Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 3704–3709, April 1996
Medical Sciences

Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance

(ascorbic acid/bioavailability)

MARK LEVINE*†, CATHY CONRY-CANTILENA‡, YAOHUI WANG*, RICHARD W. WELCH*, PHILIP W. WASHKO*, KULDEEP R. DHARIWAL*, JAE B. PARK*, ALEXANDER LAZAREV*, JAMES F. GRAUMLICH§, JEAN KING¶, AND LOUIS R. CANTILENA§

*Molecular and Clinical Nutrition Section, Building 10, Room 4D52, MSC 1372, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-1372; ‡Department of Transfusion Medicine, Warren Grant Magnuson Clinical Center, Building 10 1C/711, National Institutes of Health, Bethesda, MD 20892; §Division of Clinical Pharmacology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814; and ¶Department of Nutrition, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD 20892

Communicated by J. Edward Rall, Department of Health and Human Services, Bethesda, MD, December 14, 1995 (received for review October 31, 1995)

ABSTRACT **Determinants of the recommended dietary allowance (RDA) for vitamin C include the relationship between vitamin C dose and steady-state plasma concentration, bioavailability, urinary excretion, cell concentration, and potential adverse effects. Because current data are inadequate, an in-hospital depletion–repletion study was conducted. Seven healthy volunteers were hospitalized for 4–6 months and consumed a diet containing <5 mg of vitamin C daily. Steady-state plasma and tissue concentrations were determined at seven daily doses of vitamin C from 30 to 2500 mg. Vitamin C steady-state plasma concentrations as a function of dose displayed sigmoid kinetics. The steep portion of the curve occurred between the 30- and 100-mg daily dose, the current RDA of 60 mg daily was on the lower third of the curve, the first dose beyond the sigmoid portion of the curve was 200 mg daily, and complete plasma saturation occurred at 1000 mg daily. Neutrophils, monocytes, and lymphocytes saturated at 100 mg daily and contained concentrations at least 14-fold higher than plasma. Bioavailability was complete for 200 mg of vitamin C as a single dose. No vitamin C was excreted in urine of six of seven volunteers until the 100-mg dose. At single doses of 500 mg and higher, bioavailability declined and the absorbed amount was excreted. Oxalate and urate excretion were elevated at 1000 mg of vitamin C daily compared to lower doses. Based on these data and Institute of Medicine criteria, the current RDA of 60 mg daily should be increased to 200 mg daily, which can be obtained from fruits and vegetables. Safe doses of vitamin C are less than 1000 mg daily, and vitamin C daily doses above 400 mg have no evident value.**

The recommended dietary allowance (RDA) for vitamin C is 60 mg daily, based on threshold urinary excretion of the vitamin and on preventing the vitamin C deficiency disease scurvy with a margin of safety (1, 2). Ingestion of 60 mg daily was proposed to prevent scurvy for 30–45 days if vitamin C intake ceased (1–7). Threshold urinary excretion of vitamin C was reported at the 60-mg daily dose (3, 4, 7, 8). Tissue stores were thought to be near saturation at 60 mg, and increased excretion would occur at higher doses (1–8).

To establish an RDA for a vitamin, it is necessary to determine vitamin concentrations in plasma and tissues in relation to vitamin dose for a wide range of doses, true bioavailability or vitamin absorption at each dose, vitamin urinary excretion at each dose, and potential toxicity (1, 2, 9). In theory these data could be obtained from nutrition depletion–repletion studies in combination with pharmacokinetic principles. For vitamin C, however, the information is unavailable, incomplete, or flawed (3–8, 10–22).

Because these data are essential for an RDA for vitamin C, we conducted vitamin C depletion–repletion pharmacokinetic studies in seven healthy inpatient volunteers by using seven doses from 30 to 2500 mg.

MATERIALS AND METHODS

Study Design. The protocol (DK-92-0032) was approved by the Institutional Review Board, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health (NIH). Written informed consent was obtained from enrolled volunteers.

Volunteers were screened by questionnaire and interview. Adequate venous access, a normal history, a physical exam, laboratory tests, and a dental exam were required. Exclusion criteria were cigarette smoking, use of regular medications, history of kidney stones, glucose-6-phosphate dehydrogenase deficiency, diabetes mellitus, bleeding disorders, or family history of iron overload/hemochromatosis. Laboratory tests included a complete blood count, differential, platelet count, reticulocyte count, prothrombin and partial thromboplastin times, electrolytes, blood urea nitrogen, creatinine, fasting glucose, plasma vitamin C, serum B_{12}, folate, serum iron with percent saturation, ferritin, mineral profile, cholesterol, triglycerides, total protein, albumin, liver functions, urinalysis, hepatitis B surface antigen and core and surface antibodies, antibodies to human immunodeficiency virus and hepatitis C, syphilis serology, and glucose-6-phosphate dehydrogenase deficiency screen. Seven men ages 20–26 years were selected. Although recruitment of women was encouraged, none qualified. Efforts to study women are in progress.

Upon inpatient admission, history, physical exam, laboratory tests above, and dental evaluation were repeated. Creatinine clearance and bleeding time were obtained. Patients consumed a vitamin C-restricted diet throughout hospitalization, which contained <5 mg of vitamin C daily, and utilized a computerized 14-day-cycle selective menu design (J.K., R.W.W., Y.W., K.R.D., C.C.-C., and M.L., unpublished results). Diet composition was determined by using the DFM system (23), U.S. Department of Agriculture Handbook 8 (24), and manufacturers' nutrient label analysis for specialty foods. Composition of random diets revealed a consistent pattern of marginal deficiencies (50–95% RDA for adults) of vitamin A, vitamin B_6, vitamin D, folic acid, pantothenic acid, calcium,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RDA, recommended dietary allowance; AUC, area under curve.

