

ORIGINAL CONTRIBUTIONS

**Dietary Micronutrient Intake and Risk of Progression to Acquired Immunodeficiency Syndrome (AIDS) in Human Immunodeficiency Virus Type 1 (HIV-1)-infected Homosexual Men**

Alice M. Tang,<sup>1</sup> Neil M. H. Graham,<sup>1,2</sup> Alison J. Kirby,<sup>1</sup> L. Diane McCall,<sup>1</sup> Walter C. Willett,<sup>3</sup> and Alfred J. Saah<sup>1,2</sup>

The authors sought to determine if different levels of dietary intake of micronutrients are associated with the progression of human immunodeficiency virus type 1 (HIV-1) infection to acquired immunodeficiency syndrome (AIDS). A total of 281 HIV-1 seropositive homosexual/bisexual men were seen semiannually since 1984 at the Baltimore/Washington, DC site of the Multicenter AIDS Cohort Study. Participants completed a self-administered semiquantitative food frequency questionnaire at baseline. Levels of daily micronutrient intake at baseline were examined in relation to subsequent progression to AIDS (1987 Centers for Disease Control definition;  $n = 108$ ) during a median follow-up period of 6.8 years. For each nutrient, the authors used a Cox proportional hazards model to adjust for age, presence of symptoms, CD4+ lymphocyte count, energy intake, use of antiretrovirals, and use of *Pneumocystis carinii* pneumonia prophylaxis. The highest levels of total intake (from food and supplements) of vitamins C and B<sub>1</sub> and niacin were associated with a significantly decreased progression rate to AIDS: vitamin C (relative hazard (RH) = 0.55, 95% confidence interval (CI) 0.34–0.91), vitamin B<sub>1</sub> (RH = 0.60, 95% CI 0.36–0.98), and niacin (RH = 0.52, 95% CI 0.31–0.86). The relation between total vitamin A intake and progression to AIDS appeared to be U-shaped; the lowest and highest quartiles of intake did most poorly, while the middle two quartiles were associated with significantly slower progression to AIDS (RH = 0.55, 95% CI 0.35–0.88). Increased intake of zinc was monotonically and significantly associated with an increased risk of progression to AIDS (for highest vs. lowest quartiles, RH = 2.06, 95% CI 1.16–3.64). In a final multinutrient model, vitamin A, niacin, and zinc remained significantly associated with progression to AIDS, while vitamin C was only marginally significant. *Am J Epidemiol* 1993;138:937–51.

acquired immunodeficiency syndrome; ascorbic acid; HIV-1; niacin; nutrition; thiamine; vitamin A; zinc

Nutritional status has been studied in patients with acquired immunodeficiency syndrome (AIDS) (1, 2), and it appears to be a factor in predicting survival in this group

(3). However, little is known about nutritional status early in the course of human immunodeficiency virus type 1 (HIV-1) infection and its subsequent relation with dis-

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

<sup>1</sup> Department of Epidemiology, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD.

<sup>2</sup> Department of Medicine, The Johns Hopkins

University, School of Medicine, Baltimore, MD.

<sup>3</sup> Departments of Epidemiology and Nutrition, Harvard School of Public Health, Channing Laboratory, Department of Medicine, Harvard Medical School, and Brigham and Women's Hospital, Boston, MA.

Reprint requests to Dr. N. M. H. Graham, Department of Epidemiology, The Johns Hopkins University School of Hygiene and Public Health, 624 N. Broadway, Baltimore, MD 21205.

ease progression. There has been some suggestion that the major immunologic changes which occur in HIV-1 infection resemble the immunologic disorders seen in various stages of nutritional deficiencies (4). The effects of nutritional status and various infections on the immune system have been widely studied over the past 20 years (5-10). Human and animal studies have shown that protein-calorie, folate, iron, pyridoxine and zinc deficiencies are associated with a depression of cell-mediated immune parameters. Deficiencies of the B-group vitamins, vitamin A, and vitamin C have been found to inhibit humoral antibody formation in mammalian systems. The production and function of phagocytic cells are impaired in experimental animals with vitamin A, ascorbic acid, thiamine, or riboflavin deficiencies. Furthermore, excesses of certain nutrients in humans (e.g., iron and vitamin E) have been shown to cause functional disruptions of the immune system (9).

Serum levels of some micronutrients (vitamins A, E, B<sub>6</sub>, B<sub>12</sub>, and zinc) appear to decrease in HIV-1 infection (11-14), while levels of copper and riboflavin in serum and plasma are elevated compared with seronegative controls (11, 15). Many groups infected with HIV-1 in the United States have very high levels of micronutrient intake, in part due to supplement use. In some cases, intake may even be in the toxic range (16). What remains unknown is whether dietary intakes of these micronutrients around or above the recommended dietary allowances have any association with HIV-1 disease progression.

To date, no epidemiologic evidence has been reported on the relation between nutrient intake and the progression of HIV-1 infection to AIDS. Indeed, little information has been published on the dietary intakes of HIV-1 seropositive individuals early in the course of infection, prior to the development of symptoms. This information is potentially important because once a patient develops HIV symptoms or AIDS, dietary manipulations may have less of an impact on disease

progression. If associations are found between dietary habits early in the course of infection and subsequent progression to AIDS, then this may form the basis for rational dietary interventions.

In this study, data on dietary intake were collected in the early stages of HIV-1 infection and the subjects were followed, prospectively, to the development of AIDS. The specific goal of the study was to assess the association between the dietary intakes of selected nutrients with the rate of progression to AIDS among homosexual, HIV-1 seropositive men.

