Use of a new vitamin C-deficient diet in a depletion-repletion clinical trial1,2

Jean King, Yaohui Wang, Richard W Welch, Kuldeep R Dhariwal, Cathy Conry-Cantilena, and Mark Levine

ABSTRACT To conduct an inpatient study on the recommended dietary allowance (RDA) for vitamin C, we developed a unique vitamin C-deficient diet using a nutrient database and selective menus. Fourteen different menus were developed offering > 300 items with 0–2.4 mg vitamin C per serving. During the 4–6 mo volunteers were hospitalized, daily dietary vitamin C was restricted to ≤ 5.0 mg. The mean daily dietary vitamin C intake was < 3.9 mg for the seven study subjects. With concurrent supplementation, the diet provided ≥ 85% of the RDA for 17 essential nutrients. Within 3 wk of admission the diet induced vitamin C deficiency as indicated by plasma concentrations, which decreased from 23 ± 6.9 to 6.9 ± 2.0 μmol/L. Daily intake of vitamin C and five other nutrients was determined by nutrient database analyses. Mean energy, protein, and iron were 105–185% of the RDA and total and saturated fat were 32% and 10% of energy, respectively. Weight and nutritionally relevant indexes remained normal. Dietary adherence, calculated by the number of days with ≤ 5.0 mg vitamin C per total study days, was 88–98% per repletion dose. Computer analyses of menu selections permitted individual preferences to be met while restricting vitamin C intake to ≤ 5.0 mg/d. There were no complications from the diet during the depletion and repletion phases. With this diet, ascobic acid pharmacokinetics for escalating doses could be determined in healthy volunteers. Am J Clin Nutr 1997;65:1434–40.

KEY WORDS Ascorbic acid, vitamin C, recommended dietary allowance, men, pharmacokinetics, depletion diet

INTRODUCTION

Because of multiple mutations, primates do not synthesize the enzyme gulonolactone oxidase, which is required for vitamin C (ascorbid acid) biosynthesis (1). Vitamin C is therefore an essential vitamin for humans. Without adequate exogenous vitamin C, scurvy occurs and is fatal if untreated.

The current recommended dietary allowance (RDA) for vitamin C is 60 mg (2), which is based on three general concepts: prevention of scurvy with a margin of reserve, provision of adequate body stores as indicated by urinary excretion of the vitamin, and replacement of vitamin consumed as a result of catabolism (2).

However, there are several problems with these concepts (3, 4). Prevention of the deficiency disease scurvy does not imply the provision of the ideal amount of vitamin C for human health. Although catabolic estimates were calculated from data for vitamin C-deficient subjects, catabolism may vary in relation to repletion status (3). It is also unclear whether urinary excretion of vitamin C indicates adequate body stores or saturation of plasma and tissues.

Vitamin C allowances can be determined by using the biochemical and clinical principles of in situ kinetics (4, 5). Specifically, one clinical goal is to determine how much vitamin C is achieved in humans as a function of vitamin intake. Data available for this dose-concentration curve are incomplete or flawed (6–15). Inpatient studies used narrow dose ranges or few doses. Some outpatient studies also used narrow dose ranges (10, 13). In all outpatient studies, strict control of the amount of vitamin C intake was not possible. Such control is necessary and is best accomplished with an inpatient study design (16). If there is a steep relation between dose and resulting plasma concentration, small differences in vitamin C intake will cause large differences in plasma values (17, 18). Under such conditions, data interpretation is difficult unless intake is strictly controlled. In prior outpatient and inpatient studies, it was not possible to determine bioavailability of different doses, urine excretion of the vitamin at steady state for many different doses, and whether steady state conditions were achieved for each dose.

To obtain dose-concentration and pharmacokinetic data, it is necessary to control vitamin C intake in experimental subjects. The best experimental design would use inpatient volunteers so that the diet could be strictly controlled (16). Volunteers would consume a diet deficient only in vitamin C, and vitamin C would then be administered as a drug (17, 19, 20). Volunteers would be hospitalized 4–6 mo so that a wide range of vitamin C doses could be investigated.

