Plasma n-6 Fatty Acid Levels Are Associated With CD4 Cell Counts, Hospitalization, and Mortality in HIV-Infected Patients

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Background: Fatty acids, including n-6 series, modulate immune function, but their effect on CD4 cell counts, death, or hospitalization in HIV-infected patients on antiretroviral therapy is unknown.

Methods: In a randomized trial for effects of multivitamins in HIV-infected patients in Uganda, we used gas chromatography to measure plasma n-6 fatty acids at baseline; determined CD4 counts at baseline, 3, 6, 12, and 18 months; and recorded hospitalization or death events. The associations of fatty acids with CD4 counts and events were analyzed using repeated-measures analysis of variance and Cox regression, respectively.

Results: Among 297 patients with fatty acids measurements, 16 patients died and 69 were hospitalized within 18 months. Except for linoleic acid, n-6 fatty acids levels were positively associated with CD4 counts at baseline but not during follow-up. In models that included all 5 major n-6 fatty acids, age; sex; body mass index; anemia status; use of antiretroviral therapy, multivitamin supplements, and alcohol; and the risk of death or hospitalization decreased significantly with an increase in linoleic acid and gamma-linolenic acid levels, whereas associations for dihomo-gamma-linolenic acid, arachidonic acid, and aolrenic acid were null. The hazard ratios (95% confidence intervals) per 1 SD increase in linoleic acid and gamma-linolenic acid were 0.73 (0.56–0.94) and 0.51 (0.36–0.72), respectively. Gamma-linolenic acid remained significant (hazard ratio = 0.51; 95% confidence interval: 0.35 to 0.68) after further adjustment for other plasma fatty acids.

Conclusions: Lower levels of gamma-linolenic acid are associated with lower CD4 counts and an increased risk of death or hospitalization. These results suggest a potential for using n-6 fatty acids to improve outcomes from antiretroviral therapy.

Key Words: antiretroviral therapy, fatty acids, n-6 fatty acids, mortality, hospitalization, HIV

(*J Acquir Immune Defic Syndr* 2016;73:598–605)

INTRODUCTION

Despite the increased availability and improved potency of antiretroviral therapy (ART), a significant number of patients on ART still experience treatment failure, which results in hospitalization and mortality. Although ART-related factors such as poor adherence and development of resistance to medications are important, other factors such as nutrition may also play a role in treatment outcomes. However, several epidemiologic studies including recent randomized trials focusing on vitamins and mineral supplements or provision of food to patients have not succeeded in improving major treatment outcomes.[1–3] Other dietary components such as fatty acids, which are known to improve cardiometabolic risk and to modulate immunological responses,[4–6] may offer an opportunity for improving benefits from ART when used as adjuncts to standard therapy or alone where ART is not available.

Indeed, some studies have shown that polyunsaturated fatty acids (PUFA) improve humoral immune responses[7] and survival in mice.[8] In vitro, n-6 fatty acids such as arachidonic acid confer resistance to infection by both bacteria and viruses.[9] These benefits have also been observed in vivo. In macaque monkeys, arachidonic acid is associated with an increase in the T-helper 17 $CD4^+$ cell compartment,[2] which is known to lower viral loads for the simian immunodeficiency

Received for publication March 28, 2016; accepted July 25, 2016.

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Supported by Grant # R01HD060333 from the Eunice Kennedy Schriver National Institute of Child Health and Development, National Institutes of Health, Department of Health and Human Services. Additional funding was provided by the Division of Epidemiology, Department of Medicine, Vanderbilt University Medical Center.

The authors have no conflicts of interest to disclose.

E.K.K. and A.E.E. contributed equally.

E.K.K. and W.W.F.: conceived the idea. E.K.K.: designed the analysis plan. W.F., D.G., and A.E.E.: enrolled study participants. W.W.F.: provided biospecimens and questionnaire data for the current analyses. A.E.E.: led sample selection. H.C.: supervised fatty acid analyses. A.E.E. and E.K.K.: conducted statistical analysis. E.K.K. and A.E.E.: drafted the manuscript. All authors participated in data acquisition, interpretation, and critical revision of the manuscript for important intellectual content and approved the final draft.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

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virus.[10] Studies in humans also suggest that fatty acids may improve immunological function[11] and reduce the risk of disease transmission. For instance, in a study among pregnant women in Tanzania, higher n-6 fatty acid levels in breast milk were associated with reduced HIV shedding and with a lower risk of mother-to-child HIV transmission.[12] This benefit was mainly observed for the 2 n-6 fatty acids arachidonic acid and dihomo-gamma-linolenic acid.[12] Consistent with this finding, we recently showed that among ART-naive HIV patients in Zambia, higher plasma levels of arachidonic acid are associated with better CD4 cell counts and other markers of improved survival.[13] A positive association between plasma linoleic acid, another n-6 fatty acid, and CD4 cell counts has been reported in a study of 14 HIV-infected children.[14]

