normal or saturated status based on total intake (table 6). Disagreement occurred only among persons not taking vitamin C-containing supplements. Participants were more likely to be classified as low or deficient based on their serum level but as normal or saturated based on their intake level if they were older, African American, less educated, a current smoker, if their examination was in the winter compared with in the summer and fall, and, among women, if they used an oral contraceptive during the 6 months prior to their examination. Alcohol intake, body mass index, history of diabetes, and fasting status did not predict low or deficient serum levels occurring with normal or saturated intakes and vice versa.

TABLE 5. Number of participants in the Second National Health and Nutrition Examination Survey, 1976–1980, classified jointly by clinically based categories of serum ascorbate level and total intake of vitamin C*,†

Serum, mg/dl	Total intake, mg/day‡				
	Deficient	Low	Normal	Saturated	Total
Deficient, <0.2	167 (2)§	58 (1)	181 (2)	39 (0)	445 (5)
Low, 0.2–0.39	303 (4)	153 (2)	517 (6)	112 (1)	1,085 (13)
Normal, 0.4–0.99	353 (4)	199 (2)	1,054 (12)	458 (5)	2,064 (24)
Saturated, ≥1.0	233 (3)	164 (2)	1,434 (17)	3,031 (36)	4,862 (58)
Total	1,056 (12)	574 (7)	3,186 (38)	3,640 (43)	8,456 (100)

* Unweighted data.

† Kappa statistic = 0.25 (95% confidence interval 0.24–0.27).

‡ Definition based on total intake (estimated from a 24-hour recall and supplement use) for men and women, respectively: *deficient*: <20 (<15 mg/day); *low*: 20–29 (15–24 mg/day); *normal*: 30–149 (25–79 mg/day); *saturated*: >149 (>79 mg/day).

§ Numbers in parentheses, percentage.

TABLE 6. Odds ratios and 95% confidence intervals for predictors of disagreement between low or deficient serum ascorbate level (<0.4 mg/dl) and low or deficient total vitamin C intake (<30 mg/day and <25 mg/day for men and women, respectively) and, among individuals where disagreement between serum and intake levels was present,* for predictors of being classified as low or deficient based on serum but normal or saturated based on intake among participants in the Second National Health and Nutrition Examination Survey, 1976–1980†

Predictor	Disagreement between serum and intake level				Low serum level and normal intake			
	Men (n = 3,998)		Women (n = 4,458)		Men (n = 953)		Women (n = 845)	
	OR‡	95% CI‡	OR	95% CI	OR	95% CI	OR	95% CI
Age, years								
30–44	1.0		1.0		1.0		1.0	
45–54	0.9	0.7–1.1	0.7	0.5–0.9	1.1	0.7–1.7	0.9	0.6–1.5
55–64	0.8	0.7–1.0	0.5	0.4–0.6	1.5	1.1–2.2	1.4	0.9–2.2
65–74	0.7	0.6–0.9	0.5	0.4–0.6	1.1	0.7–1.7	1.1	0.7–1.8
Race								
White and other	1.0		1.0		1.0		1.0	
African American	1.8	1.4–2.3	1.3	1.1–1.7	1.1	0.7–1.6	2.1	1.4–3.1
Education, years								
0–8	1.0		1.0		1.0		1.0	
9–11	0.9	0.8–1.2	0.8	0.6–1.0	0.8	0.6–1.2	1.1	0.7–1.7
12	0.6	0.5–0.8	0.7	0.5–0.8	0.9	0.6–1.3	0.9	0.6–1.3
>12	0.5	0.4–0.7	0.4	0.3–0.6	0.5	0.3–0.8	0.7	0.4–1.2
Smoking status								
Never	1.0		1.0		1.0		1.0	
Past	1.1	0.9–1.3	1.0	0.8–1.2	1.5	1.1–2.3	0.6	0.3–1.0
Smoker <1 PPD‡	1.7	1.3–2.2	1.4	1.1–1.8	1.7	1.1–2.8	2.0	1.3–3.1
Smoker 1 PPD	1.9	1.4–2.5	1.6	1.2–2.1	4.2	2.6–6.9	3.6	2.2–5.8
Smoker >1 PPD	1.9	1.5–2.5	1.4	1.0–1.9	3.6	2.3–5.7	5.3	2.9–9.7
Alcohol intake, drinks/day								
0	1.0		1.0		1.0		1.0	
1–2	0.9	0.7–1.1	0.8	0.7–1.0	0.8	0.6–1.1	0.9	0.6–1.2
≥3	0.9	0.7–1.1	0.9	0.7–1.3	1.1	0.7–1.6	1.2	0.6–2.3
History of diabetes								
No	1.0		1.0		1.0		1.0	
Yes	0.8	0.6–1.2	1.0	0.7–1.4	0.8	0.4–1.5	1.0	0.5–2.0
Oral contraceptive user during past 6 months								
No			1.0				1.0	
Yes			1.1	0.7–1.6			3.1	1.4–6.8
Supplement use								
None	1.0		1.0		*		*	
Multivitamin	0.1	0–0.1	0.1	0–0.1				
Vitamin C	0.1	0–0.1	0.1	0–0.1				
Body mass index§								
Normal	1.0		1.0		1.0		1.0	
Overweight	1.0	0.9–1.3	1.0	0.8–1.3	1.0	0.7–1.4	0.8	0.6–1.2
Obese	1.0	0.7–1.3	0.9	0.7–1.0	1.0	0.6–1.7	0.9	0.6–1.3
Season of data collection								
Winter	1.0		1.0		1.0		1.0	
Spring	1.0	0.8–1.2	0.8	0.6–1.0	0.7	0.5–1.1	1.0	0.7–1.5
Summer	0.7	0.6–0.9	0.7	0.6–0.9	0.5	0.4–0.8	0.5	0.3–0.8
Fall	0.7	0.5–0.8	0.7	0.5–0.9	0.5	0.3–0.8	0.6	0.4–0.9
Fasting status								
No	1.0		1.0		1.0		1.0	
Yes	1.2	1.0–1.4	0.9	0.8–1.0	1.0	0.8–1.4	0.8	0.6–1.1