To proceed with such a study, the restriction of dietary vitamin C must not compromise the adequate provision of other essential nutrients. The diet should provide maximum palatability and variety to be suitable for the 4–6-mo study

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duration. An ideal experimental design would include daily assessment of nutrient consumption, including vitamin C, and evaluation of specific clinical indexes indicative of nutritional status throughout the study.

Although other vitamin C–deficient diets for inpatients have been described (7, 15, 21–28) they do not meet these experimental needs. We describe here the development and use of a new diet that met these criteria and contained \( \leq 5.0 \text{ mg vitamin C/d.} \) The diet was used in a study with seven male volunteers (18).

**SUBJECTS AND METHODS**

**Study design and subjects**

The study design is described in more detail elsewhere (17, 18). Seven healthy men were selected for the study after intensive outpatient screening. Written informed consent was obtained from all enrolled volunteers (protocol no. 92-DK-0033). The protocol was approved by the Institutional Review Board, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. Volunteers were hospitalized as inpatients at the National Institutes of Health Clinical Center for 116–180 d. During the entire hospitalization, volunteers received the vitamin C–deficient diet described below. Plasma vitamin C was measured every 1–3 d throughout the study.

On admission, volunteers entered the study’s depletion phase to reduce plasma vitamin C concentrations to 5–10 \( \mu \text{mol/L, without inducing signs or symptoms of scurvy.} \) At the nadir of depletion, volunteers entered the study’s repletion phase. Daily depletion doses of ascorbate were 30, 60, 100, 200, 400, 1000, and 2500 mg given sequentially as described previously (18). Half of the dose was ingested twice daily in the fasting state. Bioavailability was determined for each ingested dose amount (single doses of 15, 30, 50, 100, 200, 500, and 1250 mg). Steady state plateau concentration was defined as the mean of at least five samples drawn over \( \geq 7 \text{ d with } \leq 10\% \text{ SD. Cell isolation, urine collections, and bioavailability studies were performed as described previously (18).} \)

**Data calculation and assays**

Experimental results are given as means ± SDs. All samples for vitamin C (ascorbic acid) were analyzed by HPLC with coulometric electrochemical detection as described previously (29, 30).

**Vitamin C–deficient diet**

The diet was designed to restrict vitamin C ingestion to \( \leq 5.0 \text{ mg/d.} \) Verification of the vitamin C content of foods and recipes was determined by using four sources: the US Department of Agriculture (USDA) *Handbook No. 8* (31); manufacturers’ nutrient analyses; the DFM System, a computerized nutrient data software program based on the USDA standard reference (DFM is a trademark for proprietary food service software: DFM Systems Inc, Des Moines, IA; 32); and the National Institutes of Health (NIH) Clinical Center Nutrition Department’s modifications of DFM listings. Using DFM, the Nutrition Department created a database containing \( \approx 2500 \) recipes and food and beverage items