Some[6,15,16] but not all studies[17] suggest that exposure to higher levels of n-6 fatty acids, particularly gamma-linolenic acid and arachidonic acid, has additional beneficial effects on the immune system including modulation of gut microbiota and better epithelial function, factors that could affect nutrient utilization and translocation of pathogens. These associations suggest that n-6 fatty acids may be beneficial in improving clinical outcomes in HIV patients before or during treatment with ART. However, there are no studies that have examined plasma n-6 fatty acids in relation to major clinical outcomes (eg, change in CD4 cell counts, death, or hospitalization) in HIV/AIDS patients followed for periods lasting more than 12 months.

We hypothesized that higher n-6 fatty acid levels in plasma will be associated with higher CD4 cell counts and a lower risk of death or hospitalization in HIV-infected patients and that the associations will be independent of plasma levels of other fatty acids and established risk factors for death or hospitalization in HIV populations.

SUBJECTS AND METHODS

Participants in the present study were adult men and women selected from patients who participated in a randomized placebo-controlled clinical trial designed to determine whether supplementation with multivitamins improves outcomes from ART in Uganda. The details of the parent study design have been described elsewhere.[1] Briefly, patients who normally receive care at the Infectious Diseases Institute at Makerere University College of Health Sciences were screened by a nurse and if eligible (ie, ≥18 years old, HIV-infected, on or planning to start ART, not planning to relocate within 18 months of the commencement of the study, not pregnant, and not severely ill) were invited to participate in the study.[1] Those who gave informed consent were randomized to receive a placebo or daily multivitamin supplement containing 1 recommended daily allowance of folic acid, niacin, and vitamins B1, B2, B6, B12, C, and E. All participants received ART during the study, and only 4 people experienced a switch in their ART regimen. Investigators and staff were blinded to the treatment group. At baseline, participants provided a blood sample, and questionnaires on medical history and lifestyle attributes were administered. Participants were then followed prospectively for 18 months to collect additional blood samples at 3, 6, 12, and 18 months after enrollment to document changes in CD4 cell counts, hospitalization, or death. In the event of a missed study visit, a study nurse made an in-home visit to motivate the participant to visit the clinic if his/her medical condition permitted, or if the missed visit was because of travel, neighbors or relatives on record were contacted to monitor the patient's health. Information on hospitalization, death, and specific causes of death was obtained from the hospitals or through a standardized verbal autopsy.[1]

The study was approved by institutional review boards at the Harvard T. H. Chan School of Public Health and the Infectious Diseases Institute at Makerere University College of Health Sciences.

Laboratory Analyses

Blood samples collected at baseline and 3, 6, 12, and 18 months of follow-up were processed to measure $CD4^+$ cell counts and to extract plasma. The former were measured at each time point using FACS Calibur flow cytometer (Becton-Dickinson, San Jose, CA). Fatty acids were measured in plasma in Dr. Campos' laboratory at the Harvard T. H. Chan School of Public Health using gas chromatography.[18] Levels of fatty acids in plasma are highly correlated with those from other blood compartments, eg, the correlation coefficient for linoleic acid in whole blood and plasma was 0.88.[19] Because of budgetary constraints, we decided a priori to measure plasma fatty acids only at baseline and from 300 HIV-infected participants that included all the 16 participants who died and 284 patients randomly selected from the surviving participants (Fig. 1).

Main Study Outcomes

The main outcomes of the study were death or hospitalization during 18 months of follow-up and CD4 cell counts at baseline and 3, 6, 12, and 18 months postrandomization.

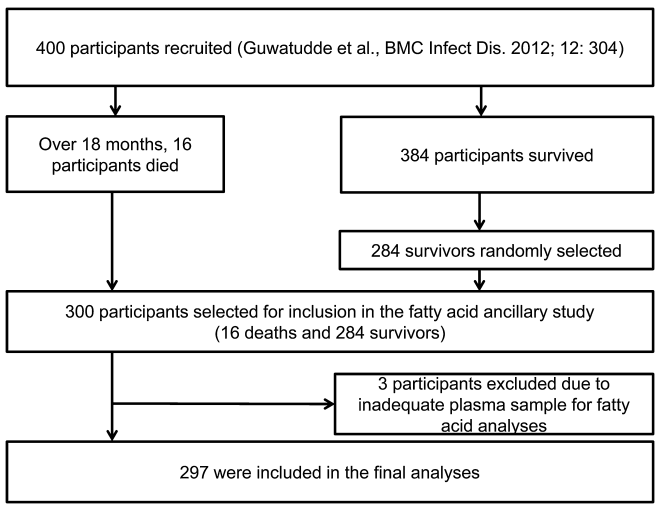

FIGURE 1. Study design for plasma fatty acids and risk of hospitalization or death in HIV/AIDS patients in Uganda.

