Supplemental Material

Supplemental Methods

Study Measures

Fatty acids were measured in 3941 of the 5565 living study participants who had blood samples collected at the 1992–1993 study visit, which we considered the baseline for this analysis. After a 12-hour fast, blood was drawn and stored at -70 °C, and shipped on dry ice for long-term storage at -80 °C. The remaining 1624 participants either did not originally provide blood samples or had insufficient stored blood available. Fatty acid was measured in 2 periods: 500 measurements were taken in 1994–1996 as part of a prior nested case– control study of incident MI [1], and 3441 measurements were taken in 2007–2010. All measurements were done by the Fred Hutchinson Cancer Research Center Biomarker Laboratory using identical techniques (see next section). We confirmed minimal laboratory drift by taking repeated measurements in 1994–1996 and 2007 on the same 1992–1993 blood samples in a subset of 163 participants. Intra-class correlations were good to excellent: 0.88 for LA, 0.61 for GLA, and 0.85 for DGLA, and 0.84 for AA. For the present analysis, we excluded 1149 participants with prevalent CVD (MI, angina, coronary revascularization, stroke, transient ischemic attack, or heart failure), resulting in 2792 participants included in this analysis.

Other risk factors including laboratory data, anthropometric measures, and resting blood pressure were assessed using standardized procedures.[2-4] Alcohol use and physical activity were assessed using validated questionnaires.[5, 6] A validated semiquantitative, picture-sort food frequency questionnaire was used to assess dietary habits 3 years earlier (1989-1990).[7]

Fatty Acid Measurements

Plasma phospholipid fatty acids were measured using previously described methods [8]. Total lipids were extracted from plasma using the Folch method, and phospholipids were separated from neutral lipids by 1-dimensional thin-layer chromatography. Fatty acid methyl esters were prepared by direct transesterification which were analyzed with gas chromatography to quantify 45 distinct fatty acid peaks. Identification, precision, and accuracy were continuously evaluated using both model mixtures of known fatty acid methyl esters and established in-house control pools, with identification

confirmed by gas chromatography– mass spectrometry at the U.S. Department of Agriculture in Peoria, Illinois. These assays had excellent precision as confirmed by the low inter-assay coefficient of variation, 0.6%, 0.8%, 6%, and 0.7% for LA, AA, GLA, and DGLA, respectively.

Similar to other physiologic measures such as blood pressure and cholesterol levels, circulating fatty acid concentrations within an individual are expected to fluctuate overtime, which reflect the combined effect of dietary changes, biologic variations, and measurement error. To evaluate the potential for such fluctuation over time, we assessed serial plasma phospholipid fatty acid measurements in a subset of 100 subjects in the CHS, using blood samples drawn in 2005-2006, 13 years after the study baseline. Within person correlations for n-6 PUFA from baseline to 13 years were 0.49 for LA, 0.34 for GLA, 0.51 for DGLA, and 0.60 for AA.

Supplement Table 1: Concentrations and Unadjusted Spearman Correlation Coefficients for Plasma Phospholipid n-6 Polyunsaturated Fatty Acids among 2792 US Adults*

	LA, 18:2n-6	GLA, 18:3n-6	DGLA, 20:3n-6	AA, 20:4n-6
Percent of total fatty acids, mean±SD†	19.7 ± 2.5	0.09 ± 0.05	3.1 ± 0.7	11.1 ± 2.0
Linoleic acid (LA), 18:2n-6	1.00			
γ-linolenic acid (GLA), 18:3n-6	-0.33	1.00		
Di-homo-γ-linolenic acid (DGLA), 20:3n-6	-0.20	0.36	1.00	
Arachidonic acid (AA), 20:4n-6	-0.65	0.17	-0.14	1.00

*All correlations were significant (P<0.001).

† Values represent percent of plasma phospholipid fatty acids.

