**Plasma Phospholipid and Dietary Alpha Linolenic Acid, Mortality, Coronary Heart Disease and Stroke: the Cardiovascular Health Study**

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**ABSTRACT**

Prior studies suggest that long-chain n-3 fatty acids derived from seafood are associated with a lower risk of mortality, coronary heart disease (CHD), and stroke. Whether alpha-linolenic acid (ALA 18:3n-3), a plant-oil-derived long chain essential n-3 fatty acid, is associated with these outcomes is unclear. The purpose of this analysis was to examine the associations of plasma phospholipid and dietary ALA with mortality, CHD, and stroke among older adults who participated in the Cardiovascular Health Study, a cohort study of adults aged 65 or older. A total of 2,709 participants were included in the plasma phospholipid analysis and 2,583 participants were included in the dietary analysis. Cox regression was used to assess the associations of plasma phospholipid and dietary ALA with mortality, and incident CHD or stroke. In minimally and-multivariable-adjusted models, plasma phospholipid ALA was not associated with mortality, incident CHD, or stroke. After adjustment for age, sex, race, clinic, education, smoking, diabetes, BMI, alcohol use, treated hypertension and total caloric intake, higher dietary intake of ALA was associated with a lower risk of total and non-cardiovascular mortality; comparing highest to lowest quintiles of dietary ALA, the odds ratios for total mortality and non-cardiovascular mortality were 0.73 (95% CI, 0.61, 0.88) and 0.64 (95% CI, 0.52, 0.80), respectively. Dietary intake of ALA was not associated with cardiovascular mortality, incident CHD or stroke. In conclusion, results from this analysis suggest that dietary, but not plasma phospholipid ALA, is associated with a lower risk of total and non-cardiovascular mortality in older adults.

**INTRODUCTION**

Prior studies suggest that long-chain n-3 fatty acids found in seafood are associated with a lower risk of mortality, coronary heart disease (CHD), and stroke ([1-7](#_ENREF_1)). Whether intake of plant and plant oil-derived α-linolenic acid (ALA 18:3n-3) is associated with a lower risk of these outcomes is less clear. Given concerns about the sustainability of fish populations and potential harm from fish contaminants ([8](#_ENREF_8), [9](#_ENREF_9)), a cheaper, plant-derived alternative source of n-3 fatty acids might be important to public health. Therefore, it is essential to understand if plant-derived ALA exhibits similar associations with cardiovascular-related morbidity and mortality as seafood-derived n-3 fatty acids.

ALA is found in selected seeds and vegetable oils, such as soybean and canola oils. These oils are used alone or in combination with other vegetable and seed oils in varying concentrations in many foods. Consequently, estimation of dietary ALA using food frequency questionnaires (FFQs) is prone to measurement error. Plasma phospholipid ALA is an objective biomarker of circulating levels of ALA over the past 1-2 months that reflects diet together with the metabolism of dietary ALA. Thus, dietary and biomarker measures provide complementary information on exposure to ALA. In this study, we investigated the associations of both plasma phospholipid and dietary ALA with mortality, CHD and stroke among adults aged 65 years or older in the Cardiovascular Health Study (CHS), a large community-based prospective cohort.

**EXPERIMENTAL METHODS**

**Design and Population.** The CHS is a prospective community-based cohort study of cardiovascular disease (CVD) and its risk factors among older adults from 4 geographical areas in the U.S. (Forsyth County, North Carolina; Sacramento County, CA; Washington County, Maryland; Allegheny County, Pennsylvania). Previous publications describe the study’s rationale, study design and data collection methodology in detail ([10](#_ENREF_10)). Briefly, non-institutionalized adults aged 65 years or older were randomly selected and enrolled in the study using Medicare eligibility lists. In total, 5,201 participants enrolled in 1989-1990 and 687 participants (predominantly African American) enrolled 3-4 years later. The study included annual clinic visits with interim phone calls through 1998-1999 and phone contact two times per year thereafter. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by each center’s institutional review board. Written informed consent was obtained for all subjects/patients.

Among the 5,565 study participants alive in 1992-1993, fatty acids were measured on 3,941. After excluding 1,232 participants with prevalent CVD (MI, angina, coronary revascularization, stroke, transient ischemic attack or heart failure) at the time of the 1992-1993 blood draw, 2,709 persons were included in the study population for the plasma phospholipid ALA analysis. Among the 3,764 participants who completed a semi-quantitative FFQ questionnaire in 1996, 1,181 had prevalent CVD at the time of the questionnaire and were excluded from the analyses. The remaining 2,583 participants comprised the analytic cohort for the dietary ALA analysis. In total, 80.1% of study participants included in the plasma phospholipid ALA analyses were also included in the dietary ALA analyses.

**Data Collection.** Standardized interviews, physical examinations, medical history review, laboratory evaluations, and diagnostic testing were performed at the annual clinic examinations. Fasting blood samples for all study participants were stored at -70 degrees Celsius.