Statistical Analyses

From the 300 participants with fatty acid measurements, we excluded 3 participants with insufficient baseline sample for fatty acid assessment leaving 297 participants for the final analyses. After examining variables for outliers and skewness, we distributed the major plasma n-6 fatty acids (linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, and aolrenic acid) into quartiles. We then computed descriptive statistics by quartiles of plasma gamma-linolenic acid as a representative fatty acid.

To test for the association between n-6 fatty acids at baseline and CD4 cell counts during follow-up, we implemented repeated-measures analysis of variance with a robust standard error estimation procedure to account for the skewed distribution of CD4 cell counts. In these analyses using the Mixed Procedure in SAS, we tested for the main effects for fatty acids (in quartiles), time, and the fatty acid × time interaction term. We adjusted for age, sex, body mass index (BMI), smoking, alcohol use, blood pressure, treatment group at randomization, and ART status.

Next, we used Cox regression to test whether plasma fatty acid levels at baseline are associated with hospitalization or death during 18 months of follow-up. Because of the low number of deaths (n = 16) and hospitalizations (n = 69), we created a composite outcome (death or hospitalization) and modeled fatty acids in 1 SD increments. Because n-6 fatty acids showed modest intercorrelations (r = 0.56 for the highest) and we are interested in estimating independent effects, we entered all the 5 major n-6 fatty acids in the model simultaneously and adjusted for covariates listed for the CD4 cell count models. We checked models for departure from the proportional hazards assumption by including the cross-product of time-at-risk and the covariate in the models.

Although all participants were on ART during the study, some (50%) were not at baseline. Thus, to explore the potential effect of ART before study enrolment, we performed a sensitivity analysis of the association between fatty acids and death or hospitalization together as the outcome within ART strata at baseline. We also conducted sensitivity analyses for death events alone and for hospitalizations alone to further understand whether the associations for both outcomes were in the same direction. The low number of events, especially death events, resulted in low statistical power and precluded thorough sensitivity analyses. Thus, the hazard ratios (HRs) [95% confidence intervals (CIs)] shown for death alone or hospitalization alone, though adjusted for fatty acids, are not adjusted for other covariates.

Associations were considered statistically significant at P-values ≤ 0.05. All analyses were conducted in SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

At baseline, the mean (±SD) age was 35.7 ± 8.7 years and BMI was 23.6 ± 4.3 kg/m^2. Most participants were women (68.7%) and ART naive (50.2%). The median (25th and 75th percentile) CD4 count was 110 (52–161) for ART-naive participants and 175 (range, 98–229 cells/μL) for ART-experienced participants. Most participants were not current alcohol consumers (80.8%) or current cigarette smokers (98.7%). A large number of participants were anemic at baseline (46.8%), and they considered themselves to have fair or poor self-rated health (52.5%).

Table 1 shows the characteristics of study participants by quartiles of gamma-linolenic acid at baseline. Participants in various gamma-linolenic acid quartiles were similar ($P > 0.05$) with respect to several variables including age, sex, mid-upper arm circumference (MUAC), ART status, and high-sensitivity C-reactive protein. Those with higher gamma-linolenic acid levels had significantly higher ($P < 0.05$) plasma levels of dihomo-gamma-linolenic acid, aolrenic acid, and saturated fatty acids but significantly lower ($P < 0.05$) plasma levels of linoleic acid and total n-3 PUFA (Table 1). Plasma fatty acid levels were not correlated with BMI or MUAC as measures of nutritional status. For instance, all correlation coefficients between MUAC and plasma levels of n-6 PUFA were <0.03 ($P > 0.05$ *for all*).

Plasma Fatty Acids and CD4 Counts

Table 2 shows associations between major n-6 fatty acids and mean CD4 cell counts at baseline and at 3, 6, 12 and 18 months of follow-up. Except for linoleic acid, CD4 cell counts at baseline increased with an increase in plasma n-6 fatty acid levels in analyses adjusted for age, sex, use of ART, multivitamin supplementation, anemia, BMI, smoking, and alcohol consumption. Also, CD4 cell counts increased with time regardless of the fatty acid levels, but the interaction between fatty acids and time of follow-up was only significant for dihomo-gamma-linolenic acid ($P < 0.05$). The positive associations between fatty acids and CD4 cell counts became attenuated during follow-up and were overall not significant ($P > 0.05$) at 18 months.