Supplement Table 2. Baseline Characteristics According to Plasma Phospholipid Omega-6 Polyunsaturated Fatty Acids

	Quintiles of Linoleic acid (LA)				
	Q1	Q2	Q3	Q4	Q5
Mean, % of total fatty acids	16.2 ± 1.2	18.4 ± 0.4	19.7 ± 0.4	21.0 ± 0.4	23.2 ± 1.3
Median, % of total fatty acids (range)	16.6 (11.4, 17.7)	18.4 (17.7, 19.1)	19.7 (19.1, 20.3)	21.0 (20.3, 21.8)	22.9 (21.8, 28.9)
n	559	558	559	558	558
Age, years	73.3 ± 4.9	73.9 ± 4.9	74.2 ± 5.1	74.3 ± 5.3	74.2 ± 5.3*
Sex, % male	28	30	36	41	46*
Race, % white	72	87	91	92	96*
Education, > high school, %	44	46	48	45	48
Income, >$25000/year	38	42	45	44	48*
Current smoking, %	11	9	9	9	9
Diabetes mellitus, %	15	13	14	11	11*
Treated hypertension, %	48	35	44	37	33*
Lipid-lowering medication, %	9	6	5	4	3*
Physical activity, mcal/day	1.4 ± 1.7	1.4 ± 1.7	1.4 ± 1.7	1.6 ± 1.8	1.6 ± 1.8*
Body mass index, kg/m^2	27.7 ± 4.8	27.4 ± 5.0	26.4 ± 4.3	26.3 ± 4.2	25.6 ± 4.3*
Waist circumference, cm	99.0 ± 13.7	98.3 ± 13.8	96.4 ± 12.6	95.6 ± 12.7	94.6 ± 13.0*
Alcohol, drinks/week	2.7 ± 11.1	2.3 ± 5.2	1.8 ± 4.2	2.2 ± 5.2	1.7 ± 4.1
Fruit, servings/day	2.3 ± 1.1	2.1 ± 1.0	2.1 ± 1.0	2.0 ± 1.0	2.1 ± 1.0*
Vegetables, servings/day	2.7 ± 1.4	2.4 ± 1.2	2.5 ± 1.2	2.5 ± 1.2	2.5 ± 1.3
Dietary fiber consumption, grams/day	31 ± 12	29 ± 12	29 ± 12	29 ± 12	31 ± 12
Use vegetable oil or margarine in cooking, %	66	66	69	66	72*
Total carbohydrate intake, % energy	52.5 ± 7.5	52.7 ± 7.6	51.6 ± 7.6	51.8 ± 7.7	52.5 ± 7.6
Total energy, mcal/day	2.1 ± 0.6	2.0 ± 0.6	2.0 ± 0.6	2.0 ± 0.6	2.1 ± 0.7

Values are mean ± SD, unless otherwise indicated.

*P for trend < 0.05 across quintiles in linear regression for continuous variables and logistic regression for binary variables.

	Quintiles of Arachidonic acid (AA)				
	Q1	Q2	Q3	Q4	Q5
Mean, % of total fatty acids	8.4 ± 0.9	10.1 ± 0.3	11.1 ± 0.3	12.1 ± 0.3	13.9 ± 1.0
Median, % of total fatty acids (range)	8.6 (5.0, 9.5)	10.1 (9.5, 10.6)	11.1 (10.6, 11.6)	12.1 (11.6, 12.7)	13.6 (12.8, 18.9)
n	561	556	560	557	558
Age, years	74.4 ± 5.2	74.1 ± 5.1	74.1 ± 5.2	74.3 ± 5.1	73.0 ± 4.8*
Sex, % male	41	41	37	32	29*
Race, % white	99	95	91	85	69*
Education, > high school, %	51	50	47	43	40*
Income, >$25000/year	47	47	43	40	39*
Current smoking, %	8	11	10	9	9
Diabetes mellitus, %	10	9	13	15	17*
Treated hypertension, %	38	31	38	43	47*
Lipid-lowering medication, %	3	4	3	6	12*
Physical activity, mcal/day	1.5 ± 1.7	1.6 ± 1.9	1.5 ± 1.6	1.4 ± 1.7	1.5 ± 1.8
Body mass index, kg/m^2	25.9 ± 4.4	26.5 ± 4.4	26.5 ± 4.4	26.9 ± 4.9	27.6 ± 4.7*
Waist circumference, cm	95.3 ± 13.1	96.6 ± 13.3	96.5 ± 12.8	97.4 ± 13.7	98.2 ± 13.3*
Alcohol, drinks/week	2.1 ± 4.5	2.2 ± 5.4	1.9 ± 4.6	2.1 ± 5.1	2.5 ± 10.7
Fruit, servings/day	2.1 ± 1.0	2.2 ± 1.0	2.1 ± 1.1	2.1 ± 1.0	2.1 ± 1.0
Vegetables, servings/day	2.5 ± 1.3	2.5 ± 1.2	2.5 ± 1.3	2.5 ± 1.2	2.6 ± 1.2
Dietary fiber consumption, grams/day	30 ± 12	30 ± 12	29 ± 12	30 ± 12	29 ± 12
Use vegetable oil or margarine in cooking, %	66	69	67	69	68
Total carbohydrate intake, % energy	52.5 ±7.2	52.3 ±7.8	52.3 ±7.8	51.6 ±7.9	52.4 ±7.2
Total energy, mcal/day	2.0 ± 0.6	2.1 ± 0.6	2.0 ± 0.6	2.1 ± 0.6	2.0 ±0.6

