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Biomarkers of Dietary Omega-6 Fatty Acids and Incident Cardiovascular Disease and Mortality: An Individual-Level Pooled Analysis of 30 Cohort Studies

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Abstract

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METHODS: We performed harmonized, de novo, individual-level analyses in a global consortium of 30 prospective observational studies from 13 countries. Multivariable-adjusted associations of circulating and adipose tissue LA and AA biomarkers with incident total CVD and subtypes (coronary heart disease, ischemic stroke, cardiovascular mortality) were investigated according to a prespecified analytic plan. Levels of LA and AA, measured as the percentage of total fatty acids, were evaluated linearly according to their interquintile range (ie, the range between the midpoint of the first and fifth quintiles), and categorically by quintiles. Study-specific results were pooled using inverse-variance–weighted meta-analysis. Heterogeneity was explored by age, sex, race, diabetes mellitus, statin use, aspirin use, omega-3 levels, and fatty acid desaturase 1 genotype (when available).

RESULTS: In 30 prospective studies with medians of follow-up ranging 2.5 to 31.9 years, 15 198 incident cardiovascular events occurred among 68 659 participants. Higher levels of LA were significantly associated with lower risks of total CVD, cardiovascular mortality, and ischemic stroke, with hazard ratios per interquintile range of 0.93 (95% CI, 0.88–0.99), 0.78 (0.70–0.85), and 0.88 (0.79–0.98), respectively, and nonsignificantly with lower coronary heart disease risk (0.94; 0.88–1.00). Relationships were similar for LA evaluated across quintiles. AA levels were not associated with higher risk of cardiovascular outcomes; in a comparison of extreme quintiles, higher levels were associated with lower risk of total CVD (0.92; 0.86–0.99). No consistent heterogeneity by population subgroups was identified in the observed relationships.

CONCLUSIONS: In pooled global analyses, higher in vivo circulating and tissue levels of LA and possibly AA were associated with lower risk of major cardiovascular events. These results support a favorable role for LA in CVD prevention.

Keywords

arachidonic acid, biomarkers, cardiovascular diseases, diet, epidemiology, linoleic acid, primary prevention

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Editorial, see p 2437

Matti Marklund, PhD
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CONCLUSIONS: In pooled global analyses, higher in vivo circulating and tissue levels of LA and possibly AA were associated with lower risk of major cardiovascular events. These results support a favorable role for LA in CVD prevention.

*Drs Lemaitre, Mozaffarian, and Risérus contributed equally to this work (see page 2433).

Full author list is available on page 2433

Key Words: arachidonic acid ■ biomarkers ■ cardiovascular diseases ■ diet ■ epidemiology ■ linoleic acid ■ primary prevention

Sources of Funding, see page 2434

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Clinical Perspective

What Is New?

- We conducted the hitherto largest pooled individual-level analysis using circulating and adipose tissue levels of linoleic acid and arachidonic acid to examine the link between omega-6 fatty acids and cardiovascular outcomes in various populations.
- Our approach increases statistical power and generalizability in comparison with individual studies; lowers the risk of publication bias and heterogeneity in comparison with meta-analyses of existing literature; and allows evaluation of the associations in key population subgroups.
- It is striking that a higher level of linoleic acid was associated with lower risks of total cardiovascular disease, ischemic stroke, and cardiovascular mortality, whereas arachidonic acid was not associated with cardiovascular risk.

What Are the Clinical Implications?

- Our findings support the potential benefits of the main dietary omega-6 fatty acid (ie, linoleic acid) for cardiovascular disease prevention.
- Furthermore, our results do not support any theorized cardiovascular harms of omega-6 fatty acids.
- Our findings provide evidence to help inform currently inconsistent global dietary recommendations on omega-6 consumption.

Recommendations for dietary consumption of omega-6 (n-6) polyunsaturated fatty acids (PUFA) for cardiovascular disease (CVD) prevention remain controversial and inconsistent.[1] For example, the American Heart Association and the Academy of Nutrition and Dietetics recommend 5% to 10%,[1,2] the United Nations Food and Agriculture Organization recommends 2.5% to 9%,[3] whereas the French national guidelines recommend 4%.[4] Pooled evidence from clinical trials and cohort studies suggests a moderate benefit of consuming n-6 PUFA, predominantly linoleic acid (LA, 18:2n-6), for coronary heart disease (CHD) risk, whether replacing saturated fat or total carbohydrate.[5–7] In contrast, recent secondary analyses of clinical trials of LA-rich corn oil (although not LA-rich soybean oil) conducted in the 1960s to 1970s suggest a possible increased risk of overall and CHD mortality.[8,9] The interpretation of these latter trials is hampered by their short duration,[8,9] small numbers of events,[8] substantial dropout,[9] and confounding by industrial trans fats.[8,9] In addition, many of the other previous trials are limited by lack of blinding or randomization and by major dietary pattern shifts; and most are decades old, creating potentially low generalizability to contemporary diets and clinical settings. Cohort studies are limited by the common reliance on self-reported dietary habits, which can be influenced by memory errors and inaccurate nutrient databases. Thus, for many scientists, clinicians, and policy makers, the role of LA in CVD risk remains uncertain.

In addition, concerns have been raised that n-6 PUFA could actually increase CVD risk because of potential proinflammatory effects.[9,10] LA is a precursor of the n-6 PUFA arachidonic acid (AA, 20:4n-6), which gives rise to a range of eicosanoids considered to be proinflammatory and prothrombotic.[10,11] Yet, stable isotope studies suggest very limited conversion of LA to AA in humans,[12] and trials show limited effects of increasing dietary LA on plasma and adipose tissue AA levels.[12–14] These findings indicate the importance of directly evaluating AA levels instead of inferring them from LA levels or intakes in relation to CVD risk. Because LA cannot be produced endogenously (making tissue levels reasonable markers of intake), biomarker (circulating and adipose tissue) levels correlate with dietary consumption.[15,16] Such objective biomarkers allow the evaluation of dietary exposure of LA status independent of self-reported food habits and estimated nutrient composition of different foods. Circulating and adipose biomarkers also allow direct evaluation of AA, which is highly metabolically regulated and for which dietary estimates correlate poorly with in vivo levels.