Plasma Fatty Acids and Death or Hospitalization

Cohortwide, the mean (SD) duration of follow-up was 16.9 (3.9) months. A total of 27 participants did not complete the study because of death (n = 16), out-migration (n = 5), loss during follow-up (n = 4), and refusal to continue participation (n = 2). Over 18 months, the 16 deaths and 69 hospitalization events observed resulted in 80 unique events of death or hospitalization. The causes of hospitalization varied greatly, the common ones being malaria (n = 22), diarrhea (n = 4), injury (n = 2), tuberculosis (n = 1), pneumonia (n = 1), and other (n = 49). Several fatty acids (linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, and arachidonic acid) showed univariate associations with either death or hospitalization but became nonsignificant when they were adjusted for each other. Higher plasma levels of dihomo-gamma-linolenic acid, which showed a significant ($P < 0.05$) positive association with CD4 cell counts at baseline, were also associated with a lower risk of death or hospitalization in unadjusted analyses (Supplemental Digital Content, Figure 1, http://links.lww.com/QAI/A875). After adjusting for age, sex, ART status, multivitamin supplementation, blood pressure, and anemia in a model with all n-6 fatty acids included simultaneously, only linoleic acid (HR = 0.73; 95% CI:

TABLE 1. Characteristics of the Study Participants by Quartiles of Plasma Gamma-Linolenic Acid at Baseline

	Quartiles of Plasma Gamma-Linolenic Acid				
	1 (n = 74)	2 (n = 75)	3 (n = 75)	4 (n = 73)	*P**
Gamma-linolenic acid, % of fatty acids	0.29 [0.23, 0.31]†	0.40 [0.37, 0.42]	0.52 [0.48, 0.54]	0.65 [0.62, 0.75]	—
Age, yrs	36.6 ± 9.1	34.5 ± 7.5	35.7 ± 10.1	33.9 ± 7.7	0.23
Sex, % women	64.9	66.7	70.7	72.6	0.73
Education, %					
Less than elementary	41.9	37.3	48.7	39.7	0.97
Elementary	10.8	12.0	12.2	13.7	
Some ordinary level	20.3	24.0	17.6	21.9	
Ordinary level or higher	27.0	26.7	21.6	24.7	
Has own income (%)	81.1	76.0	72.0	71.2	0.49
Smoking status, %					
Never smoker	77.0	84.0	85.3	83.8	0.69
Past smoker	20.3	12.0	13.3	14.9	
Current smoker	2.7	4.0	1.3	1.4	
Alcohol, %					
Never drinker	21.6	17.3	28.0	14.9	0.38
Past drinker	52.7	64.0	54.7	62.2	
Current drinker	25.7	18.7	17.3	23.0	
Anemia, %	39.2	53.3	49.3	48.0	0.36
Vitamin supplements, %	18.9	24.0	18.7	20.6	0.84
ART naive, %	60.8	50.6	41.3	46.5	0.11
BMI, kg/m^2	24.0 ± 4.3	23.3 (3.95)	23.3 (3.6)	23.6 (5.2)	0.72
MUAC, cm	28.5 ± 3.7	27.9 ± 3.3	28.2 ± 2.8	28.5 ± 3.8	0.69
WBC, ×10^9 cells/L	3.75 [3.0, 4.2]	3.85 (3.1, 4.3)	3.5 (3.0, 4.6)	3.3 (2.7, 4.0)	0.25
Ferritin, ng/mL	172.2 (59, 286)	110.6 (62, 358)	84.3 (36.9, 231.7)	87.3 (45, 238)	0.14
CD4 count, cells/μL	140 [74, 216]	118 [58, 190]	159 [82, 202]	128 [68, 183]	0.47
High-sensitivity C-reactive protein, mg/L	1.07 [0.38, 4.48]	1.82 [0.72, 6.54]	2.22 [0.72, 7.28]	1.79 [0.54, 6.44]	0.14
Systolic blood pressure, mm Hg	107 ± 13	106 ± 15	110 ± 13	106 ± 12	0.30
Diastolic blood pressure, mm Hg	69 ± 9	71 ± 10	72.5 ± 11.1	70 ± 11	0.22
Linoleic acid, % fatty acids	23.1 ± 3.94	23.0 ± 3.4	23.2 ± 3.5	21.0 ± 3.5	0.01
Dihomo-gamma-linolenic acid, % fatty acids	1.91 ± 0.44	2.20 ± 0.50	2.56 ± 0.54	2.69 ± 0.60	<0.01
Arachidonic acid, % fatty acids	8.28 ± 1.92	8.7 ± 1.6	8.6 ± 1.7	8.8 ± 1.9	0.34
Aolrenic acid, % fatty acids	0.32 ± 0.07	0.35 ± 0.09	0.38 ± 0.08	0.43 ± 0.10	<0.01
Total saturated fat, % fatty acids	29.6 ± 2.24	29.7 ± 2.0	29.4 ± 1.7	30.5 ± 2.6	0.01
Total MUFA, % fatty acids	30.4 ± 4.2	29.5 ± 3.2	29.6 ± 3.7	30.4 ± 3.1	0.22
Total PUFA (n-3), % fatty acids	4.90 ± 1.4	4.97 ± 1.4	4.5 ± 1.2	4.2 ± 0.9	<0.01
Total PUFA (n-6), % fatty acids	34.2 ± 5.0	35.0 ± 4.2	35.5 ± 4.4	33.9 ± 4.7	0.12
Total trans fatty acids, % fatty acids	0.74 ± 0.30	0.72 ± 0.23	0.68 ± 0.19	0.72 ± 0.26	0.51