Values are mean ± SD, unless otherwise indicated.

*P for trend < 0.05 across quintiles in linear regression for continuous variables and logistic regression for binary variables.

	Quintiles of Gamma-linoleic acid (GLA)				
	Q1	Q2	Q3	Q4	Q5
Mean, % of total fatty acids	0.04 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.16 ± 0.01
Median, % of total fatty acids (range)	0.05 (0.01, 0.06)	0.06 (0.06, 0.07)	0.08 (0.07, 0.09)	0.10 (0.09, 0.12)	0.14 (0.12, 1.51)
n	575	565	545	558	549
Age, years	74.7 ± 5.3	74.2 ± 5.5	74.0 ± 5.0	73.5 ± 5.0	73.3 ± 4.5*
Sex, % male	46	42	33	30	29*
Race, % white	83	87	87	89	93*
Education, > high school, %	47	48	45	46	44
Income, >$25000/year	45	46	42	45	39
Current smoking, %	9	8	10	11	10
Diabetes mellitus, %	10	14	12	13	15
Treated hypertension, %	34	39	41	43	40*
Lipid-lowering medication, %	3	6	5	6	7*
Physical activity, mcal/day	1.5 ± 1.9	1.6 ± 1.6	1.3 ± 1.5	1.5 ± 1.8	1.5 ± 1.7
Body mass index, kg/m^2	25.8 ± 4.6	26.1 ± 4.4	26.9 ± 4.6	27.2 ± 4.6	27.4 ± 4.6*
Waist circumference, cm	94.3 ± 13.6	95.4 ± 12.9	97.5 ± 13.4	97.9 ± 12.9	98.9 ± 13.0*
Alcohol, drinks/week	1.6 ± 3.7	2.1 ± 10	2.0 ± 4.5	2.3 ± 5.2	2.7 ± 6.7*
Fruit, servings/day	2.1 ± 1.0	2.1 ± 1.0	2.2 ± 1.0	2.1 ± 1.0	2.2 ± 1.1
Vegetables, servings/day	2.5 ± 1.2	2.5 ± 1.3	2.5 ± 1.2	2.5 ± 1.3	2.6 ± 1.3
Dietary fiber consumption, grams/day	30 ± 12	30 ± 12	30 ± 12	30 ± 12	30 ± 12
Use vegetable oil or margarine in cooking, %	63	72	68	68	67
Total carbohydrate intake, % energy	51.8 ± 6.8	52.7 ± 7.8	52.3 ± 7.6	51.9 ± 7.9	52.5 ± 7.7
Total energy, mcal/day	2.1 ± 0.6	2.0 ± 0.6	2.0 ± 0.6	2.0 ± 0.6	2.0 ± 0.6*

Values are mean ± SD, unless otherwise indicated.

*P for trend < 0.05 across quintiles in linear regression for continuous variables and logistic regression for binary variables.