†To whom reprint requests should be addressed.

magnesium, and zinc, which were corrected by commercial supplements. The menu contained >300 foods and beverages. Fifty-six items contained 0.1–2.4 mg of vitamin C, and the rest had none. Vitamin C contents were based on published values (23, 24) or were verified with the manufacturers.

On admission patients began vitamin C depletion to reduce plasma vitamin C concentrations to 5–10 μm without scurvy. Plasma vitamin C was measured every 1–4 days. At the nadir of depletion, clinical reevaluation included history/physical examination, repeat of laboratory tests above, and bleeding time.

For repletion, daily ascorbate doses were 30, 60, 100, 200, 400, 1000, and 2500 mg given sequentially (see below). Half of the dose was ingested twice daily to simulate vitamin C consumption in foods. Doses were given in the fasting state (≥1.5 h before meals or ≥2 h after meals and morning vitamin C prior to breakfast). Bioavailability was studied for the ingested dose (single doses of 15, 30, 50, 100, 200, 500 and 1250 mg).

Repletion began with investigation of bioavailability and circadian rhythm over 35 h. At 0800 hours, 15 mg of vitamin C was administered by mouth in the fasting state. Thirty minutes before administration, an intravenous catheter was inserted. Blood samples (0.5-ml) for vitamin C were drawn at −0.033, 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 21, and 24 h after the oral dose. Vitamin C at 15 mg was then administered by intravenous injection in the opposite arm. Samples were drawn at −0.033, 0. 0.033, 0.083, 0.167, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, and 9 h after the intravenous dose. Twenty-four-hour urine collections were saved for creatinine clearance, oxalate, and uric acid assays; aliquots from each urination were saved for vitamin C determination. Thereafter, a 30-mg daily dose (15 mg *po bid*) was begun and continued until a steady-state (plateau) vitamin C concentration was achieved. At plateau, 25 ml of blood was taken for neutrophil isolation (25). When possible, volunteers underwent apheresis for collection of lymphocytes, monocytes, platelets, and plasma (26). Laboratory tests and bleeding time were repeated. To determine bioavailability of the next vitamin C dose (30 mg), a 35-h sampling was conducted as above. After bioavailability determination, the daily vitamin C dose was increased to 60 mg (30 mg *po bid*) until a new plateau occurred. Studies above were again repeated; this cycle continued for all remaining vitamin C doses.

Vitamin C powder USP–FCC was a gift from Takeda USA Inc. Vitamin C was batch-prepared for clinical use by the NIH Clinical Center Pharmacy as a sterile solution of 50 mg/ml in water adjusted to pH 6.5 with NaOH. Batches were routinely checked for purity and stability: no degradation occurred. Vials for oral and intravenous administration were prepared from the same batch solution.

Assays and Data Calculation. Samples for vitamin C were analyzed by HPLC with coulometric electrochemical detection as described (27, 28). Every sample was divided into three aliquots and analyzed separately. Blood and urine samples were stored on ice in a dark refrigerator until prepared for assay as described (27, 28) with minor modifications. One volume of plasma or urine was mixed with 4 vol of 90% methanol/1 mM EDTA in water and centrifuged at 25,000 × *g*. The supernatant was transferred to a new tube, immediately placed on dry ice for 10 min, and frozen at −70°C until assay. No vitamin C degradation occurred under processing and storage conditions. Dehydroascorbic acid was ≤2% of plasma vitamin C and could not be distinguished from zero (29).

The pharmacokinetic model used accounts for changes in volume of distribution and clearance as a function of dose. The model incorporated nonlinear renal tubule reabsorption and multicompartment analyses utilizing the program NONMEM.

Significant differences were calculated by Student's *t* test. Experimental results are displayed as mean ± SD. When not displayed, the SD was smaller than symbol size.

RESULTS

Vitamin C Dose Absorption. Fasting plasma vitamin C concentrations as a function of study day for volunteers are shown in Fig. 1*A*. Average study duration was 146 ± 23 days.