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**Table 1**

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juice</td>
<td>Appetizers, salads, and</td>
<td>Appetizers, salads, and</td>
</tr>
<tr>
<td>Apple</td>
<td>dressings</td>
<td>dressings</td>
</tr>
<tr>
<td></td>
<td>Chicken noodle soup</td>
<td>Chicken and rice soup</td>
</tr>
<tr>
<td></td>
<td>Cream of chicken soup</td>
<td>Cottage cheese</td>
</tr>
<tr>
<td></td>
<td>Croutons</td>
<td>Croutons</td>
</tr>
<tr>
<td>Fruit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applesauce</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raisin Bran(^1)</td>
<td>Entrees</td>
<td>Entrees</td>
</tr>
<tr>
<td>Shredded Wheat(^1)</td>
<td>Escaloped chicken</td>
<td>Fried shrimp</td>
</tr>
<tr>
<td>Unprocessed bran</td>
<td>Pork chops</td>
<td>Roast beef</td>
</tr>
<tr>
<td>Hominy grits</td>
<td>Brown gravy</td>
<td>Brown gravy</td>
</tr>
<tr>
<td>Cream of Wheat(^2)</td>
<td>Garlic herb pizza</td>
<td>Macaroni and cheese</td>
</tr>
<tr>
<td></td>
<td>Grilled cheese sandwich</td>
<td>Cheeseburger</td>
</tr>
<tr>
<td></td>
<td>Vanilla yogurt</td>
<td>Chicken salad</td>
</tr>
<tr>
<td></td>
<td>Tuna chunks</td>
<td>Plain yogurt</td>
</tr>
<tr>
<td></td>
<td>Plain yogurt</td>
<td>Peach yogurt</td>
</tr>
<tr>
<td></td>
<td>Blueberry yogurt</td>
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</tr>
<tr>
<td>French toast</td>
<td></td>
<td></td>
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<tr>
<td>Syrup</td>
<td>Vegetables and starches</td>
<td>Vegetables and starches</td>
</tr>
<tr>
<td>Plain yogurt</td>
<td>Rice royale</td>
<td>Pinto beans</td>
</tr>
<tr>
<td>Strawberry yogurt</td>
<td>Black beans</td>
<td>Rice</td>
</tr>
<tr>
<td></td>
<td>Pears</td>
<td></td>
</tr>
<tr>
<td>Breads and spreads</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bran muffin</td>
<td>Pretzels</td>
<td>Diet Jello(^1)</td>
</tr>
<tr>
<td>Glazed doughnut</td>
<td>Desserts</td>
<td>Vanilla ice cream</td>
</tr>
<tr>
<td>Mini bagel</td>
<td>Applesauce</td>
<td>Angelfood cake</td>
</tr>
<tr>
<td>Cream cheese</td>
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<td></td>
</tr>
<tr>
<td>White toast</td>
<td>Diet Jello(^2)</td>
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<td>White bread</td>
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<td>Wheat toast</td>
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<tr>
<td>Margarine</td>
<td>Chocolate ice cream</td>
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</tr>
<tr>
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<td>Sugar cookie</td>
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<tr>
<td>Peanut butter</td>
<td>Breads and spreads</td>
<td>Beverages</td>
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<td></td>
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<tr>
<td>Low-fat cream cheese</td>
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</tr>
<tr>
<td>Beverages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee</td>
<td>Other</td>
<td>Coffee</td>
</tr>
<tr>
<td>Decaffeinated coffee</td>
<td>Mustard</td>
<td>Decaffeinated coffee</td>
</tr>
<tr>
<td>Tea</td>
<td>Mayonnaise</td>
<td>Cream</td>
</tr>
<tr>
<td>Decaffeinated tea</td>
<td>Sugar substitute</td>
<td>Tea</td>
</tr>
<tr>
<td>Cream</td>
<td>Relish</td>
<td>Decaffeinated tea</td>
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<tr>
<td>Milk</td>
<td>Beverages</td>
<td>Evening snacks</td>
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<tr>
<td>Whole</td>
<td></td>
<td>Peanut butter crackers</td>
</tr>
<tr>
<td>2% fat</td>
<td></td>
<td>Graham crackers</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>Chocolate chip</td>
</tr>
<tr>
<td>Chocolate</td>
<td></td>
<td>cookie</td>
</tr>
<tr>
<td>Buttermilk</td>
<td></td>
<td>Popcorn</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ginger ale</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diet cola</td>
</tr>
</tbody>
</table>

\(^1\) Kraft Foods, Inc, White Plains, NY.  
\(^2\) Nabisco, Inc, East Hanover, NJ.
TABLE 2
Nutrient contents of selections from the cycle 3 sample menu