	Quintiles of di-Homo-Gamma-linoleic acid (DGLA)				
	Q1	Q2	Q3	Q4	Q5
Mean, % of total fatty acids	2.2 ± 0.2	2.7 ± 0.1	3.0 ± 0.1	3.4 ± 0.1	4.2 ± 0.5
Median, % of total fatty acids (range)	2.2 (0.9, 2.5)	2.7 (2.5, 2.9)	3.0 (2.9, 3.2)	3.4 (3.2, 3.6)	4.0 (3.6, 6.8)
n	559	558	561	556	558
Age, years	74.7 ± 5.8	74.4 ± 5.2	73.8 ± 4.8	73.4 ± 4.9	73.5 ± 4.7*
Sex, % male	42	41	37	32	29*
Race, % white	81	84	89	90	95*
Education, > high school, %	53	45	45	44	44*
Income, >$25000/year	51	43	43	37	42*
Current smoking, %	9	11	10	9	8
Diabetes mellitus, %	9	13	13	12	16*
Treated hypertension, %	34	37	38	43	45*
Lipid-lowering medication, %	7	5	5	5	6
Physical activity, mcal/day	1.7 ± 1.9	1.6 ± 1.8	1.5 ± 1.7	1.3 ± 1.5	1.4 ± 1.7*
Body mass index, kg/m^2	24.7 ± 4.1	26.0 ± 4.2	26.8 ± 4.3	27.9 ± 4.8	27.9 ± 4.6*
Waist circumference, cm	91.2 ± 12.7	95.0 ± 12.5	97.4 ± 12.6	99.8 ± 13.3	100 ± 13.0*
Alcohol, drinks/week	2.8 ± 5.7	2.5 ± 11	2.1 ± 5.2	1.7 ± 4.2	1.6 ± 4.5*
Fruit, servings/day	2.1 ± 1.0	2.1 ± 1.0	2.1 ± 1.0	2.2 ± 1.1	2.2 ± 1.1*
Vegetables, servings/day	2.6 ± 1.2	2.4 ± 1.2	2.5 ± 1.2	2.5 ± 1.2	2.6 ± 1.4
Dietary fiber consumption, grams/day	30 ± 12	29 ± 12	30 ± 12	29 ± 12	31 ± 13
Use vegetable oil or margarine in cooking, %	66	64	67	69	72*
Total carbohydrate intake, % energy	52.2 ± 7.3	51.8 ± 7.6	52.2 ± 7.5	52.2 ± 7.8	52.7 ± 7.7
Total energy, mcal/day	2.1 ± 0.6	2.0 ± 0.6	2.0 ± 0.6	2.0 ± 0.6	2.0 ± 0.6

Values are mean ± SD, unless otherwise indicated.

*P for trend < 0.05 across quintiles in linear regression for continuous variables and logistic regression for binary variables.

Supplement Table 3. Relative Risk of Non-Cardiovascular Mortality According to Plasma Phospholipid n-6 Polyunsaturated Fatty Acids among 2792 US Adults

