Low antioxidant vitamin intakes are associated with increases in adverse effects of chemotherapy in children with acute lymphoblastic leukemia[1–3]

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ABSTRACT

Background: Chemotherapy leads to an increase in reactive oxygen species, which stresses the antioxidant defense system. Children with acute lymphoblastic leukemia rarely are overtly malnourished, which makes this population ideal for an investigation of the relations between dietary antioxidant consumption, plasma antioxidant concentrations, and chemotherapy-induced toxicity.

Objective: This study was conducted to investigate the effect of therapy on antioxidant intakes in children with acute lymphoblastic leukemia, the relation between dietary antioxidant intakes and plasma antioxidant concentrations, and the relation between the incidence of side effects due to treatment and antioxidant intake.

Design: We conducted a 6-mo observational study of 103 children with acute lymphoblastic leukemia. Plasma micronutrient concentrations, dietary intakes, and incidence of side effects of chemotherapy were ascertained at diagnosis and after 3 and 6 mo of therapy.

Results: Throughout the 6-mo study period, subjects ingested vitamin E, total carotenoid, β-carotene, and vitamin A in amounts that were 66%, 30%, 59%, and 29%, respectively, of the US recommended dietary allowance or of the amounts specified in the third National Health and Nutrition Examination Survey. Greater vitamin C intakes at 6 mo were associated with fewer therapy delays, less toxicity, and fewer days spent in the hospital. Greater vitamin E intakes at 3 mo were associated with a lower incidence of infection. Greater β-carotene intakes at 6 mo were associated with a decreased risk of toxicity.

Conclusion: A large percentage of children undergoing treatment for acute lymphoblastic leukemia have inadequate intakes of antioxidants and vitamin A. Lower intakes of antioxidants are associated with increases in the adverse side effects of chemotherapy. *Am J Clin Nutr* 2004;79:1029–36.

KEY WORDS Acute lymphoblastic leukemia, antioxidants, chemotherapy toxicity, nutrition, diet, children

INTRODUCTION

Unlike adults with cancer, children diagnosed with cancer rarely have overt malnutrition, especially if the diagnosis was made in a timely fashion. Protein energy malnutrition at diagnosis has been shown to be directly related to the duration of symptoms before a diagnosis of cancer (1, 2). The incidence of malnutrition in children with newly diagnosed cancer is similar to that in patients with benign tumors who were referred to the same institution (3). Nutritional deficiencies can develop as a result of the treatment itself or of complications that arise from the chemotherapy or radiation, such as xerostomia, mucositis, nausea, and vomiting. Patients who are malnourished at diagnosis may have a poorer outcome (3, 4), may experience more infections (2, 5), and may be less able to tolerate therapy (4).

Few research studies have investigated the nutritional status of children with cancer, and most of those few examined only anthropometric measurements of nutritional status or clinical markers such as prealbumin (3, 6–10). We examined the antioxidant status of children with acute lymphoblastic leukemia (ALL), in part because of the widespread belief among families in our practice that antioxidant supplementation may benefit the child undergoing cancer treatment.

Antioxidant use is not part of the conventional treatment of children with cancer, and therefore it is considered complementary or alternative medicine. The use of complementary or alternative medicine among cancer patients has increased dramatically during the past 30 y. A study in British Columbia showed that 42% of children with cancer were using some form of complementary or alternative medicine, and almost half of those children were taking vitamin supplements (11). In another study of 15 children with ALL, it was reported that all 15 took vitamin supplements (12). Among pediatric oncology patients at Children's Hospital in New York City, nutritional supplementation, especially with agents with antioxidant properties, was common (13).

ALL is the most common type of childhood cancer, and therefore patients with ALL represent a large homogenous population of children for study. Most children with ALL do not have overt malnutrition, which makes them a reasonable population in which to investigate the effect of antioxidant status (14). We

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TABLE 1
Chemotherapy administered to children with acute lymphoblastic leukemia during high-risk and standard-risk protocols[1]