Yet, the relations between in vivo levels of LA and AA and CHD risk have been evaluated in relatively few studies, with different study designs, outcomes, exposures (eg, lipid compartment), covariates, and statistical methodology. Results from meta-analyses of published studies using circulating or adipose tissue levels of n-6 PUFA have been contradictory.[17,18] Furthermore, associations between in vivo n-6 PUFA levels and other CVD outcomes including stroke, total CVD, and CVD mortality have been studied less frequently[19–23] and remain uncertain.

To address these major gaps in knowledge, we conducted a pooled analysis of harmonized, de novo, individual-level data across 30 cohort studies in FORCE (Fatty Acid and Outcome Research Consortium) to evaluate associations of LA and AA levels with incident total CVD and subtypes (CHD, ischemic stroke, CVD mortality).

METHODS

Data Availability

The institutional review board approvals and data-sharing agreements for the participating cohorts allowed us to share cohort results. Individual participant data are owned by individual participating cohorts and are available to researchers consented from participating cohorts. For further queries or requests, please contact force@tufts.edu. Further details are available at the FORCE website: http://force.nutrition.tufts.edu/.

Study Setting and Population: FORCE Consortium

The study was conducted within FORCE (http://force.nutrition.tufts.edu), a consortium of studies with circulating or adipose tissue fatty acid biomarker measurements and ascertained chronic disease events.[24] Studies were identified and invited to participate if assessing biomarker (circulating or adipose tissue) levels of LA and AA, and incident CVD (or subtypes thereof), based on previous FORCE projects,[24,25] expert contacts, and online searches. Studies with adult participants (≥18 years) free of CVD (myocardial infarction, angina, coronary revascularization, and stroke) at the time of fatty acid sampling were invited. Retrospective case-control studies were included in a sensitivity analysis if fatty acids were assessed in adipose tissue, which have a long half-life of exposure.[26] To minimize potential reverse causation, the main analysis included only prospective studies. Of 38 studies invited by September 2017, 31 participated (Table 1 and Tables I and II in the online-only Data Supplement), whereas 7 were ineligible, declined to participate, or failed to respond (Table III in the online-only Data Supplement). The study was approved by the institutional review boards of the participating cohorts.

Fatty Acid Measurements

Studies measured fatty acids in differing compartments, including plasma phospholipids, erythrocytes, plasma, serum, cholesterol esters, and adipose tissue. All fatty acid levels were reported as the percentage of total fatty acids. Detailed information regarding fatty acid measurements in each study is provided in the online-only Data Supplement.

Outcome Assessment

In each cohort, study participants were excluded if they were children (age <18 years) or had prevalent CVD at the time of fatty acid measurement. Among the remaining participants, we evaluated incident CVD (defined as incident CHD or stroke) and its subtypes, including CHD (fatal or nonfatal myocardial infarction, CHD death, or sudden cardiac death), ischemic stroke (fatal or nonfatal ischemic stroke), and CVD mortality (the subset of fatal events from these causes). Studies that did not separately assess ischemic stroke used total stroke (n=5 studies). Detailed information on outcomes in each study is provided in the online-only Data Supplement.

Covariates

To minimize potential confounding, the prespecified and harmonized covariates that were used included age (years), sex (male/female), race (white/non-white or study-specific), field center if applicable (categories), body mass index (kg/m^2), education (less than high school graduate, high school graduate, some college or vocational school, college graduate), smoking (current, former, never; if history not assessed, then current/not current), physical activity (quintiles of metabolic equivalents per week), alcohol intake (none, 1–6 drinks/wk, 1–2 drinks/d, >2 drinks/d), prevalent diabetes mellitus (defined as treatment with oral antihyperglycemic agents, insulin, or fasting plasma glucose >126 mg/dL), treated hypertension (defined as hypertension drug use; or if unavailable, as diagnosed/history of hypertension), treated hypercholesterolemia (defined as low-density lipoprotein–lowering drug use; if unavailable, as diagnosed/history of hypercholesterolemia), regular aspirin use (defined as ≥2 times/wk), levels of α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), sum of *trans* isomers of oleic acid (*trans*-18:1), and sum of *trans* isomers of LA (*trans*-18:2; each expressed as % total fatty acids). If data did not allow such categorization, study-specific categories were used. Imputation was allowed for linear covariates if previously established in each cohort; missing indicator categories were used for missing covariate data in categories.

Statistical Analysis and Pooling

All participating studies followed a prespecified, harmonized analysis protocol with standardized exclusions, exposures, outcomes, covariates, and analytical methods. In each study, de novo analyses of individual data were performed according to the protocol. Cox and weighted Cox proportional hazards models were used to estimate hazard ratios in cohort and nested unmatched case-cohort studies, respectively, with follow-up from the date of blood or adipose tissue sampling to date of incident event, death, loss to follow-up, or end of follow-up. In matched nested case-control studies, conditional logistic regression was used to estimate odds ratios for each outcome, considered to approximate hazard ratios. To assess potential nonlinear associations, each cohort also evaluated study-specific quintiles as indicator categories, with the lowest quintile as the reference. Studies assessing fatty acids in multiple compartments conducted separate analyses in each compartment. To investigate potential heterogeneity by other factors, associations in each study were also assessed in prespecified strata by age, sex, race, α-linolenic acid and eicosapentaenoic acid levels, prevalent diabetes mellitus, drug-treated hypercholesterolemia, and regular aspirin use. Potential interactions by genotype were examined in the 14 studies with available data for rs174547 (single-nucleotide polymorphism in the gene for fatty acid desaturase 1, a major genetic determinant of circulating LA and AA).[27] Interaction terms were constructed as a cross-product of LA or AA and rs174547 (as an additive effect: 0, 1, or 2 T-alleles) and included with the main effects in the models. Robust variance was used in all analyses.

