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Effects of Micronutrient Intake on Survival in Human Immunodeficiency Virus Type 1 Infection

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The authors examined the relation between dietary and supplemental micronutrient intake and subsequent mortality among 281 human immunodeficiency type 1 (HIV-1)-infected participants at the Baltimore, Maryland/Washington, DC, site of the Multicenter Acquired Immunodeficiency Syndrome Cohort Study. Subjects completed a semiquantitative food frequency questionnaire at their baseline visit in 1984. Levels of daily micronutrient intake were examined in relation to subsequent mortality over the 8-year follow-up period by using multivariate Cox models, adjusting for age, symptoms, CD4+ count, energy intake, and treatment. The highest quartile of intake for each B-group vitamin was independently associated with improved survival: B_1 (relative hazard (RH) = 0.60, 95% confidence interval (CI) 0.38–0.95), B_2 (RH = 0.59, 95% CI 0.38–0.93), B_6 (RH = 0.45, 95% CI 0.28–0.73), and niacin (RH = 0.57, 95% CI 0.36–0.91). In a final model, the third quartile of β-carotene intake (RH = 0.60, 95% CI 0.37–0.98) was associated with improved survival, while increasing intakes of zinc were associated with poorer survival. Intakes of B_6 supplements at more than twice the recommended dietary allowance were associated with improved survival (RH = 0.60, 95% CI 0.39–0.93), while intakes of B_1 and B_2 supplements at levels greater than five times the recommended dietary allowance were associated with improved survival (B_1: RH = 0.61, 95% CI 0.38–0.98; B_2: RH = 0.60, 95% CI 0.37–0.97). Any intake of zinc supplements, however, was associated with poorer survival (RH = 1.49, 95% CI 1.02–2.18). These data support the performance of clinical trials to assess the effects of B-group vitamin supplements on HIV-1-related survival. Further studies are needed to determine the optimal level of zinc intake in HIV-1-infected individuals. *Am J Epidemiol* 1996;143:1244–56.

carotene; diet; HIV-1; nutrition; pyridoxine; riboflavin; thiamine; zinc

It has been hypothesized that subclinical immune dysfunction caused by single or multiple nutrient abnormalities may affect the rate of human immunodeficiency virus type 1 (HIV-1) disease progression (1, 2). The complex interactions between nutritional status, host immunity, and infectious diseases have been well documented in the literature (3–6), and several micronutrients are known to play important roles in normal immune function. Among these are vitamins A, B_1, B_2, B_6, B_{12}, C, and E and folic acid, zinc, and iron (7, 8).

Several studies suggest that nutritional status is important in HIV-1 infection and may influence disease progression. Wasting and malnutrition have been routinely observed in acquired immunodeficiency syndrome (AIDS) patients since the beginning of the AIDS epidemic (9, 10). Wasting syndrome has been associated with increased opportunistic infections, lower CD4 cell counts, hyperactivation of the immune system, and anorexia (11–14). Wasting syndrome also appears to predict more rapid disease progression and decreased survival (15–18). Specific micronutrient abnormalities frequently occur in HIV-1-infected individuals compared with HIV-1-negative counterparts (19, 20). Low plasma or serum levels of many of these nutrients (most profoundly, vitamins A, B_6, B_{12}, and zinc) have been found to be associated with poor outcomes in HIV-1-infected individuals such as more rapid disease progression, increased mortality, impaired neurologic function, and increased mother-to-child transmission (20–24). In dietary intake studies, increased intakes of iron, niacin, riboflavin, and vitamins C and E, and moderate intakes of vitamin A have been associated with decreased progression to AIDS (25, 26). Finally, in small preliminary clinical trials, β-carotene given to HIV-1-infected individuals has

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Abbreviations: HIV-1, human immunodeficiency virus type 1; RH, relative hazard; CI, confidence interval; AIDS, acquired immunodeficiency syndrome; RDA, recommended dietary allowance.

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been shown to increase natural killer cell markers, total white blood cell count, percentage change in CD4+ count, and CD4/CD8 ratios (27, 28).

To our knowledge, there have been no epidemiologic studies that have longitudinally examined the relation between dietary intake and mortality rates in an HIV-1-infected population. This information is important because, in addition to the study of the effects of nutrient abnormalities on immune parameters, the impact on clinical outcomes, if any, should also be documented. In this study, we examined the association between dietary intake of selected micronutrients early in the course of infection with survival rates during 8 years of follow-up in a cohort of HIV-1-seropositive homosexual/bisexual men.

MATERIALS AND METHODS

Study population

Between April and November 1984, 1,153 men were recruited into the Baltimore/Washington, DC, site of the Multicenter AIDS Cohort Study, an ongoing prospective study of the natural history of HIV-1 infection in homosexual/bisexual men. The objectives, design, and recruitment protocol of the Multicenter AIDS Cohort Study have been described in detail elsewhere (29). Briefly, eligible men were over age 18 years, had a history of sexual activity with male partners, and had no AIDS-defining illnesses at entry into the study. At semiannual visits, participants were medically examined, and additional information on their health and medical status, therapeutic and illicit drug use, and sexual practices was obtained. Serum samples were collected at each of these visits for HIV-1 serology, laboratory studies, and repository storage. In addition, at the first follow-up visit (i.e., visit 2), subjects were asked to complete a self-administered semiquantitative food frequency questionnaire documenting their average dietary intake over the previous 12 months.

Of the 1,153 men who were enrolled in the study at baseline, 812 (70.4 percent) were HIV-1 seronegative and 341 (29.6 percent) were HIV-1 seropositive. This analysis includes subjects who were seropositive at the baseline study visit, who completed the food frequency questionnaire 6 months later (visit 2), and who did not develop AIDS within the first year after study entry. The early AIDS cases were excluded because it was not known whether the usual dietary intakes reported by these men had changed as a result of their illnesses. Reinclusion of these individuals in sensitivity analyses had little impact on the study results. Men who did not complete the questionnaire at visit 2 did not differ demographically or by disease stage from those who did (data not shown).

HIV-1 serostatus was measured by enzyme immunoassay (Genetic Systems, Seattle Washington) and a Western blot test (DuPont Co., Wilmington, Delaware, and Bio-Rad, Hercules, California) with interpretation using standard criteria. AIDS diagnoses were based on the 1987 revision of the Centers for Disease Control and Prevention surveillance case definition (30). Mortality data were obtained from physicians' reports, hospital records, death certificates, or at autopsy. Morbidity and mortality data were updated on a continuous basis.

Dietary intake

Usual dietary intake was measured by using a self-administered semiquantitative food frequency questionnaire (31). The questionnaire has been documented as being a valid and reliable instrument for measuring average intake of specific foods and beverages (31–34). It consists of 116 food items for which subjects were asked to estimate their frequency of consumption of each item over the previous 12 months in terms of a typical portion size. Nine frequency categories were available to choose from, ranging from "Never, or less than once per month" to "6+ per day". Also included on the form were sections for recording the brand name, frequency, and amount of any nutritional supplements taken, a write-in section for foods not listed, and a series of questions on the exact brands and types of fat used for frying, cooking, and baking. Upon completion, the questionnaires were reviewed and coded by a trained researcher and entered into computer data files. Nutrient estimates for the food items were determined using the nutrient analysis program developed specifically for the questionnaire (31). The nutrients assessed in this study were total calories; vitamins A, B_1, B_2, B_6, B_{12}, C, and E; folate; β-carotene; and zinc.

Other variables

Sociodemographic data and information on cigarette smoking and alcohol intake were obtained from interview records given at the baseline study visit. Body mass index was calculated as weight (kg)/height $(m)^2$.