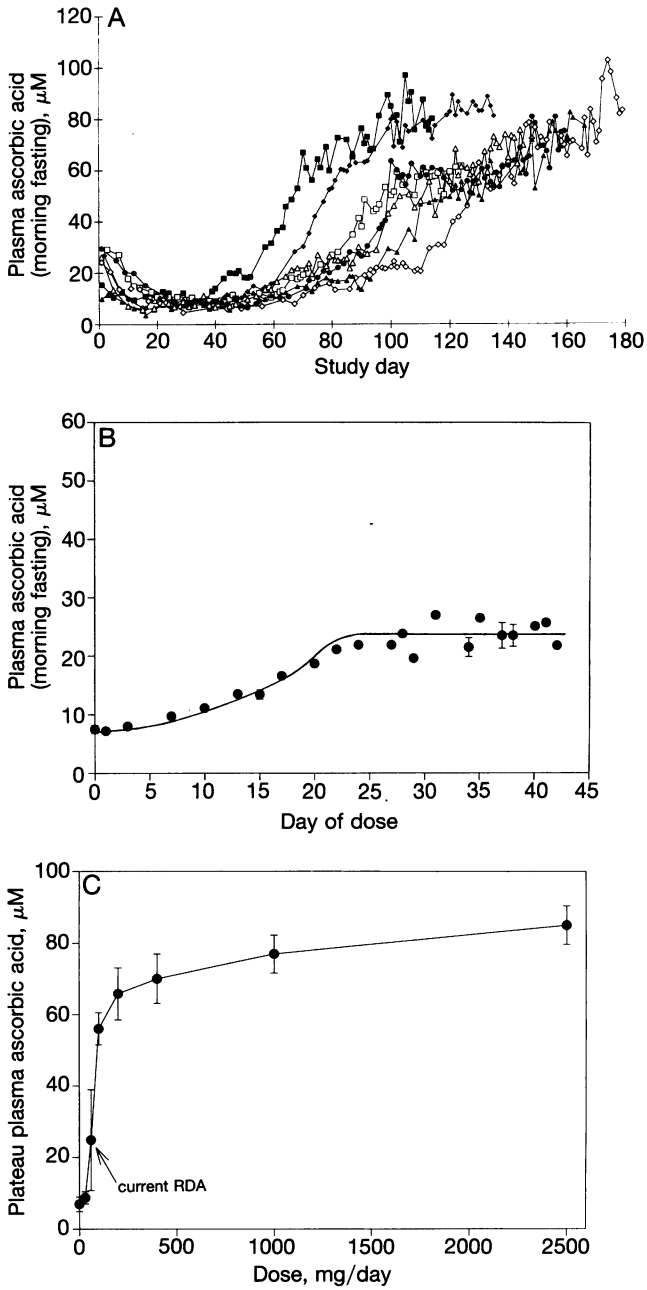

FIG. 1. Plasma ascorbic acid concentrations (μM) in volunteers as a function of daily dose. All data represent morning fasting samples. (*A*) Plasma ascorbic acid concentrations as a function of study day. Symbols represent subjects as follows: ■, volunteer 1; □, volunteer 2; ◆, volunteer 3; ◇, volunteer 4; ▲, volunteer 5; △, volunteer 6; ●, volunteer 7. (*B*) Steady-state plateau ascorbic acid concentration in plasma. Data are an example of plateau determination from volunteer 6 at the 60-mg dose. The *x* axis represents the number of days that the dose was given, and the *y* axis is plasma concentration. For all plateau determinations, plateau concentration was defined as the mean of five or more samples drawn over at least 7 days with ≤10% SD. The first sample included in all plateau determinations was ≥90% of the final plateau mean. (*C*) Steady-state plateau ascorbic acid concentrations in plasma as a function of dose. Values are the means of plateau ascorbic acid concentrations from all volunteers at all doses (see Table 1). Dose indicates the amount of vitamin C administered daily.

Table 1. Plateau plasma ascorbic acid for each vitamin C dose in each patient

Patient	Plasma ascorbic acid, μM						
	30 mg	60 mg	100 mg	200 mg	400 mg	1000 mg	2500 mg
1	11.7 ± 0.9 (6)	19.2 ± 1.2 (6)	61.9 ± 4.1 (6)	71.6 ± 3.6 (6)	80.4 ± 6.5 (6)	84.3 ± 7.7 (7)	NS
2	9.9 ± 0.7 (7)	18.3 ± 0.6 (5)	53.9 ± 3.3 (7)	57.5 ± 2.6 (8)	60.0 ± 1.4 (5)	NS	NS
3	8.8 ± 0.9 (8)	14.9 ± 1.0 (5)	62.3 ± 2.3 (8)	75.1 ± 4.4 (7)	77.3 ± 2.9 (5)	83.9 ± 3.0 (7)	84.7 ± 3.0 (7)
4	6.9 ± 0.5 (7)	21.9 ± 1.3 (13)	56.9 ± 3.8 (13)	74.7 ± 3.7 (8)	70.5 ± 2.6 (6)	75.9 ± 6.5 (6)	91.8 ± 8.5 (6)
5	7.5 ± 0.7 (7)	16.9 ± 1.6 (11)	50.4 ± 3.4 (12)	57.1 ± 5.4 (6)	62.0 ± 6.4 (6)	72.8 ± 4.9 (6)	78.6 ± 4.0 (4)
6	7.5 ± 0.7 (10)	23.6 ± 2.1 (12)	50.5 ± 4.8 (10)	60.6 ± 3.9 (7)	68.4 ± 4.2 (6)	70.5 ± 4.6 (7)	NS
7	8.7 ± 0.8 (9)	58.8 ± 3.1 (13)	55.8 ± 3.4 (12)	63.7 ± 3.2 (6)	71.1 ± 6.7 (6)	74.2 ± 4.3 (5)	NS

Steady-state plateau plasma ascorbic acid concentration for each vitamin C daily dose from 30 mg to 2500 mg in each subject. Values are the mean ± SD; values in parentheses indicate the number of samples used to calculate each mean. NS, no samples were obtained.

Because of duration, one volunteer withdrew after completion of the 400-mg dose, and three volunteers withdrew after completion of the 1000-mg dose. Calculations include data from all volunteers. The shape of the curves are different because volunteers differed in times needed to reach deficiency and to reach steady-state plateau when vitamin C was administered (see below).

Plateau steady-state plasma concentration indicated the amount of plasma vitamin C achieved by a given dose. Plateau plasma concentration was determined as shown in Fig. 1*B*. Plateau values were calculated for each dose in every patient (Table 1). At least five samples were used to calculate plateau values, and 86% were based on six or more samples (the plateau for the highest dose in one patient was based on four samples as discussed below). Display of all plateau values in all volunteers demonstrated a sigmoidal relationship between dose and steady-state plasma concentration (Fig. 1*C*). The current RDA of 60 mg daily was on the lower third of the steep portion of the curve.

Several factors could account for the sigmoid shape of the dose/plateau plasma concentration curve: vitamin distribution in cellular compartments, bioavailability, and urinary excretion. We first investigated whether dose affected intracellular vitamin C concentrations measured at the plateau of each dose. Concentrations in neutrophils reached 1.3 mM at the 100-mg dose and did not increase at higher doses (Fig. 2). Vitamin C in purified monocytes and lymphocytes also reached maximum concentration at 100 mg daily (Fig. 2).