**Plasma Phospholipid ALA Measurement.** ALA was measured using blood samples from 1992-1993. Plasma lipids were extracted using the methods of Folch, as described previously ([11](#_ENREF_11)). A one dimensional thin-layer chromatography was used to separate phospholipids from neutral lipids. Phospholipid fractions were directly trans-esterified using the Lepage and Roy method to prepare fatty acid methyl esters ([12](#_ENREF_12)). Individual fatty acid methyl esters were separated using gas chromatography, as previously described (Agilent 5890 Gas Chromatograph flame ionization detector, Agilent Technologies, Palo Alto, CA USA; fused silica capillary column SP-2560 [100m x 0.25mm, 0.2μm], Supelco Belefonte, PA USA; initial 160 degrees Celsius for 16 min, ramp 3 degrees Celcius/min to 240 degrees Celsius, hold at 160 degrees for 15 minutes) ([5](#_ENREF_5)). Laboratory inter-assay coefficient of variation was 3.1% for ALA ([13](#_ENREF_13)). All fatty acids were processed at the Biomarker Laboratory of the Fred Hutchinson Cancer Research Center (Seattle, WA USA). ALA is expressed as percent of total plasma phospholipid fatty acids analyzed.

**Dietary ALA Measurement.** Past year dietary intake was measured using a Willett 131-item semi-quantitative FFQ administered in 1996. This questionnaire has known reliability and validity ([14](#_ENREF_14)). To obtain a measure of dietary ALA, we multiplied the dietary ALA content of each food item with the participant’s frequency response, and then summed for all foods. For consistency with the measurement of plasma phospholipid ALA, which is assessed as percent of total fatty acids, dietary ALA was evaluated as percent of total fat, which is correlated better than absolute intake (grams/day) or percent of total energy with plasma phospholipid ALA (r=0.18) ([15](#_ENREF_15), [16](#_ENREF_16)).

**Mortality and CVD Assessment.** Cause of death and cardiovascular events were adjudicated by a centralized CHS events committee based on information from medical records, laboratory/diagnostic reports, death certificates and/or interviews with next of kin. Details of CHS methods for surveillance and disease classification have been reported in detail previously ([17](#_ENREF_17), [18](#_ENREF_18)). For the purposes of this analysis, we evaluated the relationships of plasma phospholipid or dietary ALA with total mortality, as well as CVD mortality, CHD mortality, and non-CVD mortality, and incident CHD and incident stroke. Total CHD mortality was further sub-classified as arrhythmic or non-arrhythmic death; and strokes as ischemic, hemorrhagic, or unknown type. The maximum length of follow-up was 16 years for the plasma phospholipid ALA analyses and 12 years for the dietary ALA analyses.

**Statistical Analyses.** Cox regression was used to examine the associations of plasma phospholipid and dietary ALA with total and cause-specific mortality, incident CHD, and incident stroke (total, ischemic, hemorrhagic). Circulating and dietary ALA were assessed both categorically (indicator quintiles) and semi-parametrically (cubic splines). Loss to follow-up was considered a censoring event. Schoenfeld’s residuals were used to evaluate the proportional hazards assumption for both plasma phospholipid and dietary ALA. For consistency with plasma phospholipid ALA (measured as a percent of total fatty acids), dietary ALA was adjusted for total energy to reduce measurement error and confounding by total reported energy ([19](#_ENREF_19)). Covariates of interest included age, sex, race (European American or African American), enrollment site (Bowman Grey, Davis, Hopkins, Pittsburg), education (no high school, high school/vocational school, college), smoking (never, past, current), diabetes (yes/no), BMI (kg/m2), waist circumference (cm), physical activity (kcal/week), alcohol use (drinks/week) and treated hypertension (yes/no).

 In sensitivity analyses, we further adjusted for linoleic acid (LA); LA is a major dietary polyunsaturated fatty acid that is present in many of the same foods as ALA and may compete with ALA for elongation into longer chain n-3 and n-6 fatty acids. We also performed sensitivity analyses truncating follow-up at 8 years after the collection of plasma phospholipid or dietary ALA measures to minimize exposure misclassification that may be higher in later years of follow-up.

We examined the potential interaction of ALA (modeled continuously) with sex, age, LA, and delta-6-desaturase (FADS2) genotype on risk of death or incident CHD or stroke. We tested the statistical significance of each multiplicative interaction term using likelihood ratio tests. We chose to examine potential interaction of ALA and LA since LA competes with ALA for the elongation and desaturation into very long chain n-3 fatty acids ([20](#_ENREF_20)). Likewise, genetic variability in FADS2 may affect the conversion of ALA to EPA and DHA ([21](#_ENREF_21)), and we also examined the interaction between FADS2 genotype and ALA. Since dietary EPA and DHA may influence the associations of ALA with the outcomes of interest ([22](#_ENREF_22)), we stratified the analyses at the 25th percentile of fish intake (0.6 servings/day) in sensitivity analyses.

We used single imputation to impute missing values for covariates (<2% missing values for all covariates) using data on age, gender, smoking, education, race, BMI, physical activity, self-reported health status, and diabetes at the time of the ALA measure. All statistical analyses were conducted using STATA version 10.0 (Stata Corp, College Station, Texas).