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>RDA†</th>
<th>Selection 1</th>
<th>Selection 2</th>
<th>Selection 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>58</td>
<td>134</td>
<td>129</td>
<td>130</td>
</tr>
<tr>
<td>Vitamin A (RE)</td>
<td>1000</td>
<td>1129</td>
<td>1133</td>
<td>1111</td>
</tr>
<tr>
<td>Vitamin D (mg)</td>
<td>10</td>
<td>20.4</td>
<td>16.0</td>
<td>9.9</td>
</tr>
<tr>
<td>Ascorbate (mg)</td>
<td>60</td>
<td>4.4</td>
<td>3.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>1.5</td>
<td>2.9</td>
<td>2.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.7</td>
<td>2.2</td>
<td>2.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>19</td>
<td>26.8</td>
<td>16.9</td>
<td>26.0</td>
</tr>
<tr>
<td>Vitamin B-6 (mg)</td>
<td>2.0</td>
<td>2.2</td>
<td>1.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Folacin (µg)</td>
<td>200</td>
<td>485.0</td>
<td>503.0</td>
<td>421.2</td>
</tr>
<tr>
<td>Vitamin B-12 (µg)</td>
<td>20</td>
<td>5.5</td>
<td>5.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1200</td>
<td>1177</td>
<td>1688</td>
<td>694</td>
</tr>
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<td>Phosphorus (mg)</td>
<td>1200</td>
<td>2204</td>
<td>2210</td>
<td>1686</td>
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<tr>
<td>Magnesium (mg)</td>
<td>350</td>
<td>441</td>
<td>461</td>
<td>328</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>10</td>
<td>61.3</td>
<td>58.2</td>
<td>55.9</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>15</td>
<td>15.6</td>
<td>15.7</td>
<td>13.6</td>
</tr>
<tr>
<td>Pantothenate (mg)</td>
<td>42</td>
<td>3.5</td>
<td>2.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>1.5</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>2.0</td>
<td>4.4</td>
<td>4.8</td>
<td>4.0</td>
</tr>
</tbody>
</table>

† Recommended dietary allowances (2) based on standards for males aged 11–24 y.

‡ No RDA limit has been set for pantothenic acid.

(data on file). More than 500 potential items from the above four sources were scrutinized for vitamin C content. Foods and beverages that were good sources of vitamin C were immediately omitted. Approximately 300 different foods and beverages were chosen for final menu inclusion. Fifty-six items contained 0.1–2.4 mg vitamin C; the remainder had no vitamin C.

Food content and recipe calculations were based on the reference cooked value for vitamin C (31, 32). When data for a specific manufactured product differed from those listed for its generic form (31, 32), nutrient information was obtained directly from the manufacturer.

Nutrient analysis for 18 essential nutrients was completed for all menus and was compared with the RDA for adult males. On the basis of this comparison, potential deficiencies were identified. With concurrent use of oral supplements (see results), the final diet provided ≥85% of the RDA for 17 essential nutrients. Vitamin C intake was restricted to ≤5.0 mg/d.

To provide variety and palatability, different menu items were offered each day in a 14-d cycle, after which time the menus were repeated. All foods were prepared by using standardized recipes and were served from the Nutrition Department’s main kitchen. Of the 56 items containing vitamin C, 5 were not prepared or prepackaged as individual portions. Therefore, these items were measured to a specified gram weight when served.

Menus were selected 1 d in advance by each subject and were scanned by computer. A nutrient limit report was generated and reviewed for vitamin C content. When the 5.0-mg maximum was exceeded, selections were revised. Meals were delivered to the nursing unit and, on completion, returned to the kitchen. Observational and weighed measurements were taken. Nutrient intake was estimated for energy, protein, fat, saturated fat, iron, and vitamin C. Snacks were offered as part of the diet according to the selection criteria described. Subjects recorded intake of snacks, and nutrients were measured similarly. Dietary intakes were documented in each subject’s medical record daily. Nutritional indexes were reviewed every 2 wk.

To reduce in-hospital depletion time, subjects were instructed to limit daily dietary vitamin C to 60 mg for the 3 wk before admission. Written materials delineating the vitamin C content of foods and sample menus were provided and weekly telephone contact was maintained.