Subjects were placed into one of three categories on the basis of their baseline CD4+ T lymphocyte count. The groups were defined as less than 500, 500–750, and greater than 750 cells/μl to give approximately equal numbers in each category. CD4+ cell counts were missing for 30 subjects, and these men were assigned to a category on the basis of their CD4 counts from subsequent adjacent visits. The mean decline of CD4+ lymphocyte counts in this cohort is 30 cells/

liter/6 months, so we were confident of estimating the appropriate CD4 category for these individuals.

Participants were also categorized as HIV-1 symptomatic or asymptomatic. Subjects were considered symptomatic if there were reports of any of the following over the 6 months prior to study entry: oral thrush, fatigue, weight loss (more than 4.5 kg unintentional weight loss), fever (greater than 38.5°C for 2 weeks), or diarrhea (daily for more than 2 weeks).

Data on use of antiretroviral drugs (zidovudine, didanosine, dideoxycytidine) and *Pneumocystis carinii* pneumonia prophylaxis (aerosolized pentamidine, trimethoprim-sulfamethoxazole, dapsone) during the 8 years of follow-up were also included in the analyses.

Statistical methods

Baseline characteristics of those who survived the follow-up period were univariately compared with those who did not survive by using *t* tests for continuous variables and chi-square analyses for categorical variables. For each nutrient, intakes were normalized by using the natural log transformation, calorie adjusted, and then divided into quartiles. Calorie-adjusted nutrient values were computed by obtaining the residuals from a linear model regressing nutrient intake on total caloric intake. The predicted nutrient intake at the median total caloric intake was added to these residuals to bring the residuals back into the biologic range of the raw nutrient values. Calorie adjustment of nutrient intake values is recommended in order to account for the positive correlation between nutrient intakes and total energy intake. This method is described in more detail elsewhere (35).

Univariate analyses of calorie-adjusted nutrient intakes were performed by using Kaplan-Meier survival curves. For subjects who died prior to the cutoff date for analysis (December 15, 1992), survival time was calculated as the time from study entry to the date of death. For subjects who died after the cutoff date or who remained alive, survival time was calculated as the time from study entry to the analysis cutoff date. Subjects who were lost to follow-up ($n = 14$) were censored at the midpoint between their last contact date and the analysis cutoff date. The survival probability for each nutrient intake category was then estimated from the Kaplan-Meier curves.

To assess the effect of total nutrient intake on survival, Cox proportional hazards models were fit for each nutrient by using a single variable for intake from food and supplements combined (total intake). Calorie-adjusted total nutrient intake values were divided into quartiles and entered as dummy variables into the Cox models for initial analyses. The lowest quartile of nutrient intake was always used as the reference category. Some of the quartiles were subsequently combined, if it was judged necessary, for the final analyses. Pearson's correlation coefficients were computed to determine the level of intercorrelation between micronutrients, and subsequently, models with more than one nutrient (multinutrient models) were fit to assess the degree of confounding among the different nutrients. If micronutrient intakes were highly correlated ($r > 0.85$), they were not modeled together in order to avoid collinearity.

For separation of the effects of intake from food and intake from supplements on survival, Cox proportional hazards models were fit, using a separate variable for each. In these models, quartiles of food intake were entered as a single ordinal covariate, while levels of supplement intake (in multiples of the recommended dietary allowance (RDA)) were entered as dummy variables. Again, the lowest levels of supplement intake were used as the reference category. The above models were all adjusted for age, presence of symptoms, CD4+ cell count, total energy intake, use of antiretrovirals, and use of *P. carinii* pneumonia prophylaxis. These covariates were included in the models because they were proven or putative confounding variables in the relation between nutrient intake and survival.

RESULTS

Of the 341 men who were HIV-1 seropositive at baseline, 286 (84 percent) completed the semiquantitative food frequency questionnaire at their first follow-up visit (visit 2). Five of these men were subsequently excluded from the analyses due to the early onset of AIDS-defining illnesses. Thus, the final analyses were based on 281 seropositive participants who had complete dietary intake information at visit 2. A total of 162 (58 percent) of these subjects survived the 8-year follow-up period (April-November 1984 to December 1992). Of the 119 deaths, 109 (91.6 percent) were AIDS related, eight (6.7 percent) were not AIDS-related, and two (1.7 percent) were due to unknown causes. Table 1 shows the comparison of baseline characteristics between those who survived and those who did not. Those who died tended to have a slightly higher mean age and more symptoms, and a higher percentage were white, although none of these were statistically significant. As might be expected, those who died had a significantly lower mean CD4+ cell count (592 vs. 713 cells/μl; $p = 0.003$). No differences were observed in level of education, body mass index, cigarette smoking, or alcohol consumption.

Except for vitamins B_1 and E and zinc, the median intake levels achieved from food alone were at or above the recommended levels. With the addition of

TABLE 1. Baseline characteristics of 281 human immunodeficiency virus type 1 (HIV-1) seropositive, acquired immunodeficiency syndrome (AIDS)-free men in the Baltimore/Washington, DC, Multicenter AIDS* Cohort Study, 1984

	Alive (n = 162)		Deceased (n = 119)		p value
	Mean ± SE*	%	Mean ± SE	%	
Age at baseline (years)	42.7 ± 6.4		44.0 ± 6.2		0.09
Race (% white)		81.5		89.1	0.08
Education (% college graduate)		65.4		63.0	0.68
Quetelet index	23.1 ± 2.6		23.2 ± 2.7		0.76
% cigarette smokers		34.2		33.6	0.92
Alcohol (% >3–4 drinks/week)		50.0		42.3	0.23
CD4+ cell count/μl	713 ± 344		592 ± 294		0.003
% with symptoms†		16.0		21.8	0.11

* AIDS, acquired immunodeficiency syndrome; SE, standard error.
† Presence of oral thrush, fatigue, unintentional weight loss, fever, or diarrhea within previous 6 months.

vitamin supplements, median intakes reached or surpassed the RDA level for all of the micronutrients. For vitamins B_1, B_6, and E and for zinc, greater than 20 percent of the population had intake levels below the RDA. Nearly 50 percent of the subjects had zinc intake levels below the recommended amount.

The distribution of CD4+ T lymphocyte counts according to quartiles of total nutrient intake (from food and supplements) of specific micronutrients is shown in table 2. Median CD4+ cell counts were lowest in the highest quartiles of intake for all micronutrients examined, except for β-carotene and niacin.

Impact of total intake on survival

Kaplan-Meier survival curves were computed for each of the nutrients of interest. From these curves, 70th percentile survival times were estimated for each of the nutrients. The highest quartiles of intake of vitamins B_1, B_2, and B_6 and niacin and the third quartile of β-carotene were associated with increased 70th percentile survival time of up to 1.3 years compared with men with intakes in the lower quartiles. However, higher intakes of zinc were monotonically associated with poorer survival in these univariate analyses. The Kaplan-Meier curves for the remaining nutrients (vitamin B_{12}, folate, vitamin C, and vitamin E) did not show large differences in survival probability between the different quartiles of intake. Figure 1 shows the Kaplan-Meier curves for vitamins B_6 and niacin.

Multivariate analyses were then performed using Cox proportional hazards models on the nutrients found to be significantly associated with survival in the Kaplan-Meier analyses or where near significant trends were observed (vitamins A, B_1, B_2, B_6, and B_{12}, niacin, β-carotene, and zinc). In the baseline Cox model (before the nutrients were added), higher CD4+ lymphocyte counts at study entry were significantly associated with improved survival time (relative hazard (RH) of death = 0.54 for 500–750 cells/μl vs. <500 cells/μl, $p = 0.006$) and RH = 0.49 for greater than 750 vs. less than 500 cells/μl, $p = 0.002$). Use of antiretroviral drugs during the follow-up period was also strongly associated with improved survival (RH of death = 0.44, $p < 0.001$). Age, use of *P. carinii* pneumonia prophylaxis, presence of symptoms, and total energy intake were not associated with mortality after adjustment for other covariates at baseline.