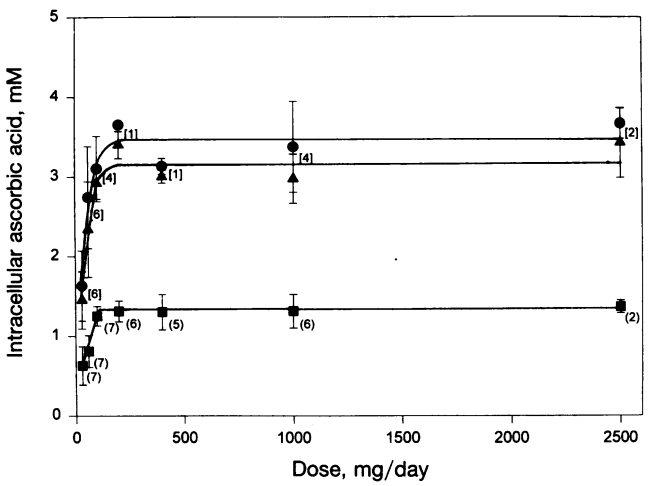

FIG. 2. Intracellular ascorbic acid concentration (mM) in circulating immune cells as a function of dose. Dose is expressed as mg of vitamin C administered daily. Neutrophils (■), monocytes (▲), and lymphocytes (●) were isolated as described (25, 26) when the plateau was achieved for each dose. Numbers in parentheses at each dose indicate the number of volunteers from whom neutrophils were obtained; numbers in brackets at each dose indicate the number of volunteers from whom lymphocytes and monocytes were obtained.

Bioavailability and Urinary Excretion. Bioavailability was determined from oral and i.v. administration of vitamin C with sampling before and after dose; an example is shown in Fig. 3. Bioavailability was 100% after a single dose of 200 mg but decreased to ≈33% after a single dose of 1250 mg. Bioavailability calculations using area under curve (AUC) ratios assume that kinetics are linear and that AUCs are proportional to dose (30, 31). To test these assumptions, we determined the time needed to reach the first value included in the plateau calculation for each patient at every dose. If kinetics were linear, all times to reach plateau would be similar. Times were consistent with linear kinetics only for doses ≥200 mg (data not shown). These data and those in Fig. 1*C* suggest that vitamin C kinetics are nonlinear at lower doses. By using the linear trapezoidal method, bioavailability was calculated only for vitamin C doses ≥200 mg (Table 2). Bioavailability was complete for 200 mg as a single dose, corresponding to the upper portion of the sigmoid dose curve (Fig. 1*C*). At higher vitamin C doses, bioavailability declined: <50% of the 1250-mg dose was absorbed.

Plateau plasma vitamin C was close to maximum at 200 mg daily (Fig. 1*C*), but at higher doses bioavailability did not

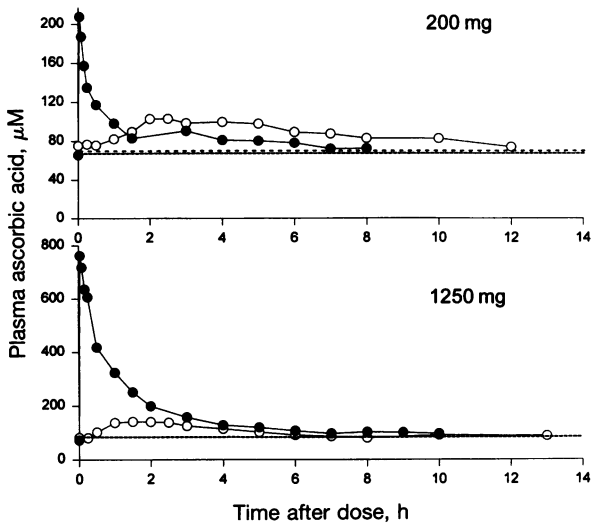

FIG. 3. Ascorbic acid bioavailability in plasma. (*Upper*) Bioavailability in subject 3 for 200 mg. (*Lower*) Bioavailability in subject 3 for 1250 mg. For each dose, ascorbic acid was administered at zero time (0800 hours) orally and sample values (○) are shown for the times indicated. Baseline is indicated by a dashed line with larger spaces. After 24 h, the same dose was given intravenously and samples were taken for the time indicated (●). Baseline is indicated by dashed line with smaller spaces. For oral doses, samples taken before zero time and between 13 and 24 h are not shown for clarity. Bioavailability was calculated by using the linear trapezoidal method (30, 31). Bioavailability (F) was the ratio of the area of the oral dose (AUC_{po}) divided by the area of the i.v. dose (AUC_{iv}). The AUC after the curve returned to baseline was assumed to equal zero.

Table 2. Ascorbic acid bioavailability

Parameter	Dose, mg	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Mean ± SD
AUC_{po}	200	25.53	20.85	29.20	NS	24.57	22.21	25.96	24.72 ± 2.95
AUC_{iv}		31.83	13.55	30.80	NS	21.04	20.43	22.64	23.38 ± 6.90
F, %		80	154	95	NS	117	109	115	112 ± 25
AUC_{po}	500	15.22	NS	33.22	23.46	47.54	41.24	43.64	34.05 ± 12.59
AUC_{iv}		38.10	NS	54.70	34.06	39.48	52.97	60.09	46.57 ± 10.66
F, %		40	NS	61	69	120	78	73	73 ± 27
AUC_{po}	1250	NS	NS	48.63	44.43	70.68	NS	NS	54.58 ± 14.10
AUC_{iv}		NS	NS	147.53	121.56	90.93	NS	NS	120.01 ± 28.33
F, %		NS	NS	33	37	78	NS	NS	49 ± 25