Standard-risk protocol	High-risk protocol
Day 0–28	Day 28
Vincristine, IV, on days 0, 7, 14, and 21	Vincristine, IV, on days 0, 7, 14, and 21
Prednisone, PO, on days 0–28	Prednisone, PO, on days 0–28
Asparaginase, IM, on M, W, and F for 3 wk	Asparaginase, IM, on M, W, and F for 3 wk
Cytarabine, IT, on day 0	Cytarabine, IT, on day 0
Methotrexate, IT, on days 7 and 28	Methotrexate, IT, on days 7 and 28
Consolidation and interim maintenance 1	Consolidation
Vincristine, IV, on days 0, 28, and 56	Cranial radiation over 14–16 d
Prednisone, PO, on days 0–9, 28–32, and 56–60	Methotrexate, IT, on days 1, 8, 15, and 22
Methotrexate, PO, on days 28, 35, 42, 49, 56, 63, 70, and 77	Cyclophosphamide, IV, on days 0 and 14
Mercaptopurine or thioguanine, PO, daily for 11 wk	Cytarabine, IV, on days 1–4, 8–11, 15–18, and 22–25
Methotrexate, IT, or methotrexate/cytarabine/hydrocotisone, IT, on days 7, 14, and 21	6-Mercaptopurine, PO, on days 0–27
	Prednisone, PO, for 10 d
	Interim maintenance 1
	Methotrexate, PO, on days 7, 14, 21, and 35
	Methotrexate, IT, on days 0 and 28
	6-Mercaptopurine, PO, on days 0–41
First 28 d of delayed intensification	First 28 d of delayed intensification
Vincristine, IV, on days 0, 7, and 14	Vincristine, IV, on days 0, 7, and 14
Dexamethasone, PO, on days 0–6 and 14–20	Dexamethasone, PO, on days 0–20
Asparaginase, IM, on M, W, and F for 2 wk	Asparaginase, IM, for 7 doses
Doxorubicin, IV, on days 0, 7, and 14	Cyclophosphamide, IV on day 28
Cyclophosphamide, IV, on day 28	Methotrexate, IT, on days 0 and 28
Methotrexate, IT, or methotrexate/cytarabine/hydrocortisone, IT, on days 0 and 28	Doxorubicin, IV, on days 0, 7, and 14

[1] IV, intravenous; PO, by mouth; IM, intramuscular; IT, intrathecal; M, Monday; W, Wednesday; F, Friday.

hypothesized that, despite adequate dietary antioxidant intakes, children would develop inadequate plasma antioxidant concentrations as a result of the increased free radical load from the chemotherapy and the cancer. We also hypothesized that patients with sufficient antioxidant intakes while undergoing chemotherapy would have better tolerance of the treatment and would experience fewer treatment-related adverse effects than would those with insufficient antioxidant intakes.

SUBJECTS AND METHODS

Subjects

Children and adolescents between the ages of 1 and 18 y with newly diagnosed ALL were recruited for this study. The study was open to patients being treated according to the standard-risk or high-risk protocols of the Children's Cancer Group, a National Cancer Institute-sponsored research consortium for the study of childhood cancer (Internet: www.childrensoncologygroup.org). According to the standards of the institutional review board of the New York Presbyterian Hospital and all other participating centers (see **Appendix A** for participating institutions), which approved the study, written informed consent was obtained from the parents or guardians of participating subjects <18 y old. Subjects aged 18–21 y signed their own consent forms. One hundred three children and adolescents with ALL were enrolled in the study. Data were available for follow-up on 93 subjects at the second time point and on 90 subjects at the third time point.

Design

The study was a prospective observational study of antioxidant status in children and adolescents with ALL who were undergoing treatment. Blood (14 mL) was collected at 3 regularly scheduled visits. The first sample collection occurred at diagnosis before any chemotherapy was given (time 1), the second on day 28 of interim maintenance therapy (3 mo after diagnosis; time 2), and the third on day 28 of delayed intensification therapy (6 mo after diagnosis; time 3). These time points were selected to reflect various amounts of chemotherapy exposure; none at time 1, low dose at time 2, and high dose at time 3. The schedule and type of chemotherapy administered during this study are shown in **Table 1**. Quality of life was assessed by using the Pediatric Quality of Life Scale (15) at all 3 time points.

Dietary intake

A 24-h food recall and a food-frequency questionnaire (FFQ) (16) were administered on the same days as the blood collection for most of the subjects. A nutritionist at each of the sites conducted the 24-h food recall and distributed the FFQ. Interviewers followed a script to probe for details about intakes. During the 24-h recall, parents were asked about their use of nutritional or herbal supplements or both. If the 24-h recall was not done within a 3-d period after blood sampling, the recall was excluded from the study. The FFQ, named the Youth/Adolescent Questionnaire (16), was used to assess intakes, both from the diet and through supplement use, over the previous 3 mo. In most cases, the parent or guardian completed the FFQ.

Nutrient intake analysis

Nutrient calculations for the 24-h dietary recall were performed by using Minnesota NUTRITION DATA SYSTEM (NDS) software (FOOD DATABASE, version 11a, and NUTRIENT

DATABASE, version 26; Nutrition Coordinating Center, University of Minnesota, Minneapolis). The Youth/Adolescent Questionnaire was analyzed at the Channing Laboratory, Boston.