Table 3 shows the results of the Cox models for quartiles of total nutrient intake after adjustment for the covariates mentioned above. All of the B-group vitamins except vitamin B_{12} showed a trend toward increased survival time with the highest quartiles of intake. The middle two quartiles of total vitamin A intake and the third quartile of β-carotene also showed improved survival over the other quartiles. Higher intakes of zinc (quartiles 3 and 4), however, were significantly associated with poorer survival, showing a dose-response effect.

On the basis of these results, we then collapsed quartiles of intake where the relative hazards did not vary much. By doing so, we were able to observe some of the associations more clearly. Table 4 shows the results of the single- and multinutrient Cox models for total intake of each of the B-group vitamins, after the lowest three quartiles of intake for each were collapsed. In the single-nutrient Cox models, vitamins B_1, B_2, and niacin continued to show a trend toward increased risk of survival with the highest quartiles of intake. The highest quartile of total vitamin B_6 intake was significantly associated with improved survival (RH = 0.58, 95 percent confidence interval (CI) 0.37–0.92). Since quartiles of these B-group vitamins were highly intercorrelated with each other ($r > 0.8$), we did not place them together in multinutrient models.

TABLE 2. Distribution of CD4+ T lymphocyte counts* by quartiles of total nutrient intake (from food supplements), Baltimore/Washington, DC, AIDS† Cohort Study, 1984

Quartiles (Q) of total intake	CD4+ T lymphocyte count (cells/μl)	
	Median	Interquartile range
Vitamin B_1 (mg/day)		
Q1: (<1.4)	608	454–884
Q2: (1.4–2.4)	699	408–860
Q3: (>2.4–5.3)	573	413–847
Q4: (>5.3)	525	347–799
Vitamin B_2 (mg/day)		
Q1: (<2.0)	611	475–793
Q2: (2.0–3.1)	682	437–892
Q3: (>3.1–6.3)	533	407–847
Q4: (>6.3)	525	371–799
Vitamin B_6 (mg/day)		
Q1: (<2.0)	614	473–885
Q2: (2.0–3.2)	688	406–881
Q3: (>3.2–5.9)	532	401–742
Q4: (>5.9)	525	390–912
Niacin (mg/day)		
Q1: (<25)	582	438–806
Q2: (25–37)	746	515–928
Q3: (>37–64)	507	376–707
Q4: (>64)	562	371–954
Vitamin B_{12} (mg/day)		
Q1: (<7.7)	689	475–885
Q2: (7.7–14.0)	614	410–865
Q3: (>14.0–20.2)	559	406–751
Q4: (>20.2)	533	388–839
Vitamin A (IU/day)		
Q1: (<9,098)	620	462–867
Q2: (9,098–13,221)	613	407–854
Q3: (>13,221–20,762)	629	447–860
Q4: (>20,762)	514	356–845
β-carotene (IU/day)		
Q1: (<4,960)	631	438–858
Q2: (4,960–7,622)	581	413–849
Q3: (>7,622–11,179)	612	447–857
Q4: (>11,179)	591	385–865
Zinc (mg/day)		
Q1: (<12)	612	490–903
Q2: (12–14)	619	413–812
Q3: (>14–20)	623	393–860
Q4: (>20)	520	367–815

* CD4+ T cell counts from the baseline study visit were missing on 30 subjects.
† AIDS, acquired immunodeficiency syndrome.

Therefore, we fit four separate multinutrient Cox models, adjusting each B-group vitamin simultaneously for β-carotene and zinc intakes. The results showed that the highest quartile of intake for each of the B-group vitamins was significantly associated with increased survival time: vitamin B_1 (RH = 0.60, 95 percent CI 0.38–0.95), vitamin B_2 (RH = 0.59, 95 percent CI 0.38–0.93), vitamin B_6 (RH = 0.45, 95 percent CI 0.28–0.73), and niacin (RH = 0.57, 95 percent CI 0.36–0.91).

Since the highest quartile of total B_6 intake showed the strongest association with survival time in the multinutrient models, we have shown the results of this model in table 5. In a single-nutrient Cox model, the third quartile of β-carotene intake showed an improvement in survival over the lower two quartiles combined and the upper quartile of intake. After adjustment for B_6 and zinc intakes, this U-shaped relation became even more apparent (RH = 0.60 for the third quartile of intake; 95 percent CI 0.37–0.98). For zinc, higher quartiles of intake were significantly and monotonically associated with poorer survival. In the multinutrient model, the relative hazard of death was 1.84 (95 percent CI 1.16–2.93) for the third quartile of total zinc intake versus the lower two quartiles and 2.44 (95 percent CI 1.51–3.95) for the fourth quartile compared with the lowest quartile. Also shown in table 5 are the results obtained when we put vitamin A into the model in place of β-carotene. In this model, we saw the same U-shaped relation between vitamin A and mortality as we did with β-carotene, however the results were not as strong. The relative hazards for vitamin B_6 and zinc remained similar to what we observed with β-carotene in the model.

Impact of micronutrient supplements on survival

The independent effect of supplemental micronutrient intake on survival was then examined in a series of Cox models that included levels of supplemental intake and quartiles of food intake as separate variables. In this way, we could examine the effects of vitamin supplements after adjusting for each subject's vitamin intake from food. Data on supplemental intake were not available for niacin or β-carotene, so these nutrients were excluded from further analyses. Table 6 shows the results of the analyses on the remaining nutrients. Supplemental intakes of vitamins B_1 and B_2 at levels above five times the RDA were significantly associated with improved survival: For B_1, RH = 0.61 (95 percent CI 0.38–0.98), and for B_2, RH = 0.60 (95 percent CI 0.37–0.97). Supplemental intakes of vitamin B_6 at more than twice the RDA were also associated with decreased mortality (RH = 0.60, 95 percent CI 0.39–0.93). In these models, quartiles of food intake showed no association with survival. The analysis for zinc revealed that any amount of supplemental zinc intake was associated with a significantly increased risk of mortality (RH = 1.49, 95 percent CI 1.02–2.18). Quartiles of zinc intake from food were