**RESULTS**

**Plasma Phospholipid ALA and Risk of Mortality, CHD or Stroke** There were 2,709 CHS participants free of CVD and with available plasma phospholipid ALA measures in 1992-1993. Of these participants, 36.1% were male, 90.0% were Caucasian, and the median age was 73.0 years (interquartile range: 71.0, 98.0 years).Baseline characteristics of study participants according to quintile of plasma phospholipid ALA are described in the online supplement (**supplementary table 1**). There were 1,757 deathsduring 32,111 person-years of follow-up. In both age-and-sex and fully adjusted analyses, plasma phospholipid ALA was not associated with total or cause-specific mortality (**Table 1**). Similarly, plasma phospholipid ALA was not associated with incident CHD, stroke, or stroke subtypes (**Table 2).** Using restricted cubic splines to model plasma phospholipid ALA, further adjustment for plasma phospholipid LA or fish intake, truncating follow-up at 8 years, or performing subgroup analyses by sex or fish intake had no meaningful effect on reported hazard ratios (data not shown). We found no evidence of interactions of ALA with age, sex, FADS2 genotype, or plasma phospholipid LA on risk of death, CHD, or stroke (data not shown).

**Dietary ALA and Risk of Mortality, CHD or Stroke** In general, characteristics of study participants according to quintile of dietary ALA were similar to those of plasma phospholipid ALA, except for no observed differences in lipid-lowering medication use or BMI according to quintile of dietary ALA (**supplementary Table 2**). In total, 1,517 deaths occurred during 25,849 person-years of follow-up among participants with dietary data. In both minimally-and-fully-adjusted analyses, higher dietary ALA was associated with a lower risk of total mortality and non-CVD mortality (**Table 3**). Hazard ratios were not materially changed after additionally adjusting for other dietary factors, such as dietary LA or fish intake. There were no statistically significant associations of dietary ALA with total CVD mortality, total CHD mortality, or CHD-related non-arrhythmic or arrhythmic deaths after adjustment for age, sex, energy intake, race, clinic, education, smoking, alcohol, BMI, diabetes, and treated hypertension (**Table 3**). We found no significant association of dietary ALA with incident CHD or stroke (**Table 4**). Similar to the plasma phospholipid results, further adjustment for dietary LA or fish intake, truncating follow-up at 8 years, or performing subgroup analyses by fish intake did not affect results (data not shown). There was also no evidence of interactions between dietary ALA and age, sex, FADS2 genotype, or LA on risk of death, CHD, or stroke (data not shown).

In exploratory secondary analyses, we further examined the association of dietary ALA with sub-types of non-CVD mortality (i.e., deaths from dementia, cancer, infection, trauma/fracture, and respiratory diseases). Approximately 60% of participants who died from non-CVD causes had cancer or dementia listed as causes of death in death records. Higher dietary ALA was associated with a lower risk of death from dementia and cancer. We found no statistically significant associations of dietary ALA with deaths from respiratory diseases, infection, or trauma/fracture, although power was limited due to a small number of deaths from these causes (**Supplementary Table 3**).

**DISCUSSION**

In this large prospective cohort study of older adults, we found no significant associations of plasma phospholipid ALA with total or cause-specific mortality, CHD, or stroke. Dietary ALA was associated with a lower risk of total mortality, which appeared related to significantly lower non-CVD deaths. Dietary ALA was not associated with CVD mortality, CHD, or stroke.

It is interesting that higher dietary ALA was associated with lower total mortality in our cohort. When specific types of death were evaluated, this inverse association was only statistically significant for non-CVD deaths, in particular deaths due to cancer and dementia. Biological mechanisms by which dietary ALA may reduce non-CVD mortality are not well-established. In vitro and rodent studies of cancer suggest that ALA may suppress cancer cell proliferation, inhibit tumor growth, and increase apoptosis ([23-25](#_ENREF_23)). Additionally, animal models suggest that ALA deficiency may promote abnormalities in cerebral structures, and it is hypothesized that low levels of ALA may be associated with poor cognitive function in humans ([26](#_ENREF_26)). However, the results of epidemiological studies that have examined the relationships of ALA with cancer or dementia in human populations are conflicting ([26-30](#_ENREF_26)). More research is needed to better understand the mechanisms by which ALA may be associated with lower non-CVD mortality.

On the other hand, the inverse associations of dietary ALA level with total mortality and non-CVD mortality might also be due to chance or to residual confounding by other poorly measured or unmeasured factors related to both dietary ALA and risk of death. Our findings support the need for further investigation of the relationship of habitual dietary ALA consumption and total-and-cause-specific mortality.

Very few prior studies have evaluated circulating ALA with incident CVD, stroke, or mortality. Results of a small prospective nested case-control study among 192 men in the U.S. indicated that ALA in cholesterol esters was associated with lower risk of stroke ([31](#_ENREF_31)), while a retrospective case-control study among 134 South Koreans found no significant association of ALA in erythrocytes with stroke ([32](#_ENREF_32)). Of four previous prospective studies have examined circulating ALA and incident CHD, stroke, or mortality, circulating ALA was not significantly associated with development of CHD ([33](#_ENREF_33), [34](#_ENREF_34)), stroke ([35](#_ENREF_35)), or total/CVD mortality ([36](#_ENREF_36)). Our findings are also consistent with a recent meta-analysis that found no statistically significant association of circulating ALA with fatal and non-fatal CHD or stroke ([37](#_ENREF_37)).