## MATERIALS AND METHODS

### Study population

Between April and November 1984, 1,153 homosexual men from the Baltimore/Washington, DC area were recruited into the SHARE (Study to Help the AIDS Research Effort). These men comprise the Baltimore/Washington, DC site of the Multicenter AIDS Cohort Study, a prospective study of the natural history of HIV-1 infection in homosexual and bisexual men. The objectives, design, and recruitment protocol have been described in detail elsewhere (17). In brief, eligible men were over age 18 years, had a history of sexual activity with male partners, and had no AIDS defining illness at study entry. At baseline and subsequent semiannual follow-up visits, participants provided information about health and medical status, therapeutic and illicit drug use, and sexual practices. The subjects were all examined by a medical provider and serum samples were collected for HIV-1 serology, laboratory studies, and repository storage. In addition, at follow-up visit 2 (6 months after baseline), participants 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 into the study at baseline, 1,014 (88 percent) completed the nutrition questionnaire at the first follow-up visit (October

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1984 to March 1985, inclusive). Of these, 718 (70.8 percent) were HIV-1 seronegative, 292 (28.8 percent) were HIV-1 seropositive, and 4 (0.4 percent) were indeterminate by Western blot. Included in this analysis are men who were HIV-1 seropositive at baseline (visit 1), who completed the food frequency questionnaire 6 months later (visit 2), and who did not develop AIDS in the first 12 months after baseline. This analysis excludes four cases who were diagnosed with AIDS within the first 12 months of follow-up, because it was not known whether the usual dietary intakes reported by these men had changed over the previous 12 months as a result of their illness.

### Laboratory tests

HIV-1 serostatus was measured by enzyme immunoassay (Genetic Systems, Seattle, Washington) and a Western blot (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 surveillance case definition (18).

### Dietary intake

Usual dietary intake was measured using a semiquantitative food frequency questionnaire (19) at study visit 2. The validity and reproducibility of the questionnaire for measuring average intake of specific foods and beverages has been documented elsewhere (19-22). For each of the 116 food items listed, subjects were asked to estimate their use of that food in terms of a typical portion size. Participants reported the frequency of consumption of each food by selecting one of nine frequency categories, ranging from "Never, or less than once per month" to "6+ per day". The questionnaire also includes a section 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 type of margarine and the exact brand

and type of fat used for frying, cooking, and baking.

Prior to data entry, the completed questionnaires were reviewed and coded by a research dietician. Nutrient estimates for the semiquantitative food frequency questionnaire were analyzed using the nutrient analysis program developed specifically for the questionnaire (19). Among the nutrients assessed were total calories, protein, fiber, fat, cholesterol, vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, C, D, E, niacin, calcium, folate, iron, zinc, and copper.

### Other independent variables

Other independent variables used in this study were based on data collected at the baseline clinic visit (study visit 1). Body mass index was calculated as weight (kg)/height (m)<sup>2</sup>. Sociodemographic and health status data were obtained by interview, together with information on cigarette smoking and alcohol intake. Immunologic indices used were CD4+ and CD8+ T-lymphocyte counts.

Participants were placed into one of three categories based on their baseline CD4+ T-lymphocyte count. The groups were defined as <500, 500-750, and >750 cells/ $\mu$ liter to give approximately equal numbers in each category. In the case of missing data on CD4+ cell counts ( $n = 30$ ), subjects were assigned to a category based on data from adjacent semiannual visits. Since the mean decline of CD4+ lymphocyte counts in our cohort is 30 cells/ $\mu$ liter per 6 months, it is possible to estimate the appropriate CD4 category for these individuals with a reasonably high degree of accuracy. Participants were also categorized as HIV-1 symptomatic or HIV-1 asymptomatic at the baseline visit. Subjects were considered symptomatic if there was a report of any of the following symptoms or signs within the previous 6 months: oral thrush, fatigue, weight loss (>4.5 kg unintentional weight loss between study visits), fever (>38.5° C for 2 weeks), or diarrhea (daily for >2 weeks).

Data on the use of antiretroviral drugs (zidovudine, didanosine, dideoxycytidine) and *Pneumocystis carinii* pneumonia prophylaxis (aerosolized pentamidine, trimethoprim-sulfamethoxazole, dapsone) were also recorded for each subject during the follow-up period. In order to be highly conservative in our analysis, "ever use" of antiretrovirals or *Pneumocystis carinii* pneumonia prophylaxis at any time during the follow-up period were included as baseline covariates in all regression models (see below).

### Statistical methods

The baseline characteristics of those who developed AIDS were compared univariately with those who did not develop AIDS using *t* tests for continuous variables and chi-square analyses for categorical variables. Because nutrient intakes are positively correlated with total energy intake, it is usually desirable to use a measure of nutrient intake which is adjusted for the total energy intake. In order to do this, calorie-adjusted nutrient values were computed by taking the residuals from a simple linear model, regressing nutrient intake on total caloric intake, and adding to these the predicted response at the median total caloric intake. This method is described in more detail elsewhere (23).

Analyses were performed using both nutrient intakes from food alone and nutrient intakes from food and supplements combined (total intake). The relation between AIDS and intake from supplements alone was also assessed by dividing into tertiles the reported supplement intakes for thiamine, riboflavin, vitamins A, B<sub>6</sub>, B<sub>12</sub>, C, D, E, calcium, folate, iron, retinol, and zinc.

Cox proportional hazards models were fit for the micronutrients of interest (thiamine, riboflavin, vitamins A, B<sub>6</sub>, B<sub>12</sub>, C, D, E, calcium, folate, iron, zinc, niacin, and copper) adjusting for CD4+ lymphocyte count, age, presence of symptoms, total calorie intake, use of antiretrovirals, and use of *Pneumocystis carinii* pneumonia prophylaxis.

Smoking and alcohol intake were not included in the final Cox model since neither was related to disease progression nor were they confounders in any initial models. Furthermore, seven AIDS cases were excluded from this survival analysis because the date of their AIDS diagnosis was not known, although death from an AIDS-related condition was recorded on each of their death certificates. These analyses were therefore based on 173 AIDS-free subjects and 108 AIDS subjects. Models with more than one nutrient included were fit in order to assess the degree of confounding among the different nutrients.

Pearson's correlation coefficients were calculated to determine the level of intercorrelation between micronutrients. The Kaplan-Meier method was used in univariate analyses to calculate the proportion surviving AIDS-free for each nutrient intake category. For the Kaplan-Meier analyses and Cox proportional hazards models, survival time was calculated beginning at visit 2 when the food frequency questionnaire was administered.

