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Plasma n6-fatty acid levels are associated with CD4 cell counts, hospitalization and mortality in HIV-infected patients

Edmond K Kabagambe, DVM, MS, PhD^{#*}, Amara E Ezeamama, PhD^{#†}, David Guwatudde, PhD[‡], Hannia Campos, PhD[§], and Wafaie Fawzi, MD, DrPH[§]

* Division of Epidemiology, Department of Medicine and the Department of Otolaryngology, Vanderbilt University Medical Center, Nashville, TN 37203 †Department of Epidemiology and Biostatistics, The University of Georgia, Athens, GA 30602 ‡Department of Epidemiology and Biostatistics (DG), School of Public Health, Makerere University College of Health Sciences, P.O. Box 7072, Kampala, Uganda §Department of Nutrition and Departments of Global Health and Population and Epidemiology, Harvard T.H. Chan School of Public Health, Harvard University, Boston, MA 02115.

These authors contributed equally to this work.

Abstract

Background—Fatty-acids, including n6 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 n6-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 ANOVA 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, n6-fatty acids levels were positively associated with CD4 counts at baseline but not during follow-up. In models that included all five major n6-fatty acids, age, sex, BMI, anemia status and use of antiretroviral therapy, multivitamin supplements and alcohol, the risk of death or hospitalization decreased significantly with increase in linoleic acid and gamma-linolenic acid levels while associations for dihomo-gamma-linolenic acid, arachidonic acid and aolenic acid were null. The hazard ratios (95% confidence intervals)

Correspondence to: Wafaie Fawzi.

Address correspondence to EKK, Division of Epidemiology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37203, USA; edmond.kabagambe@vanderbilt.edu; Phone: (615) 322-8033; Fax: (615) 343-5938.

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The authors' responsibilities were as follows – EKK and WF: conceived the idea; EKK: designed the analysis plan; WF, DG and AEE enrolled study participants; WF: provided biospecimens and questionnaire data for the current analyses; AEE: led sample selection; HC: supervised fatty acid analyses; AEE and EKK conducted statistical analysis; All authors: participated in data acquisition and interpretation; EKK and AEE: drafted the manuscript; All authors: participated in critical revision of the manuscript for important intellectual content and approved the final draft.

per one 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 (HR=0.51; 95% CI: 0.35-0.68) after further adjustment for other plasma fatty acids.

Conclusion—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 n6-fatty acids to improve outcomes from antiretroviral therapy.

Keywords

Antiretroviral therapy; fatty acids; n6-fatty acids; mortality; hospitalization; HIV

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. While ART-related factors such as poor adherence and development of resistance to medications are important factors, 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 responses⁷ and survival in mice.⁸ *In vitro*, n6-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 (T_H17) CD4+ cell compartment,² which is known to lower viral loads for the simian immunodeficiency virus.¹⁰ Studies in humans also suggest that fatty acids may improve immunological function¹¹ and reduce the risk of disease transmission. For instance, in a study among pregnant women in Tanzania, higher n6-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 n6-fatty acids arachidonic acid and dihomo-gamma-linolenic acid.¹² Consistent with this finding, we recently showed that among ART-naïve 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 n6-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 studies¹⁷ suggest that exposure to higher levels of n6-fatty acids, particularly gamma-linolenic acid and arachidonic acid, have 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 n6-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 n6-fatty acids in relation to major clinical outcomes (e.g., change in CD4 cell counts, death or hospitalization) in HIV/AIDS patients followed for periods lasting more than 12 months.

We hypothesized that higher n6-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 current 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 antiretroviral therapy 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 (i.e., 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 one recommended daily allowance for 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 due to 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 (HTHCSPH) and the Infectious Diseases Institute at Makerere University College of Health Sciences.

Laboratory analyses

Blood samples collected at baseline, 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 HTHCSPH using gas chromatography.¹⁸ Levels of fatty acids in plasma are highly correlated with those from other blood compartments e.g., the correlation coefficient for linoleic acid in whole blood and plasma was 0.88.¹⁹ Due to 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 (Figure 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, 3, 6, 12 and 18 months post-randomization.