* All cases with disagreement between serum and intake level were nonusers of supplements. Therefore, this variable was excluded from the model predicting low or deficient based on serum ascorbate level but normal or saturated based on total vitamin C intake.

† Unweighted data.

‡ OR, odds ratio; CI, confidence interval; PPD, pack per day.

§ Categories were defined, using Survey data for 20- to 29-year-olds, for men and women, respectively: *normal weight*: <27.8, <27.3 (i.e., <85th percentile); *overweight*: 27.8–31.0, 27.3–32.2 (i.e., 85th–95th percentile); *obese*: ≥31.1, ≥32.3 (i.e., >95th percentile).

DISCUSSION

Agreement among the three indicators of vitamin C status examined in this study varied depending upon how vitamin C status was classified. Agreement may have been affected by differing reference periods with respect to vitamin C status, errors in one or more of these indicators, and the ability to estimate intake level versus body pool. The reference period for the dietary methods varied from a single day for the 24-hour recall to 3 months for the food frequency question. The reference period for a single serum ascorbate measure may vary from 1 month, for chronically low levels of intake, to 1 week, for higher intake levels (14, 22, 31, 32). In spite of differing reference periods, the median serum levels for quintiles of both dietary measures showed good agreement at the group level among persons who were not taking vitamin C supplements. Similarly, group level comparisons of the two dietary measures showed good agreement with each other.

However, agreement was inconsistent when assessed at the individual level. Agreement between pairs of the three indicators, classified as continuous measures, was moderately good, and correlation coefficients were remarkably similar for all pairs of indicators among persons not taking supplements. In contrast, agreement of vitamin C indicators, classified as quartiles, was uniformly poor across indicators even though the pattern of disagreement varied. More individuals were classified into a higher quartile based on fruit and vegetable intake rather than serum level or dietary intake. Moreover, most disagreement occurred among persons in the highest quartile of fruit and vegetable intake. This pattern of disagreement probably reflected the multimodal distribution of fruit and vegetable intake with a large mode occurring at once per day. Because of this, the upper quartile had a much larger range of variation than did the lower three quartiles. Thus, the lack of agreement of fruit and vegetable intake with the other two indicators probably had more to do with the shape of its frequency distribution than actual differences in relative validity.

In contrast, disagreement between quartiles of serum level and total intake was evenly distributed and differed mostly by one quartile. The agreement between clinically based categories of serum and total intake levels, although poor, was slightly better than the corresponding agreement for quartiles of these two measures. These categories account for the plateau observed in serum levels with higher intakes and, thus, should have demonstrated better agreement than quartiles. More individuals were categorized favorably based on serum level rather than total intake, and disagreement was greatest among individuals with saturated serum levels but normal vitamin C intakes. The longer reference period for serum level compared with total intake may account for this disagreement.