RESULTS

The mean (± SD) plasma vitamin C concentration was 67.0 ± 17.6 µmol/L when volunteers were screened for study participation. As a consequence of limiting vitamin C intake 3 wk before admission, the mean plasma concentration at admission was 23.0 ± 6.9 µmol/L. With administration of the vitamin C inpatient diet, the rate of plasma vitamin C depletion was 1.3 ± 0.5 µmol·L⁻¹·d⁻¹. At the nadir of depletion, the plasma vitamin C concentration was 6.9 ± 2.0 µmol/L. Vitamin C administration began at this time because of a clinical concern that scurvy would otherwise occur with the study diet. These data indicate that the diet induced vitamin C depletion in patients. The data also suggest that when patients were repleted with vitamin C, the diet would not confound the results at each dose.

A replica of one of the 14 menu cycles, which shows the wide variety of items offered, is shown in Table 1. Although the diet provided ≤5.0 mg vitamin C, nutrient analyses of menus before patient enrollment revealed a consistent pattern of marginal deficiencies for the following nutrients: vitamin A, vitamin D, vitamin B-6, folic acid, pantothenate, calcium, magnesium, and zinc. For these nutrients the diet provided 50–95% of the RDA, depending on the cycle and the particular nutrient. Minimal deficiencies of copper, niacin, thiamine, and riboflavin were seen less frequently with each menu. The nutrient contents of the three different menu items shown in Table 1 are shown in Table 2. These data provide the range of nutrient consumption achievable from a selective menu design. Similar information was obtained for all menu cycles (data not...
shown). On the basis of these menu analyses, supplements were prescribed for those nutrients with the greatest potential for depletion over the study duration (Table 3). A vitamin B–complex tablet was used to reduce the number of pills subjects ingested daily.

The mean intake of the six nutrients monitored daily over the 4–6 mo is shown in Table 4. These data show that estimated nutrient needs were met. To further assess the nutritional adequacy of this diet, fat, saturated fat, and serum lipids were monitored. With an average intake of 32% of energy as fat and 10% of energy as saturated fat, mean serum cholesterol decreased 13% and triacylglycerols by 38% (Table 4 and Table 5). Although hemoglobin and hematocrit decreased 8.5% and 11.0%, respectively, acceptable values were maintained. A decline in hemoglobin and hematocrit values was likely due to ~600 mL of blood taken for study purposes during hospitalization rather than to inadequate dietary intake. Mean iron intake was 27 mg, 270% of the RDA for adult males, and came predominantly from heme-iron sources that are not influenced by vitamin C (2).

The daily dietary intake of vitamin C for all subjects was calculated and averaged 3.87 ± 0.64 mg over the entire study. As shown in Table 6, subjects ingested ≥ 5.0 mg vitamin C 91% of the time and 5.1–6.0 mg for approximately one half of the remaining days. We further evaluated the dietary intake of vitamin C at each repletion dose. Intakes ≤ 5.0 mg were achieved 88–98% of the time (Table 7). During the RDA dose (60 mg), adherence was 93%. These data indicate that, as predicted, the diet succeeded in providing ≤ 5.0 mg vitamin C/d, and that this goal could be accomplished by using a selective menu design.

With the use of selective menus, it was possible that vitamin C intake from the diet could have varied at different repletion doses. Such variation could affect dose-concentration data. Therefore, to be certain dietary vitamin C depletion was uniform for all patients, we calculated the dietary intake of vitamin C at every dose for every patient (Table 8). These data indicate that similar vitamin C intakes from the diet were achieved at each vitamin C dose.

Intake analysis was completed for 98.5% of all study days. Data omission occurred only 1.5% of the time and was random for dose and subject. These omissions did not skew the results presented.

**DISCUSSION**

We created a new diet that induced vitamin C depletion, provided dietary variety, and provided ≥ 85% of the RDA for 17 essential nutrients. A computerized nutrient database has the unique advantage of permitting individual intake to be calculated by using a selective menu design. The 14 menus offered 300 different items, as many as 35 items at each meal, from which personal preferences were chosen. During the 4–6 mo, vitamin C ingestion was restricted to ≤ 5.0 mg vitamin C for 91.4 ± 8.7% of all study days. Mean daily intake of vitamin C was 3.87 ± 0.64 mg. With the use of this diet, ascorbic acid pharmacokinetic studies were performed for the first time in healthy volunteers for doses of 30–2500 mg (18).