### RESULTS

One hundred and eight (38 percent) of the 281 seropositive men developed AIDS during a median follow-up period of 6.8 years. The baseline characteristics of those who later did and did not develop AIDS are presented in table 1. No significant differences were found between those who subsequently developed AIDS and those who did not with respect to race, body mass index, level of education, caloric intake, cigarette smoking, or alcohol use. Illicit drug use was uncommon in the cohort and did not vary between the outcome groups (data not shown). As would be expected, those who later developed AIDS were slightly older (35.2 years vs. 33.7 years;  $p = 0.05$ ), had significantly lower mean CD4+ lymphocyte counts (586 cells/ $\mu$ liter vs. 722 cells/ $\mu$ liter;  $p = 0.0006$ ), and had a significantly higher percentage showing symptoms (25 percent vs. 14 percent,  $p = 0.02$ ). AIDS cases were predominantly due to *Pneumo-*

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

	Age (years)		Race (% white)		Body mass index†		Education (% college graduate)		Calorie intake (kcal)		Weight (kg)		Cigarette smoking‡ (%)		Alcohol consumption§ (% ≥3-4 drinks/week)		CD4+ cell count/μliter		% with symptoms¶	
	Mean	(SE)*	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)
No AIDS (n = 173)	33.7	(6.2)	82.7	(2.7)	23.3	(2.7)	63.6	(9.44)	2,320	(944)	73.6	(10.5)	35.5	49.1	722	(359)	13.9			
AIDS (n = 108)	35.2	(6.4)	89.7	(2.7)	23.1	(2.7)	65.2	(9.33)	2,345	(933)	74.2	(10.0)	31.3	40.2	586	(277)	25.2			
p value	0.05		0.10		0.66		0.78		0.82		0.65		0.47	0.15	0.0006		0.02			

\* SE, standard error.  
 † Data available for 171 AIDS-free subjects and 108 AIDS+ subjects.  
 ‡ Data available for 172 AIDS-free subjects and 108 AIDS+ subjects.  
 § Data available for 165 AIDS-free subjects and 107 AIDS+ subjects.  
 || Data available for 156 AIDS-free subjects and 104 AIDS+ subjects.  
 ¶ Presence of oral thrush, fatigue, unintentional weight loss, fever, or diarrhea within previous 6 months.

*cystis carinii* pneumonia (44 percent) and Kaposi's sarcoma (29 percent).

Nutrient intake according to degree of immunosuppression is presented in table 2. Total energy intake and intake of most macronutrients varied little by CD4 category, although carbohydrate intake decreased with increasing immunosuppression. Micronutrient intake from food was relatively constant across all CD4 categories, but for several micronutrients (e.g., vitamin C, niacin, and vitamin B<sub>6</sub>), men with <500 CD4+ cells/μliter had the highest levels of intake from supplements.

Single-nutrient analyses

Non-nutrient covariates were included in a baseline Cox proportional hazards model if they were related to progression rate to AIDS in the univariate analysis or were considered to be a putative confounding factor from other analyses. In this model, higher CD4+ lymphocyte counts (>750 cells/μliter vs. <500 cells/μliter; relative hazard (RH) = 0.48, p = 0.002) and use of antiretrovirals before AIDS (RH = 0.51, p = 0.005) were associated with a lower likelihood of progressing to AIDS. However, subjects who were older (per 10 year increase, RH = 1.34, p = 0.046) and had more symptoms (≥1 symptom, RH = 1.79, p = 0.009) were significantly more likely to develop AIDS. Use of *Pneumocystis carinii* pneumonia prophylaxis and calorie intake did not significantly differ between subjects who developed AIDS and those who did not. After adjustment for the above baseline covariates, intakes of protein, fat, cholesterol, fiber, vitamins B<sub>12</sub>, D, and E, calcium, folate, iron, and copper were unrelated to subsequent progression to AIDS.

Table 3 shows the results of the Cox models for those micronutrients that had statistically significant associations with progression to AIDS or where marginally significant trends were observed. The nutrients were entered as quartiles of intake. For vitamin A, the third quartile of total intake was associated with a significantly de-

THE BALTIMORE COHORT

TABLE 2. Median values and interquartile ranges (IQR) for crude intakes of selected nutrients at baseline, stratified by category of CD4+ lymphocyte count, in 281 human immunodeficiency virus type 1 (HIV-1) seropositive men from the Baltimore/Washington, DC Multicenter AIDS Cohort Study

Nutrients	CD4+ lymphocyte count		
	<500 cells/μliter (n = 100)	500-750 cells/μliter (n = 86)	>750 cells/μliter (n = 95)
	Median	Median	Median
	IQR	IQR	IQR
Total calories (kcal)	2,112.0 (1,606.2-2,833.6)	2,194.8 (1,669.0-2,737.7)	2,201.4 (1,705.9-2,751.9)
Protein	83.2 (66.3-109.1)	82.7 (63.1-110.7)	83.7 (65.1-103.3)
Animal fat (g)	45.5 (30.5-62.5)	42.4 (29.0-60.5)	43.5 (31.9-56.6)
Vegetable fat (g)	29.4 (19.8-41.4)	31.6 (22.1-47.3)	29.5 (21.2-40.4)
Carbohydrate (g)	244.5 (184.4-350.0)	263.7 (194.0-337.5)	270.5 (185.2-366.9)
Vitamin A, total* (IU)	13,882.5 (8,912.8-22,238.1)	12,930.0 (8,692.0-18,952.6)	14,291.1 (9,002.0-21,083.8)
Vitamin A, food (IU)	10,046.8 (7,067.6-15,357.8)	10,607.7 (6,984.7-13,014.5)	10,396.4 (7,286.9-14,996.7)
Vitamin B <sub>1</sub> , total† (mg)	2.6 (1.5-6.9)	2.1 (1.3-9.2)	2.5 (1.4-4.5)
Vitamin B <sub>2</sub> , food (mg)	1.3 (1.0-1.7)	1.3 (1.0-1.8)	1.3 (0.9-1.8)
Vitamin B <sub>2</sub> , total† (mg)	3.4 (2.2-7.8)	2.9 (1.9-9.6)	3.2 (2.0-5.7)
Vitamin B <sub>2</sub> , food (mg)	1.9 (1.5-2.5)	1.8 (1.4-2.6)	1.9 (1.4-2.4)
Vitamin B <sub>6</sub> , total‡ (mg)	3.7 (2.4-5.8)	3.0 (2.0-6.3)	3.3 (2.2-5.5)
Vitamin B <sub>6</sub> , food (mg)	2.1 (1.5-2.6)	1.9 (1.4-2.5)	2.0 (1.4-2.5)
Vitamin C, total§ (mg)	400.7 (159.0-1,106.5)	221.2 (141.0-598.9)	251.8 (150.2-617.9)
Vitamin C, food (mg)	139.7 (95.9-209.7)	135.7 (94.2-200.5)	153.4 (87.3-224.6)
Niacin (mg)	40.4 (27.4-62.9)	34.3 (22.9-60.6)	35.7 (26.1-63.4)
Zinc, total   (mg)	16.2 (11.3-27.3)	15.0 (10.2-22.7)	14.3 (11.4-21.8)
Zinc, food (mg)	13.0 (10.2-16.9)	12.7 (9.7-16.9)	13.2 (10.1-15.6)