Ascorbic acid bioavailability (*F*) at single doses of 200, 500, and 1250 mg. AUC represents area under the curve and above baseline after administration of each dose either orally (AUC_{po}) or i.v. (AUC_{iv}). Units for AUC_{po} and AUC_{iv} are mg of vitamin C per h per liter. Bioavailability (*F*) was the ratio of AUC_{po} divided by AUC_{iv} expressed as percent. NS, no samples were available. See Fig. 3 for details.

decrease proportionally (Table 2). The explanation could be vitamin C urinary excretion (32). Urine was collected throughout bioavailability sampling, during both oral and intravenous administration of vitamin C. Less than 0.4 mg of vitamin C appeared in urine of all volunteers after single doses of 15 and 30 mg (Fig. 4). After single doses of 50 mg, six of seven subjects had <0.4 mg of vitamin C in urine. At the plateau for the 60-mg daily dose (immediately prior to the 50-mg bioavailability sampling), vitamin C excretion was <0.4 mg in 24-h urine collections from several volunteers (data not shown). From 100 mg of vitamin C as a single dose administered orally or i.v., urine excretion was ≈25 mg. At 500- and 1250-mg single doses, urine excretion was greater for i.v. than oral vitamin C, consistent with decreased bioavailability at these doses (Table 2). Fractional excretion was determined for vitamin C administered intravenously (Fig. 4 *Inset B*). The 500- and 1250-mg doses were entirely excreted in urine.

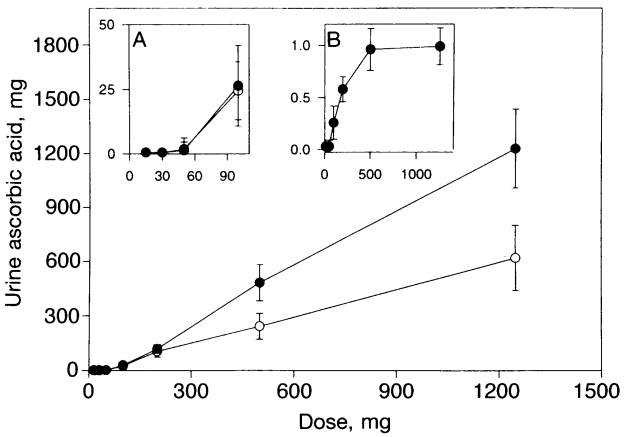

FIG. 4. Ascorbic acid excretion as a function of single vitamin C doses. Ascorbic acid excretion in urine was determined after administration of single doses of vitamin C given either orally (○) or i.v. (●). Urine was collected during determination of ascorbic acid bioavailability for each dose. The collection time for oral sampling was 24 h, and the collection time for i.v. sampling was 9–10 h, the intervals required to be certain plasma ascorbate returned to baseline (see Fig. 3 and text for details). (*Inset A*) Ascorbic acid excretion for single oral (○) or i.v. (●) doses of 15–100 mg. The condition times and conditions are as described above. The *x* axis indicates ascorbic acid dose, and *y* axis indicates ascorbic acid excretion in urine (mg). (*Inset B*) Fractional excretion (the fraction of the dose excreted) of ascorbic acid. Urine samples were collected after i.v. administration of single doses of vitamin C and were those obtained during bioavailability sampling (see above and Fig. 3). The *x* axis indicates ascorbic acid dose and *y* axis indicates fractional excretion, defined as ascorbate excreted in urine (mg) divided by the dose administered i.v. (mg). The minimum amount of ascorbate excreted was ≤0.4 mg. Fractional excretion was not determined for vitamin C administration orally because of decreasing bioavailability at doses >200 mg.

Diurnal variation of plasma vitamin C has been proposed to occur, with regulation by the pituitary–adrenal axis (33). To test this possibility, plasma vitamin C concentrations were measured over 24 h in conjunction with bioavailability sampling. No evidence of circadian rhythm or diurnal variation of vitamin C was found at any dose (not shown).

To be certain that plateau did not change and to verify the plateau concept (Fig. 1*B*), some volunteers were kept at plateau for several weeks on the lower doses (Table 1). For example, volunteer 6 had 12 samples drawn to determine plateau for 60 mg (Table 1 and Fig. 1*B*). Since samples were not drawn daily, the volunteer remained on the dose for >3 weeks after plateau was achieved (Fig. 1*B*). The different shape of the curves in Fig. 1*A* is explained by the varying amounts of time some subjects were kept at plateau. A plateau value was based on four samples over 1 week for volunteer 5 at the highest dose (Table 1). Because only 1 day was required to achieve plateau at the highest dose (data not shown), these data were included in the calculations.

Adverse Effects. Oxalate and urate excretion could increase in relation to vitamin C dose, as a consequence of either vitamin C metabolism or reabsorption (34–37). At the plateau of each dose, 24-h urine samples were collected to measure

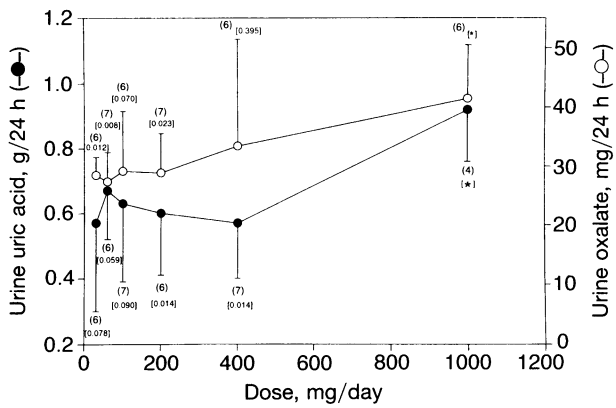

FIG. 5. Twenty-four-hour urine uric acid and oxalate excretion as a function of daily vitamin C dose. Urine uric acid (●) (left *y* axis) and urine oxalate (○) (right *y* axis) were measured in 24-h urine samples at plateau of each vitamin C dose. Values in brackets are *P* values for excretion compared to excretion at the 1000-mg vitamin C dose. Numbers in parentheses are the number of subjects whose urine was analyzed. Samples were not available for vitamin C daily doses of 2500 mg. Uric acid was analyzed by a coupled measurement of hydrogen peroxide formed by uricase (38) when urine vitamin C was ≤5 mg/dl (vitamin C doses of 30, 60, and 100 mg). In this assay vitamin C at 1 mg/dl produces a decrease of 1 mg/dl in measured uric acid. For higher vitamin C doses, urinary uric acid was analyzed by a modified uricase method (39). Urine oxalate was analyzed by a coupled assay using oxalate oxidase and horseradish peroxidase; samples were treated with activated charcoal to avoid vitamin C interference (40)