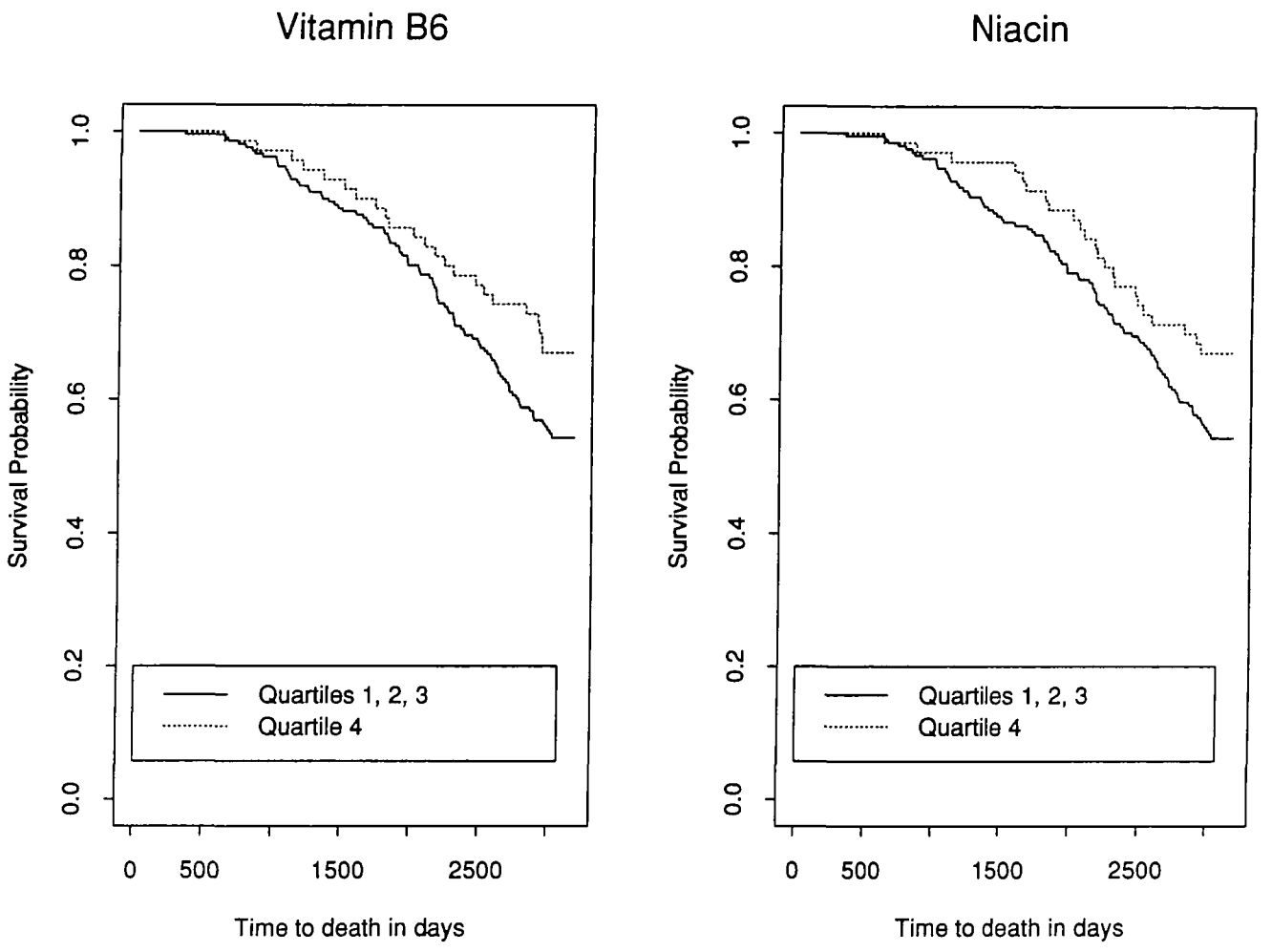

FIGURE 1. Kaplan-Meier survival curves for vitamins B_6 and niacin, comparing the upper quartile of total daily intake (>5.9 mg/day for B_6 and >64 mg/day for niacin) with the lower three quartiles combined (≤5.9 mg/day for B_6 and ≤64 mg/day for niacin) for 281 HIV-1-seropositive men enrolled in the Baltimore/Washington, DC, site of the Multicenter AIDS Cohort Study, October 1984 to December 1992.

also monotonically associated with poorer survival in the same model. Figures 2 and 3 show the Kaplan-Meier survival curves for supplemental intake of these four micronutrients.

DISCUSSION

In this study, we examined the association of dietary micronutrient intake with survival among largely asymptomatic HIV-1-seropositive men during an 8-year follow-up period. In univariate analyses using Kaplan-Meier survival curve methods, we found that the highest quartiles of total intake of several B-group vitamins (vitamins B_1, B_2, B_6, and niacin) were associated with increased survival time of up to 1.3 years. We also found that higher total intake of zinc was associated with decreased survival time. In multinutrient analyses, after adjusting each B-group vitamin for β-carotene and zinc intakes, along with several other covariates, we found that the highest quartiles of B_1, B_2, B_6, and niacin intake were each significantly associated with improved survival. In a multinutrient model that included vitamin B_6, β-carotene, and zinc, we found that the highest quartile of vitamin B_6 intake was associated with improved survival by approximately 55 percent compared with the lower three quartiles combined, while the third quartile of β-carotene intake was associated with an improved survival of about 40 percent compared with the lower two quartiles combined. In contrast, increasing intake of zinc was associated with poorer survival.

Much of the protective effects observed from the B-group vitamins (i.e., B_1, B_2, B_6, and niacin) seemed to be due to the intake of vitamin supplements. In this cohort, after adjustment for food intake, the intake of vitamin B_6 supplements at levels greater than twice the RDA of 2.0 mg/day were associated with a decrease in

TABLE 3. Cox proportional hazards models* using quartiles of total daily nutrient intake (from food and supplements) as the independent variable and time to death as the outcome variable, Baltimore/Washington, DC, Multicenter AIDS† Cohort Study, 1984–1992

Quartiles (Q) of total intake	No. of deaths/ total no. in stratum	RH†	95% CI†
Vitamin B_1 (mg/day)			
Q1: (<1.4)	31/71	1.00	
Q2: (1.4–2.4)	31/70	1.10	0.66–1.84
Q3: (>2.4–5.3)	32/70	0.97	0.59–1.61
Q4: (>5.3)	25/70	0.71	0.42–1.21
Vitamin B_2 (mg/day)			
Q1: (<2.0)	31/71	1.00	
Q2: (2.0–3.1)	31/70	1.21	0.73–2.01
Q3: (>3.1–6.3)	32/70	0.97	0.59–1.62
Q4: (>6.3)	25/70	0.71	0.42–1.21
Vitamin B_6 (mg/day)			
Q1: (<2.0)	31/71	1.00	
Q2: (2.0–3.2)	30/70	1.10	0.66–1.84
Q3: (>3.2–5.9)	35/70	1.17	0.71–1.92
Q4: (>5.9)	23/70	0.63	0.37–1.09
Niacin (mg/day)			
Q1: (<25)	32/71	1.00	
Q2: (25–37)	26/70	0.79	0.47–1.35
Q3: (>37–64)	38/70	1.12	0.74–1.95
Q4: (>64)	23/70	0.66	0.38–1.13
Vitamin B_{12} (mg/day)			
Q1: (<7.7)	29/71	1.00	
Q2: (7.7–14.0)	26/70	0.78	0.45–1.34
Q3: (>14.0–20.2)	32/70	1.25	0.75–2.08
Q4: (>20.2)	32/70	1.23	0.73–2.05
Vitamin A (IU/day)			
Q1: (<9,098)	37/71	1.00	
Q2: (9,098–13,221)	25/70	0.80	0.48–1.34
Q3: (>13,221–20,762)	25/70	0.72	0.43–1.21
Q4: (>20,762)	32/70	1.21	0.74–1.99
β-carotene (IU/day)			
Q1: (<4,960)	29/71	1.00	
Q2: (4,960–7,622)	36/70	1.39	0.85–2.29
Q3: (>7,622–11,179)	24/70	0.78	0.45–1.35
Q4: (>11,179)	30/70	1.15	0.69–1.93
Zinc (mg/day)			
Q1: (<12)	23/71	1.00	
Q2: (12–14)	26/70	1.08	0.61–1.90
Q3: (>14–20)	33/70	1.73	1.01–2.97
Q4: (>20)	37/70	1.91	1.12–3.26

* Adjusted for CD4+ cell count, age, presence of symptoms, use of antiretroviral drugs, use of *Pneumocystis carinii* prophylaxis, and total energy intake.
† AIDS, acquired immunodeficiency syndrome; RH, relative hazard; CI, confidence interval.

mortality risk of approximately 40 percent. Similar protective effects were seen with supplemental intakes of vitamins B_1 and B_2 at levels greater than five times the RDA. Conversely, intake of zinc supplements at any level above the RDA was associated with significantly poorer survival, confirming the results seen with food intake.