\* Includes 161 men taking supplements.  
 † Includes 175 men taking supplements.  
 ‡ Includes 177 men taking supplements.  
 § Includes 186 men taking supplements.  
 || Includes 88 men taking supplements.

creased hazard of developing AIDS, while the second quartile was also associated with a lower, but not significant, hazard ratio. The highest quartile of total vitamin A intake had a relative hazard for AIDS very close to 1.0, which suggests that a U-shaped relation may exist between intake of this nutrient and risk of AIDS.

To determine if this U-shaped relation was significant, a quadratic term was added to a baseline linear model for vitamin A intake. The quadratic term was highly significant ( $p = 0.006$ ), supporting the observation of a U-shaped relation. To determine if this relation was largely explained by intakes of either  $\beta$ -carotene or

**TABLE 3. Single nutrient Cox proportional hazards models\* using quartiles of daily nutrient intake as independent variables and acquired immunodeficiency syndrome (AIDS) as the outcome variable for 281 human immunodeficiency virus type 1 (HIV-1) seropositive men in the Baltimore/Washington, DC Multicenter AIDS Cohort Study, 1984-1992**

Model no.	Nutrient and quartile of intake†	No. of AIDS events/total no. of subjects in intake stratum	Relative hazard	95% confidence interval
1	Vitamin A (IU/day)			
	<9,062	33/71	1.00	
	9,062-13,267	24/70	0.65	0.38-1.12
	13,267-20,268	20/70	0.47	0.27-0.83
2	Vitamin B <sub>1</sub> (mg/day)			
	<1.4	30/71	1.00	
	1.4-2.4	29/70	1.13	0.67-1.90
	2.4-4.9	28/70	0.80	0.47-1.36
3	Vitamin B <sub>2</sub> (mg/day)			
	<2.0	29/71	1.00	
	2.0-3.0	27/70	0.99	0.59-1.68
	3.0-5.9	32/70	0.98	0.58-1.66
4	Vitamin B <sub>6</sub> (mg/day)			
	<2.0	31/71	1.00	
	2.0-3.2	27/70	0.87	0.51-1.47
	3.2-5.7	28/70	0.81	0.48-1.38
5	Vitamin C (mg/day)			
	<157	27/71	1.00	
	157-254	33/70	1.43	0.85-2.40
	254-715	28/70	1.06	0.62-1.83
6	Niacin (mg/day)			
	<24.9	33/71	1.00	
	24.9-36.3	21/70	0.58	0.33-1.01
	36.3-61.0	36/70	1.07	0.65-1.75
7	Zinc (mg/day)			
	<11.6	18/70	0.45	0.25-0.80
	11.6-14.1	21/71	1.00	
	14.1-20.0	26/70	1.33	0.75-2.38
	>20.0	28/70	1.69	0.95-3.02
		33/70	2.06	1.16-3.64

\* Adjusted for age, symptoms, CD4+ lymphocyte count, energy intake, use of antiretrovirals, and use of *Pneumocystis carinii* pneumonia prophylaxis.

† Includes nutrient intake from food and micronutrient supplements.

**TABLE 4. Comparison of single nutrient Cox proportional hazards models\* and final multinutrient model\* using combined quartiles of daily nutrient intake, from food and supplements, as the independent variables and acquired immunodeficiency syndrome (AIDS) as the outcome variable for 281 human immunodeficiency virus type 1 (HIV-1) seropositive men in the Baltimore/Washington, DC Multicenter AIDS Cohort Study, 1984-1992**

Total intake	No. of AIDS events/total no. of subjects in intake stratum	Single nutrient models		Final multinutrient model	
		Relative hazard	95% CI†	Relative hazard	95% CI
Vitamin A (IU/day)					
<9,062	33/71	1.00		1.00	
9,062-20,268	44/140	0.55	0.35-0.88	0.57	0.35-0.91
>20,268	31/70	0.94	0.56-1.57	0.95	0.54-1.69
Vitamin B <sub>1</sub> (mg/day)					
<2.4	59/141	1.00			
2.4-4.9	28/70	0.75	0.47-1.21		
>4.9	21/70	0.60	0.36-0.98		
Vitamin B <sub>2</sub> (mg/day)					
≤5.9	88/211	1.00			
>5.9	20/70	0.61	0.38-1.00		
Vitamin B <sub>6</sub> (mg/day)					
≤2.0	31/71	1.00			
2.1-5.7	55/140	0.84	0.54-1.32		
>5.7	22/70	0.60	0.35-1.05		
Vitamin C (mg/day)					
≤715	88/211	1.00		1.00	
>715	20/70	0.55	0.34-0.91	0.59	0.34-1.03
Niacin (mg/day)					
≤61.0	90/141	1.00		1.00	
>61.0	18/70	0.52	0.31-0.86	0.51	0.29-0.92
Zinc (mg/day)					
<11.7	21/71	1.00		1.00	
11.7-14.2	26/70	1.33	0.75-2.38	1.49	0.83-2.68
14.2-20.2	28/70	1.69	0.95-3.02	1.85	1.03-3.31
>20.2	33/70	2.06	1.16-3.64	2.97	1.59-5.56

\* Adjusted for age, symptoms, CD4+ lymphocyte count, energy intake, use of antiretrovirals, and use of *Pneumocystis carinii* pneumonia prophylaxis.