Statistical analyses

From the 300 participants with fatty acid measurements, we excluded three 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 n6-fatty acids (linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid and aolenic 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 n6-fatty acids at baseline and CD4 cell counts during follow-up, we implemented repeated-measures ANOVA 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, 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 one standard deviation (SD) increments. Since n6-fatty acids showed modest inter-correlations ($r=0.56$ for the highest) and we are interested in estimating independent effects, we entered all the five major n6-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 prior to 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 HRs (95% 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 ($\pm$ SD) age was 35.7 ± 8.7 years and BMI was 23.6 ± 4.3 kg/m². Most participants were women (68.7%) and ART naïve (50.2%). The median (25th, 75th percentile) CD4 count was 110 (52-161) for ART-naïve participants and 175 (98-229 cells/ μ L) 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 (CRP). Those with higher gamma-linolenic acid levels had significantly higher ($P < 0.05$) plasma levels of dihomo-gamma-linolenic acid, aolenic acid and saturated fatty acids but significantly lower ($P < 0.05$) plasma levels of linoleic acid and total n3-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 n6-PUFA were < 0.03 ($P > 0.05$ for all).

Plasma fatty acids and CD4 counts

Table 2 shows associations between major n6-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 increase in plasma n6-fatty acid levels in analyses adjusted for age, sex, use of antiretroviral therapy, 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

Cohort-wide, the mean (SD) duration of follow-up was 16.9 (3.9) months. A total of 27 participants did not complete the study due to 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 non-significant 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 Figure 1**). After adjusting for age, sex, ART status, multivitamin supplementation, blood pressure and anemia in a model with

all n6-fatty acids included simultaneously, only linoleic acid (HR=0.73; 95% CI: 0.57-0.94) and gamma-linolenic acid (HR=0.52; 95% CI: 0.37-0.72) retained significant inverse associations with death or hospitalization (**Table 3**). The association for gamma-linolenic acid remained statistically significant (HR=0.51; 95% CI: 0.35-0.68) even after further adjustment for BMI, alcohol use, and plasma levels of total n3-fatty acids, total saturated fat, total monounsaturated fat and total trans fat. In models containing variables for individual n6-fatty acids, total n3-fatty acids, total trans-fat and total monounsaturated fat, total n3-fatty acids (HR=0.86; 95% CI: 0.65-1.14) and total trans fat (HR=0.89; 95% CI: 0.70-1.14) were not significantly associated with death or hospitalization. In the same model, one standard deviation increase in total monounsaturated fat was marginally associated with a decreased risk of death/hospitalization (HR=0.60; 95% CI: 0.36-1.00). On the other hand, each standard deviation increase in total saturated fat was marginally associated with elevated risk of death or hospitalization (HR=1.32; 95% CI: 1.00-1.75) in multivariable models adjusted for individual n6-fatty acids, total trans-fatty acid and total n3-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 in gamma-linolenic acid was 0.52 (0.36, 0.74) when MUAC was adjusted for and 0.51 (0.35, 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 naïve groups. For instance, the HR (95% CIs) for death/hospitalization associated with a 1 SD increase in linoleic acid was 0.89 (0.55, 1.44) in ART experienced and 0.74 (0.54, 1.02) in ART-naïve participants. The corresponding HR (95% CIs) for gamma-linolenic acid was 0.84 (0.51, 1.41) in ART experienced and 0.41 (0.24, 0.68) in ART-naïve participants.

In a sensitivity analysis restricted to mortality as an outcome (n=16 events) and with all five n6-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 (0.63-2.41) for linoleic acid, 0.31 (0.13, 0.71) for gamma-linolenic acid, 0.71 (0.33-1.54) for dihomo-gamma-linolenic acid, 0.48 (0.23-0.98) for arachidonic acid and 2.00 (0.92-4.36) for aolenic acid. The corresponding HR (95% CIs) for hospitalization alone were 0.74 (0.56-0.97) for linoleic acid, 0.57 (0.40-0.81) for gamma-linolenic acid, 1.00 (0.72-1.36) for dihomo-gamma-linolenic acid, 1.13 (0.85-1.50) for arachidonic acid and 0.97 (0.68-1.38) for aolenic acid. Associations between respective n6-fatty acids and time to first hospitalization were robust to further 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 n6-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 n3-fatty acids, trans fatty acids and monounsaturated fatty acids. However, it became non-significant after further adjusting for total saturated fatty acids. Total plasma n3-fatty acid levels were not significantly associated with death or hospitalization, probably because of the low plasma n3-fatty acid levels in our study sample (4.7% of total fatty acids in plasma). Our previous studies in African Americans in the US²⁰ show that total n3-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 non-significant after adjusting for various covariates.