*The *P*-value is from an analysis of variance model or the χ^2 test assessing whether baseline characteristics are significantly different across quartiles of gamma-linoleic acid also measured at baseline.

†Values are median [25th, 75th percentile], mean (SD), or %. MUFA = monounsaturated fatty acids.

0.57 to 0.94) and gamma-linolenic acid (HR = 0.52; 95% CI: 0.37 to 0.72) retained significant inverse associations with death or hospitalization (Table 3). The association for gamma-linolenic acid remained statistically significant (HR = 0.51; 95% CI: 0.35 to 0.68) even after further adjustment for BMI, alcohol use, and plasma levels of total n-3 fatty acids, total saturated fat, total monounsaturated fat, and total trans fat. In models containing variables for individual n-6 fatty acids, total n-3 fatty acids, total trans-fat and total monounsaturated fat, total n-3 fatty acids (HR = 0.86; 95% CI: 0.65 to 1.14), and total trans fat (HR = 0.89; 95% CI: 0.70 to 1.14) were not significantly associated with death or hospitalization. In the same model, 1 SD increase in total monounsaturated fat was marginally associated with a decreased risk of death/hospitalization (HR = 0.60; 95% CI: 0.36 to 1.00). On the other hand, each SD increase in total saturated fat was marginally associated with elevated risk of death or hospitalization (HR = 1.32; 95% CI: 1.00 to 1.75) in multivariable models adjusted for individual n-6 fatty acids, total trans-fatty acid, and total n-3 fatty acids.

Sensitivity Analyses

As shown in Table 4, replacing BMI with MUAC as a measure of nutritional status yielded results similar to those in Table 3 when BMI was used. For instance, the HR (95% CIs) for death/hospitalization associated with a 1 SD increase

TABLE 2. Mean CD4 Cell Counts by Baseline Plasma n-6 Fatty Acids Quartiles and Study Visit

		Month of Follow-up					*P*†	
Fatty Acid*	**Quartile***	**0**	**3**	**6**	**12**	**18**	**FA**	**FA × Time**
Linoleic acid	1	139 ± 13	213 ± 12	218 ± 11	260 ± 16	293 ± 20	0.29	0.89
	2	167 ± 11	242 ± 15	257 ± 14	284 ± 14	320 ± 23		
	3	148 ± 11	225 ± 18	229 ± 13	248 ± 14	279 ± 14		
	4	141 ± 12	235 ± 17	226 ± 16	254 ± 18	276 ± 18		
Gamma-linolenic acid	1	118 ± 10	226 ± 15	223 ± 12	259 ± 15	307 ± 22	0.72	0.08
	2	144 ± 12	235 ± 21	243 ± 17	254 ± 16	284 ± 19		
	3	160 ± 14	225 ± 14	227 ± 13	259 ± 17	277 ± 17		
	4	176 ± 11	231 ± 12	239 ± 13	275 ± 15	303 ± 18		
Dihomo-gamma-linolenic	1	130 ± 10	220 ± 15	221 ± 12	259 ± 15	287 ± 19	0.76	0.01
	2	130 ± 12	234 ± 14	237 ± 14	254 ± 13	302 ± 19		
	3	159 ± 12	242 ± 20	238 ± 15	265 ± 17	277 ± 18		
	4	177 ± 12	221 ± 14	235 ± 13	268 ± 18	303 ± 21		
Arachidonic acid	1	147 ± 12	223 ± 13	234 ± 12	267 ± 14	290 ± 16	0.51	0.47
	2	140 ± 12	218 ± 15	226 ± 14	272 ± 16	307 ± 21		
	3	148 ± 12	225 ± 15	221 ± 14	236 ± 16	267 ± 19		
	4	162 ± 12	250 ± 20	249 ± 15	272 ± 16	305 ± 19		
Aolrenic acid	1	137 ± 12	245 ± 22	232 ± 14	265 ± 15	306 ± 22	0.16	0.09
	2	122 ± 10	205 ± 14	215 ± 14	244 ± 16	267 ± 17		
	3	169 ± 12	234 ± 14	240 ± 14	255 ± 14	270 ± 15		
	4	167 ± 12	231 ± 13	242 ± 14	281 ± 18	325 ± 21		