urate and oxalate excretion (Fig. 5). Excretion of both was significantly higher at plateau of the 1000-mg daily dose compared to lower doses. There were no differences when other doses were compared to each other.

Laboratory tests were unchanged from baseline during depletion and repletion at all doses, except for ferritin. Ferritin decreased for each patient presumably due to study-related blood loss. Bleeding times were normal.

High doses of vitamin C had no adverse clinical impact. For example, these doses did not cause diarrhea or abdominal cramps (11). At the nadir of depletion, mild but consistent feelings of fatigue and/or irritability were elicited from six of seven volunteers. These symptoms resolved in three volunteers within 1 week after the 30-mg daily dose was begun, and in the other three volunteers within 1 week after the 60-mg daily dose was begun. At the lowest and highest vitamin C doses, there were no differences in psychometric testing for each volunteer (data not shown). At the nadir of depletion, one volunteer had gum pain but no gingival signs of scurvy; another volunteer developed dry eyes and hyperkeratosis that was distinct from hair follicles. Both volunteers were immediately begun on the repletion part of the study with disappearance of signs and symptoms in 2–3 days. No volunteer developed scurvy.

DISCUSSION

The novel data in this paper describe vitamin C concentrations as a function of dose in humans and have three important clinical implications.

A New RDA for Vitamin C by Using Institute of Medicine Criteria. The Food and Nutrition Board of the Institute of Medicine described criteria for determining an RDA (1, 2, 9). Criteria studied here are the relationship between dose and concentration in plasma and tissues, bioavailability, urinary excretion, and potential toxicity. Vitamin C plasma concentration as a function of daily dose followed a steep sigmoidal curve in healthy young men. Sixty milligrams was on the bottom third of the curve, 100 mg was on the upper third of the curve, and 200 mg was the first dose beyond the sigmoid portion. Neutrophil vitamin C concentration was similar at the 30- and 60-mg daily doses, while 100 mg resulted in saturation of neutrophils, monocytes, and lymphocytes. Bioavailability was complete at 200 mg but not at higher doses. Urinary excretion did not occur until the 100-mg dose, while nearly all of the absorbed vitamin was excreted at the 500-mg dose. Complete plasma saturation occurred at 1000 mg at the expense of decreased bioavailability and increased urinary excretion. Potentially adverse effects did not occur until the 1000-mg daily dose (see below). The Institute of Medicine concept of the RDA includes the criteria above. The RDA is the amount of vitamin that yields the least risk of inadequacy and the least risk of toxicity (1, 2, 9). Based on these guidelines and our data, especially the sigmoid curve characteristics of vitamin C pharmacokinetics, 200 mg daily is a suitable RDA for vitamin C. The current RDA falls on the sigmoid part of the curve and is not desirable because small changes in ingestion produce large changes in plasma vitamin concentration.

Other Institute of Medicine RDA criteria are nutrient intakes and availability, biochemical and molecular function in relation to intake, and epidemiologic observations (9). Although these criteria were not studied here, they provide additional support for an RDA of 200 mg. (*i*) Vitamin C is available in the diet, but only if appropriate foods are consumed. Based on cancer prevention, the U.S. Department of Agriculture and the National Cancer Institute recommend that five servings of fruits and vegetables should be eaten daily, providing ≥200 mg of vitamin C (41). However, many people ingest much less: 20–30% of adults in the United States ingest ≤60 mg daily (42, 43). (*ii*) Indirect but revealing information is available regarding function. Vitamin C is accumulated in cells in part by a sodium-dependent transporter with saturable kinetics (44, 45). The transporter achieves V_{max} at ≈70 μM (25, 44, 45), the same plasma concentration achieved by ingestion of 200 mg daily. In contrast, the current RDA yielded a plasma concentration of ≈24 μM, similar to transporter K_m of 5–30 μM (25, 44, 45). Small changes in concentration at transporter K_m yield large changes in amount transported, behavior predicted by Michaelis–Menten kinetics. Kinetic and biochemical data (11, 25, 45–48) imply that ideal vitamin C ingestion should yield a plasma concentration above the K_m of the transporter: 200 mg daily produced this plasma concentration. The 200-mg daily dose also might prevent formation of harmful nitrosamines in the gastrointestinal tract (49) and produces a plasma concentration that might inhibit low density lipoprotein oxidation (50).

The present data have implications for epidemiologic studies that test benefits of vitamin C. Recent controversial results (51–55) might be explained by the sigmoid curve of vitamin C dose vs. plateau concentration (Fig. 1*C*). If control subjects ingested ≥100 mg of vitamin C from foods (54), plasma vitamin C would be two-thirds of maximum. Supplements would not affect plasma concentrations sufficiently to test benefit. Future epidemiologic studies must account for the steep relationship between vitamin C dose and plasma/tissue concentrations at doses between 30 and 100 mg.