† CI, confidence interval.

retinol, separate Cox models were run for each of these two variables. No relation was found between retinol intake and AIDS. However, a U-shaped relation was observed between  $\beta$ -carotene and risk of AIDS (relative hazard (RH) for third quartile = 0.63, RH for other quartiles 1.00-1.14). This relation was similar to that seen for total vitamin A, but did not reach the same level of significance. The highest quartile of total niacin intake (>61.0 mg/day) was significantly associated with an approximate twofold decrease in the relative hazard of AIDS. The progression rate to AIDS was also decreased in subjects in the highest quartile of total intake for vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, and C, though none reached statistical significance. However,

the patterns of association were somewhat clearer for these micronutrients than for niacin since a clear threshold effect was apparent where most of the benefit was limited to the upper quartile of intake. Higher intakes of zinc from food alone and from total intake (food and supplements) were associated with an increased hazard of developing AIDS. A dose response pattern was evident for total zinc, from lowest to highest quartile of intake, but was less apparent for zinc from food only.

Tests for heterogeneity (chi-square on 3 degrees of freedom) across the quartiles of nutrient intake were significant ( $p < 0.05$ ) for vitamins A and C, and niacin, while for zinc, the result was statistically marginal ( $p$

= 0.075). For vitamins B<sub>1</sub>, B<sub>2</sub>, and B<sub>6</sub>, the test for heterogeneity was not significant ( $p > 0.1$ ).

For all nutrients, total intake levels appeared to be more clearly associated with risk of AIDS than intake levels from food only. The analysis on intake of supplements alone did not reveal any significant relations with development of AIDS in this group of subjects. However, the highest tertile of supplemental zinc intake (15–155 mg/day) did show an increased risk of developing AIDS (RH = 1.8, 95 percent confidence interval (CI) 0.9–3.5).

Based on the above results of possible threshold effects for several of the nutrients, we then combined certain levels of nutrient intake where the relative hazards did not vary from the reference category. Table 4 shows the results of this analysis. By means of this approach, the highest quartiles of intake of vitamin C (>715 mg/day) and niacin (>61.0 mg/day) were associated with a significant protective effect against the development of AIDS, relative to the lower three quartiles of intake combined. The highest quartile of intake of vitamin B<sub>1</sub> (>4.9 mg/day) was also associated with a significantly decreased hazard of developing AIDS, relative to the lowest two quartiles of intake combined ( $\leq 2.4$  mg/day). Protective trends were also seen for the highest quartiles of intake of vitamins B<sub>2</sub> and B<sub>6</sub>. In addition, the second and third quartiles of vitamin A intake combined (9,062–20,268 IU/day) were associated with reduced progression to AIDS, in comparison with the lower ( $\leq 9,062$  IU/day) and upper (>20,268 IU/day) quartiles.

#### Multinutrient analyses and nutrient correlations

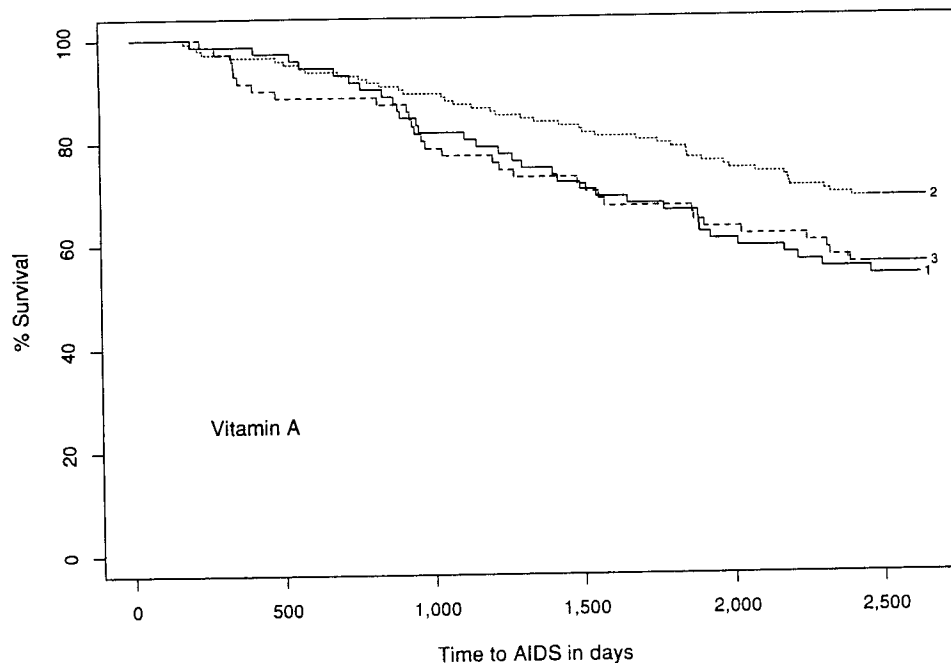
The correlations among the four micronutrients which showed the strongest associations with risk of progression to AIDS (vitamins A and C, zinc, and niacin) ranged from 0.31 (zinc and niacin) to 0.58 (vitamin C and niacin). B-group vitamins were all highly intercorrelated ( $r > 0.90$ ). In order to

assess the degree of confounding among these nutrients, we fit a series of Cox proportional hazards models including pairs and triplets of micronutrients as independent variables and AIDS as the outcome variable. In these multinutrient analyses, vitamin A, vitamin C, and zinc remained as independent predictors of AIDS. Finally, to a model including these three micronutrients, we added each of the individual B-group vitamins and niacin. Table 4 shows the results of these analyses and a comparison with the single nutrient models. Vitamin A, niacin, and zinc remained significantly associated with risk of progression to AIDS, while vitamin C was marginally significant. None of the other B-group vitamins significantly added to the model.