Access to a nutrient database permitted potential deficiencies to be identified and corrected with supplements before feeding subjects. On the basis of individual intake data and nutritional indexes, sufficient amounts of essential nutrients, excluding vitamin C, were provided. Weight and visceral protein stores were maintained. Hyperlipidemia, iron deficiency anemia, and macrocytic anemia did not occur and serum magnesium remained stable.

An inpatient diet providing < 5 mg vitamin C/d was essential for determining steady state pharmacokinetics of vitamin C at each different dose, including steady state plasma concentrations, bioavailability, urine excretion, and cell content. Prior intake studies were incomplete, and steady state plasma concentrations for doses > and < 60 mg were not clear (6–15). Because the current RDA is 60 mg/d, it was necessary to study doses both > and < 60 mg. Outpatient studies provided data for intakes < 60 mg, but these data were variable (10, 12–14). Detected variation in baseline dietary intake in some of these studies was as much as 40 mg, and undetected variation could be larger (13, 16). If small changes in dose result in large changes in plasma concentrations, variations in intake make it very difficult to interpret results (17, 18). It is therefore essential to control intake rigorously, especially at low doses. The best way to determine complete pharmacokinetics is to deplete inpatient subjects as much as possible but without inducing scurvy, and then to provide ascorbic acid in its simplest form in a range of doses (17–20). In this way, steady state can be achieved for doses > and < 60 mg/d. Steady state conditions are necessary for determining pharmacokinetics. The inpatient diet was essential for reducing plasma concentrations of vitamin C to < 10 μmol/L, so that complete pharmacokinetics could be determined for different daily doses.

The mean plasma vitamin C concentration at entrance to the study was 23 μmol/L, achieved by asking subjects to follow an outpatient diet limiting vitamin C intake to 60 mg/d. Subjects were not at steady state on the outpatient diet. The outpatient diet was not intended to produce steady state but rather to shorten in-hospital depletion time. The outpatient diet was not used as a tool to test the effect of dose on plasma concentrations or renal threshold. Previously published plasma concentrations at an intake of 60 mg/d varied ~10-fold (6–13, 15). To avoid the problems that may have contributed to this wide

**TABLE 4**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/d)</td>
<td>12 667 ± 1 142 [105]$^2$</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>120 ± 21 [182]$^1$</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>109 ± 27 [32 total energy]</td>
</tr>
<tr>
<td>Saturated fat (g/d)</td>
<td>33 ± 12 [10 total energy]</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>27 ± 7 [151]</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>3.87 ± 0.64 [6.5]</td>
</tr>
</tbody>
</table>

$^1$ ± SD; n = 7. RDA, recommended dietary allowance (2). Percentage estimated need or RDA in brackets; based on actual mean intake compared with upper limit of estimated requirement range.

$^2$ Estimated energy needs based on the Harris–Benedict equation—basal energy expenditure × 1.5 for moderately high activity level—ranged from 10 815 to 12 075 kJ/d (2575 to 2875 kcal/d). To convert kilojoules to kilocalories, divide by 4.2.

$^3$ Estimated needs, based on an RDA of 0.8 g/kg, ranged from 56 to 66 g/d.
TABLE 5
Mean nutritional indexes for all subjects