Results from each study were provided to the lead author in standardized electronic forms and pooled using inverse-variance–weighted meta-analysis. The results were pooled overall and within each specific type of fatty acid compartment including phospholipids (erythrocyte phospholipids or plasma phospholipids), total plasma, cholesterol esters, and adipose tissue. To allow comparison and pooling of results across different compartments, LA and AA concentrations were standardized to study-specific interquintile range defined as the range between the midpoint of the first and fifth quintiles (ie, range between 10th and 90th percentiles). Potential semiparametric associations were assessed by meta-regression with restricted cubic splines constructed from study-specific quintiles.[28]

Overall heterogeneity was assessed by the I^2 statistic, with values of ≈ 25%, 50%, and 75%, considered to

Table 1. Characteristics of 31 Studies and Baseline Characteristics of Individual Study Participants With Linoleic Acid (18:2n6) and Arachidonic Acid (20:4n6) Biomarker Measures and Follow-Up for Cardiovascular Disease Incidence or Mortality

Study*	Country	Study Design	Age, y (Mean)	Sex (% Male)	BMI, kg/m² (Mean)	Biomarker Compartment	Year of Biomarker Sampling	Outcome Assessed
AGES-Reykjavik	Iceland	PC	77	39	27.1	PP	2002–2006	All†
ARIC	USA	PC	54	52	27.0	PP	1987–1989	All
CCCC	Taiwan	PC	61	55	23.3	TP	1992–2000	All
CHS	USA	PC	73	36	26.7	PP	1992–1993	All
CRS	Costa Rica	RCC	58	73	26.2	AT	1994–2004	Nonfatal MI
DCH	Denmark	PNC	57	61	26.6	AT‡	1993–1997	Total CHD
EPIC-Norfolk	UK	PCC	63	49	26.5	PP	1993–1997	All
EPIC-Potsdam	Germany	PC	50	37	26.0	RBC	1994–1998	Total CVD
FHS	USA	PC	66	43	28.2	RBC	2005–2008	All
HPFS	USA	PCC	65	100	25.8	RBC, TP	1993–1995	Total CVD, CHD, and stroke
HS	Japan	PC	61	42	23.1	TP	2002–2003	All
KIHD	Finland	PC	52	100	26.7	TP	1984–1989	All
MCCS	Australia	PC	56	46	27.2	PP	1990–1994	Fatal CVD, CHD, and ischemic stroke
MESA	USA	PC	62	47	28.3	PP	2000–2002	All
METSIM	Finland	PC	55	100	26.5	CE, PP, RBC	2006–2010	Total CVD
MORGEN (*CHD*)	Netherlands	PCC	52	79	26.2	CE	1993–1997	Fatal CHD
MORGEN (*Stroke*)	Netherlands	PCC	50	53	25.9	CE	1993–1997	Ischemic stroke
MPCDRF	Netherlands	PCC	51	70	25.9	CE	1987–1991	Fatal CHD
NHS	USA	PCC	60	0	25.6	RBC, TP	1989–1990	Total CVD, CHD, and stroke
NSHDS I	Sweden	PCC	54	79	26.2	PP	1987–1994	Total CHD
NSHDS II	Sweden	PCC	54	76	26.4	PP	1987–1999	Total CHD
NSHDS III	Sweden	PCC	55	61	26.7	PP	1987–1995	Ischemic stroke
PHS	USA	PCC	69	100	25.7	RBC	1995–2001	Total CHD
PIVUS	Sweden	PC	70	47	26.9	CE, PP	2001–2004	All
SCHS	Singapore	PCC	66	65	23.0	TP	1994–2005	Total CHD
SHHEC	UK	PC	49	52	25.6	AT	1985–1986	All
60YO	Sweden	PC	60	48	26.8	CE	1997–1998	All
3C Study	France	PC	75	39	26.0	TP	1999–2000	All
ULSAM-50§	Sweden	PC	50	100	25.0	CE	1970–1973	All
ULSAM-70§	Sweden	PC	71	100	26.4	AT	1991–1995	All
WHIMS	USA	PC	70	0	28.2	RBC	1996	All

AA indicates arachidonic acid; AT, adipose tissue; BMI, body mass index; CE, cholesterol ester; CHD, coronary heart disease; CVD, cardiovascular disease; LA, linoleic acid; MI, myocardial infarction; PC, prospective cohort; PCC, prospective nested case–control; PNC, prospective nested case–cohort; PP, plasma phospholipid; RBC, erythrocyte phospholipid; RCC, retrospective case–control; and TP, total plasma.

*AGES-Reykjavik indicates Age, gene/environment susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CCCC, Chin-Shan Community Cardiovascular Cohort Study; CHS, Cardiovascular Health Study; CRS, Costa Rica study on adults; DCH, Diet, Cancer, and Health study; EPIC, European Prospective Investigation into Cancer; FHS, Framingham Heart Study; HPFS, Health Professionals Follow-up Study; HS, The Hisayama Study; KIHD, Kuopio Ischemic Heart Disease Risk Factor Study; MCCS, Melbourne Collaborative Cohort Study; MESA, Multi-Ethnic Study of Atherosclerosis; METSIM, Metabolic syndrome in men study; MORGEN, Monitoring Project on Risk Factors for Chronic Diseases; MPCDRF, Monitoring Project on Cardiovascular Disease Risk Factors; NHS I, Nurses' Health Study I; NSHDS I-III, Northern Sweden Health and Disease Study; PHS, Physicians' Health Study; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; SCHS, Singapore Chinese Health Study; SHHEC, Scottish Heart Health Extended Cohort; 60YO, 60-year-old Swedish men and women; 3C Study, Three City Study; and ULSAM-50 &-70, Uppsala Longitudinal Study of Adult Men investigations at ages 50 y and 70 y, respectively.

†All specified outcomes (total CVD, CVD mortality, total CHD, and ischemic stroke) were assessed.

‡In DCH, the association of adipose tissue arachidonic acid, but not linoleic acid, with total CHD was evaluated.

§Fatty acids were measured in cholesterol ester and adipose tissue at the first and third ULSAM investigation, respectively.

indicate low, medium, and high heterogeneity, respectively.[29] Heterogeneity between prespecified subgroups was explored by meta-analyzing study-specific effect estimates from each stratum, with statistical differences between subgroups tested by meta-regression. Potential interactions by desaturase genotype were examined by meta-analyzing

study-specific interaction terms. For each study, associations of n-6 PUFA with CVD per genotype at rs174547 (ie, CC, CT, or TT) were calculated from β-coefficients and the variance-covariance matrix of the main and interaction terms.[24] The genotype-specific estimates were pooled using inverse-variance–weighted meta-analysis. Although subgroups were prespecified, all heterogeneity analyses were considered exploratory and Bonferroni corrected for multiple comparisons (10 subgroups; corrected α=0.005).