Relatively few studies have assessed the relationship of dietary ALA with mortality, stroke, or CHD, and the findings are inconsistent. Our results support the findings from the Nurses’ Health Study that suggest dietary ALA is associated with a lower risk of all-cause mortality ([38](#_ENREF_38)). They are also consistent with findings from seven prospective studies and a small meta-analysis that indicate no association of ALA with non-fatal or fatal heart disease ([39-46](#_ENREF_39)), and with two prospective studies that found no association of dietary ALA with stroke ([47](#_ENREF_47), [48](#_ENREF_48)). Our findings are also consistent with a recent meta-analysis of prior observational studies, which demonstrate no association of dietary ALA with CHD or stroke ([37](#_ENREF_37)). On the other hand, results of the meta-analysis showed a modest inverse association of dietary ALA with fatal CHD ([37](#_ENREF_37)). Our results are inconsistent with the results from two studies in which higher ALA was associated with a lower risk of fatal ischemic heart disease ([49](#_ENREF_49)) and sudden cardiac death ([43](#_ENREF_43)) among women who participated in the Nurses’ Health Study. Although our analyses did not specifically assess the relationship of dietary ALA with fatal ischemic heart disease or sudden cardiac death, we found no statistically significant association of dietary ALA with cardiovascular-related deaths. Our findings are also discordant with the findings of a large Dutch study that suggests dietary ALA is inversely associated with stroke risk. In that study, participants in the upper four quintiles of dietary ALA had a 35%-50% lower risk of stroke when compared to participants in the lowest dietary ALA quintile([40](#_ENREF_40)). Inconsistencies of these studies’ results may be due to underlying differences between the populations studied (e.g., differences in age, background diet or other health factors). For instance, participants in the Dutch study that reported an inverse association of dietary ALA with stroke were younger (mean (sd): 41.5±11.1 years at baseline) and had higher reported dietary ALA intake (mean dietary ALA for women and men: 1.2±0.5 g/day and 1.6±0.6 g/day, respectively) when compared to CHS study participants. Inconsistencies between studies may also be due to measurement error.

Our analysis has several strengths. The prospective analysis and cohort design reduced potential for both recall bias and selection bias. We assessed both plasma phospholipid ALA and dietary ALA, providing complementary measures of exposure to this plant-derived n-3 fatty acid. We focused on older adults—a population at high risk for mortality, CHD, and stroke. Detailed information on demographics, clinical risk factors, and lifestyle habits were collected using standardized instruments, increasing our ability to adjust for confounding. The community-based enrollment of the cohort increased generalizability.

The present analysis also has several limitations. Plasma phospholipid and dietary ALA were assessed once, and levels may have changed during the follow-up. Nevertheless, terminating follow-up at 8 years, which would minimize effects of misclassification, did not change the results. For the purposes of this analysis, we considered dietary and plasma phospholipid ALA as complimentary measures of ALA exposure. However, plasma phospholipid ALA levels were low (<1% total fatty acids), and the correlation of plasma phospholipid and dietary ALA was modest. It is possible that other tissue compartments with higher proportions of ALA (e.g., adipose tissue) may be a better marker of dietary ALA intake, and more studies are needed to examine the association of other biomarkers of ALA with mortality, CHD, and stroke. Additional limitations include error in dietary ALA measurement, which if random could attenuate findings toward the null. Although we included several major risk factors as covariates in our analyses of ALA with mortality, CHD, and stroke, residual confounding by unknown or poorly measured factors is possible. Since ALA competes with LA for elongation/desaturation, the absolute intakes of ALA and LA are important determinants of ALA metabolism, and the association of ALA with mortality, CHD or stroke may differ in populations with other diets (e.g., populations with very low intakes of LA). Likewise, all study participants were aged 65 years or older, and the generalizability of these findings to younger populations in unknown. On the other hand, we found little evidence for effect modification in analyses stratified by LA consumption or age.

In summary, in this large prospective cohort of older adults, neither plasma phospholipid nor dietary ALA were associated with CVD mortality, CHD, or stroke. The inverse association of dietary ALA with total mortality and non-CVD mortality requires further investigation and replication in other studies.

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**Conflicts of Interest.** Dr. Mozaffarian reports receiving research grants from GlaxoSmithKline, Sigma Tau, Pronova, and the National Institutes of Health for an investigator-initiated, not-for-profit clinical trial; travel reimbursement, honoraria, or consulting fees related to conferences on diet and chronic diseases from the International Life Sciences Institute, Aramark, Unilever, SPRIM, Bunge, Foodminds, McKinsey Health Systems Institute, and Nutrition Impact; and royalties from UpToDate. Dr. Psaty serves on the DSMB for a clinical trial of a device funded by the manufacturer (Zoll LifeCor) and on the Steering Committee for the Yale Open Data Access Project funded by Medtronic. There are no other conflicts of interest to report.

**Authorship.** This work comprises the contribution of eleven authors. Amanda Fretts performed the literature review and data analysis for the project, as well as writing the manuscript. Rozenn Lemaitre, Dariush Mozaffarian and David Siscovick were the senior investigators on the project. They supervised all activities, and aided in all aspects of the project, including development of the research question and writing the manuscript. Colleen Sitlani, Barbara McKnight and Donna Spiefelman were the biostatisticians on the project, and supervised the statistical methods of the paper, as well as reviewed all drafts of the manuscript. Irena King, Bruce Psaty, Eric Rimm, and Xiaoling Song obtained funding, collected the data, and reviewed and edited all drafts of the manuscript.