Nutrient intake data from the FFQ adjusted by 24-h dietary recall data was used to ascertain changes in intake over time, to evaluate relations between nutrient intake and toxicities, and to determine whether there was a correlation between plasma concentrations and dietary intakes of the nutrient. Analyses were performed for total intakes of vitamin A, E and C, which included dietary and supplement contributions, and separately for dietary intakes alone, adjusted for supplement use as appropriate. The FFQ and the 24-h dietary recall asked about supplement use.

The 24-h dietary recalls were used to adjust the FFQ values when exploring changes in intakes over time as well as when determining deficient intakes. Because the FFQ may overestimate the intake of a single nutrient, the FFQ value was adjusted according to the percentage difference between the 2 diet analysis methods. For each micronutrient, the following method of adjustment was used: the mean intake from 24-h recalls for the group at each time point was calculated and compared with the FFQ means at each time point. The percentage difference between the FFQ and 24-h mean values was calculated, and then the FFQ was adjusted according to the percentage difference:

$$\text{Adjusted value} = \text{FFQ} \times (\text{group mean 24-h/mean FFQ}) \quad (1)$$

Side effects and disease status

Data on adverse effects of chemotherapy were abstracted from each patient's medical chart at time 1, time 2, and time 3. The data collected for each of the 3 time points included the number of days of hospitalization, chemotherapy dose reductions because of hematologic and nonhematologic toxicity, amount of delay in scheduled therapy, use of total parenteral nutrition, infection, and other grade 3 or 4 hematologic and nonhematologic toxicity as defined by the second version of the National Cancer Institute common toxicity criteria (Internet: http://ctep.cancer.gov/forms/CTCManual_v4_10-4-99.pdf). In addition, the number of patients requiring a major change in the treatment protocol (termed going "off study") because of progressive disease, relapse, or severe toxicity was recorded for each time point.

Rapidity of response to chemotherapy was evaluated after the first 7 d (high-risk patients) or 14 d (standard-risk patients) of therapy. Remission status was ascertained at the end of 28 d of treatment. The percentage of lymphoblasts present in a 100-cell count of the bone marrow was calculated. If $<5\%$ of cells in the day 7 (high-risk patients) or day 14 (standard-risk patients) bone marrow were lymphoblasts, then the bone marrow was classified as M1 (rapid early responder); a bone marrow with 5–25% lymphoblasts was classified as M2 (intermediate responder); and a bone marrow with a lymphoblast count $>25\%$ was classified as M3 (slow early responder). Remission was attained if the day 28 bone marrow was classified as M1.

Blood collection

Blood was collected into heparin-containing tubes that were protected from light. Samples were brought to the laboratory or shipped overnight for processing as soon as possible after collection. Blood was kept under subdued light until mononuclear cells were isolated by centrifugation for 25 min at $1600 \times g$ and at room temperature over Histopaque 1077 (Sigma Chemical Co, St Louis).

Antioxidant measurements

Total ascorbate

Total ascorbic acid was measured by using HPLC analysis of deproteinized plasma in which complete reduction of the dehydroascorbic acid was accomplished by the addition of homocysteine according to the procedure of Behrens et al (17). Samples were injected onto a 150×4-mm reverse-phase column (Bio-Sil ODS 5S; Bio-Rad, Richmond, CA) and analyzed by HPLC (Millennium System; Waters Associates, Milford, MA) with the use of an amperometric electrochemical detector (model LC4B; Bioanalytical Systems, West Lafayette, IN). Ascorbic acid (10 μg/mL) was used as a standard.

Vitamins A and E

Retinol and α-tocopherol concentrations in plasma were measured simultaneously by using HPLC according to the procedure of Bieri et al (18). Plasma samples were deproteinized, extracted in hexane, dried, and then reconstituted in an ethanol and methanol solution. This sample was injected onto a J'sphere ODS reverse-phase column (YMC, Wilmington, NC) and analyzed by HPLC (Waters Associates) with the use of a multiwavelength detector (model 490; Waters Associates). Concentrations of retinol and α-tocopherol standards (Sigma Chemical Co) were measured on a spectrophotometer (model DU640; Beckman Instruments, Fullerton, CA) with an extinction coefficient of 1835 (1%, 1 cm) for retinol and 71 (1%, 1 cm) for α-tocopherol.

Total carotenoids

Carotenoids in plasma were extracted into hexane and measured by using a colorimetric procedure according to Roels et al (19) on a model DU640 spectrophotometer with an absorbance of 452 nm and using an extinction coefficient of 2550 (1%, 1 cm).