Kaplan-Meier plots for these final four micronutrients are presented in figures 1–4. The proportions surviving AIDS-free after the median follow-up period of 6.8 years, for each nutrient category, were calculated. For vitamin A, 53.4 percent and 55.7 percent survived AIDS-free in groups 1 and 3 ( $\leq 9,062$  IU/day and >20,268 IU/day, respectively) compared with 68.6 percent in group 2 (9,062–20,268 IU/day). Of those with a vitamin C intake of >715 mg/day, 71.4 percent remained AIDS-free compared with 58.3 percent in the lower intake group ( $\leq 715$  mg/day). The proportion AIDS-free in the high intake niacin group (>61.0 mg/day) was 74.3 percent compared with 57.3 percent in the low intake group ( $\leq 61.0$  mg/day). Three zinc groupings were used in the Kaplan-Meier plots to allow for a clearer graphical representation ( $\leq 14.1$  mg/day; 14.1–20.0 mg/day; >20.0 mg/day). The proportion surviving AIDS-free in the three groups were 70.4 percent, 61.4 percent, and 52.9 percent, respectively.

#### DISCUSSION

In this study, we related dietary micronutrient intakes of asymptomatic HIV-1-infected individuals with subsequent rates of progression to AIDS. To our knowledge, this is the first reported epidemiologic study



**FIGURE 1.** Kaplan-Meier plot of the proportions of HIV-1 seropositive men surviving AIDS-free according to different levels of total dietary intake of vitamin A: Baltimore/Washington, DC Multicenter AIDS Cohort Study. Group 1,  $<9,062$  IU/day; group 2,  $9,062$ – $20,268$  IU/day; group 3 =  $>20,268$  IU/day.

to address this question. After adjustment for important potential confounding variables, we found that the highest quartiles of intake of vitamin C, niacin, and vitamin B<sub>1</sub> were each significantly associated with a decreased progression rate to AIDS. These results suggest that a protective threshold may exist for intake of these three water-soluble vitamins. Vitamin A intake appeared to have a U-shaped relation with progression of HIV-1 infection to AIDS, with the middle two quartiles of intake having the most benefit. Furthermore, we found higher intakes of zinc to be monotonically and significantly associated with an increased rate of progression to AIDS. In multinutrient analyses, vitamin A, niacin, zinc, and vitamin C (to a lesser extent) remained important predictors of progression to AIDS. The antioxidant and immune stimulatory effects of several of these micronutrients may provide a biologic basis for some of these findings.

Vitamin A deficiency is known to be associated with increased susceptibility to

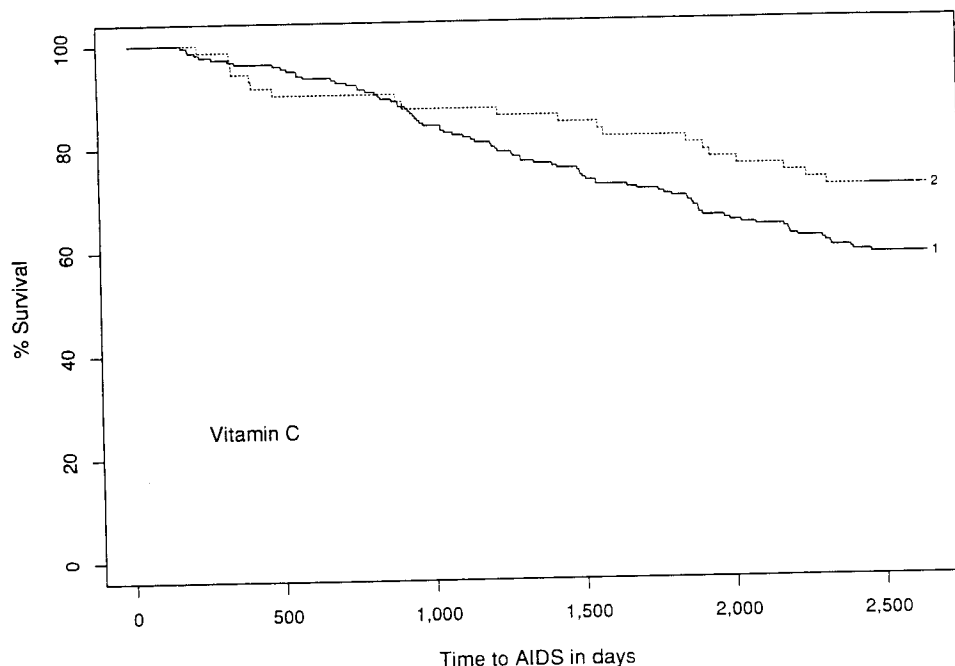
acute respiratory and gastrointestinal infections in children (24). In developing countries, clinical trials of vitamin A supplementation have been shown to reduce childhood mortality, presumably by improving immune response to endemic infections (25). Vitamin A deficiency causes atrophy of lymphoid tissues, decreases lymphocyte counts, suppresses antibody production after immunization, reduces lymphocyte response to mitogens in vitro, suppresses delayed dermal hypersensitivity reactions, reduces mobilization of peripheral macrophages, and increases serum concentration of hemolytic complement (26, 27). More recently, in a cohort of 179 HIV-1 seropositive injection drug users in Baltimore, a low plasma level of vitamin A ( $<1.05$   $\mu\text{mol/liter}$ ) was independently associated with subsequent risk of mortality (odds ratio = 4.3, 95 percent CI 1.1–17.8) after adjustment for baseline CD4+ T-lymphocyte count and other clinical markers (28).