These results are important and to our knowledge the first to show that n6-fatty acids are associated with survival/hospitalization in HIV/AIDS patients. Previous studies on the long-term effects plasma n6-fatty acids on death were done in patients not known to be HIV-infected and showed that higher linoleic acid levels, but not other n6-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 n6-fatty acids and the metabolic syndrome or its components in HIV-uninfected individuals.²³ Studies on effects of n6-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 dihome-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-naïve Zambian adults followed for 90 days showed that n6-fatty acids, particularly higher plasma levels of arachidonic acid, were positively associated with clinical markers of improved patient survival (e.g., 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 studies²⁵ show that addition of gamma-linolenic acid to cells significantly reduces production of inflammatory cytokines, particularly interleukin-1 β and tumor necrosis factor (TNF)- α . Higher levels of the latter have been associated with enhanced HIV replication,²⁶ suggesting that interventions that lower inflammatory cytokines such as TNF- 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 n6-fatty acids on medium- and long-term clinical outcomes in HIV/AIDS patients, studies from elderly

non-HIV participants (>65 years) in the US²¹ and Sweden (age>60 years)²² show that higher plasma levels of linoleic acid, but not gamma-linolenic acid or 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 n6-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 n6-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 n6-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 n6-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 n3-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. Secondly, 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 n6-fatty acids are significantly and inversely associated with these events; specifically, per SD increment, gamma-linolenic acid was associated with 70% lower risk of death and 30% lower risk of hospitalization, when these events were modeled separately. Our study has 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. Secondly, 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 Thirdly, our study had near complete follow-up, an attribute that greatly reduced the risk of selection bias.

While these results are interesting they leave some unanswered questions. For example, would adding n6-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 n6-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.

Conclusion

In this prospective study of objectively measured plasma n6-fatty acids, we show that higher plasma levels of gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid and aolenic 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 n6-fatty acids on the immune system, suggest a potential for use of n6-fatty acids in improving therapeutic outcomes from antiretroviral therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ART	Antiretroviral therapy
FA	Fatty acid
GLA	Gamma linolenic acid
DGLA	Dihomogamma-linolenic acid

References

1. Guwatudde D, Ezeamama AE, Bagenda D, et al. Multivitamin supplementation in HIV infected adults initiating antiretroviral therapy in Uganda: the protocol for a randomized double blinded placebo controlled efficacy trial. *BMC Infect Dis.* 2012; 12:304. [PubMed: 23151221]
2. Filteau S, PrayGod G, Kasonka L, et al. Effects on mortality of a nutritional intervention for malnourished HIV-infected adults referred for antiretroviral therapy: a randomised controlled trial. *BMC Med.* 2015; 13:17. [PubMed: 25630368]
3. Tang AM, Quick T, Chung M, Wanke CA. Nutrition assessment, counseling, and support interventions to improve health-related outcomes in people living with HIV/AIDS: a systematic review of the literature. *Journal of acquired immune deficiency syndromes.* 2015; 68(Suppl 3):S340–349. [PubMed: 25768873]
4. Kim YS, Sayers TJ, Colburn NH, Milner JA, Young HA. Impact of dietary components on NK and Treg cell function for cancer prevention. *Molecular Carcinogenesis.* 2015 n/a/n/a.
5. Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol.* 2015; 36(2):81–91. [PubMed: 25592731]
6. Ardeshtir A, Narayan NR, Mendez-Lagares G, et al. Breast-fed and bottle-fed infant rhesus macaques develop distinct gut microbiotas and immune systems. *Sci Transl Med.* 2014; 6(252):252ra120.