HDL or LDL cholesterol and triacylglycerols

Total cholesterol and triacylglycerol concentrations were analyzed by using standardized enzymatic methods (Boehringer Mannheim, Mannheim, Germany) and an automated spectrophotometer (model 704; Hitachi, Tokyo, Japan). HDL-cholesterol concentrations were analyzed in the same manner after precipitation of apolipoprotein B–containing lipoproteins with phosphotungstic acid (20) with the use of reagents supplied by Boehringer Mannheim (St Louis). LDL-cholesterol concentrations were calculated by using the Friedewald formula (21).

Statistical analysis

The primary outcomes of this study were the 6-mo changes in plasma concentrations of vitamin E and vitamin C. The power to detect a change of 0.85 in vitamin E/total cholesterol with 100 subjects was 82% and the power to detect a change of 0.2 mg in vitamin C/total cholesterol was 93%. Plasma total carotenoids, plasma vitamin A, and dietary intakes were secondary endpoints.

Changes in nutrient intakes over time were investigated with repeated-measures analysis of variance by using the mixed procedure in SAS software (version 8.2; SAS Inc, Cary, NC). Analysis was performed together on adjusted total intakes of vitamins A, E, and C, which included dietary and supplement contribu-

TABLE 2
Demographic and clinical characteristics of participants at study entry

	Standard-risk protocol (n = 41 M, 27 F)	High-risk protocol (n = 19 M, 16 F)
Age (y)	4.7 ± 2.6[1]	10.5 ± 4.6
BMI (kg/m^2)	16.3	19.1
z Score	−0.2	0.2
Race or ethnicity (n)		
African	11	3
Asian	4	0
Non-Hispanic European	41	27
Hispanic	9	4
Other	3	1

[1] $\bar{x}$ ± SD (all such values).

tions, and separately on adjusted dietary intake alone for vitamins A, C, and E; total carotenoids; and β-carotene. The potential confounders energy, age, sex, race, and supplement use (yes or no; for dietary intake only) were included in the models. Data from the adjusted FFQ was used for both total and dietary intakes to investigate changes in dietary intakes of vitamins A, C, and E; total carotenoids; and β-carotene. Data on the use of supplements for vitamins A, E, and C were obtained from the FFQ. Tukey's adjusted test was used for post hoc analyses.

To explore the relation between antioxidants in plasma and dietary intake, we employed regression analysis with adjustment for the confounding factors of age, sex, race, energy intake, and supplement use (for dietary intake only). Regression analysis was also used to investigate the relation between dietary intakes and the risk of toxicity, response to therapy, quality of life, and disease status with the use of age, sex, race, BMI z score in relation to current recommendations for age (22), energy, and supplement intake as covariates. The distributions of measured nutrient intakes were highly skewed for all but total vitamin C intake; therefore, we analyzed square root–transformed values for energy, β-carotene, total carotenoids, vitamin E, and vitamin A. Analysis was performed on both total intake and dietary intake alone and adjusted for supplement consumption by using SPSS software (version 10.0; SPSS Inc, Chicago).

Subjects were classified as having adequate or inadequate nutrient plasma concentrations as compared with clinical chemistry standards for vitamins A, C, and E (23). A low plasma concentration of total carotenoid was defined as <40 μg/dL. Low intake was defined as less than the recommended dietary allowance (RDA) for the specific age group for vitamins C, A, and E (24) and less than the age-specific mean intake data from the third National Health and Nutrition Examination Survey (NHANES III; 25) for β-carotene and total carotenoids, obtained with the use of the adjusted values.

We investigated the relation of FFQ data, which asked about intakes over the previous 3 mo, to plasma concentrations by using SPSS version 10.0 software package (Chicago). As an internal validity check of the food intake instruments, we assessed the correlation between the FFQ and the 24-h dietary recall.

RESULTS

The study population consisted of 103 patients with newly diagnosed ALL (**Table 2**). FFQ data were available for 100 patients at time 1, for 84 patients at time 2, and for 85 patients at time 3. Forty-two percent were female and 58% were male; most (66%) were white. Fourteen percent were African American, 13% were Hispanic, and 4% were Asian. The median age was 6.7 y (range: 1–18 y).

Antioxidant intake

Changes in intakes from the diagnosis of ALL through phases of chemotherapy at different intensities were analyzed separately for each nutrient. Adjusted mean intakes for each of the nutrients are presented in **Table 3**. On average, total vitamin A intake decreased significantly between time 1 [724 ± 29 retinol equivalents (RE)] and time 2 (645 ± 31 RE; $P = 0.04$) and between time 1 and time 3 (612 ± 31 RE; $P = 0.009$). When vitamin A intake from the diet was investigated, there was an increase between time 1 and time 2 ($P < 0.001$) and between time 1 and time 3 ($P < 0.001$). There was also a decrease between time 2 and time 3 ($P < 0.001$).