In sensitivity analyses, we evaluated compartment-specific associations using the absolute percentage of total fatty acids as the unit of exposure, instead of study-specific interquintile range. In other sensitivity analyses, we censored events at a maximum 10 years of follow-up, to minimize bias by changes in fatty acid levels over time; used alternative blood compartments in the overall pooled analysis for studies having more than one measure; included one retrospective study; and excluded studies assessing only fatal outcomes.

Meta-analyses were performed using Stata 13 (StataCorp), with 2-tailed α=0.05 for the primary analyses.

RESULTS

The pooled analyses included 76 356 fatty acid measurements from 68 659 participants in 30 prospective studies from 13 countries (Table 1). The studies included 18 cohort and 12 nested case-control or case-cohort studies. Most studies assessed fatty acids in blood compartments (plasma phospholipids, n=11 studies; erythrocyte phospholipids, total plasma, or cholesterol esters, n=7 studies each), whereas adipose tissue was less commonly used (n=3 studies). One retrospective case-control study measuring adipose tissue biomarkers was included in a sensitivity analysis, but not in the primary analyses.

Across studies, mean age at baseline ranged from 49 to 77 years (Table 1 and Table IV in the online-only Data Supplement). Overall proportions of women and men were comparable, although some studies included one sex only (Table 1). Most participants were white, but several studies included sizable numbers of blacks, Asians, and Hispanics (Table V in the online-only Data Supplement). In most studies, up to 30% of the participants smoked, and alcohol intake was moderate, in general (<1 drink/d). Education level, diabetes mellitus prevalence, and medication use varied across studies. As would be expected, levels of fatty acids varied between different compartments (Figure 1 and Tables IV and VI in the online-only Data Supplement).

Median study follow-up durations ranged from 2.5 to 31.9 years. Among the 30 prospective studies, 10 477 total incident CVD events, 4508 CVD deaths, 11 857 incident CHD events, and 3705 incident ischemic strokes occurred (Table VII in the online-only Data Supplement).

Per interquintile range, higher LA levels were associated with 7% (95%CI, 1%–12%), 22% (15%–

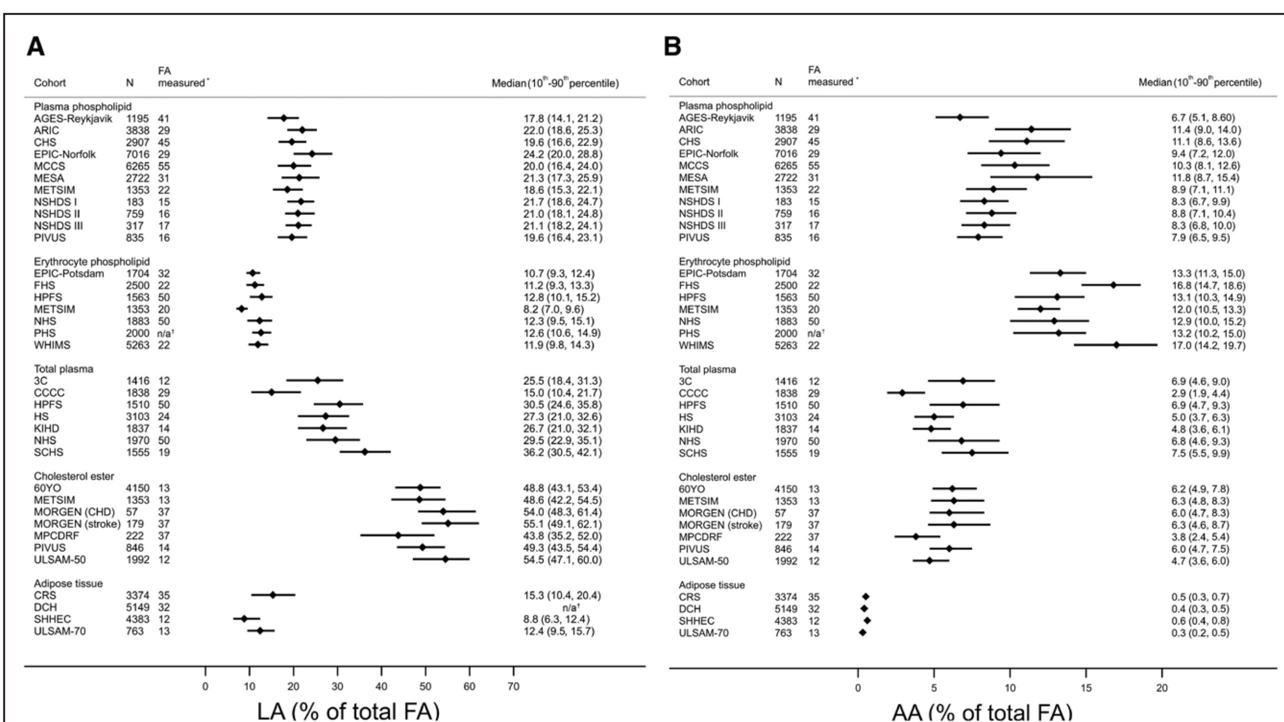

Figure 1. Circulating and tissue levels of linoleic acid (LA; 18:2n6) and arachidonic acid (AA; 20:4n6).

Concentration of linoleic acid (**A**) and arachidonic acid (**B**) across different biomarker compartments measured in the 31 contributing studies. Arachidonic acid and linoleic acid concentrations are expressed as % of total fatty acids (FAs), and indicated as median (circles) and interquintile range (lines; defined as the range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]), respectively. For Monitoring Project on Cardiovascular Disease Risk Factors (MPCDRF) and Monitoring Project on Risk Factors for Chronic Diseases (MORGEN) values are only shown for controls. *Total number of individual FAs measured in the biomarker compartment. †Not reported.

30%), and 12% (2%–21%) lower incidence of total CVD, CVD mortality, and ischemic stroke, respectively (Figures 2 and 3, Table 2). LA levels were also nonsignificantly (*P*=0.065) associated with lower incidence of total CHD. Overall heterogeneity was moderate (I^2=28%–63%). Associations of LA with total CVD, total CHD, and CVD mortality varied by compartment (*P*-interaction≤0.031), with generally less prominent inverse associations in studies using phospholipids (Figures 2 and 3).

In comparison with the lowest quintile, participants in the highest quintile of LA levels experienced lower risk of CVD mortality (hazard ratio [HR], 0.77; 95% CI, 0.69–0.86), with nonsignificant trends toward lower risk of total CVD (0.94; 0.87–1.01), CHD (0.92; 0.85–1.00), and ischemic stroke (0.90; 0.79–1.02; Table VIII in the online-only Data Supplement). There was no significant evidence of nonlinear associations between LA and each outcome (*P*-nonlinearity>0.05 each).