*Quartile 1 indicates lowest and quartile 4 the highest amount of fatty acid in plasma; FA = fatty acid. Values show the mean (standard error of the mean) CD4 cell counts adjusted for age, sex, use of ART, vitamin supplementation and anemia, BMI, and smoking and alcohol consumption.

†*P*-values for the main effects (FA and time) and interaction terms (FA × time) are from a repeated-measures analysis of variance with a robust estimator of variance.

in gamma-linolenic acid was 0.52 (0.36 to 0.74) when MUAC was adjusted for and 0.51 (0.35 to 0.68) when BMI was used in the final model (Tables 3 and 4).

Sensitivity analyses stratifying by ART status at baseline to capture ART experience before cohort enrolment halved the sample size for each analysis and overall showed similar associations in the ART-experienced and ART-naive groups. For instance, the HR (95% CIs) for death/hospitalization associated with a 1 SD increase in linoleic acid was 0.89 (0.55 to 1.44) in ART-experienced and 0.74 (0.54 to 1.02) in ART-naive participants. The corresponding HR (95% CIs) for gamma-linolenic acid was 0.84 (0.51 to 1.41) in ART-experienced and 0.41 (0.24 to 0.68) in ART-naive participants.

In a sensitivity analysis restricted to mortality as an outcome (n = 16 events) and with all 5 n-6 fatty acids entered in the model simultaneously, the HRs (95% CIs) for death associated with 1 SD increase in a given fatty acid at baseline were 1.16 (0.63 to 2.41) for linoleic acid, 0.31 (0.13 to 0.71) for gamma-linolenic acid, 0.71 (0.33 to 1.54) for dihomo-gamma-linolenic acid, 0.48 (0.23 to 0.98) for arachidonic acid, and 2.00 (0.92 to 4.36) for aolrenic acid. The corresponding HRs (95% CIs) for hospitalization alone were 0.74 (0.56 to 0.97) for linoleic acid, 0.57 (0.40 to 0.81) for gamma-linolenic acid, 1.00 (0.72 to 1.36) for dihomo-gamma-linolenic acid, 1.13 (0.85 to 1.50) for arachidonic acid, and 0.97 (0.68 to 1.38) for aolrenic acid. Associations between respective n-6 fatty acids and time to first hospitalization were robust to further

TABLE 3. Hazard Ratios (95% CIs) for the Associations Between Major Plasma n-6 Fatty Acids and Time to Hospitalization or Death

n-6 Fatty Acid	**Model 1***	**Model 2†**	**Model 3‡**
Linoleic acid	**0.73 (0.57 to 0.94)**	**0.73 (0.56 to 0.94)**	0.82 (0.60 to 1.14)
Gamma-linolenic acid	**0.52 (0.37 to 0.72)**	**0.51 (0.36 to 0.72)**	**0.51 (0.35 to 0.68)**
Dihomo-gamma-linolenic acid	0.94 (0.70 to 1.27)	0.94 (0.69 to 1.28)	0.98 (0.71 to 1.33)
Arachidonic acid	1.08 (0.81 to 1.42)	1.07 (0.81 to 1.50)	1.15 (0.82 to 1.62)
Aolrenic acid	1.07 (0.77 to 1.49)	1.08 (0.77 to 1.51)	1.08 (0.75 to 1.53)

*Hazard ratios (95% CIs) for 1 SD increase in a given fatty acid from a Cox regression model in which all the 5 major n-6 fatty acids were entered simultaneously. The HRs (95% CIs) are adjusted for age, sex, use of ART, vitamin supplementation, anemia, and systolic and diastolic blood pressures.