**References**

1. Harris WS, Kris-Etherton PM, Harris KA. Intakes of long-chain omega-3 fatty acid associated with reduced risk for death from coronary heart disease in healthy adults. *Curr Atheroscler Rep* 2008;10(6):503-9.

2. Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis* 2007;193(1):1-10.

3. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;346(15):1113-8.

4. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. *JAMA* 1998;279(1):23-8.

5. Lemaitre RN, King IB, Mozaffarian D, et al. n-3 Polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the Cardiovascular Health Study. *Am J Clin Nutr* 2003;77(2):319-25.

6. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 1989;2(8666):757-61.

7. Wang C, Harris WS, Chung M, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr* 2006;84(1):5-17.

8. Bushkin-Bedient S, Carpenter DO. Benefits versus risks associated with consumption of fish and other seafood. *Rev Environ Health* 2010;25(3):161-91.

9. Lenihan-Geels G, Bishop KS, Ferguson LR. Alternative sources of omega-3 fats: can we find a sustainable substitute for fish? *Nutrients* 2013;5(4):1301-15.

10. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol* 1991;1(3):263-76.

11. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957;226(1):497-509.

12. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res* 1986;27(1):114-20.

13. Djousse L, Biggs ML, Lemaitre RN, et al. Plasma omega-3 fatty acids and incident diabetes in older adults. *Am J Clin Nutr* 2011;94(2):527-33.

14. Feskanich D, Rimm EB, Giovannucci EL, et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc* 1993;93(7):790-6.

15. Lemaitre RN, Sitlani C, Song X, et al. Circulating and dietary alpha-linolenic acid and incidence of congestive heart failure in older adults: the Cardiovascular Health Study. *Am J Clin Nutr* 2012;96(2):269-74.

16. Fretts AM, Mozaffarian D, Siscovick DS, et al. Associations of plasma phospholipid and dietary alpha linolenic Acid with incident atrial fibrillation in older adults: the cardiovascular health study. *J Am Heart Assoc* 2013;2(1):e003814.

17. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol* 1991;1(3):263-76.

18. Ives DG, Fitzpatrick AL, Bild DE, et al. Surveillance and ascertainment of cardiovascular events. The Cardiovascular Health Study. *Ann Epidemiol* 1995;5(4):278-85.

19. Willet W. *Nutritional Epidemiology*. New York: Oxford University Press; 1998.

20. Goyens PL, Spilker ME, Zock PL, et al. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. *Am J Clin Nutr* 2006;84(1):44-53.

21. Baylin A, Ruiz-Narvaez E, Kraft P, et al. alpha-linolenic acid, Delta(6)-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. *Am J Clin Nutr* 2007;85(2):554-60.

22. Mozaffarian D, Ascherio A, Hu FB, et al. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation* 2005;111(2):157-64.

23. du Toit PJ, van Aswegen CH, du Plessis DJ. The effect of essential fatty acids on growth and urokinase-type plasminogen activator production in human prostate DU-145 cells. *Prostaglandins Leukot Essent Fatty Acids* 1996;55(3):173-7.

24. Kamano K, Okuyama H, Konishi R, et al. Effects of a high-linoleate and a high-alpha-linolenate diet on spontaneous mammary tumourigenesis in mice. *Anticancer Res* 1989;9(6):1903-8.

25. Fritsche KL, Johnston PV. Effect of dietary alpha-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two murine mammary adenocarcinomas. *J Nutr* 1990;120(12):1601-9.

26. Bourre JM. Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing. *J Nutr Health Aging* 2004;8(3):163-74.

27. Gerber M. Omega-3 fatty acids and cancers: a systematic update review of epidemiological studies. *Br J Nutr* 2012;107 Suppl 2:S228-39.

28. Geleijnse JM, Giltay EJ, Kromhout D. Effects of n-3 fatty acids on cognitive decline: a randomized, double-blind, placebo-controlled trial in stable myocardial infarction patients. *Alzheimers Dement* 2012;8(4):278-87.

29. Kim M, Nam JH, Oh DH, et al. Erythrocyte alpha-linolenic acid is associated with the risk for mild dementia in Korean elderly. *Nutr Res* 2010;30(11):756-61.

30. Cherubini A, Andres-Lacueva C, Martin A, et al. Low plasma N-3 fatty acids and dementia in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci* 2007;62(10):1120-6.

31. Simon JA, Fong J, Bernert JT, Jr., et al. Serum fatty acids and the risk of stroke. *Stroke* 1995;26(5):778-82.

32. Park Y, Park S, Yi H, et al. Low level of n-3 polyunsaturated fatty acids in erythrocytes is a risk factor for both acute ischemic and hemorrhagic stroke in Koreans. *Nutr Res* 2009;29(12):825-30.

33. Khaw KT, Friesen MD, Riboli E, et al. Plasma Phospholipid Fatty Acid Concentration and Incident Coronary Heart Disease in Men and Women: The EPIC-Norfolk Prospective Study. *Plos Med* 2012;9(7).

34. Wang L, Folsom AR, Eckfeldt JH. Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Nutr Metab Cardiovasc Dis* 2003;13(5):256-66.