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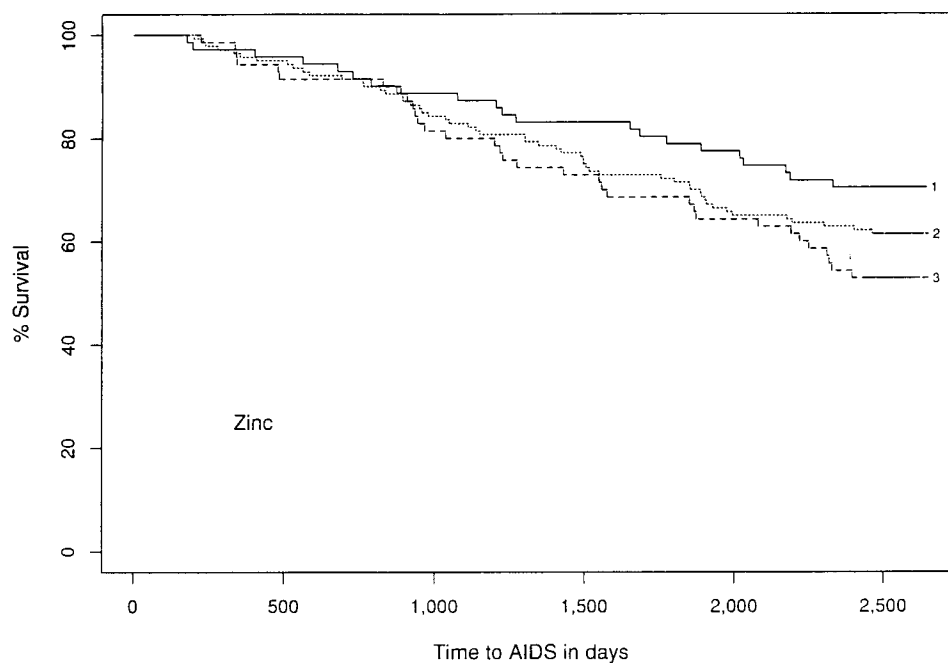


**FIGURE 2.** Kaplan-Meier plot of the proportions of HIV-1 seropositive men surviving AIDS-free according to different levels of total dietary intake of vitamin C: Baltimore/Washington, DC Multicenter AIDS Cohort Study. Group 1,  $\leq 715$  mg/day; group 2,  $> 715$  mg/day.

center AIDS Cohort Study, we were unable to assess the effects of low intakes of vitamin A on the natural history of HIV-1 infection because only a small number of subjects consumed less than the recommended dietary allowance. In fact, well over 75 percent of the men in our population consumed vitamin A, from food and supplements, in doses over 180 percent of the recommended dietary allowance. Over one quarter of the men were consuming over 400 percent of the recommended levels. Our results showed that men who consumed two to four times the recommended dietary allowance for vitamin A had a slower progression rate to AIDS. Previous studies have shown that excesses of vitamin A may diminish susceptibility to infection, enhance antibody production after immunization, increase *in vitro* lymphocyte response to mitogens, and shorten allograft survival (26, 27). However, we found that daily intakes in excess of four times the recommendation appeared to diminish the protective effect of this nutrient.

Vitamin A intakes in this range may begin to approach toxic levels, so it is possible that the beneficial effects of vitamin A may be limited to a narrower band of doses than that seen in some of the water-soluble vitamins. Our study seems to suggest that 9,000 to 20,000 IU daily may slow progression to AIDS, and this result remained after adjustment for other micronutrients. Our finding that  $\beta$ -carotene may play a more important role than retinol is supported by a recent clinical trial of  $\beta$ -carotene supplementation in 21 HIV-1-infected patients (29). In this study, the supplemented individuals had a significant rise in CD4+ percent of lymphocytes compared with placebo, but duration of effect, clinical outcomes, and vitamin A status were not measured.

Less is known about the effects of niacin on immune function. There is some evidence that deficiencies of niacin and other B-group vitamins disrupt the tissue barriers to infection (5), and may moderately depress humoral antibody responses in mammalian

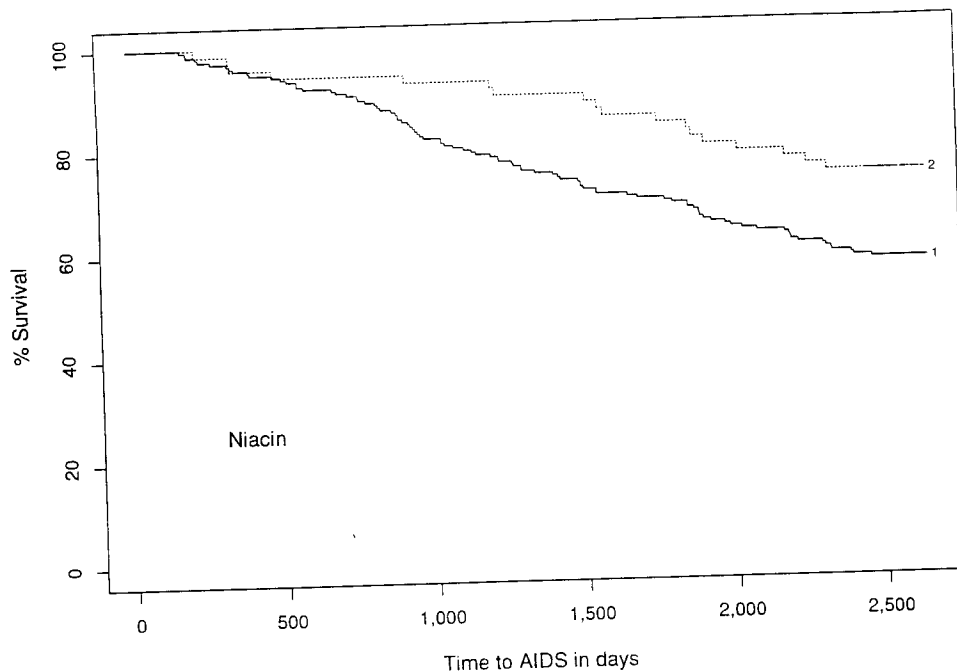


**FIGURE 3.** Kaplan-Meier plot of the proportions of HIV-1 seropositive men surviving AIDS-free according to different levels of total dietary intake of zinc: Baltimore/Washington, DC Multicenter AIDS Cohort Study. Group 1, <14.2 mg/day; group 2, 14.2–20.2 mg/day; group 3, >20.2 mg/day.

systems (5, 6). In our study, the highest quartiles of intake of niacin and thiamine were associated with a slower progression rate to AIDS in single-nutrient analysis but only niacin remained significantly associated with AIDS after adjusting for intakes of vitamin A, vitamin C, and zinc. Nonsignificant protective trends were also seen for the other B-group vitamins (riboflavin, pyridoxine). Since intakes of B-group vitamins are highly intercorrelated, further research is needed to determine if one or more of these nutrients is related to HIV-1 disease progression. Under these circumstances, niacin may represent a marker of overall intake of B-group vitamins rather than having any direct effect on immune function.