†Additionally adjusted for BMI and alcohol use.

‡Further adjusted for total n-3 fatty acids, total saturated fat, total monounsaturated fat, and total trans fat, all measured in plasma.

Values in bold are significant at $P \leq 0.05$.

TABLE 4. Hazard Ratios (95% CIs) for the Associations Between Major Plasma n-6 Fatty Acids and Time to Hospitalization or Death Obtained From Sensitivity Analyses That Adjust for MUAC in the Total Sample or Within ART Strata at Baseline*

n-6 Fatty Acid	All Participants (n = 297)	ART Experienced (n = 149)	ART Naive (n = 148)
Linoleic acid	0.89 (0.64 to 1.23)	0.89 (0.55 to 1.44)	0.74 (0.54 to 1.02)
Gamma-linolenic acid	**0.52 (0.36 to 0.74)**	0.84 (0.51 to 1.41)	**0.41 (0.24 to 0.68)**
Dihomo-gamma-linolenic acid	0.95 (0.69 to 1.32)	0.99 (0.59 to 1.67)	0.83 (0.52 to 1.31)
Arachidonic acid	1.03 (0.72 to 1.47)	0.94 (0.59 to 1.49)	1.02 (0.68 to 1.54)
Aolrenic acid	1.06 (0.72 to 1.56)	0.82 (0.44 to 1.52)	1.22 (0.77 to 1.93)

*Hazard ratios (95% CIs) for 1 SD increase in a given fatty acid from a Cox regression model in which all the 5 major n-6 fatty acids were entered simultaneously. The HRs (95% CIs) are adjusted for age, sex, use of ART, vitamin supplementation, anemia, systolic and diastolic blood pressures, MUAC, alcohol use, total n-3 fatty acids, total saturated fat, total monounsaturated fat, and total trans fat, all measured in plasma.

Values in bold are significant at $P \leq 0.05$.

adjustment for baseline characteristics, such as age, sex, ART status, multi-vitamin use, anemia, BMI and systolic and diastolic blood pressure.

DISCUSSION

We have shown that at baseline, higher plasma levels of most major n-6 fatty acids are positively associated with higher CD4 cell counts, but these associations became attenuated and no longer significant during follow-up. We also observed that linoleic acid and gamma-linolenic acid are significantly associated with a reduced risk of death/hospitalization, independent of age, sex, BMI, and ART, among other variables associated with survival in HIV-infected patients. The association for linoleic acid and death or hospitalization was robust to adjustment for total n-3 fatty acids, trans fatty acids, and monounsaturated fatty acids. However, it became nonsignificant after further adjusting for total saturated fatty acids. Total plasma n-3 fatty acid levels were not significantly associated with death or hospitalization, probably because of the low plasma n-3 fatty acid levels in our study sample (4.7% of total fatty acids in plasma). Our previous studies in African Americans in the United States[20] show that total n-3 fatty acids make about 5.1%–6.6% of fatty acids in plasma. Dihomo-gamma-linolenic acid was inversely associated with death/hospitalization and arachidonic acid was inversely associated with death in models that only included fatty acids, but these associations became nonsignificant after adjusting for various covariates.

These results are important and to our knowledge the first to show that n-6 fatty acids are associated with survival/hospitalization in HIV/AIDS patients. Previous studies on the long-term effects of plasma n-6 fatty acids on death were done in patients not known to be HIV-infected and showed that higher linoleic acid levels, but not other n-6 fatty acids, were associated with a reduced risk of all-cause mortality.[21,22] These studies are consistent with short-term or cross-sectional studies showing inverse associations between n-6 fatty acids and the metabolic syndrome or its components in HIV-uninfected individuals.[23] Studies on effects of n-6 fatty acids on outcomes of HIV-infection have been conducted either in vitro or have focused on short-term clinical measures such as inflammation or pregnancy outcomes in HIV-infected patients. For instance, a study from Tanzania showed that higher levels of dihomo-gamma-linolenic acid in breast milk are associated with reduced HIV shedding and a lower risk of mother-to-child HIV transmission.[12] Our study among ART-naive Zambian adults followed for 90 days showed that n-6 fatty acids, particularly higher plasma levels of arachidonic acid, were positively associated with clinical markers of improved patient survival (eg, CD4 cell counts and serum albumin).[13]