Earlier studies did not meet Institute of Medicine criteria for RDA determination because of narrow dose ranges, few doses, no measurements of true bioavailability, insufficient dietary control in outpatients, insensitive nonspecific assays, and study designs (1–21). The current RDA is based on depletion–repletion data from nine inpatients (1, 2, 4–7). Since four were fed a diet with multiple vitamin deficiencies (4, 5), the appropriate inpatient data base for the current RDA is five inpatients. We studied seven inpatients.

Saturation, Potential Adverse Effects, and Upper Safe Ingestion. At the vitamin C dose of 1000 mg daily, urine uric acid and oxalate were elevated. Earlier results were variable, perhaps due to differences in assays, duration of vitamin C administration, patient subsets, or patients with prior history of oxalate stones (35–37). If high doses were administered longer, it is unclear whether the elevations would remain and whether renal calculi would result. When patients ingested ≥1000 mg of vitamin C daily, unexpected calculi were not reported (51, 54–56). Based on our new data, recent urinary excretion findings (36, 37), and Institute of Medicine criteria (1, 2, 9), upper safe doses of vitamin C are <1000 mg daily in healthy people. We do not recommend higher doses because there is no clear benefit of excess excreted or unabsorbed vitamin C, plasma concentrations were near saturation at 400 mg daily, and there may be adverse consequences at doses ≥1000 mg.

Subclinical Vitamin C Deficiency: Clinical Application. Six of seven volunteers noted mild but distinct fatigue and/or irritability at depletion, without scurvy. Symptoms disappeared within several days of the 30- or 60-mg daily dose. Although fatigue and irritability have myriad causes, vitamin C deficiency without scurvy should be an additional consideration. Since fatigue and irritability are common symptoms and were so easily reversible, physicians should ask patients with these symptoms about vitamin C ingestion from foods or supplements.

Methodologic Considerations. The findings here are for seven healthy men under age 27. Vitamin C pharmacokinetics are incomplete for women, ill patients, smokers, and the elderly. Pharmacokinetics studies in progress will provide new data for healthy women. Until then, findings here can be applied to women because it is possible that they achieve higher plasma concentrations at lower doses compared to men (14, 15). The data here are also relevant to subjects over a wide

age range, although it is possible that the elderly will require more ascorbate than younger subjects (19, 20, 22).

Bioavailability of the 200-mg dose was complete when calculated by using the linear trapezoidal method (30, 31). Although bioavailability was also probably complete at lower doses, it could not be determined precisely. Bioavailability calculations assume constant volume of distribution and constant clearance. Based on the data here and elsewhere (8), these assumptions are not met at vitamin C doses <200 mg. Precise methods to determine bioavailability depend on a pharmacokinetic model that accounts for changes in these parameters as a function of dose. Such a model for vitamin C is under development.

We are indebted to the 8 West Nursing Staff for their hard work and dedication, to Dr. Thomas Ludden for his thoughtful comments, to Dr. Edythe Wiggs for performing psychometric testing, and most of all to the volunteers who made this study possible.