	Multivariable hazard ratio (95% CI), by quintiles of plasma phospholipid fatty acid levels*					
	1	2	3	4	5	P_{trend}†
Cancer (411 cases)						
LA	1.0 (Reference)	1.20 (0.88, 1.64)	1.06 (0.78, 1.46)	0.85 (0.60, 1.19)	0.84 (0.59, 1.19)	0.08
AA	1.0 (Reference)	0.95 (0.70, 1.29)	1.17 (0.87, 1.57)	1.05 (0.77, 1.43)	0.84 (0.60, 1.17)	0.54
GLA	1.0 (Reference)	1.07 (0.79, 1.44)	1.00 (0.73, 1.37)	0.92 (0.67, 1.27)	1.02 (0.74, 1.40)	0.84
DGLA	1.0 (Reference)	1.21 (0.88, 1.67)	1.23 (0.89, 1.69)	1.11 (0.79, 1.55)	1.19 (0.85, 1.67)	0.54
Dementia (328 cases)						
LA	1.0 (Reference)	1.23 (0.85, 1.79)	1.21 (0.85, 1.74)	0.92 (0.62, 1.36)	1.03 (0.69, 1.53)	0.63
AA	1.0 (Reference)	0.75 (0.53, 1.04)	0.91 (0.66, 1.27)	0.77 (0.54, 1.08)	0.66 (0.45, 0.95)	0.05
GLA	1.0 (Reference)	0.91 (0.64, 1.28)	1.09 (0.78, 1.51)	0.83 (0.58, 1.20)	1.10 (0.77, 1.57)	0.67
DGLA	1.0 (Reference)	0.97 (0.69, 1.36)	0.97 (0.69, 1.37)	0.99 (0.69, 1.41)	0.83 (0.57, 1.21)	0.39
Infection (156 cases)‡						
LA	1.0 (Reference)	1.33 (0.80, 2.23)	0.83 (0.47, 1.45)	1.11 (0.65, 1.89)	1.12 (0.64, 1.95)	0.91
AA	1.0 (Reference)	1.05 (0.64, 1.73)	1.08 (0.66, 1.77)	0.86 (0.51, 1.44)	0.81 (0.47, 1.39)	0.35
GLA	1.0 (Reference)	1.05 (0.65, 1.69)	0.83 (0.49, 1.38)	0.86 (0.52, 1.43)	0.88 (0.52, 1.48)	0.49
DGLA	1.0 (Reference)	0.96 (0.58, 1.58)	0.82 (0.49, 1.39)	1.11 (0.67, 1.83)	0.70 (0.40, 1.23)	0.34
Respiratory (116 cases)§						
LA	1.0 (Reference)	1.30 (0.72, 2.37)	0.93 (0.50, 1.74)	0.96 (0.52, 1.79)	0.42 (0.20, 0.87)	0.008
AA	1.0 (Reference)	1.24 (0.69, 2.24)	1.25 (0.68, 2.30)	1.54 (0.85, 2.78)	1.34 (0.71, 2.53)	0.26
GLA	1.0 (Reference)	1.66 (0.96, 2.87)	0.98 (0.53, 1.81)	1.14 (0.62, 2.09)	0.75 (0.38, 1.48)	0.14
DGLA	1.0 (Reference)	0.80 (0.44, 1.43)	0.97 (0.55, 1.71)	0.78 (0.43, 1.43)	0.81 (0.44, 1.48)	0.53
Trauma/fracture (107 cases)						
LA	1.0 (Reference)	1.41 (0.72, 2.78)	1.07 (0.53, 2.15)	1.33 (0.67, 2.61)	1.06 (0.52, 2.17)	0.94
AA	1.0 (Reference)	0.84 (0.48, 1.48)	0.56 (0.29, 1.10)	1.06 (0.60, 1.88)	1.03 (0.57, 1.88)	0.82

GLA	1.0 (Reference)	0.90 (0.49, 1.67)	0.70 (0.36, 1.37)	1.22 (0.68, 2.21)	1.40 (0.78, 2.54)	0.11
DGLA	1.0 (Reference)	1.52 (0.86, 2.70)	1.23 (0.68, 2.25)	0.78 (0.39, 1.56)	0.89 (0.45, 1.75)	0.26
Other (196 cases)‖						
LA	1.0 (Reference)	1.21 (0.75, 1.95)	1.27 (0.80, 2.01)	1.15 (0.71, 1.86)	1.04 (0.62, 1.74)	0.99
AA	1.0 (Reference)	0.80 (0.51, 1.26)	0.90 (0.57, 1.40)	1.09 (0.71, 1.67)	0.69 (0.42, 1.12)	0.37
GLA	1.0 (Reference)	1.25 (0.81, 1.90)	1.18 (0.76, 1.82)	0.90 (0.57, 1.44)	0.75 (0.45, 1.25)	0.09
DGLA	1.0 (Reference)	1.57 (1.02, 2.43)	0.75 (0.45, 1.26)	1.13 (0.69, 1.84)	1.32 (0.82, 2.12)	0.67

LA, linoleic acid, AA, arachidonic acid, GLA, γ-linolenic acid, DGLA, di-homo-γ-linolenic acid.