The finding that higher levels of linoleic acid and gamma-linolenic acid are significantly associated with a lower risk of death or hospitalization before and after adjusting for covariates is particularly interesting, given the known roles of these fatty acids in the functioning of the immune system.[24] In vitro studies[25] show that addition of gamma-linolenic acid to cells significantly reduces production of inflammatory cytokines, particularly interleukin-1β and tumor necrosis factor-α. Higher levels of the latter have been associated with enhanced HIV replication,[26] suggesting that interventions that lower inflammatory cytokines such as tumor necrosis factor-α could be beneficial in HIV/AIDS management. These inverse associations between gamma-linolenic acid and cytokines have also been reported in short-term human studies in which gamma-linolenic acid was administered orally.[24,25] We are not aware of any long-term studies investigating the role of linoleic acid or gamma-linolenic acid on survival or hospitalization in HIV/AIDS patients. Therefore, future long-term studies are needed to confirm or refute our findings.

Although there are no epidemiologic studies investigating the direct effect of n-6 fatty acids on medium- and long-term clinical outcomes in HIV/AIDS patients, studies from elderly non-HIV participants (>65 years) in the United States[21] and Sweden (age >60 years)[22] show that higher plasma levels of linoleic acid, but not gamma-linolenic acid, dihomo-gamma-linolenic acid, or arachidonic acid, are inversely associated with reduced risk of all-cause mortality, in part supporting the inverse association observed for linoleic acid in our study. Another study that pooled 83,349 women from the Nurses' Health Study and 42,884 men from the Health Professionals Follow-up Study also showed that total n-6 PUFA, linoleic acid, and arachidonic acid estimated from food frequency questionnaires were associated with a 10%–15% lower risk of all-cause mortality.[27] Although the reason why other n-6 fatty acids such as gamma-linolenic acid did not show significant associations in non–HIV-infected populations is not clear, it is possible that the non–HIV-infected populations studied had adequate levels of these fatty acids,

an attribute that would make the associations null. As shown in our previous studies,[23] n-6 fatty acids may also protect against metabolic complications that are common in HIV/AIDS patients,[28] thus contributing to overall survival and reduced risk of hospitalization.

We are not aware of any studies on n-6 fatty acids and incidence of hospitalization in HIV/AIDS patients; however, existing randomized studies on enteral supplementation of septic shock patients with eicosapentaenoic acid and gamma-linolenic acid combination show that this intervention significantly reduces the number of days spent in the intensive care unit but not mortality.[29] This is consistent with our finding that gamma-linolenic acid is inversely associated with a reduced risk of hospitalization even after adjusting for various covariates including n-3 fatty acids.

Our study has a number of limitations. First, fatty acids were measured only at baseline and we do not know whether patients changed their dietary habits in a way that could affect fatty acid profiles during follow-up. Second, we had a relatively small number of deaths, which precluded adequately powered analyses that are restricted to mortality. Nonetheless, our sensitivity analyses restricted to death alone (16 deaths) or hospitalization alone (69 events) showed that n-6 fatty acids are significantly and inversely associated with these events; specifically, per SD increment, gamma-linolenic acid was associated with a 70% lower risk of death and 30% lower risk of hospitalization, when these events were modeled separately. Our study has a number of strengths. First, fatty acids, our main exposure, were measured objectively in plasma samples collected at baseline, an attribute that reduces measurement error and potential for exposure misclassification. Second, our study was prospective and patients were followed for 18 months, a period of follow-up that is considerably longer than follow-up periods in most studies on dietary factors in HIV/AIDS patients in Africa.[13] Third, our study had near-complete follow-up, an attribute that greatly reduced the risk of selection bias.

Although these results are interesting, they leave some unanswered questions. For example, would adding n-6 fatty acids to existing HIV treatment regimens improve long-term clinical outcomes such as death or hospitalization? By what mechanisms do fatty acids affect HIV outcomes? Would a cocktail of n-6 fatty acids work better than individual fatty acids? These questions are the subject of our future studies in which we seek to determine whether fatty acids, particularly linoleic acid and gamma-linolenic acid, could be used as adjunct therapies to antiretroviral drugs.

CONCLUSIONS

In this prospective study of objectively measured plasma n-6 fatty acids, we show that higher plasma levels of gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, and aolrenic acid are associated with higher CD4 cell counts at baseline and that higher plasma levels of gamma-linolenic acid are independently associated with reduced risk of death or hospitalization in HIV/AIDS patients. These data, together with known roles of these n-6 fatty acids on the immune system, suggest a potential for use of n-6 fatty acids in improving therapeutic outcomes from ART.

ACKNOWLEDGMENTS

We are grateful to study participants for volunteering their time. We also thank Dr. Jeremy Furtado and his staff for running fatty acid analyses.

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