AA levels evaluated linearly were not significantly associated with CVD events, with a hazard ratio of 0.95 (0.90–1.01) for total CVD (Table 2, Figures 4 and 5). When different lipid compartments were assessed, AA levels in total plasma, but not other compartments, were associated with lower risk of total CVD (HR, 0.81; 95% CI, 0.70–0.94; Table 2, Figure 4). Overall heterogeneity was low to moderate (I^2≤54%). When AA levels were evaluated in quintiles (Table IX in the online-only Data

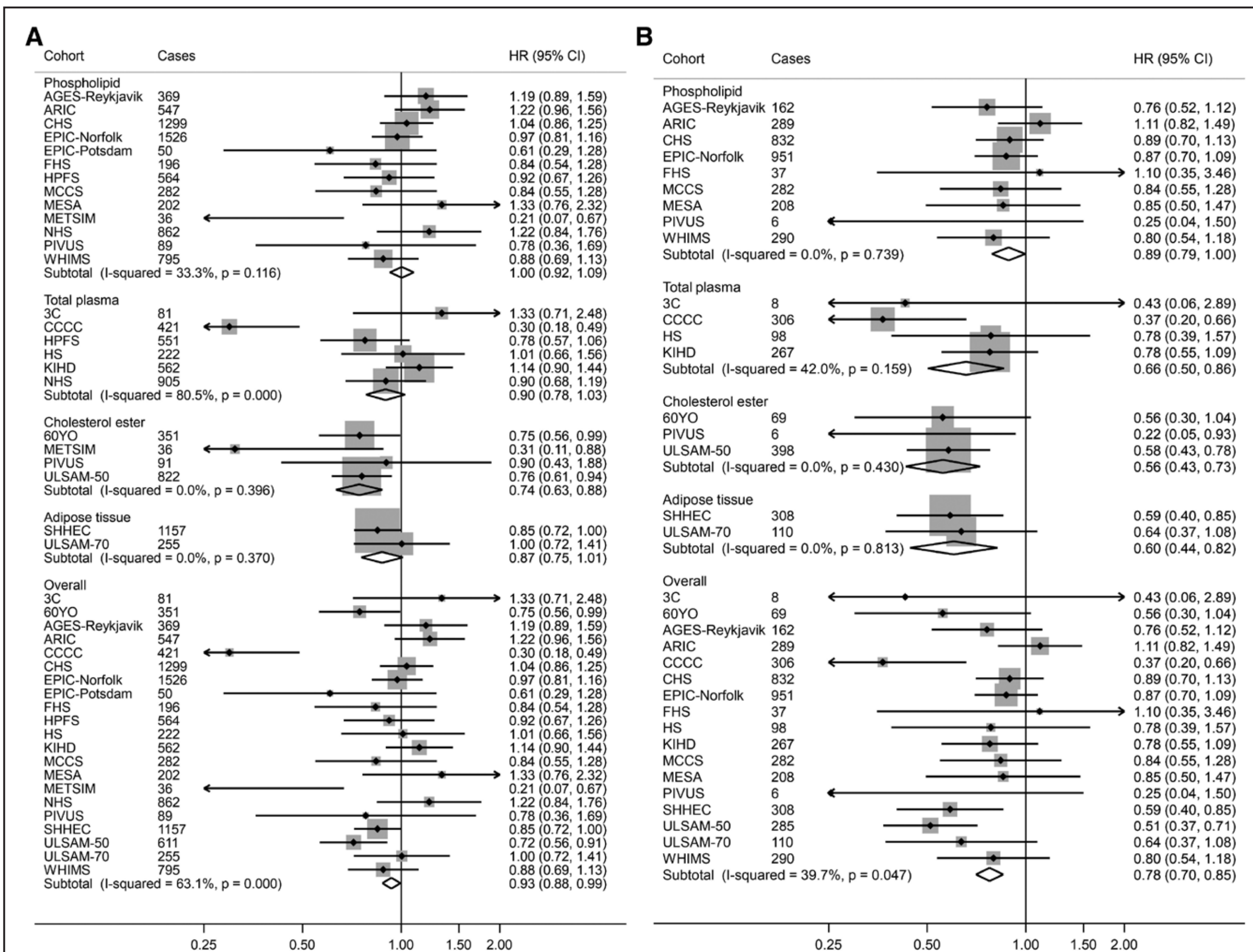

Figure 2. Circulating and tissue linoleic acid (18:2n6) and cardiovascular disease incidence and mortality.
Associations of linoleic acid with total cardiovascular disease (**A**) and cardiovascular disease mortality (**B**) in pooled analysis of 30 prospective studies. Study-specific estimates for hazard ratio (HR) per interquintile range (ie, range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]) of biomarker linoleic acid were pooled based on the following order: (1) adipose tissue, (2) erythrocyte phospholipid, (3) plasma phospholipid, (4) cholesterol ester, and (5) total plasma. Study weights are indicated (gray squares) by individual biomarker compartment and overall. Study-specific analyses were conducted using models that included the following covariates: age (years), sex (male/female), race (white/non-white, or study-specific), field center if applicable (categories), body mass index (kg/m^2), education (less than high school graduate, high school graduate, some college or vocational school, college graduate), smoking (current, former, never; if history not assessed, then current/not current), physical activity (quintiles of metabolic equivalents per week), alcohol intake (none, 1–6 drinks/wk, 1–2 drinks/d, >2 drinks/d), prevalent diabetes mellitus (defined as treatment with oral antihyperglycemic agents, insulin, or fasting plasma glucose >126 mg/dL), treated hypertension (defined as hypertension drug use; or if unavailable, as diagnosed/history of hypertension), treated hypercholesterolemia (defined as low-density lipoprotein–lowering drug use; if unavailable, as diagnosed/history of hypercholesterolemia), regular aspirin use (defined as ≥2 times/wk), levels of α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), sum of *trans* isomers of oleic acid (*trans*-18:1), and sum of *trans* isomers of linoleic acid (*trans*-18:2; each expressed as % total fatty acids). If data did not allow such categorization, study-specific categories were used. See Table 1 footnote for abbreviations of cohorts.