β-carotene intakes remained constant over time. Intakes at time 1, time 2, and time 3 were 1647 ± 92, 1594 ± 98, and

TABLE 3
Micronutrient intakes for adjusted total intake and adjusted dietary intake alone at diagnosis and during 2 phases of chemotherapy[1]

Micronutrient	Diagnosis, time 1 (n = 100)	Interim maintenance, time 2 (n = 84)	Delayed intensification, time 3 (n = 85)
Vitamin A (μg RE)			
Total intake (includes carotenoid intake)	724 ± 29^a	645 ± 31^b	612 ± 31^b
Dietary intake	450 ± 30^a	941 ± 33^b	690 ± 33^c
β-Carotene (μg)	1647 ± 92^a	1594 ± 98^a	1465 ± 98^a
Total carotenoids (μg)	17259 ± 830^a	17638 ± 875^a	17377 ± 877^a
Vitamin C (mg)			
Total intake	92 ± 5^a	84 ± 6^a	83 ± 6^a
Dietary intake	73 ± 4^a	100 ± 5^b	106 ± 5^b
Vitamin E (mg αTE)			
Total intake	6.7 ± 0.2^a	6.2 ± 0.2^b	6.3 ± 0.2^{a,b}
Dietary intake	5.2 ± 0.1^a	8.3 ± 0.1^b	7.6 ± 0.1^c

[1] All values are $\bar{x}$ ± SE. RE, retinol equivalents; αTE, α-tocopherol equivalents; adjusted total intake, adjusted intake of nutrient from the diet plus supplement; adjusted dietary intake, adjusted intake solely from the diet. Values are adjusted by 24-h dietary recalls from patients represented in the entire data set. Repeated-measures ANOVA was used to determine change over time with Tukey's adjustment for post hoc analyses. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

TABLE 4
Percentage of children with estimated below-recommendation nutrient intakes as measured by using an adjusted food-frequency questionnaire[1]

Nutrient	Diagnosis (time 1)	Interim maintenance (time 2)	Delayed intensification (time 3)
		%	
Vitamin A (μg RE)[2]	49[a] (54[a])[3]	12[b] (12[b])	29[c] (23[c])
Total carotenoid (μg)[4]	33[5]	24	29
β-Carotene (μg)[4]	88[a]	28[b]	58[c]
Vitamin C (mg)[2]	10 (13[a])	7 (8)	7 (4[b])
Vitamin E (mg α-tocopherol)[2]	83[a] (85[a])	54[b] (50[b])	64[c] (61[c])

[1] RE, retinol equivalents. Values were adjusted according to the percentage difference between the food-frequency questionnaire and the 24-h dietary recalls. Repeated-measures ANOVA was used to determine change over time with Tukey's adjustment for post hoc analyses. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

[2] Recommended values are from the 2000 recommended dietary allowance (24).

[3] Percentage of children below recommended total intake; percentage of children below recommended dietary intake in parentheses (all such values).

[4] Recommended values are the mean intake data from the third National Health and Nutrition Examination Survey (25).

[5] Percentage of children below recommended total intake (all such values).

1465 ± 98 μg, respectively. Intakes of total carotenoids remained constant over time. Intakes at time 1, time 2, and time 3 were 17 259 ± 830, 17 638 ± 875, and 17 377 ± 877 μg, respectively.

There was no significant change in vitamin C intakes, both total and dietary, over the 6-mo period. Intakes at time 1, time 2, and time 3 were 92 ± 5, 84 ± 6, and 83 ± 6 mg, respectively.

Total vitamin E intakes decreased from time 1 [6.7 ± 0.2 mg α-tocopherol equivalents (αTE)] to time 2 (6.2 ± 0.2 mg αTE; $P = 0.03$) and remained unchanged at time 3 (6.3 ± 0.2 mg αTE; $P = 0.94$). When vitamin E intakes from the diet alone were examined separately, there was an increase from time 1 (5.2 ± 0.1 mg αTE) to time 2 (8.3 ± 0.1 mg αTE; $P < 0.001$) and a decrease between time 1 and time 3 (7.6 ± 0.1 mg αTE; $P < 0.001$) and between time 2 and time 3 ($P < 0.001$).