35. Wiberg B, Sundstrom J, Arnlov J, et al. Metabolic risk factors for stroke and transient ischemic attacks in middle-aged men - A community-based study with long-term follow-up. *Stroke* 2006;37(12):2898-903.

36. Warensjo E, Sundstrom J, Vessby B, et al. Markers of dietary fat quality and fatty acid desaturation as predictors of total and cardiovascular mortality: a population-based prospective study. *Am J Clin Nutr* 2008;88(1):203-9.

37. Pan A, Chen M, Chowdhury R, et al. alpha-Linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. *Am J Clin Nutr* 2012;96(6):1262-73.

38. Folsom AR, Demissie Z. Fish intake, marine omega-3 fatty acids, and mortality in a cohort of postmenopausal women. *Am J Epidemiol* 2004;160(10):1005-10.

39. Oomen CM, Ocke MC, Feskens EJ, et al. alpha-Linolenic acid intake is not beneficially associated with 10-y risk of coronary artery disease incidence: the Zutphen Elderly Study. *Am J Clin Nutr* 2001;74(4):457-63.

40. de Goede J, Verschuren WM, Boer JM, et al. Alpha-linolenic acid intake and 10-year incidence of coronary heart disease and stroke in 20,000 middle-aged men and women in the Netherlands. *PLoS One* 2011;6(3):e17967.

41. Pietinen P, Ascherio A, Korhonen P, et al. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol* 1997;145(10):876-87.

42. Ascherio A, Rimm EB, Giovannucci EL, et al. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. *BMJ* 1996;313(7049):84-90.

43. Albert CM, Oh K, Whang W, et al. Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary heart disease. *Circulation* 2005;112(21):3232-8.

44. Vedtofte MS, Jakobsen MU, Lauritzen L, et al. Dietary alpha-linolenic acid, linoleic acid, and n-3 long-chain PUFA and risk of ischemic heart disease. *Am J Clin Nutr* 2011;94(4):1097-103.

45. Laaksonen DE, Nyyssonen K, Niskanen L, et al. Prediction of cardiovascular mortality in middle-aged men by dietary and serum linoleic and polyunsaturated fatty acids. *Arch Intern Med* 2005;165(2):193-9.

46. Brouwer IA, Katan MB, Zock PL. Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. *J Nutr* 2004;134(4):919-22.

47. Larsson SC, Virtamo J, Wolk A. Dietary fats and dietary cholesterol and risk of stroke in women. *Atherosclerosis* 2012;221(1):282-6.

48. He K, Rimm EB, Merchant A, et al. Fish consumption and risk of stroke in men. *JAMA* 2002;288(24):3130-6.

49. Hu FB, Stampfer MJ, Manson JAE, et al. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. *Am J Clin Nutr* 1999;69(5):890-7.

\* Additionally adjusts for race, clinic, education, smoking, diabetes, BMI, waist circumference, physical activity, alcohol use & treated hypertension

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| Table 1. Hazard Ratios (95% CI) for Association of Plasma Phospholipid ALA with Risk of Total and Cause-Specific Mortality among 2,709 US Adults |
| Quintile | I | II | III | IV | V | *p-trend* |
| Total Mortality |  |  |  |  |  |  |
| Person-Years | 6483 | 6025 | 6315 | 6352 | 6936 |  |
| No. of deaths | 360 | 354 | 359 | 331 | 353 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 1.08 | (0.93-1.25) | 1.07 | (0.92-1.23) | 0.94 | (0.81-1.10) | 0.91 | (0.79-1.06) | 0.06 |
| Additionally adjusted model\* | 1.0 (ref) | 1.09 | (0.93-1.26) | 1.09 | (0.94-1.27) | 0.95 | (0.81-1.11) | 0.93 | (0.79-1.08) | 0.11 |
| Non-CVD Mortality |  |  |  |  |  |  |
| No. of deaths | 235 | 229 | 236 | 209 | 229 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 1.07 | (0.89-1.28) | 1.08 | (0.90-1.29) | 0.92 | (0.76-1.11) | 0.91 | (0.76-1.09) | 0.10 |
| Additionally adjusted model\* | 1.0 (ref) | 1.09 | (0.90-1.31) | 1.11 | (0.92-1.34) | 0.92 | (0.76-1.11) | 0.90 | (0.75-1.09) | 0.09 |
| Total CVD Mortality |  |  |  |  |  |  |
| No. of deaths | 101 | 108 | 102 | 102 | 106 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 1.16 | (0.88-1.52) | 1.07 | (0.81-1.41) | 1.02 | (0.78-1.35) | 0.96 | (0.73-1.27) | 0.50 |
| Additionally adjusted model\* | 1.0 (ref) | 1.15 | (0.87-1.53) | 1.08 | (0.81-1.44) | 1.05 | (0.79-1.40) | 1.02 | (0.77-1.36) | 0.87 |
| Total CHD Mortality |  |  |  |  |  |  |
| No. of deaths | 68 | 64 | 69 | 64 | 66 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 1.03 | (0.74-1.46) | 1.11 | (0.79-1.56) | 0.99 | (0.70-1.40) | 0.92 | (0.66-1.30) | 0.59 |
| Additionally adjusted model\* | 1.0 (ref) | 1.06 | (0.75-1.51) | 1.16 | (0.82-1.65) | 1.02 | (0.71-1.45) | 1.03 | (0.72-1.46) | 0.98 |
|  Arrhythmic Death |  |  |  |  |  |  |
| No. of deaths | 35 | 30 | 38 | 34 | 33 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 0.93 | (0.57-1.52) | 1.16 | (0.73-1.83) | 1.00 | (0.62-1.60) | 0.88 | (0.55-1.42) | 0.72 |
| Additionally adjusted model\* | 1.0 (ref) | 1.02 | (0.62-1.67) | 1.23 | (0.76-1.99) | 1.05 | (0.64-1.71) | 0.98 | (0.60-1.62) | 0.99 |
|  Non-Arrhythmic Death |  |  |  |  |  |  |
| No. of deaths | 33 | 34 | 31 | 30 | 33 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 1.14 | (0.71-1.85) | 1.06 | (0.65-1.74) | 0.98 | (0.60-1.62) | 0.97 | (0.59-1.57) | 0.70 |
| Additionally adjusted model\* | 1.0 (ref) | 1.12 | (0.68-1.83) | 1.09 | (0.65-1.81) | 0.99 | (0.59-1.66) | 1.07 | (0.65-1.76) | 0.97 |