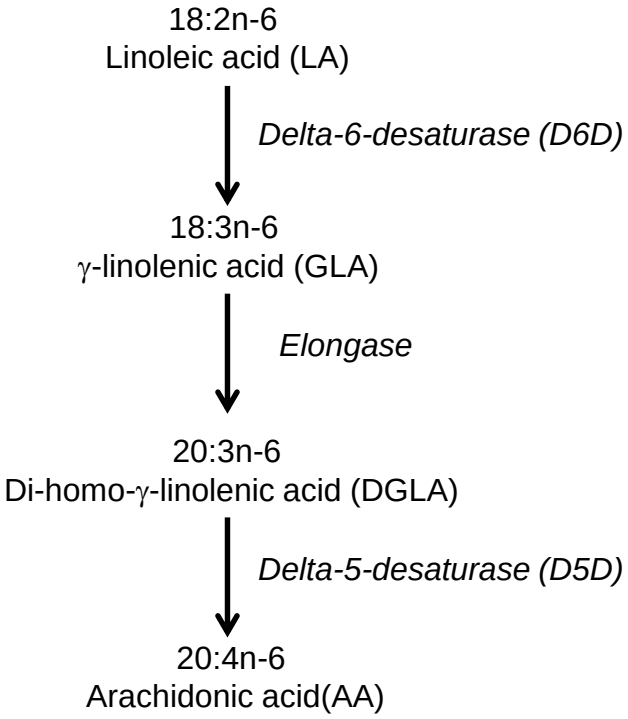
*See Table 1 for median fatty acid levels in each quintile. Adjusted for age (years), gender (male or female), race (white or non white), enrollment site (4 sites), education (<high school, high school, some college, or college graduate), smoking status (never, former, or current), prevalent diabetes (yes or no), atrial fibrillation (yes or no), and hypertension (yes or no), leisure-time physical activity (mcal/wk), body mass index (kg/m^2), waist circumference (cm), alcohol use (6 categories), and plasma phospholipid long-chain n-3 PUFA (sum of EPA+DPA+DHA, % of total fatty acids).

† Linear trend was tested by assigning to participants the median value in each quartile and assessing this as a continuous variable. Findings were similar when fatty acid concentrations were evaluated in their natural units as continuous exposures.

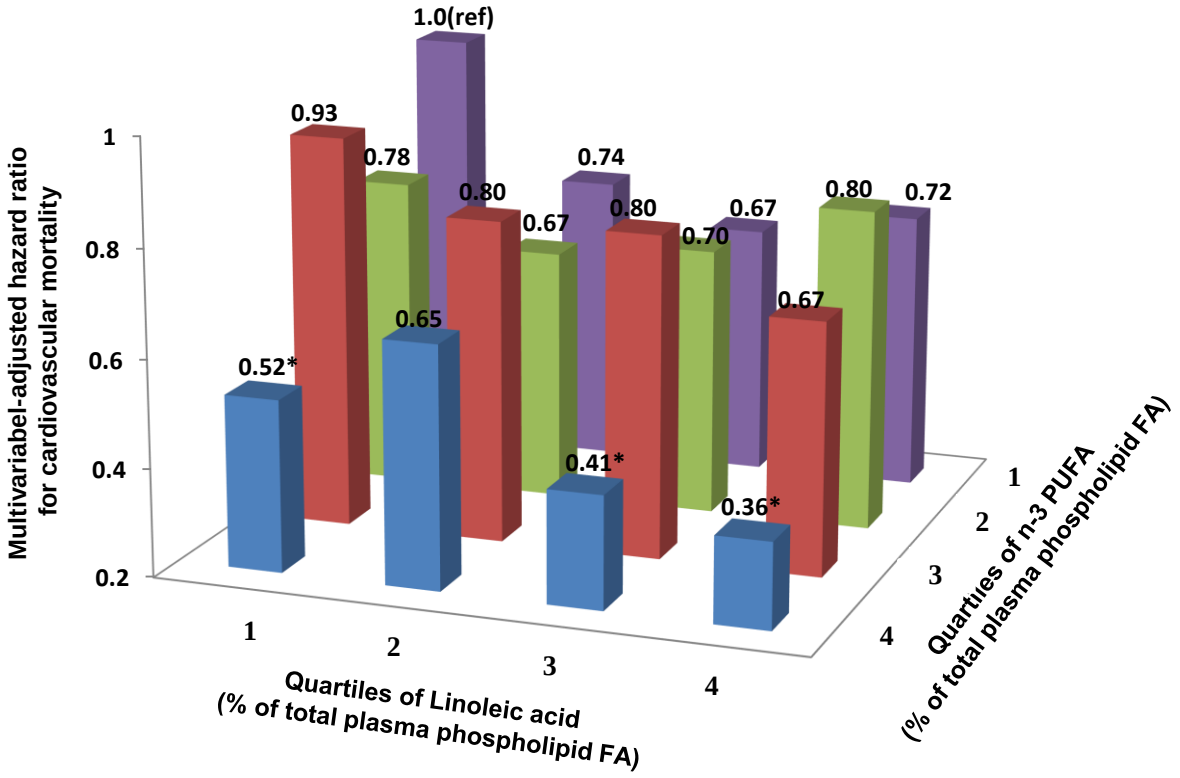
‡ Includes deaths due to pneumonia, sepsis, and other infection.