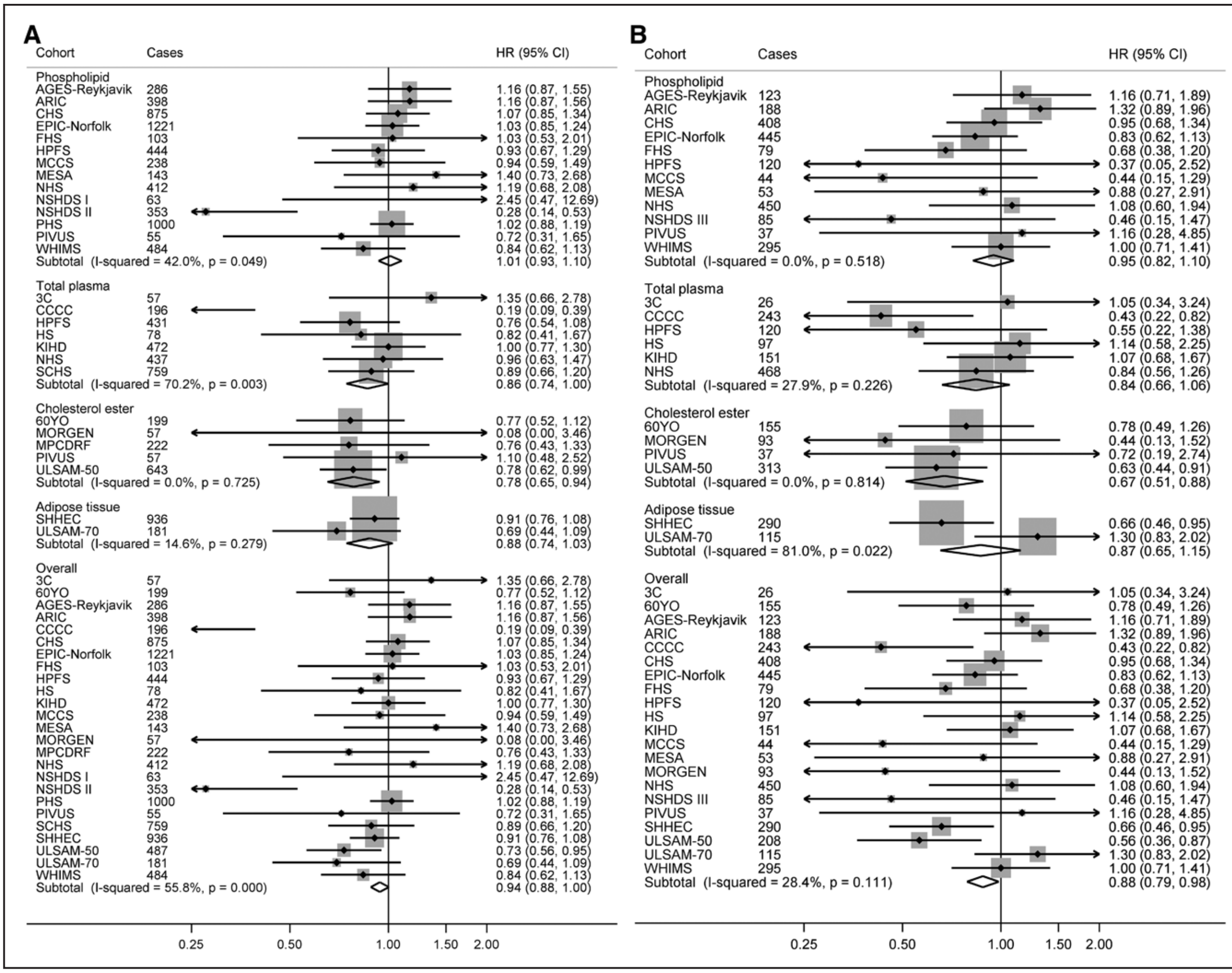

Figure 3. Circulating and tissue linoleic acid (18:2n6) and incident coronary heart disease and ischemic stroke.

Associations of linoleic acid with total coronary heart disease (**A**) and ischemic stroke (**B**) in pooled analysis of 30 prospective studies. Study-specific estimates for hazard ratio (HR) per interquintile range (ie, range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]) of biomarker linoleic acid were pooled based on the following order: (1) adipose tissue, (2) erythrocyte phospholipid, (3) plasma phospholipid, (4) cholesterol ester, and (5) total plasma. Study weights are indicated (gray squares) by individual biomarker compartment and overall. Study-specific analyses were conducted using models that included the following covariates: age (years), sex (male/female), race (white/non-white, or study-specific), field center if applicable (categories), body mass index (kg/m^2), education (less than high school graduate, high school graduate, some college or vocational school, college graduate), smoking (current, former, never; if history not assessed, then current/not current), physical activity (quintiles of metabolic equivalents per week), alcohol intake (none, 1–6 drinks/wk, 1–2 drinks/d, >2 drinks/d), prevalent diabetes mellitus (defined as treatment with oral antihyperglycemic agents, insulin, or fasting plasma glucose >126 mg/dL), treated hypertension (defined as hypertension drug use; or if unavailable, as diagnosed/history of hypertension), treated hypercholesterolemia (defined as low-density lipoprotein–lowering drug use; if unavailable, as diagnosed/history of hypercholesterolemia), regular aspirin use (defined as ≥2 times/wk), levels of α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), sum of *trans* isomers of oleic acid (*trans*-18:1), and sum of *trans* isomers of linoleic acid (*trans*-18:2; each expressed as % total fatty acids). If data did not allow such categorization, study-specific categories were used. See Table 1 footnote for abbreviations of cohorts.

Supplement), participants in the highest quintile, in comparison with the lowest, experienced significantly lower incidence of total CVD (0.92; 0.86–0.99). There was evidence for a borderline nonlinear association (*P*-nonlinearity=0.039) between total plasma AA and ischemic stroke (Figure I in the online-only Data Supplement).

Associations of LA and AA with CVD outcomes did not significantly differ according to subgroups defined by age, sex, race, n-3 PUFA levels, diabetes mellitus status, statin use, aspirin use, or baseline year of fatty acid measurement (Table X in the online-only Data Supplement). In 14 studies with genotype data (Table XI in the online-only Data Supplement), a significant interaction (*P*-interaction=0.002) was observed between LA and rs174547 genotype in relation to the risk of ischemic stroke (Table XII in the online-only Data Supplement), with inverse associations appearing stronger in carriers of the major T-allele. The associations of AA with cardiovascular outcomes did not significantly vary by rs174547 genotype.