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| Table 2. Hazard Ratios (95% CI) for Association of Plasma Phospholipid ALA with Risk of Incident Stroke and CHD among 2,709 US Adults |
|  Quintile | I | II  | III  | IV  | V  | *p-trend* |
| Total Stroke |  |  |  |  |  |  |
| Person-Years | 6208 | 5792 | 6026 | 6132 | 6589 |  |
| No. of cases | 85 | 80 | 94 | 80 | 91 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 1.00 | (0.74-1.36) | 1.11 | (0.83-1.50) | 0.91 | (0.67-1.24) | 0.98 | (0.73-1.32) | 0.68 |
| Additionally adjusted model\* | 1.0 (ref) | 0.96 | (0.70-1.31) | 1.10 | (0.81-1.49) | 0.88 | (0.64-1.20) | 0.97 | (0.71-1.31) | 0.66 |
| Ischemic Stroke |  |  |  |  |  |  |
| No. of cases | 69 | 63 | 70 | 62 | 73 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 0.97 | (0.69-1.37) | 1.03 | (0.74-1.43) | 0.88 | (0.62-1.24) | 0.97 | (0.70-1.35) | 0.69 |
| Additionally adjusted model\* | 1.0 (ref) | 0.92 | (0.65-1.30) | 1.01 | (0.72-1.43) | 0.84 | (0.59-1.20) | 0.97 | (0.69-1.36) | 0.72 |
| Hemorrhagic Stroke |  |  |  |  |  |  |
| No. of cases | 11 | 10 | 15 | 11 | 12 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 0.97 | (0.41-2.28) | 1.38 | (0.63-3.02) | 0.96 | (0.41-2.22) | 0.99 | (0.44-2.26) | 0.96 |
| Additionally adjusted model\* | 1.0 (ref) | 1.01 | (0.42-2.43) | 1.45 | (0.65-3.27) | 0.94 | (0.39-2.26) | 0.95 | (0.40-2.25) | 0.83 |
| Total CHD |  |  |  |  |  |  |  |
| Person-Years | 6124 | 5756 | 5995 | 5928 | 6406 |  |
| No. of cases | 83 | 80 | 81 | 92 | 90 |  |
| Age-and-sex-adjusted | 1.0 (ref) | 1.08 | (0.79-1.47) | 1.09 | (0.80-1.49) | 1.25 | (0.93-1.68) | 1.14 | (0.85-1.54) | 0.23 |
| Additionally adjusted model\* | 1.0 (ref) | 1.10 | (0.80-1.50) | 1.10 | (0.80-1.52) | 1.21 | (0.88-1.64) | 1.22 | (0.90-1.68) | 0.16 |

\* Additionally adjusts for race, clinic, education, smoking, diabetes, BMI, waist circumference, physical activity, alcohol use, & treated hypertension