For most of the children, vitamin E intakes were below the RDA recommendation and β-carotene intakes were below the US mean intake as measured by NHANES III (**Table 4**). Up to 88% of the children reported intakes below the recommendation for at least one antioxidant nutrient. The percentage of children whose intakes of total vitamin C and total carotenoids were below the recommendation did not change over time, but there was an improvement over time in the percentage of those whose vitamin E intakes were originally below the recommendation. For vitamin A and β-carotene, there was a decrease in the percentage of children whose intakes were below recommendation between time 1 and time 2, but that percentage rose again between time 2 and time 3. When nutrient intake values from the diet alone were investigated, the percentage of children consuming amounts below the standard intakes did not change appreciably. The percentage of children whose intakes were below recommendation, when the dietary intakes of vitamin C were taken into account, decreased between time 1 and time 3.

Plasma antioxidant concentrations

A repeated-measures analysis of variance, after adjustment for age, sex, race, and fasting status, was conducted for vitamins A and C, for vitamin E/total lipids, and for total carotenoids. Plasma vitamin A concentrations increased both between time 1 and time 2 ($P < 0.001$) and between time 1 and time 3 ($P < 0.001$). Total plasma carotenoids increased between time 1 and time 3 ($P = 0.008$). When total carotenoids were expressed per total cholesterol, no change in plasma concentrations over time was observed. Total plasma cholesterol concentrations increased from 3.6 mmol/L at time 1 to 4.1 mmol/L at time 2 ($P = 0.02$) and to 4.8 mmol/L at time 3 ($P < 0.001$).

Plasma vitamin C concentrations increased from time 1 to time 2 ($P = 0.04$) but then decreased at time 3 ($P = 0.007$) to the original concentrations. Vitamin E expressed per total lipid was the only nutrient that decreased between time 1 and time 2 ($P = 0.02$). The ratio remained low at time 3 ($P = 0.009$), even though increases in total lipid between time 1 and time 3 ($P = 0.001$) and between time 2 and time 3 ($P = 0.006$) were observed. Plasma vitamin E concentrations decreased between time 1 and time 2 ($P = 0.005$) and remained lower at time 3 ($P = 0.02$).

Relation of antioxidant intakes to plasma concentrations

The relation between the consumption of a specific nutrient from the FFQ and its plasma concentration was analyzed for vitamins A, C, and E and total carotenoids by using regression analysis adjusted for age, sex, race, energy intake, and fasting status. β-Carotene was not measured in the plasma. Total vitamin E intakes were associated with plasma α-tocopherol ($\beta = 0.296$, $R^2 = 0.083$, $P = 0.014$) at time 1 and time 2 ($\beta = 0.284$, $R^2 = 0.136$, $P = 0.014$). Total vitamin E intakes approached significance for plasma α-tocopherol/total lipid at time 1($\beta = 0.240$, $R^2 = 0.071$, $P = 0.064$) and were significant at time 2 ($\beta = 0.263$, $R^2 = 0.184$, $P = 0.021$) and time 3 ($\beta = 0.242$, $R^2 = 0.110$, $P = 0.044$). Total vitamin C intakes were associated with plasma vitamin C ($\beta = 0.292$, $R^2 = 0.207$, $P = 0.013$) at time 1 and approached significance at time 2 ($\beta = 0.196$, $R^2 = 0.112$, $P = 0.086$). Total carotenoid and vitamin A intakes were not associated with their respective plasma concentrations at any time point according to the FFQ.

Relation of antioxidant intakes to chemotherapy regimen toxicity

Regression analysis was conducted with total vitamin intakes and dietary vitamin intakes alone to determine whether either was associated with side effects. Greater vitamin intakes were associated with a lower incidence of measured side effects (**Table 5**).

Those with greater β-carotene intakes at time 3 had a lower risk of hematologic or nonhematologic toxicity ($P = 0.04$) at this

TABLE 5
Relation between nutrient intake and side effects[1]

Nutrient (unit)	Total intake	Dietary intake
	OR (95% CI)	*OR (95% CI)*
Vitamin A (square root μg RE)		
Response to chemotherapy		
At diagnosis	—	1.08 (1.00, 1.17)
β-Carotene (square root μg)		
Toxicity		
Average intake of 3 time points	0.87 (0.80, 0.96)	—
At 6 mo	0.96 (0.92, 0.99)	—
Total carotenoids (square root μg)		
Toxicity		
Average intake of 3 time points	1.00 (1.00, 1.00)	—
Vitamin C (mg)		
Days in hospital		
At 6 mo	—	−0.31 (−1.26, −0.02)
Therapy delay		
At 6 mo	0.991 (0.98, 0.99)	0.77 (0.59, 0.98)
Toxicity		
At 6 mo	0.99 (0.98, 1.00)	0.70 (0.54, 0.92)
Average intake of 3 time points	0.98 (0.97, 0.99)	—
Vitamin E (square root mg α-tocopherol)		
Response to chemotherapy		
At diagnosis	0.55 (0.31, 0.99)	—