In sensitivity analyses, results of compartment-specific analysis that used units of the percentage of total fatty acids, rather than study-specific interquintile ranges, were not appreciably different from the main findings (Table XIII in the online-only Data Supplement). Results were also similar across all other sensitivity analyses (Table XIV in the online-only Data Supplement).

Table 2. Risk of Incident CVD According to Objective Biomarker Levels of Linoleic Acid (18:2n6) and Arachidonic Acid (20:4n6) in 30 Pooled Prospective Cohort Studies

Outcome	Biomarker	Studies, n	Cases, n	Multivariable-Adjusted Hazard Ratio (95% CI) per Interquintile Range*	
				Linoleic Acid	Arachidonic Acid
Total CVD	Phospholipid	14	6853	1.00 (0.92–1.09)	0.95 (0.87–1.03)
	Total plasma	6	2742	0.90 (0.78–1.03)	0.81 (0.70–0.94)
	Cholesterol esters	4	1300	0.74 (0.63–0.88)	1.03 (0.88–1.20)
	Adipose tissue	2	1412	0.87 (0.75–1.01)	0.98 (0.87–1.10)
	Overall†	21	10 477	0.93 (0.88–0.99)	0.95 (0.90–1.01)
CVD mortality	Phospholipid	9	3057	0.89 (0.79–1.00)	0.93 (0.83–1.05)
	Total plasma	4	679	0.66 (0.50–0.86)	0.85 (0.66–1.09)
	Cholesterol esters	3	473	0.56 (0.43–0.73)	0.99 (0.76–1.29)
	Adipose tissue	2	418	0.60 (0.44–0.82)	1.02 (0.84–1.23)
	Overall†	17	4508	0.78 (0.70–0.85)	0.94 (0.86–1.02)
Total CHD	Phospholipid	14	6075	1.01 (0.93–1.10)	0.96 (0.90–1.03)
	Total plasma	7	2430	0.86 (0.74–1.00)	0.86 (0.74–1.01)
	Cholesterol esters	5	1178	0.78 (0.65–0.94)	1.02 (0.85–1.23)
	Adipose tissue	3‡	3255	0.88 (0.74–1.03)	1.10 (0.98–1.23)
	Overall†	26‡	11 857	0.94 (0.88–1.00)	0.99 (0.94–1.04)
Ischemic stroke	Phospholipid	12	2327	0.95 (0.82–1.10)	0.98 (0.85–1.13)
	Total plasma	6	1105	0.84 (0.66–1.06)	0.93 (0.73–1.18)
	Cholesterol esters	4	598	0.67 (0.51–0.88)	1.13 (0.89–1.43)
	Adipose tissue	2	405	0.87 (0.65–1.15)	0.91 (0.74–1.11)
	Overall†	21	3705	0.88 (0.79–0.98)	0.99 (0.90–1.10)

AA indicates arachidonic acid; CHD, coronary heart disease; CVD, cardiovascular disease; and LA, linoleic acid.

*Based on harmonized, de novo individual-level analyses in each cohort, pooled using inverse-variance weighted meta-analysis. Risk was assessed according to the interquintile range (ie, range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]) of each fatty acid, corresponding to the difference between the midpoint of the first and fifth quintiles. Study-specific analyses were adjusted for age (years), sex (male/female), race (white/non-white, or study-specific), field or clinical center if applicable (study-specific categories), body mass index (kg/m^2), education (less than high school graduate, high school graduate, some college or vocational school, college graduate), smoking (current, former, or never; if former not assessed, then current or not current), physical activity (quintiles of metabolic equivalents per week; or if metabolic equivalents unavailable, quintiles of study-specific definitions of physical or leisure activity), alcohol intake (none, 1–6 drinks/week, 1–2 drink/d, >2 drink/d [14 g alcohol=1 standard drink]), diabetes mellitus (yes/no; defined as treatment with oral hypoglycemic agents, insulin, or fasting plasma glucose >126 mg/dL), treated hypertension (yes/no; defined as hypertension drug use; or if unavailable, as diagnosed/history of hypertension according to study-specific definitions), treated hypercholesterolemia (yes or no; defined as lipid-lowering drug use; if unavailable, as diagnosed/history of hypercholesterolemia according to study-specific definitions), regular aspirin use (yes/no), biomarker concentrations of α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), sum of *trans*-18:1 fatty acids, and sum of *trans*-18:2 fatty acids (each expressed as % total fatty acids).

†For studies that assessed LA and AA levels in >1 biomarker compartment, the primary compartment for that study was preselected for pooled analyses based on the following order: (1) adipose tissue, (2) erythrocyte phospholipid, (3) plasma phospholipid, (4) cholesterol ester, and (5) total plasma.

‡Because the Diet, Cancer and Health study assessed associations of AA, but not LA, with total CHD (n cases=2138), a total of 2 studies (n cases=1117) evaluated adipose tissue LA and 25 studies (n cases=9719) assessed any biomarker level of LA in relation to total CHD.

DISCUSSION

In this harmonized, individual-level pooled analysis across 30 prospective studies from 13 countries, higher in vivo levels of the n-6 PUFA LA were associated with lower risk of CVD events, in particular, CVD mortality and stroke. AA levels were not associated with higher risk, and were associated with lower CVD risk in some analyses. To our knowledge, this is the largest pooled analysis of fatty acid levels and CVD end points, including ≈70 000 individuals and 10 000 total CVD events.