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| Table 3. Hazard Ratios (95% CI) for Association of Dietary ALA with Risk of Total and Cause-Specific Mortality among 2,583 US Adults |
| Quintile | I | II | III | IV | V |  *p-trend* |
| Total Mortality |  |   |  |  |  |   |
| Person-Years | 4875 | 4987 | 5096 | 5291 | 5600 |  |
| No. of deaths | 328 | 328 | 301 | 298 | 262 |  |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.93 | (0.80-1.09) | 0.85 | (0.73-0.99) | 0.83 | (0.71-0.97) | 0.70 | (0.59-0.82) | <0.0001 |
| Additionally adjusted model\* | 1.0 (ref) | 0.98 | (0.84-1.15) | 0.88 | (0.75-1.03) | 0.86 | (0.73-1.02) | 0.73 | (0.61-0.88) | <0.0001 |
| Non-CVD Mortality |  |  |  |  |  |  |
| No. of deaths | 225 | 225 | 204 | 186 | 161 |  |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.94 | (0.78-1.13) | 0.84 | (0.69-1.02) | 0.75 | (0.62-0.92) | 0.62 | (0.51-0.77) | <0.0001 |
| Additionally adjusted model\* | 1.0 (ref) | 0.98 | (0.81-1.18) | 0.87 | (0.71-1.05) | 0.79 | (0.65-0.97) | 0.64 | (0.52-0.80) | <0.0001 |
| Total CVD Mortality |  |  |  |  |  |  |
| No. of deaths | 89 | 83 | 76 | 92 | 89 |  |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.86 | (0.64-1.17) | 0.79 | (0.58-1.07) | 0.94 | (0.70-1.26) | 0.88 | (0.65-1.18) | 0.61 |
| Additionally adjusted model\* | 1.0 (ref) | 0.93 | (0.68-1.25) | 0.83 | (0.61-1.14) | 0.97 | (0.72-1.31) | 0.96 | (0.71-1.32) | 0.92 |
| Total CHD Mortality |  |   |   |  |   |  |
| No. of deaths | 61 | 55 | 50 | 62 | 52 |  |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.84 | (0.59-1.22) | 0.77 | (0.53-1.11) | 0.95 | (0.66-1.36) | 0.77 | (0.53-1.13) | 0.35 |
| Additionally adjusted model\* | 1.0 (ref) | 0.89 | (0.62-1.29) | 0.83 | (0.57-1.21) | 0.94 | (0.65-1.36) | 0.85 | (0.58-1.26) | 0.54 |
|  Arrhythmic Death |  |   |   |  |   |  |
| No. of deaths | 30 | 28 | 23 | 34 | 20 |  |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.87 | (0.52-1.45) | 0.72 | (0.42-1.24) | 1.05 | (0.64-1.73) | 0.61 | (0.34-1.08) | 0.26 |
| Additionally adjusted model\* | 1.0 (ref) | 0.93 | (0.55-1.58) | 0.80 | (0.46-1.38) | 1.10 | (0.66-1.84) | 0.68 | (0.38-1.23) | 0.42 |
|  Non-Arrhythmic Death |  |   |   |  |  |  |
| No. of deaths | 31 | 27 | 27 | 28 | 32 |  |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.82 | (0.49-1.38) | 0.81 | (0.49-1.36) | 0.85 | (0.50-1.42) | 0.93 | (0.56-1.54) | 0.85 |
| Additionally adjusted model\* | 1.0 (ref) | 0.86 | (0.51-1.44) | 0.87 | (0.52-1.45) | 0.79 | (0.46-1.36) | 1.02 | (0.61-1.71) | 0.94 |

\* Additionally adjusts for race, clinic, education, smoking, diabetes, BMI, alcohol use & treated hypertension

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| Table 4. Hazard Ratios (95% CI) for Association of Dietary ALA with Risk of Incident Stroke and CHD among 2,583 Adults |
| Quintile | I | II | III | IV | V | *p-trend* |
| Total Stroke |   |  |  |  |  |   |
| Person-Years | 4691 | 4785 | 4891 | 4997 | 5380 |   |
| No. of cases | 70 | 64 | 75 | 81 | 68 |   |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.86 | (0.61-1.21) | 1.00 | (0.72-1.39) | 1.05 | (0.76-1.45) | 0.84 | (0.60-1.17) | 0.69 |
| Additionally adjusted model\* | 1.0 (ref) | 0.89 | (0.64-1.26) | 0.97 | (0.70-1.35) | 1.09 | (0.78-1.51) | 0.86 | (0.60-1.21) | 0.80 |
| Ischemic Stroke |   |  |  |  |   |   |
| No. of cases | 59 | 52 | 54 | 67 | 46 |   |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.85 | (0.59-1.23) | 0.85 | (0.59-1.24) | 1.03 | (0.72-1.47) | 0.67 | (0.45-1.01) | 0.19 |
| Additionally adjusted model\* | 1.0 (ref) | 0.89 | (0.61-1.30) | 0.84 | (0.58-1.22) | 1.08 | (0.75-1.54) | 0.70 | (0.47-1.04) | 0.29 |
| Hemorrhagic Stroke |   |  |   |  |   |   |
| No. of cases |   |   |   |   |   |   |
| Age-sex-and-energy adjusted | 1.0 (ref) | 1.26 | (0.44-3.63) | 2.36 | (0.91-6.08) | 1.57 | (0.57-4.36) | 2.29 | (0.88-5.99) | 0.09 |
| Additionally adjusted model\* | 1.0 (ref) | 1.19 | (0.41-3.44) | 2.12 | (0.81-5.54) | 1.52 | (0.54-4.24) | 1.96 | (0.73-5.27) | 0.16 |
| Total CHD |   |  |   |  |   |   |
| Person-Years | 4631 | 4740 | 4815 | 4960 | 5321 |  |
| No. of cases | 77 | 71 | 67 | 92 | 71 |   |
| Age-sex-and-energy adjusted | 1.0 (ref) | 0.92 | (0.67-1.27) | 0.87 | (0.63-1.21) | 1.25 | (0.92-1.70) | 0.92 | (0.66-1.28) | 0.67 |
| Additionally adjusted model\* | 1.0 (ref) | 0.97 | (0.70-1.34) | 0.88 | (0.63-1.23) | 1.25 | (0.91-1.70) | 0.93 | (0.67-1.30) | 0.75 |

\* Additionally adjusts for race, clinic, education, smoking, diabetes, BMI, alcohol use & treated hypertension