With regard to the B-group vitamins (B_6, in particular), the results of this study are consistent with the hypothesis that in an HIV-1-infected individual intake of these micronutrients may help improve immunologic control of HIV-1, perhaps enough to prolong survival. There is considerable in vitro and in vivo evidence in the literature on the effects of vitamin B_6

TABLE 4. Four separate multinutrient Cox proportional hazards models*, Baltimore/Washington, DC, Multicenter AIDS† Cohort Study, 1984–1992

Quartiles (Q) of total intake	Single nutrient		Multinutrient‡	
	RH†	95% CI†	RH	95% CI
Vitamin B_1 (mg/day)				
Q1–3: (≤5.3)	1.00		1.00	
Q4: (>5.3)	0.70	0.45–1.08	0.60	0.38–0.95*
Vitamin B_2 (mg/day)				
Q1–3: (≤6.3)	1.00		1.00	
Q4: (>6.3)	0.68	0.44–1.06	0.59	0.38–0.93*
Vitamin B_6 (mg/day)				
Q1–3: (≤5.9)	1.00		1.00	
Q4: (>5.9)	0.58	0.37–0.92*	0.45	0.28–0.73*
Niacin (mg/day)				
Q1–3: (≤64)	1.00		1.00	
Q4: (>64)	0.66	0.42–1.04	0.57	0.36–0.91*

* $p < 0.05$.
† AIDS, acquired immunodeficiency syndrome; RH, relative hazard; CI, confidence interval.
‡ Each multinutrient model includes a B-group vitamin adjusted for total intakes of β-carotene and zinc. Time to death is the outcome variable. All models adjusted for CD4+ cell count, age, presence of symptoms, use of antiretroviral drugs, use of *Pneumocystis carinii* prophylaxis, and total energy intake.

TABLE 5. Two multinutrient Cox proportional hazards models† including vitamin B_6, zinc, and β-carotene/vitamin A, Baltimore/Washington, DC, Multicenter AIDS‡ Cohort Study, 1984–1992

Quartiles (Q) of total intake	Single nutrient		Final multinutrient model	
	RH‡	95% CI‡	RH†	95% CI
Model 1				
Vitamin B_6 (mg/day)				
Q1–3: (≤5.9)	1.00		1.00	
Q4: (>5.9)	0.58	0.37–0.92*	0.45	0.28–0.73*
β-carotene (IU/day)				
Q1–2: (<7,621)	1.00		1.00	
Q3: (7,621–11,179)	0.67	0.41–1.08	0.60	0.37–0.98*
Q4: (>11,179)	0.98	0.63–1.52	0.82	0.52–1.28
Zinc (mg/day)				
Q1–2: (<14)	1.00		1.00	
Q3: (14–20)	1.67	1.06–2.64*	1.84	1.16–2.93*
Q4: (>20)	1.84	1.17–2.90*	2.44	1.51–3.95*
Model 2				
Vitamin B_6 (mg/day)				
Q1–3: (≤5.9)	1.00		1.00	
Q4: (>5.9)	0.58	0.37–0.92*	0.46	0.28–0.74*
Vitamin A (IU/day)				
Q1: (<9,098)	1.00		1.00	
Q2–3: (9,098–20,762)	0.76	0.49–1.17	0.69	0.44–1.08
Q4: (>20,762)	1.21	0.74–1.98	1.05	0.62–1.80
Zinc (mg/day)				
Q1–2: (<14)	1.00		1.00	
Q3: (14–20)	1.67	1.06–2.64*	1.87	1.17–2.99*
Q4: (>20)	1.84	1.17–2.90*	2.28	1.38–3.78*

* $p < 0.05$.
† All models adjusted for CD4+ cell count, age, presence of symptoms, use of antiretroviral drugs, use of *Pneumocystis carinii* prophylaxis, and total energy intake. Time to death is the outcome variable.
‡ AIDS, acquired immunodeficiency syndrome; RH, relative hazard; CI, confidence interval.

TABLE 6. Cox proportional hazards models† using levels of supplemental intake, Baltimore/Washington, DC, Multicenter AIDS‡ Cohort Study, 1984–1992

Nutrient and levels of supplemental intake	No. of deaths/ total in stratum	RH‡	95% CI‡
Vitamin B_1			
<5 × RDA‡	97/216	1.00	
≥5 × RDA	22/65	0.61	0.38–0.98*
Vitamin B_2			
<5 × RDA	98/218	1.00	
≥5 × RDA	21/63	0.60	0.37–0.97*
Vitamin B_6			
<2 × RDA	91/202	1.00	
≥2 × RDA	28/79	0.60	0.39–0.93*
Zinc			
No supplements	74/192	1.00	
Supplements	45/89	1.49	1.02–2.18*

* $p < 0.05$.

† Adjusted for quartiles of nutrient intake from food, CD4+ cell count, age, presence of symptoms, use of antiretroviral drugs, use of *Pneumocystis carinii* prophylaxis, and total energy intake. Time to death is the outcome variable.

‡ AIDS, acquired immunodeficiency syndrome; RH, relative hazard; CI, confidence interval; RDA, Recommended Daily Allowance.

deficiency on immune function (36–41). Vitamin B_6, or pyridoxine, is required for the synthesis of DNA (39), and therefore, deficiencies will adversely affect rapidly proliferating cell populations, such as those of the immune system. Studies have also looked at the effects of pyridoxine supplementation in the elderly, who are at increased risk of both low vitamin B_6 status and reduced immunocompetence. Talbott et al. (42) found that in vitro lymphocyte proliferation from both T cell and B cell mitogens increased after supplementation with 50 mg pyridoxine per day for 8 weeks compared with a control group who received placebos. In another study of the elderly, Chandra (43) demonstrated that optimum intake of all essential micronutrients resulted in an improvement in immune responses and reduced the frequency of infection.

Studies have found that HIV-1-infected individuals are also at an increased risk of vitamin B_6 deficiency (19, 23, 44). One of these studies (23) has extensively examined the effects of vitamin B_6 status in early HIV-1 infection. Using a cross-sectional study design, the authors looked at the association of vitamin B_6 status with different parameters of immune function. They found that 64 percent of the clinically asymptomatic HIV-1-infected subjects were vitamin B_6 deficient while only 33 percent of the HIV-1-seronegative control subjects were deficient. Their final results showed that immune parameters, including lymphocyte proliferative response to mitogens and natural killer cell cytotoxicity, were significantly lower in HIV-1-infected subjects who were deficient in B_6 than in HIV-1-infected subjects with adequate vitamin B_6 status. In light of these studies, our results add to the evidence that supplementation with vitamin B_6 soon after infection with HIV-1 may have some beneficial effects. However, since we do not yet know the actual vitamin B_6 status of the men in our study, we cannot be sure if the subjects who were deficient in vitamin B_6 were the ones who profited from having taken the supplements. In other words, we do not know whether the beneficial effects observed were due to the correction of B_6 deficiencies or to further supplementation beyond already adequate B_6 levels.

To a lesser extent, the other B-group vitamins (B_1, B_2, and niacin) have also been found to play important roles in immune function (7, 45), and this may be evidenced by the significantly protective effects of these vitamins that we observed in our study. However, because all of the B-group vitamins were so highly intercorrelated and all had similar effects on mortality, it is not possible to tell which one(s) may actually be associated with improved survival. Nonetheless, these and other data add further support for the hypothesis that B-group vitamins taken in moderately high doses may slow HIV-1 disease progression and improve immune function. Given this growing body of evidence, the low cost and toxicity of micronutrient supplementation, and the devastating effects of HIV-1 disease, the time appears right to have these hypotheses formally tested using a large-scale randomized study design.