Leukocytes contain a high concentration of ascorbate, which is rapidly expended during infection and phagocytosis (10). This seems to suggest that vitamin C could play an important role in immune function during HIV-1 infection. There is evidence that a deficiency of vitamin C leads to reduced de-

layed cutaneous hypersensitivity reactions and phagocytic cell function (7). Well over three quarters of the men in our study consumed over 250 percent of the recommended dietary allowance of vitamin C. Because of this, we did not have enough data to study the effects of vitamin C deficiency on subsequent progression to AIDS. Our results did show that men who consumed over 12 times the recommended allowance for vitamin C (those who were in the highest quartile of intake) had a significantly slower progression rate to AIDS, however, this result became marginally significant when simultaneously adjusting for zinc, niacin, and vitamin A. There has been some experimental evidence that an excess of vitamin C may be associated with increased phagocytic cell mobility through the synthesis and assembly of the microtubular structures of these cells (26). Other investigators, however, have reported that megadoses of vitamin C may depress some mechanisms of phagocytic bacterial killing (7). It has also been suggested



**FIGURE 4.** Kaplan-Meier plot of the proportions of HIV-1 seropositive men surviving AIDS-free according to different levels of total dietary intake of niacin: Baltimore/Washington, DC Multicenter AIDS Cohort Study. Group 1,  $\leq 61.0$  mg/day; group 2,  $>61.0$  mg/day.

that some of the benefits of vitamin C may be derived through its antioxidant properties. Other antioxidants (e.g., glutathione) may slow HIV-1 production by inhibiting transcription of the virus (30). This may occur by suppressing the production of certain cytokines, such as tumor necrosis factor. Our results seem to suggest that levels of vitamin C intake at several times the recommended dietary allowance may slow progression of HIV-1 infection, but it is still unclear through which mechanisms this occurs.

Zinc deficiency in humans can result in impairment of several immune parameters such as atrophy of lymphoid tissue, reduced antibody response to thymus-dependent antigens, loss of cytotoxic T-lymphocyte responses to tumor cells, and loss of natural killer cell function (7, 9, 31). There is evidence that low serum levels of zinc are common in HIV-1 infection and may be a useful marker of progression to AIDS (14, 15, 32-34). Over half of the men in our study popu-

lation consumed less than the recommended daily allowance of 15 mg for zinc. Nonetheless, our results showed that those who consumed only 1.3 times the recommended levels (approximately 25 percent of the population) had an increased progression rate to AIDS. The dose-response relation that we observed became even stronger when we simultaneously adjusted for the potential confounding effects from niacin, vitamin A, and vitamin C. One study has shown that excessive intakes of zinc (at a level of 10-20 times the recommendation) can substantially impair lymphocyte and neutrophil functions in healthy adults (35). Whether lower levels of intake can adversely affect immune function in HIV-1 seropositive patients remains unknown. We found no relation between zinc intake and serum zinc levels in our populations, which suggests that persons who have higher intakes may sequester more of the zinc in liver and other tissues (assuming absorption is not yet impaired). One could speculate that

higher tissue concentrations may lead to toxicity under this scenario, but in preliminary studies, we have not found higher zinc levels in toenail samples taken from HIV-1 seropositive patients (15, 36).

Although many of our findings appear to have some biologic plausibility on the basis of the experimental studies noted above and the recent clinical studies in other infections (37), several caveats should be mentioned. One limitation of our data is that the semi-quantitative food frequency questionnaire for quantifying usual dietary intake has not been specifically validated in any HIV-1 seropositive population, but it has been found to be reasonably valid and reliable in other study populations (19–22). The underlying principle of the food frequency questionnaire is that average long-term diet is the important exposure, not intake on a few specific days. Thus, for each nutrient, individuals can be ranked according to the intake levels they report over the previous 12 months. In our study, we ranked individuals according to quartiles of nutrient intake in order to target possible ranges which might impact the progression of HIV-1 infection to AIDS.

A second limitation is that diet was measured in a seroprevalent cohort where the duration of infection is not known. It is possible that the infection may have influenced dietary habits, but we feel that this was unlikely to have had a major impact on our results. At the time that the questionnaire was administered in our population, the men did not yet know their HIV-1 status, so this knowledge could not have influenced their dietary habits. Also, we collected and used considerable data on age, CD4+ lymphocyte levels, and clinical symptoms and signs which, together, are good surrogates for duration of infection and disease stage. Adjustment for these factors in the multivariate analyses had little effect on our findings.

A third caveat is that dietary intake was measured at a point relatively early in the course of HIV-1 infection and it is possible that diet changed markedly for some men as

their disease progressed. Such changes in diet might be a result of disease progression, rather than a cause. Using data at baseline, before their HIV-1 status was known, would therefore tend to reduce any bias introduced on that basis.

In summary, we have found that high intakes of several nutrients (niacin, vitamin C, and vitamin B<sub>1</sub>) were associated with slower progression to AIDS, after adjustment for confounding variables. Vitamin A may have a U-shaped relation with risk of AIDS, and increasing zinc intake was associated with more rapid disease progression. These results need to be replicated before any firm conclusions can be drawn about their relevance to the natural history of HIV-1 infection.

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