[1] RE, retinol equivalents. Appearance of side effects (yes or no) was regressed on individual nutrients [total intake and intake from diet adjusted for supplement use (yes or no)]. Logistic regression was used for all models except days in hospital, which used linear regression. Models were adjusted for age, sex, race, BMI *z* score, supplement use, and energy intake. Dose reduction was also studied but was not significant. $P < 0.05$.

time. At time 3, greater intakes of vitamin C were associated with lower risk of hematologic or nonhematologic toxicity (total intake: $P = 0.04$; dietary intake: $P = 0.01$), fewer delays in administration of scheduled chemotherapy (total intake: $P = 0.03$; dietary intake: $P = 0.04$), and fewer days spent in the hospital (dietary intake: $P = 0.04$).

When the nutrient intakes were averaged over the 3 time points, greater average intakes of vitamin C, β-carotene, and total carotenoids were associated with a lower risk of hematologic or nonhematologic toxicity at time 2. After Bonferroni's adjustment for multiple side effect comparisons for each nutrient intake, only greater average intakes of β-carotene remained significant at a *P* value < 0.008 at time 2 ($P = 0.002$), for a lower risk of toxicity.

Antioxidant intakes and responses to chemotherapy

Data on the rapidity of response to chemotherapy were available for 87 patients. In 57 patients, the bone marrow morphology (ie, the proportion of cells that were lymphoblasts) was classified as M1; in 19 patients the classification was M2; and in 11 patients the classification was M3. At diagnosis, those with higher vitamin A intakes ($P = 0.05$) were more likely to have a slow response to treatment, whereas those with higher vitamin E intakes ($P = 0.05$) were more likely to have a rapid response to treatment (Table 5).

Nutritional support

As determined by the FFQ, multivitamin and mineral supplementation decreased between time 1 and time 2 ($P < 0.001$) and between time 1 and time 3 ($P < 0.001$) with 41%, 20%, and 17% of patients using supplements at time 1, time 2, and time 3, respectively. Antioxidant supplementation was rare, with 4%, 3%, and 1% of patients using supplements at time 1, time 2, and time 3, respectively. Supplementation was not associated with side effects at any of the time points.

Data from the 24-h dietary recalls showed that energy intake was only 3.19 mJ at time 1, compared with 5.74 mJ at time 2 and 7.25 mJ at time 3. Average body mass index (BMI; in kg/m^2) increased from time 1 (17.2) to time 3 (18.2; $P = 0.02$). BMI *z* scores also increased between time 1 (−0.02) and time 2 (0.84; $P = 0.01$). Nutritional intervention was rarely prescribed during the 6-mo course of the study: at time 1, only 2% of patients were receiving total parenteral nutrition (TPN), and 2% were receiving oral nutrition supplementation such as Pediasure or Ensure (both: Ross Products Division, Abbott Laboratories, Columbus OH). At time 2, 7% of the subjects had received oral nutrition supplementation during the previous 3 mo, but none had received TPN. Between time 2 and time 3, 6% of the patients received TPN and 6% received oral nutrition supplementation.

DISCUSSION

Results from this study indicate that antioxidant intakes are inadequate in the children and adolescents with ALL. In relation to the RDA and NHANES III mean intake data, vitamin intakes remained inadequate throughout the study for all the nutrients measured except vitamin C. Despite adequate intakes of vitamin C, almost half of the patients had inadequate plasma concentrations. In addition, we observed a significant decrease in plasma vitamin E concentrations over the 6-mo study period, whereas plasma total carotenoid and vitamin A concentrations increased despite consistent or decreased intakes.

We observed that children were eating less at diagnosis than is usual for their age. When we conducted the 24-h dietary recalls,

it was common for the parent to comment that intake was not usual because the child was in the hospital and not feeling well. Another study of 15 children with ALL found low energy intakes at diagnosis, but intakes returned to normal after 36 d of treatment (26). Low energy intakes were also short-term in our study. Despite the increase in energy intakes, consumption of antioxidant nutrients remained low, and little improvement in their consumption was observed with the initiation of chemotherapy and through the 6-mo study period. Supplement use prevented declines in vitamin E and vitamin A intakes between time 1 and time 3.