It has been reported that zinc deficiency can have profound effects on practically all aspects of the immune system, including hypoplasia of lymphoid tissues, lowered lymphocyte counts, depressed humoral and cell-mediated immunity, impaired delayed-type hypersensitivity reactions, and decreased phagocytic function of neutrophils (7, 46, 47). In many of these studies, correction of the deficiency managed to improve these functions. In HIV-1 infection, low serum zinc levels have been correlated with decreased immune parameters (48) and increased risk of progression to AIDS (22). Our findings seem to suggest, however, that levels of zinc intake above the RDA may begin to exhibit some harmful effects. In a previous paper (26), we found that increasing intake of zinc was also associated with an increased risk of progression to AIDS. Other studies have demonstrated that excess levels of zinc intake can have toxic effects. In a study by Chandra (49), 11 healthy adult men were given 150 mg of oral zinc twice a day for 6 weeks. By the end of this period, Chandra found that this relatively large amount of zinc supplementation was as-

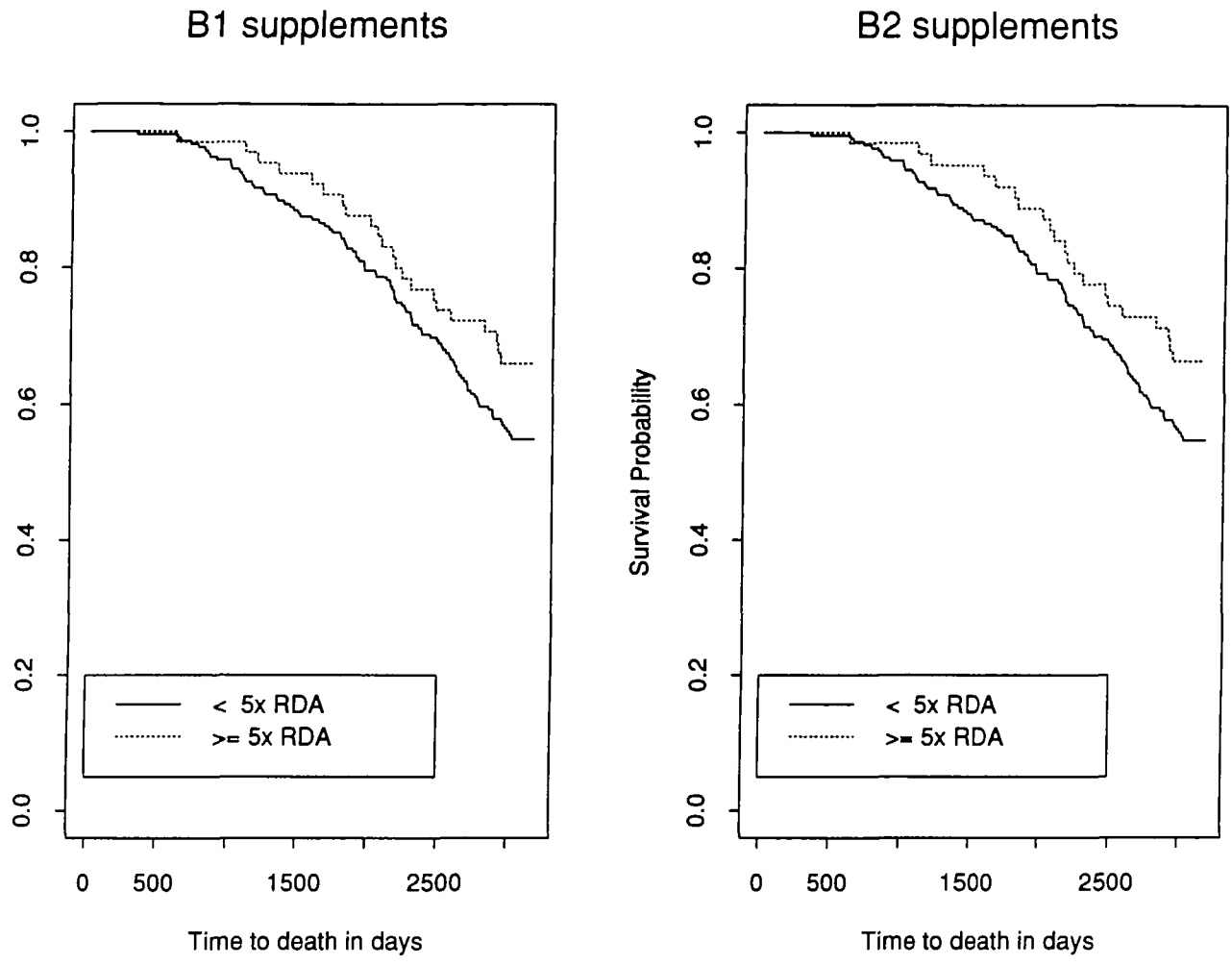

FIGURE 2. Kaplan-Meier survival curves for supplemental intake of vitamins B_1 and B_2 according to multiples of the recommended dietary allowance (RDA). RDAs for men aged 29–50 years = 1.5 mg/day for B_1 and 1.7 mg/day for B_2 for 281 HIV-1-seropositive men enrolled in the Baltimore/Washington, DC, site of the Multicenter AIDS Cohort Study, October 1984 to December 1992.

sociated with significant impairment of lymphocyte and polymorphonuclear leukocyte function. Other studies indicate that use of zinc supplements at more moderate levels, between 15 and 100 mg per day (amounts commonly used in self-supplementation and levels that are seen in our cohort), may have adverse effects (50).

In recent studies of HIV-1, researchers have raised the possibility that the HIV-1 nucleocapsid protein binds zinc and forms retroviral-type zinc finger structures within the gag protein that are essential for RNA packaging and infectivity (51, 52). It is unknown whether excess zinc influences this process, but, given the conflicting results of studies on the effects of zinc in HIV-1 infection, further study is clearly needed to determine optimal levels of zinc intake in HIV-1 infection.

There is accumulating evidence that both β-carotene and vitamin A may play important roles in HIV infection (20, 24, 28). In this analysis, we observed a U-shaped relation between both vitamin A and β-carotene intake and survival, with the association being stronger for β-carotene (table 5). Our study seems to suggest that excessive intake of β-carotene and/or vitamin A may actually diminish the protective effect seen at more moderate levels of intake. Vitamin A deficiency is associated with more rapid HIV-1 disease progression (20) and perinatal transmission of HIV-1 (24). However, serum vitamin A status is relatively replete in our cohort (A. M. Tang, unpublished data). Thus, supplementation in this population may have less of an impact than in more deficient groups.

β-Carotene is not known to have major toxic effects at high dosages. Nonetheless, in two recently published clinical trials on the effects of β-carotene on the incidence of cancer in male smokers (53) and in preventing colorectal adenoma (54), it was found, unexpectedly, that daily supplementation with β-carotene