§ Includes deaths due to chronic pulmonary diseases.

‖ Includes deaths due to liver disease, gastrointestinal disease, renal failure, amyotrophic lateral sclerosis, Parkinson disease, bladder disease, metabolic conditions, amyloid,
failure to thrive, the myelodysplastic syndrome, and other musculoskeletal diseases.



Supplement Figure 1



Supplement Figure 2

Supplemental Figure Legends

Supplement Figure 1. Pathways for endogenous metabolism of linoleic acid (LA) to downstream n-6 PUFA, including γ-linolenic acid (GLA), di-homo-γ-linolenic acid (DGLA), and arachidoic acid (AA), by desaturase and elongase enzymes.

Supplement Figure 2. Multivariable hazard ratios for CVD mortality by joint levels of plasma phospholipid linoleic acid and long-chain n-3 PUFA, adjusted for age, gender, race, enrollment site, education, smoking status, prevalent diabetes, atrial fibrillation, and hypertension, leisure-time physical activity, body mass index, waist circumference, and alcohol use, *P<0.05 compared with the referent category. Associations appeared independent, with little evidence for significant interaction between linoleic acid and long-chain n-3 PUFA (Wald test for multiplicative interaction: P=0.39).

Supplemental References

1. Lemaitre RN, King IB, Mozaffarian D, Kuller LH, Tracy RP, Siscovick DS. N-3 polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: The cardiovascular health study. *The American journal of clinical nutrition*. 2003;77:319-325
2. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the cardiovascular health study. *Clin Chem*. 1995;41:264-270
3. Psaty BM, Kuller LH, Bild D, Burke GL, Kittner SJ, Mittelmark M, Price TR, Rautaharju PM, Robbins J. Methods of assessing prevalent cardiovascular disease in the cardiovascular health study. *Annals of epidemiology*. 1995;5:270-277
4. Tell GS, Fried LP, Hermanson B, Manolio TA, Newman AB, Borhani NO. Recruitment of adults 65 years and older as participants in the cardiovascular health study. *Annals of epidemiology*. 1993;3:358-366
5. Mukamal KJ, Chung H, Jenny NS, Kuller LH, Longstreth WT, Jr., Mittleman MA, Burke GL, Cushman M, Psaty BM, Siscovick DS. Alcohol consumption and risk of coronary heart disease in older adults: The cardiovascular health study. *J Am Geriatr Soc*. 2006;54:30-37
6. Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between physical activity and markers of inflammation in a healthy elderly population. *Am J Epidemiol*. 2001;153:242-250
7. Kumanyika SK, Tell GS, Shemanski L, Martel J, Chinchilli VM. Dietary assessment using a picture-sort approach. *The American journal of clinical nutrition*. 1997;65:1123S-1129S
8. Wu JH, Lemaitre RN, King IB, Song X, Sacks FM, Rimm EB, Heckbert SR, Siscovick DS, Mozaffarian D. Association of plasma phospholipid long-chain omega-3 fatty acids with incident atrial fibrillation in older adults: The cardiovascular health study. *Circulation*. 2012;125:1084-1093