7. Ramon S, Baker SF, Sahler JM, et al. The specialized proresolving mediator 17-HDHA enhances the antibody-mediated immune response against influenza virus: a new class of adjuvant? *J Immunol.* 2014; 193(12):6031–6040. [PubMed: 25392529]
8. Svahn SL, Grahnemo L, Palsdottir V, et al. Dietary polyunsaturated fatty acids increase survival and decrease bacterial load during septic *Staphylococcus aureus* infection and improve neutrophil function in mice. *Infect Immun.* 2015; 83(2):514–521. [PubMed: 25404025]
9. Chouinard F, Turcotte C, Guan X, et al. 2-Arachidonoyl-glycerol- and arachidonic acid-stimulated neutrophils release antimicrobial effectors against *E. coli*, *S. aureus*, HSV-1, and RSV. *J Leukoc Biol.* 2013; 93(2):267–276. [PubMed: 23242611]
10. Hartigan-O'Connor DJ, Abel K, Van Rompay KK, Kanwar B, McCune JM. SIV replication in the infected rhesus macaque is limited by the size of the preexisting TH17 cell compartment. *Sci Transl Med.* 2012; 4(136):136ra169.
11. Harbige LS. Dietary n-6 and n-3 fatty acids in immunity and autoimmune disease. *The Proceedings of the Nutrition Society.* 1998; 57(4):555–562. [PubMed: 10096116]
12. Villamor E, Koulinska IN, Furtado J, et al. Long-chain n-6 polyunsaturated fatty acids in breast milk decrease the risk of HIV transmission through breastfeeding. *Am J Clin Nutr.* 2007; 86(3): 682–689. [PubMed: 17823433]
13. Nyirenda CK, Kabagambe EK, Koethe JR, et al. Plasma Fatty Acids in Zambian Adults with HIV/AIDS: Relation to Dietary Intake and Cardiovascular Risk Factors. *Journal of Nutrition and Metabolism.* 2015; 2015:8.
14. Agostoni C, Zuccotti GV, Riva E, et al. Low levels of linoleic acid in plasma total lipids of HIV-1 seropositive children. *Journal of the American College of Nutrition.* 1998; 17(1):25–29. [PubMed: 9477386]
15. Kaku S, Ohkura K, Yunoki S, et al. Dietary gamma-linolenic acid dose-dependently modifies fatty acid composition and immune parameters in rats. *Prostaglandins Leukot Essent Fatty Acids.* 2001; 65(4):205–210. [PubMed: 11728173]
16. Kaku S, Yunoki S, Ohkura K, et al. Interactions of dietary fats and proteins on fatty acid composition of immune cells and LTB4 production by peritoneal exudate cells of rats. *Biosci Biotechnol Biochem.* 2001; 65(2):315–321.
17. Benoit B, Bruno J, Kayal F, et al. Saturated and Unsaturated Fatty Acids Differently Modulate Colonic Goblet Cells In Vitro and in Rat Pups. *The Journal of Nutrition.* 2015
18. Kabagambe EK, Baylin A, Allan DA, Siles X, Spiegelman D, Campos H. Application of the Method of Triads to Evaluate the Performance of Food Frequency Questionnaires and Biomarkers as Indicators of Long-term Dietary Intake. *Am J Epidemiol.* 2001; 154(12):1126–1135. [PubMed: 11744518]
19. Baylin A, Kim MK, Donovan-Palmer A, et al. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. *Am J Epidemiol.* 2005; 162(4):373–381. [PubMed: 16014782]
20. Lemaitre RN, Tanaka T, Tang W, et al. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet.* 2011; 7(7):e1002193. [PubMed: 21829377]
21. Wu JHY, Lemaitre RN, King IB, et al. Circulating Omega-6 Polyunsaturated Fatty Acids and Total and Cause-Specific Mortality: The Cardiovascular Health Study. *Circulation.* 2014; 130(15):1245–1253. [PubMed: 25124495]
22. Marklund M, Leander K, Vikstrom M, et al. Polyunsaturated Fat Intake Estimated by Circulating Biomarkers and Risk of Cardiovascular Disease and All-Cause Mortality in a Population-Based Cohort of 60-Year-Old Men and Women. *Circulation.* 2015
23. Kabagambe EK, Tsai MY, Hopkins PN, et al. Erythrocyte Fatty Acid Composition and the Metabolic Syndrome: A National Heart, Lung, and Blood Institute GOLDN Study. *Clin Chem.* 2008; 54(1):154–162. [PubMed: 17998266]
24. Harbige LS. Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. *Lipids.* 2003; 38(4):323–341. [PubMed: 12848277]

25. DeLuca P, Rossetti RG, Alavian C, Karim P, Zurier RB. Effects of gammalinolenic acid on interleukin-1 beta and tumor necrosis factor-alpha secretion by stimulated human peripheral blood monocytes: studies in vitro and in vivo. *J Investig Med*. 1999; 47(5):246–250.
26. Biswas P, Mantelli B, Delfanti F, et al. Tumor necrosis factor-alpha drives HIV-1 replication in U937 cell clones and upregulates CXCR4. *Cytokine*. 2001; 13(1):55–59. [PubMed: 11145843]
27. Wang DD, Li Y, Chiuve SE, et al. Association of Specific Dietary Fats With Total and Cause-Specific Mortality. *JAMA Intern Med*. 2016
28. Kiage JN, Heimbarger DC, Nyirenda CK, et al. Cardiometabolic risk factors among HIV patients on antiretroviral therapy. *Lipids in Health and Disease*. 2013;12. [PubMed: 23391102]
29. Pontes-Arruda A, Martins LF, de Lima SM, et al. Enteral nutrition with eicosapentaenoic acid, gamma-linolenic acid and antioxidants in the early treatment of sepsis: results from a multicenter, prospective, randomized, double-blinded, controlled study: the INTERSEPT study. *Crit Care*. 2011; 15(3):R144. [PubMed: 21658240]

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

Table 1

Characteristics of the study participants by quartiles of plasma gamma-linolenic acid at baseline.