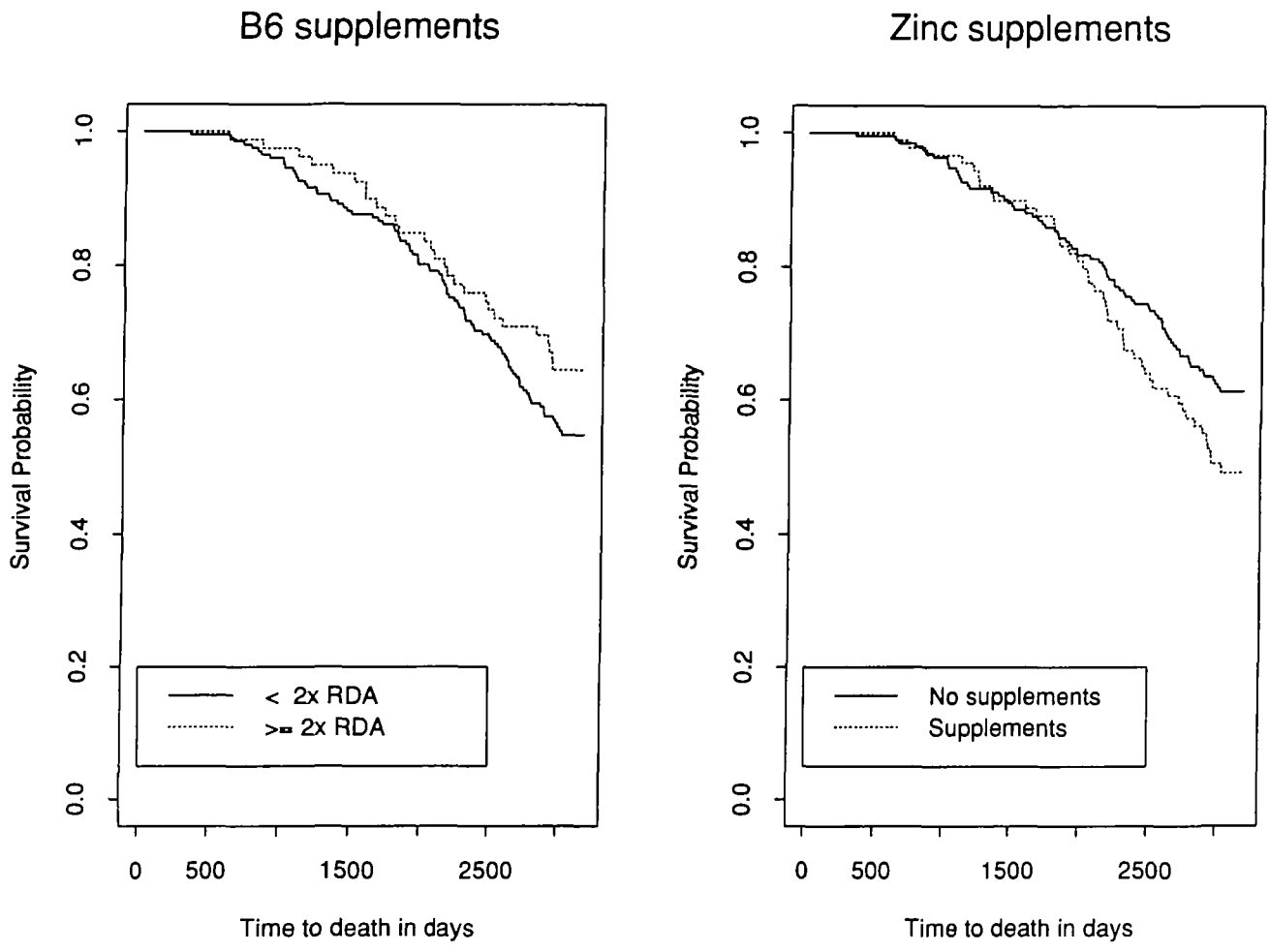

FIGURE 3. Kaplan-Meier survival curves for supplemental intake of vitamins B_6 and zinc according to multiples of the recommended dietary allowance (RDA). RDAs for men aged 29–50 years = 2.0 mg/day for B_6 and 15 mg/day for zinc for 281 HIV-1-seropositive men enrolled in the Baltimore/Washington, DC, site of the Multicenter AIDS Cohort Study, October 1984 to December 1992.

at doses of 20 mg (33,400 IU) and 25 mg (41,750 IU) did not appear to protect against either of the two outcomes. In one of these studies (53), β-carotene supplementation was associated with a significant increase in incidence of lung cancer by 18 percent (95 percent CI 3–36 percent). Whether we are observing a similar phenomenon in our HIV-1-infected population remains to be seen. However, the level of β-carotene intake in the highest quartile in our population was similar to the doses used in those clinical trials.

In studies of this nature, the question arises as to whether the relation between dietary intake and survival is confounded by duration of infection or stage of illness. Table 2 shows a slight negative association between CD4+ cell counts at baseline (a well-established marker of disease progression) and total intake of the micronutrients. For all analyses, we adjusted for CD4+ cell count category. For micronutrients that showed an increase in survival with the highest quartiles of total intake (i.e., vitamins B_1, B_2, B_6, and niacin), CD4+ T cell count was an apparent negative confounder. Any residual confounding after the adjustments for category of CD4 count that were made in the multivariate Cox models would only serve to enhance the protective effects seen. Therefore, duration of infection and/or disease stage were very unlikely to have confounded these relations. CD4 count was, however, lower in the highest quartile of zinc intake compared with the lower three quartiles. Adjustment for CD4 category should have dealt with this imbalance, and the consistent dose-response associations that we observed across all quartiles of zinc intake would tend to discount any important role of residual confounding as an explanation for the relation between higher zinc intakes and poorer survival.

Currently, we have only examined dietary intake in relation to AIDS progression and survival. Of course, there are many other facets to this question that we have not addressed in our studies, such as the link

between reported dietary intake of these men, their actual intakes, and the degree of absorption and utilization of these nutrients by the body. Studies are under way that will examine both the changes in reported nutrient intake across time and the serum levels of these nutrients in the same group of subjects. Preliminary results from studying changes in dietary intake over time show that seropositive subjects with CD4 counts of less than 350 at study entry, on average, had higher intakes of many macronutrients and micronutrients (e.g., protein, calories, carbohydrates, vitamins A and B_{12}, β-carotene, and glutathione) at baseline compared with seronegatives and/or seropositives with CD4 counts equal to or above 350 at study entry. Over the first 2 years of follow-up, however, the seropositives with CD4 counts of less than 350 showed a rapid decline in dietary intake of these nutrients, while, on average, the intake levels for seronegatives and seropositives with CD4 counts of 350 or greater remained relatively stable. Differences in dietary intake over time may be a result of disease progression, rather than a cause. In this study, we have used data at baseline, before participants knew their HIV-1 status, to reduce any biases that might result after HIV-1 status was revealed. Nonetheless, changes in diet during more severe immunosuppression in later-stage disease will be an important area for further research. Until more of these studies are performed, caution should be used in the interpretation of these results.

In summary, these data suggest that high intakes of several B-group micronutrient supplements, particularly vitamin B_6, may be associated with improved survival in HIV-1 infection, while higher intakes of zinc may be associated with poorer survival. These data support the need for definitive clinical trials to assess the effects of B-group vitamins on HIV-1 disease progression and/or survival. In addition, more studies are needed to determine the optimal level of zinc intake in the early stages of HIV-1 infection.