1. Subcommittee on the 10th Edition of the RDAs, Food and Nutrition Board, Commission on Life Sciences, National Research Council (1989) *Recomended Dietary Allowances* (National Academy Press, Washington, DC), 10th Ed., pp. 115–124.
2. Subcommittee on the 9th Edition of the RDAs, Food and Nutrition Board, Commission on Life Sciences, National Research Council (1980) *Recomended Dietary Allowances* (National Academy Press, Washington, DC), 9th Ed., pp. 72–82.
3. Baker, E. M., Saari, J. C. & Tolbert, B. M. (1966) *Am. J. Clin. Nutr.* **19,** 371–378.
4. Baker, E. M., Hodges, R. E., Hood, J., Sauberlich, H. E. & March, S. C., (1969) *Am. J. Clin. Nutr.* **22,** 549–558.
5. Hodges, R. E., Baker, E. M., Hood, J., Sauberlich, H. E. & March, S. C. (1969) *Am. J. Clin. Nutr.* **22,** 535–548.
6. Hodges, R. E., Hood, J., Canham, J. E., Sauberlich, H. E. & Baker, E. M. (1971) *Am. J. Clin. Nutr.* **24,** 432–443.
7. Baker, E. M., Hodges, R. E., Hood, J., Sauberlich, H. E., March, S. C. & Canham, J. E. (1971) *Am. J. Clin. Nutr.* **24,** 444–454.
8. Kallner, A., Hartman, D. & Hornig, D. (1979) *Am. J. Clin. Nutr.* **32,** 530–539.
9. Food and Nutrition Board, Institute of Medicine, U.S. National Academy of Sciences (1994) *How Should the Recommended Dietary Allowances be Revised?* (National Academy Press, Washington, DC), pp. 7–15.
10. Levine, M. (1986) *N. Engl. J. Med.* **314,** 892–902.
11. Levine, M., Dhariwal, K. R. & Cantilena, C. C. (1993) *World Rev. Nutr. Diet.* **72,** 114–127.
12. Jacob, R. A., Skala, J. H. & Omaye, S. T. (1987) *Am. J. Clin. Nutr.* **46,** 818–826.
13. Jacob, R. A., Pianalto, F. S. & Agee, R. E. (1992) *J. Nutr.* **122,** 1111–1118.
14. Vanderjagt, D. J., Garry, P. J. & Bhagavan, H. N. (1987) *Am. J. Clin. Nutr.* **46,** 290–294.
15. Garry, P. J., Goodwin, J. S., Hunt, W. C. & Gilbert, B. A. (1982) *Am. J. Clin. Nutr.* **36,** 332–339.
16. Mayersohn, M. (1972) *Eur. J. Pharmacol.* **19,** 140–142.
17. Melethil, S. I., Mason, W. E. & Chiang, C.-J. (1986) *Int. J. Pharmacol.* **31,** 83–89.
18. Zetler, G., Seidel, G., Siegers, C. P. & Iven, H. (1976) *Eur. J. Clin. Pharmacol.* **10,** 273–282.
19. Blanchard, J., Conrad, K. A., Mead, R. A. & Garry, P. J. (1990) *Am. J. Clin. Nutr.* **51,** 837–845.
20. Blanchard, J. (1991) *J. Nutr.* **121,** 170–176.
21. Washko, P. W., Welch, R. W., Dhariwal, K. R., Wang, Y. & Levine, M. (1992) *Anal. Biochem.* **204,** 1–14.
22. Heseker, H. & Schneider, R. (1994) *Eur. J. Clin. Nutr.* **48,** 118–127.
23. DFM System, Inc. (1991) *DFM System* (DFM System, Des Moine, IA).
24. U.S. Department of Agriculture, Human Nutrition Information Service (1992) *Agriculture Handbook Number 8* (U.S. Dept. of Agriculture, Washington, DC), Vol. 1–21.
25. Washko, P., Rotrosen, D. & Levine, M. (1989) *J. Biol. Chem.* **264,** 18996–19002.
26. Bergsten, P., Amitai, G., Kehrl, J., Dhariwal, K., Klein, H. & Levine, M. (1990) *J. Biol. Chem.* **265,** 2584–2587.
27. Dhariwal, K., Hatzell, W. & Levine, M. (1991) *Am. J. Clin. Nutr.* **54,** 712–716.
28. Washko, P., Hartzell, W. O. & Levine, M. (1989) *Anal. Biochem.* **181,** 276–282.
29. Dhariwal, K., Washko, P. W. & Levine, M. (1990) *Anal. Biochem.* **189,** 18–23.
30. Gibaldi, M. & Perrier, D. (1982) *Pharmacokinetics* (Dekker, New York), pp. 294–297.
31. Rowland, M. & Tozer, T. N. (1989) *Clinical Pharmacokinetics: Concepts and Applications* (Lea & Febiger, Philadelphia), pp. 459–461.
32. Friedman, G. J., Sherry, S. & Ralli, E. P. (1940) *J. Clin. Invest.* **19,** 685–689.
33. Lahiri, S. & Lloyd, B. B. (1962) *Biochem. J.* **84,** 478–483.
34. Stein, H. B., Hasan, A. & Fox, I. H. (1976) *Ann. Intern. Med.* **84,** 385–388.
35. Mitch, W. E., Johnson, M. W., Kirshenbaum, J. M. & Lopez, R. E. (1981) *Clin. Pharmacol. Ther.* **29,** 318–321.
36. Urivetzky, M., Kessaris, D. & Smith, A. D. (1992) *J. Urol.* **147,** 1215–1218.
37. Wandzilak, T. R., D'Andre, S. D., Davis, P. A. & Williams, H. E. (1994) *J. Urol.* **151,** 834–837.
38. Tamaoku, K., Ueno, K., Akiura, K. & Ohkura, Y. (1982) *Chem. Pharmacol. Bull.* **30,** 2492–2497.
39. Henry, R. J., Cannon, D. C. & Winkleman, J. W. (1984) *Clinical Chemistry Principles and Techniques* (Harper & Row, New York), pp. 538–539.
40. Li, M. G. & Madappally, M. M. (1989) *Clin. Chem.* **35,** 2330–2333.
41. LaChance, P. & Langseth, L. (1994) *Nutr. Rev.* **52,** 266–270.
42. Koplan, J. P., Annest, J. L., Layde, P. M. & Rubin, G. L. (1986) *Am. J. Public Health* **76,** 287–290.
43. Murphy, S. P., Rose, D., Hudes, M. & Viteri, F. E. (1992) *J. Am. Diet. Assoc.* **92,** 1352–1357.
44. Zhou, A., Nielsen, J. H., Farver, O. & Thorn, N. A. (1991) *Biochem. J.* **274,** 739–744.
45. Welch, R. W., Bergsten, P., Butler, J. & Levine, M. (1993) *Biochem. J.* **294,** 505–510.
46. Levine, M. (1986) *J. Biol. Chem.* **261,** 7347–7356.
47. Dhariwal, K. R., Washko, P., Hartzell, W. O. & Levine, M. (1989) *J. Biol. Chem.* **264,** 15404–15409.
48. Dhariwal, K., Shirvan, M. & Levine, M. (1991) *J. Biol. Chem.* **266,** 5384–5387.
49. Helser, M. A., Hotchkiss, J. H. & Roe, D. A. (1992) *Carcinogenesis* **13,** 2277–2280.
50. Jialal, I., Vega, G. L. & Grundy, S. M. (1990) *Atherosclerosis* **82,** 185–191.
51. Enstrom, J. E., Kanim, L. E. & Klein, M. A. (1992) *Epidemiology* **3,** 194–202.
52. Gey, K. F., Stahelin, H. B. & Eichholzer, M. (1993) *Clin Invest.* **71,** 3–6.
53. Riemersma, R. A., Wood, D. A., Macintyre, C. C. A., Elton, R. A., Gey, K. F. & Oliver, M. F. (1991) *Lancet* **337,** 1–5.
54. Greenberg, E. R., Baron, J. A., Tosteson, T. D., Tor, D., Freeman, D. H. *et al.* (1994) *N. Engl. J. Med.* **331,** 141–147.
55. Rimm, E. B., Stampfer, M. J., Ascherio, A., Giovannucci, E., Colditz, G. A. & Willett, W. C. (1993) *N. Engl. J. Med.* **328,** 1450–1456.
56. Cook, J. D., Watson, S. S., Simpson, K. M., Lipschitz, D. A. & Skikne, B. S. (1984) *Blood* **64,** 721–726.