In a subset analysis of a larger study evaluating serum antioxidant concentrations in children undergoing chemotherapy for a wide range of cancers, a trend toward reductions in serum β-carotene and α-tocopherol concentrations after 6 mo of treatment was observed in 62 children with ALL (27). However, the relation between dietary intakes and plasma antioxidant concentrations was not previously investigated in children with ALL. Whereas intakes of vitamin E remained below the RDA for most patients in the current study, few had inadequate plasma vitamin E concentrations, and vitamin A and total carotenoid concentrations increased over the course of therapy, despite either a reduction or no change in consumption. Vitamin C concentrations in the plasma varied by the phase of therapy, whereas consumption remained constant. With a reduction in leukemic cell burden, there is less utilization of vitamin C by lymphoblasts, which possibly resulted in an increase in the plasma concentrations at time 2. During delayed intensification therapy, children underwent more intensive chemotherapy and thus received a greater free radical load. This greater free radical load and a limited supply of vitamin C may account for the decrease in plasma vitamin C concentrations between time 2 and time 3. Thus, the requirement for vitamin C may be greater in children with ALL, as reflected by the number of children with low plasma concentrations despite apparently adequate intakes.

Previous studies investigated the association of anthropometric measures of nutritional status and disease outcome. Children with ALL who were undernourished, as defined by low BMI, had a lower 5-y survival, increased relapse rate, and more frequent reductions in the chemotherapy dose (4). A shorter period of remission was observed in malnourished children, as defined by height-for-age and weight-for-age (28). A lower weight-for-height was also associated with shorter remission (29), although that was not seen in a follow-up study (30). None of these studies investigated the occurrence of side effects according to micronutrient status.

The association of antioxidant intakes and the presence of hematologic or nonhematologic toxicity at time 2 and time 3 appeared to be the most sensitive of all the side effects measures that we examined. Higher average intakes of vitamin C, β-carotene, and total carotenoids over time and higher intakes of vitamin C and β-carotene at time 3 were associated with a lower incidence of chemotherapy-related toxicity. It is possible, however, that some of these significant findings were due to the multiple comparisons performed in the analyses, although the pattern of results suggests a benefit of vitamin intakes with respect to side effects. Further study is needed to confirm these findings.

Low intakes of vitamin C were the most predictive of all the nutrient intakes studied with respect to the occurrence of side effects, especially during delayed intensification therapy. Because lower consumption of vitamin C at the end of the study was found to be related to an increased risk of a delay in therapy or of experiencing hematologic or nonhematologic toxicity and because only a small minority (7%) of subjects were not meeting the RDA, an increase in the requirement for vitamin C in these patients should be considered.

Our results suggest that children are not meeting the requirement for vitamin E and that the high intakes of vitamin C during this study may not be sufficient. Because there are concerns about potential adverse interactions with chemotherapy (31), we do not support antioxidant supplementation at this time. Although we had expected antioxidant use to be common, our results did not confirm this in the children with ALL.

Limitations of this study include the lack of national standards for antioxidant status and of intake requirements for β-carotene and total carotenoids. It is possible that the cutoffs chosen were too conservative and that more children than we identified had deficiencies. Another limitation is the inadequacy of the instruments used to predict dietary intake: the 24-h dietary recall tends to underestimate and the FFQ tends to overestimate nutrient intakes. This was especially true in this study, as portion sizes remained constant in the FFQ despite a large age range for subjects. We made an adjustment to compensate for this shortcoming, but we acknowledge its limitations for quantitative assessment. However, the adjusted FFQ measures should adequately rank subject intakes.

Although subjects were consuming more energy at the later time points, they were not meeting the established antioxidant requirements set for healthy children. With the increase in free radical exposure from ALL and the chemotherapy, it would be reasonable to assume that a child with cancer would have greater nutrient requirements.

Further studies of micronutrient intakes and plasma status and their relation to side effects over the entire treatment period in children with ALL are warranted. Future studies might include an investigation of nutritional counseling and its effect on increasing antioxidant intakes from the diet and a study of the effectiveness of antioxidant supplementation. Adding more time points for data collection during the treatment period may help to better delineate the effect of antioxidant status on side effects. Our results suggest that it would be prudent for children with ALL to receive nutritional counseling to ensure that they are meeting their needs for antioxidant nutrients.

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DDK, KLT, JB, and KMK were responsible for the study design; DDK, KLT, EDL, SRR, and KMK were responsible for the data collection; DDK, JB, and KMK were responsible for the data analysis; and DDK, KLT, EDL, SRR, JB, and KMK were responsible for writing the manuscript. None of the authors had any financial or personal conflicts of interest.

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APPENDIX A

Participating institutions

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Children's Hospitals and Clinics, Minneapolis and St Paul	Susan Sencer
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