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REFERENCES

1. Jain VK, Chandra RK. Does nutritional deficiency predispose to acquired immune deficiency syndrome? Nutr Res 1984;4:537–43.
2. Leonard-Green TK, Watson RR. The AIDS-immunocompetence-nutrition-infection cycle. In: Watson RR, ed. Cofactors in HIV-1 infection and AIDS. Boca Raton, FL: CRC Press, 1989:187–99.
3. Scrimshaw NS. Effect of infection on nutrient requirements. Am J Clin Nutr 1977;30:1536–44.
4. Gershwin ME, Beach RS, Hurley LS. Nutrition and immunity. Orlando, FL: Academic Press, Inc., 1985.
5. Chandra RK, Newberne P. Nutrition, immunity, and infection: mechanisms of interaction. New York, NY: Plenum Press, 1977.
6. Axelrod AE. Immune processes in vitamin deficiency states. Am J Clin Nutr 1971;24:265–71.
7. Beisel WR. Single nutrients and immunity. Am J Clin Nutr 1982;35 (Suppl. 2):417–68.
8. Dreizen S. Nutrition and the immune response—a review. Int J Vitam Nutr Res 1979;49:220–8.
9. Kotler D, Goetz H, Lange M, et al. Enteropathy associated with the acquired immunodeficiency syndrome. Ann Intern Med 1984;101:421–8.
10. Kotler D, Wang J, Pierson R. Body composition studies in patients with the acquired immunodeficiency syndrome. Am J Clin Nutr 1985;42:1255–65.
11. Graham NMH, Muñoz A, Bacellar H, et al. Clinical factors associated with weight loss related to infection with human immunodeficiency virus type 1 in the Multicenter AIDS Cohort Study. Am J Epidemiol 1993;137:439–46.
12. Graham NMH, Rubb S, Hoover DR, et al. Beta$_2$-microglobulin and other early predictors of human immunodeficiency virus type 1-related wasting. Ann Epidemiol 1994;4:32–9.
13. Zangerle R, Reibnegger G, Wachter H, et al. Weight loss in HIV-1 infection is associated with immune activation. AIDS 1993;7:175–81.
14. Grunfeld C, Feingold KR. Metabolic disturbances and wasting in the acquired immunodeficiency syndrome. N Engl J Med 1992;427:329–37.
15. Kotler D, Tierney AR, Wang J, et al. Magnitude of body-cell-mass depletion and the timing of death from AIDS. Am J Clin Nutr 1989;50:444–7.
16. Palenicek J, He D, Graham NMH, et al. Weight loss as a predictor of survival after AIDS among HIV infected gay men enrolled in the MACS. (Abstract WS-B34–1). Presented at the IXth International Conference on AIDS, Berlin, Germany, June 1993.
17. Swanson CE, Cooper DA. Factors influencing outcome of treatment with zidovudine of patients with AIDS in Australia. AIDS 1990;4:749–57.
18. Guenter P, Muurahainen N, Simons G, et al. Relationships among nutritional status, disease progression, and survival in HIV infection. J Acquir Immune Defic Syndr 1993;6:1130–8.
19. Beach RS, Mantero-Atienza E, Shor-Posner G, et al. Specific nutrient abnormalities in asymptomatic HIV-1 infection. AIDS 1992;6:701–8.
20. Semba RD, Graham NMH, Caiaffa WT, et al. Increased mortality associated with vitamin A deficiency during HIV-1 infection. Arch Intern Med 1993;153:2149–54.
21. Beach RS, Cabrejos C, Shor-Posner G, et al. Nutritional aspects of early HIV infection. In: Chandra RK, ed. Nutrition and immunology. St. John's, Newfoundland, Canada: ARTS Biomedical Publishers and Distributors, 1992:241–53.
22. Graham NMH, Sorenson D, Odaka N, et al. Relationship of serum copper and zinc levels to HIV-1 seropositivity and progression to AIDS. J Acquir Immune Defic Syndr 1991;4:976–80.
23. Baum MK, Mantero-Atienza E, Shor-Posner G, et al. Association of vitamin B6 status with parameters of immune function in early HIV-1 infection. J Acquir Immune Defic Syndr 1991;4:1122–32.
24. Semba RD, Miotti PG, Chiphangwi JD, et al. Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. Lancet 1994;343:1593–7.

25. Abrams B, Duncan D, Hertz-Picciotto I. A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. J Acquir Immune Defic Syndr 1993;6:949–58.
26. Tang AM, Graham NMH, Kirby AJ, et al. Dietary micronutrient intake and risk of progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men. Am J Epidemiol 1993;138:937–51.
27. Garewal HS, Ampel NM, Watson RR, et al. A preliminary trial of beta-carotene in subjects infected with the human immunodeficiency virus. J Nutr 1992;122 (3 suppl.):728–32.
28. Coodley GO, Nelson HD, Loveless MO, et al. Beta-carotene in HIV infection. J Acquir Immune Defic Syndr 1993;6: 272–6.
29. Kaslow RA, Ostrow DG, Detels R, et al. for the Multicenter AIDS Cohort Study. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. Am J Epidemiol 1987;126:310–18.
30. Centers for Disease Control. Revision of CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR Morb Mortal Wkly Rep 1987;36 (Suppl. 1):1S-15S.
31. Willett WC, Sampson L, Brown ML, et al. The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol 1988;127:188–99.
32. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
33. Willett WC, Reynolds RD, Cottrell-Hoehner S, et al. Validation of a semi-quantitative food frequency questionnaire: comparison with a 1-year diet record. J Am Diet Assoc 1987;87: 43–7.
34. Salvini S, Hunter DJ, Sampson L, et al. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int J Epidemiol 1989;18:858–67.
35. Willett WC. Nutritional epidemiology. New York, NY: Oxford University Press, 1990:245–71.
36. Ha C, Kerkvliet NI, Miller LT . The effect of vitamin B6 deficiency on host susceptibility to Moloney sarcoma virus-induced tumor growth in mice. J Nutr 1984;114:938–45.
37. Hodges RE, Bean WB, Ohlson MA, et al. Factors affecting human antibody response. IV. Pyridoxine deficiency. Am J Clin Nutr 1962;11:180–6.
38. Ha C, Miller LT, Kerkvliet NI. The effect of vitamin B6 deficiency on cytotoxic immune responses of T cells, antibodies, and natural killer cells, and phagocytosis by macrophages. Cell Immunol 1984;85:318–29.
39. Axelrod AE, Trakatellis AC. Relationship of pyridoxine to immunological phenomena. Vitam Horm 1964;22:591–607.
40. Cheslock KE, McCully MT. Response of human beings to a low-vitamin B6 diet. J Nutr 1960;70:507–13.
41. Robson LC, Schwarz MR, Perkins WD. Vitamin B6 and immunity. In: Human vitamin B6 requirements: proceedings of a workshop. Letterman Army Institute of Research, June 11–12, 1976. San Francisco, CA: National Academy of Sciences, 1978:162–79.
42. Talbott MC, Miller LT, Kerkvliet NI. Pyridoxine supplementation: effect on lymphocyte responses in elderly persons. Am J Clin Nutr 1987;46:659–64.
43. Chandra RK. Effect of vitamin and trace element supplementation on immune responses and infection in elderly subjects. Lancet 1992;340:1124–7.
44. Bogden JD, Baker H, Frank O, et al. Micronutrient status and human immunodeficiency virus (HIV) infection. In: Bendich A, Chandra RK, eds. Micronutrients and immune functions. Ann N Y Acad Sci 1990;587:189–95.
45. Coodley GO. Vitamins in HIV infection. In: Watson RR, ed. Nutrition and AIDS. Boca Raton, FL: CRC Press, 1994: 89–115.
46. Keen CL, Gershwin ME. Zinc deficiency and immune function. Annu Rev Nutr 1990;10:415–31.
47. Sherman AR. Zinc, copper, and iron nutriture and immunity. J Nutr 1992;122:604–9.
48. Falutz J, Tsoukas C, Gold P. Zinc as a cofactor in human immunodeficiency virus-induced immunosuppression. (Letter). J Am Med Assoc 1988;259:2850–1.
49. Chandra RK. Excessive intake of zinc impairs immune responses. J Am Med Assoc 1984;252:1443–6.
50. Fosmire GJ. Zinc toxicity. Am J Clin Nutr 1990;51:225–7.
51. South TL, Blake PR, Sowder RC III, et al. The nucleocapsid protein isolated from HIV-1 particles binds zinc and forms retroviral-type zinc fingers. Biochemistry 1990;29:7786–9.
52. South TL, Kim B, Hare DR, et al. Zinc fingers and molecular recognition. Structure and nucleic acid binding studies of an HIV zinc finger-like domain. Biochem Pharmacol 1990;40: 123–9.
53. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–35.
54. Greenberg ER, Baron JA, Tosteson TD, et al. A clinical trial of antioxidant vitamins to prevent colorectal adenoma. N Engl J Med 1994;331:141–7.