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Admission</th>
<th>Weeks 2–3</th>
<th>Weeks 16–22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>74.7 ± 6.6</td>
<td>74.1 ± 7.2</td>
<td>75.4 ± 6.2</td>
</tr>
<tr>
<td>BMI (19–25 kg/m²)</td>
<td>24.3 ± 2.5</td>
<td>24.0 ± 2.7</td>
<td>24.5 ± 2.5</td>
</tr>
<tr>
<td>Hemoglobin (127–167 g/L)</td>
<td>154 ± 8</td>
<td>146 ± 9</td>
<td>141 ± 3</td>
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<tr>
<td>Hematocrit (0.367–0.483)</td>
<td>0.448 ± 0.026</td>
<td>0.428 ± 0.019</td>
<td>0.400 ± 0.012</td>
</tr>
<tr>
<td>Vitamin B-12 (147.6–664.2 pmol/L)</td>
<td>386.0 ± 128.4</td>
<td>448.7 ± 149.8</td>
<td>467.9 ± 174.2</td>
</tr>
<tr>
<td>Folate (679.5–2491.5 mmol/L)</td>
<td>1449.6 ± 611.6</td>
<td>1774.1 ± 702.2</td>
<td>1812.0 ± 724.8</td>
</tr>
<tr>
<td>Cholesterol (2.59–5.18 mmol/L)</td>
<td>4.35 ± 0.57</td>
<td>3.94 ± 0.65</td>
<td>3.78 ± 1.22</td>
</tr>
<tr>
<td>Triglycerol (0.11–1.58 mmol/L)</td>
<td>1.30 ± 0.84</td>
<td>0.77 ± 0.32</td>
<td>0.80 ± 0.26</td>
</tr>
<tr>
<td>Magnesium (0.65–1.05 mmol/L)</td>
<td>0.78 ± 0.04</td>
<td>0.78 ± 0.05</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td>Albumin (37–47 g/L)</td>
<td>43 ± 2</td>
<td>42 ± 2</td>
<td>42 ± 3</td>
</tr>
</tbody>
</table>

† x ± SD; n = 7.
1 Acceptable laboratory ranges based on NIH clinical laboratory standards for adult males are in parentheses.
4 Volume red cells/volume whole blood.

variation, it was necessary to have strict control of intake and to verify that steady state conditions were present. These conditions were best met with an inpatient diet.

Although other investigators of vitamin C status in humans also controlled vitamin C intake, the diets used were not suitable for our study. Experimental scurvy was investigated in four prisoner subjects (7). Depletion was achieved and maintained over 113 d by using a liquid-formula diet devoid of vitamin C. Liquid supplements were added to meet the RDAs for other vitamin and minerals (7). The formula was administered three times daily via a nasogastric tube because of its poor palatability. During the following 100-d repletion phase, a 2.5-mg/kg vitamin C diet was served according to a 2-d cycle, nonselective menu. This diet was deficient in calcium, vitamins A and D, and possibly other B vitamins, but supplements were not given (7). Today, use of a modified enteral product to deplete serum ascorbic acid concentrations is not necessary given the flexibility of planning oral diets with expanded nutrient database programs.

In a 40-d study with 10 males, another 2-d cycle, nonselective menu was used to limit daily vitamin C to 6–7 mg (21). The diet was not supplemented because of reported, but unspecified, nutritional adequacy. The nonselectivity, lack of variety, and vitamin C range prohibited its use in our study.

A 4-d nonselective menu was used in a 13-wk study with 12 males (15). Daily vitamin C intake averaged 5.1 mg, with a range of 4.8–5.3 mg. Oral supplements were not administered because the diet was reported to provide > 80% of the RDA for all other essential nutrients. The repetitiveness of nonselective menus would not provide sufficient variety for our study, which lasted nearly twice as long.

In 1986, a 14-wk study used a 7-d cycle, nonselective menu design during two consecutive 4-wk periods (22–24). The mean vitamin C content was 4.9 mg, with a range of 4.8–5.0 mg/d. Copper, magnesium, and folic acid supplements were given because of the deficiencies identified with food-composition tables. Although this cycle was longer than that of other studies (7, 15, 21), risk of diet monotony was still more likely to occur with nonselective menus repeating weekly over 4–6 mo.

An outpatient diet was used in 8–10-wk studies (25–28). Subjects were provided with written materials to help them restrict vitamin C ingestion to < 10 mg. Weekly food records were used not to assess actual intake, but to improve compliance. Adherence rates, however, were not reported (25–28).