	Quartiles of plasma gamma-linolenic acid				P [†]
	1 (n = 74)	2 (n = 75)	3 (n = 75)	4 (n = 73)	
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, years	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, %					
< 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
Mid upper arm circumference, 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, mmHg	107 ± 13	106 ± 15	110 ± 13	106 ± 12	0.30

	Quartiles of plasma gamma-linolenic acid				<i>P</i> [†]
	1 (n = 74)	2 (n = 75)	3 (n = 75)	4 (n = 73)	
Diastolic blood pressure, mmHg	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
Adrenic 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 (n3), % fatty acids	4.90 ± 1.4	4.97 ± 1.4	4.5 ± 1.2	4.2 ± 0.9	<0.01
Total PUFA (n6), % 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

* Values are median [25th, 75th percentile], mean (SD) or %. MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids.

[†]The *P*-value is from an ANOVA model or the chi square test assessing whether baseline characteristics are significantly different across quartiles of gamma-linolenic acid also measured at baseline.

Table 2

Mean CD4 cell counts by baseline plasma n-6 fatty acids quartiles and study visit.

Fatty acid*	Quartile*	Month of follow-up					P†	
		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		
Aolenic 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 while 4 the highest amount of fatty acid in plasma; FA = Fatty acid; Values show the mean (SEM) CD4 cell counts adjusted for age, sex, use of antiretroviral therapy, vitamin supplementation and anemia, BMI, smoking and alcohol consumption.

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

Table 3

Hazard ratios (95% CIs) for the associations between major plasma n6-fatty acids and time to hospitalization or death.

N6-fatty acid	Model 1[*]	Model 2[†]	Model 3[‡]
Linoleic acid	0.73 (0.57, 0.94)	0.73 (0.56, 0.94)	0.82 (0.60, 1.14)
Gamma-linolenic acid	0.52 (0.37, 0.72)	0.51 (0.36, 0.72)	0.51 (0.35, 0.68)
Dihomogammalinolenic acid	0.94 (0.70, 1.27)	0.94 (0.69, 1.28)	0.98 (0.71, 1.33)
Arachidonic acid	1.08 (0.81, 1.42)	1.07 (0.81, 1.50)	1.15 (0.82, 1.62)
Adrenic acid	1.07 (0.77, 1.49)	1.08 (0.77, 1.51)	1.08 (0.75, 1.53)

^{*} Hazard ratios (95% CIs) for one standard deviation increase in a given fatty acid from a Cox regression model in which all the five major n6-fatty acids were entered simultaneously. The HRs (95% CIs) are adjusted for age, sex, use of antiretroviral therapy, vitamin supplementation, anemia and systolic and diastolic blood pressure.

[†] Additionally adjusted for BMI and alcohol use.

[‡] Further adjusted for total n3-fatty acids, total saturated fat, total monounsaturated fat and total trans fat, all measured in plasma.

Hazard ratios (95% CIs) for the associations between major plasma n6-fatty acids and time to hospitalization or death obtained from sensitivity analyses that adjust for mid-upper arm circumference in the total sample or within ART strata at baseline

Table 4

N6-fatty acid	All Participants (n = 297)	ART Experienced (n = 149)	ART naïve (n = 148)
Linoleic acid	0.89 (0.64, 1.23)	0.89 (0.55, 1.44)	0.74 (0.54, 1.02)
Gamma-linolenic acid	0.52 (0.36, 0.74)	0.84 (0.51, 1.41)	0.41 (0.24, 0.68)
Dihomogammalinolenic acid	0.95 (0.69, 1.32)	0.99 (0.59, 1.67)	0.83 (0.52, 1.31)
Arachidonic acid	1.03 (0.72, 1.47)	0.94 (0.59, 1.49)	1.02 (0.68, 1.54)
Adrenic acid	1.06 (0.72, 1.56)	0.82 (0.44, 1.52)	1.22 (0.77, 1.93)

* Hazard ratios (95% CIs) for one standard deviation increase in a given fatty acid from a Cox regression model in which all the five major n6 fatty acids were entered simultaneously. The HRs (95% CIs) are adjusted for age, sex, use of antiretroviral therapy, vitamin supplementation, anemia and systolic and diastolic blood pressure, MUAC, alcohol use, total n3-fatty acids, total saturated fat, total monounsaturated fat and total trans fat, all measured in plasma.