The differences in the quality of the agreement between categorical and continuous measures can be partially explained by the statistical methods used. Kappa measures exact agreement, whereas Spearman's correlation coefficient measures the degree of agreement in the same direction (33, 34). Linearly weighted kappas were higher than unweighted kappas; however, most yielded only fair agreement (data not shown). Thus, the nonlinear relation between the serum ascorbate level and vitamin C intake (21, 35) is probably better assessed with a Spearman's correlation coefficient than with a kappa statistic.

Additionally, error resulting from misclassification in one or more indicators may have contributed to the discrepant findings. Individuals were ranked similarly using the three indicators as continuous measures, whereas they were ranked differently by these same indicators when classified into quartiles. The ranking of percentiles, such as quartiles, can be profoundly affected by misclassification resulting from imperfect exposure measures (36). In particular, the difference in quartile ranking of serum ascorbate level and total intake may have been due to misclassification near the quartile cutpoints because most disagreements were in adjacent quartiles.

Misclassification of total vitamin C intake could have resulted from the use of a single recall based on weekdays only, which does not capture day-to-day variation in intake (37–41). Thus, associations may have been better if a measure of total intake was used that typified intake over a longer period. Another potential source of error in intakes is how well the food composition database actually reflected the actual vitamin C content of foods consumed by NHANES II participants. Variability in the vitamin C content of foods is averaged in such databases. Additionally, error could have resulted from the use of a single serum ascorbate level. Higher intakes have been associated with more intraindividual variation in serum levels than low intakes, although no quantitative estimates were published (21, 22). Thus, these studies suggested that a single serum ascorbate measure was a good indicator of chronically low levels of vitamin C intake (21, 22).

In contrast, we found poor agreement between low levels of serum ascorbate and total vitamin C intake. Disagreement between these measures was associated with smoking status, oral contraceptive use, age, education level, race, and season of data collection. Other studies have found that serum levels were related to body weight or body mass index (21, 42), smoking,

age, and education level (21) after controlling for vitamin C intakes. Thus, the agreement between serum and intake levels may be modified by factors that affect absorption or metabolic turnover of vitamin C, such as smoking and oral contraceptive use, confirming that these indicators measure two different aspects of vitamin C status, available pool and intake levels, respectively. Age, education level, and race or ethnicity may affect the reporting of intakes (43–45) and contribute to measurement error, thereby affecting the agreement between serum and intake levels.

Among participants not taking supplements, intake and serum levels were both lower in winter when fresh fruits and vegetables with a high vitamin C content are less available compared with summer and fall (data not shown). Similar seasonal variation in vitamin C intakes and serum levels has been observed in studies in other countries (22, 46). In individuals with normally high ascorbic acid intakes, a seasonal drop in intake may cause plasma ascorbate levels to fluctuate, resulting in more disagreement between intake and serum levels during winter months. Thus, seasonal fluctuations in intake may also have contributed to disagreement between serum and intake levels.

The inconsistency between our findings and those from two experimental studies showing that chronically low intakes are correlated with low serum ascorbate levels may be explained by differences in study design. Both experimental studies had longer-term measures of vitamin C intake than did NHANES II. Additionally, factors that alter the relation between vitamin C intake and body pool may have affected fewer subjects in the experimental studies than in the free-living NHANES II subjects. One study was conducted in healthy, nonsmoking, young men (21), whereas the other study was carried out in healthy, elderly men and women and fewer than one quarter smoked (22). In contrast, over one third of the NHANES II cohort were smokers, and subjects were recruited regardless of their health status so that they represent the noninstitutionalized US population.

In conclusion, depending upon how these vitamin C indicators are used in statistical analyses, they may classify individuals differently, which, in turn, may influence findings from epidemiologic studies. Categorical measures, more commonly used in such studies than continuous measures, may have been affected more by errors and misclassification. Within each statistical method, however, agreement between indicators of vitamin C status was equivalent after accounting for supplement use. Thus, overall agreement was no better between any two indicators than any other pair, suggesting that their relative validity is equivalent or that similar errors exist in these indicators. Nevertheless, predictors of differences in serum level and total intake confirmed that these indicators measure two different aspects of vitamin C status, available pool and intake levels, respectively. Thus, the choice of an indicator will depend upon the question being asked. Until we know whether intake level or available pool is the more important aspect of vitamin C status with respect to chronic diseases, it may be optimal to assess both since they cannot be used interchangeably.

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