TABLE 6
Dietary vitamin C intake for all subject days

<table>
<thead>
<tr>
<th>Vitamin C intake (mg)</th>
<th>Percentage days at range†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0–5.0</td>
<td>91.4 [828]</td>
</tr>
<tr>
<td>5.1–6.0</td>
<td>4.3 [39]</td>
</tr>
<tr>
<td>6.1–7.0</td>
<td>1.66 [15]</td>
</tr>
<tr>
<td>7.1–8.0</td>
<td>1.21 [11]</td>
</tr>
<tr>
<td>8.1–9.0</td>
<td>0.66 [6]</td>
</tr>
<tr>
<td>9.1–10.0</td>
<td>0 [0]</td>
</tr>
<tr>
<td>10.1–15.0</td>
<td>0.55 [5]</td>
</tr>
<tr>
<td>15.1–20.0</td>
<td>0.22 [2]</td>
</tr>
</tbody>
</table>

† Adherence rate determined by dividing the number of days with < 5.0 mg ingested by the total number of study days. Vitamin C intake was based on observational and weighed measurements of plate waste compared with amount of foods initially served; the number of total subject days at adherence rate in brackets.

TABLE 7
Dietary adherence per oral dose for all subjects

<table>
<thead>
<tr>
<th>Vitamin C dose</th>
<th>Percentage dietary adherence</th>
<th>Intake on days when &gt; 5.0 mg vitamin C consumed†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg</td>
</tr>
<tr>
<td>Nadir</td>
<td>88.20</td>
<td>6.7 ± 2.05 [14]</td>
</tr>
<tr>
<td>30 mg</td>
<td>92.35</td>
<td>6.5 ± 2.19 [15]</td>
</tr>
<tr>
<td>60 mg</td>
<td>92.95</td>
<td>7.69 ± 4.09 [16]</td>
</tr>
<tr>
<td>100 mg</td>
<td>88.83</td>
<td>6.58 ± 1.56 [23]</td>
</tr>
<tr>
<td>200 mg</td>
<td>94.74</td>
<td>6.69 ± 1.01 [4]</td>
</tr>
<tr>
<td>400 mg</td>
<td>96.49</td>
<td>7.95 ± 4.39 [4]</td>
</tr>
<tr>
<td>1000 mg</td>
<td>97.73</td>
<td>6.0 ± 0 [1]</td>
</tr>
<tr>
<td>2500 mg</td>
<td>86.96</td>
<td>5.47 ± 0.21 [3]</td>
</tr>
</tbody>
</table>

† x ± SD; n = 7. Values in brackets represent the number of total subject days on which there was a dietary vitamin C intake > 5.0 mg/d.


Vitamin B complex and calcium supplements were given. An outpatient design was not considered for our study because of the risk of decreased dietary control (16).

For the repletion part of the study, vitamin C doses were given in the fasted state. This is the most straightforward and consistent condition for achieving steady state and for determining true bioavailability (19, 20). To learn whether these indexes are affected by dietary substances, data for vitamin C administered alone must be obtained first. These data were published recently (18). Because bioavailability of vitamin C is complex (18), a new bioavailability model for vitamin C is under development. Once available, it will become possible to determine true bioavailability of vitamin C in relation to other substances in the diet and compare the data with bioavailability of vitamin C given in the fasted condition. Perhaps the most important substance to test is glucose because glucose inhibits both ascorbic acid and dehydroascorbic acid transport in human cells (33, 34). The diet described here can be very useful for these studies. Prior bioavailability studies described indirect measurements of bioavailability or relative bioavailability (35, 36). From these studies it was not clear whether vitamins interacted during absorption. Other dietary substances did not appear to affect relative bioavailability (36), but relative bioavailability for vitamin C may not be the same as true bioavailability (4).

The new diet described in this paper restricted daily vitamin C ingestion to \( 5.0 \) mg while substantially expanding the variety of items provided. The extended 14-d cycle minimized diet monotony over the 4–6 mo of the study. The database and updated references now available were essential for recipe evaluation and verification (31, 32). Because individual intakes could vary when selective menus are used, specific supplements were necessary to provide \( 85\% \) of the RDA.

The computerized design of our vitamin C–depleted diet can be applied to other nutrients. This will be advantageous for studies requiring specific nutrient modification and control in the inpatient setting, especially for studies of long duration.

REFERENCES