Our findings provide evidence to help inform currently inconsistent global dietary recommendations on n-6 PUFA consumption. LA, an essential fatty acid not synthesized by humans, is the main dietary PUFA, comprising ≈85% to 90% of the total. Although circulating and adipose tissue LA levels can be influenced by metabolism,[27,30] they are established and useful markers of diet because they increase in a dose-response manner in response to dietary LA in controlled feeding trials[15,26,30] and consistently correlate with self-reported dietary estimates in large cohort studies,[26] including a considerable number of studies participating in the current analysis (Table

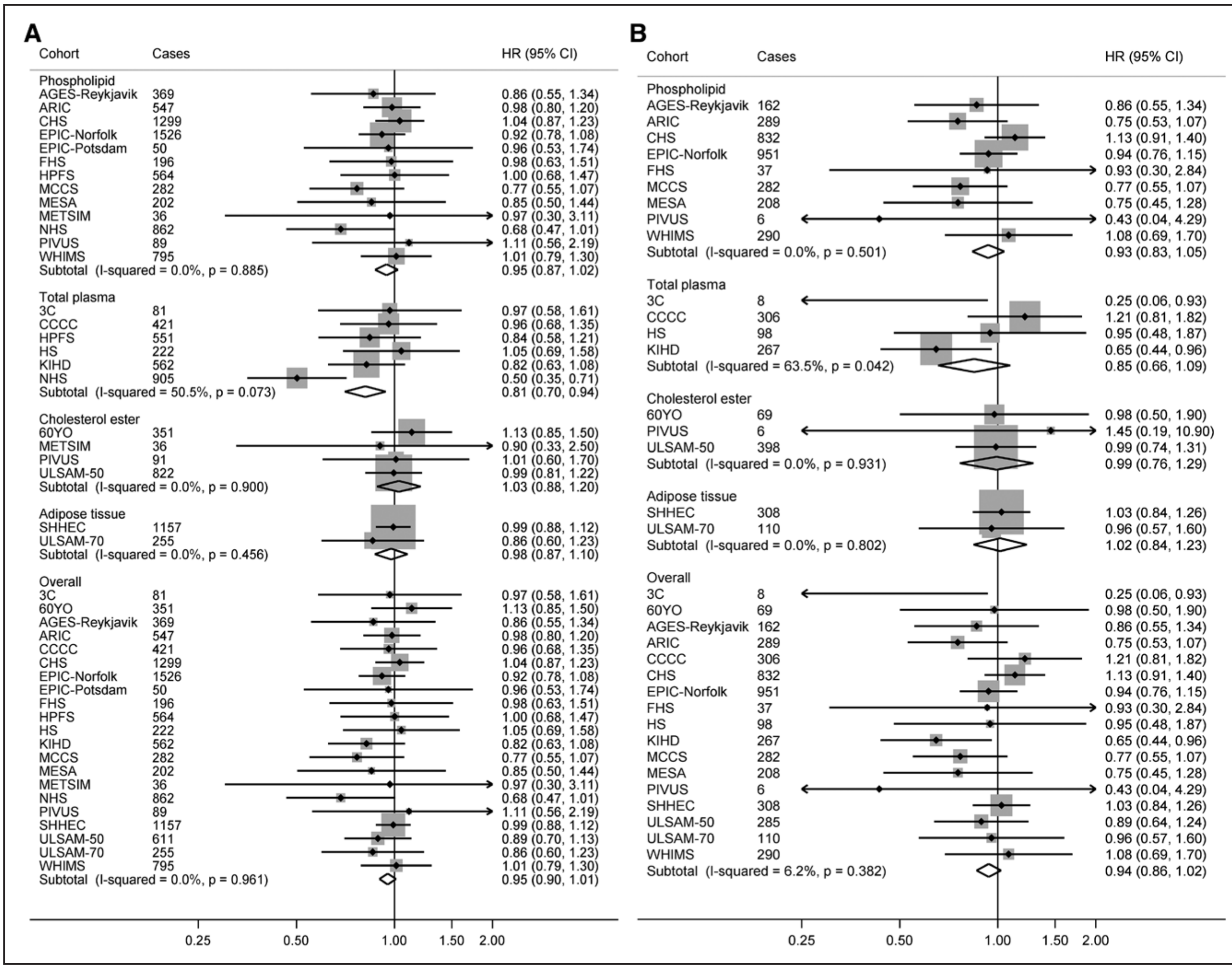

Figure 4. Circulating and tissue arachidonic acid (20:4n6) and cardiovascular disease incidence and mortality.
Associations of arachidonic acid with total cardiovascular disease (**A**) and cardiovascular disease mortality (**B**) in pooled analysis of 30 prospective studies. Study-specific estimates for hazard ratio (HR) per interquintile range (ie, range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]) of biomarker linoleic acid were pooled based on the following order: (1) adipose tissue, (2) erythrocyte phospholipid, (3) plasma phospholipid, (4) cholesterol ester, and (5) total plasma. Study weights are indicated (gray squares) by individual biomarker compartment and overall. Study-specific analyses were conducted using models that included the following covariates: age (years), sex (male/female), race (white/non-white, or study-specific), field center if applicable (categories), body mass index (kg/m[2]), education (less than high school graduate, high school graduate, some college or vocational school, college graduate), smoking (current, former, never; if history not assessed, then current/not current), physical activity (quintiles of metabolic equivalents per week), alcohol intake (none, 1–6 drinks/week, 1–2 drinks/d, >2 drinks/d), prevalent diabetes mellitus (defined as treatment with oral antihyperglycemic agents, insulin, or fasting plasma glucose >126 mg/dL), treated hypertension (defined as hypertension drug use; or if unavailable, as diagnosed/history of hypertension), treated hypercholesterolemia (defined as low-density lipoprotein–lowering drug use; if unavailable, as diagnosed/history of hypercholesterolemia), regular aspirin use (defined as ≥2 times/wk), levels of α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), sum of *trans* isomers of oleic acid (*trans*-18:1), and sum of *trans* isomers of linoleic acid (*trans*-18:2; each expressed as % total fatty acids). If data did not allow such categorization, study-specific categories were used. See Table 1 footnote for abbreviations of cohorts.

XV in the online-only Data Supplement). Several lines of evidence support mechanisms by which dietary LA may reduce CVD. In randomized controlled feeding trials, dietary PUFA (primarily LA) as a replacement for either carbohydrates or saturated fat lowers low-density lipoprotein cholesterol, triglycerides, and apolipoprotein B levels, and raises high-density lipoprotein cholesterol,[14,31] and also lowers hemoglobin A1c and insulin resistance and potentially augments insulin production.[32] Other potential cardiometabolic benefits of dietary LA may include favorable effects on inflammation,[14] blood pressure,[33] and body composition, including prevention and reduction of visceral and liver fat.[14,34] In a pooled analyses of prospective cohort studies, self-reported estimates of LA consumption are associated with lower CHD risk.[6] Similarly, in meta-analyses of older, limited clinical trials, increased consumption of LA-rich vegetable oils, especially soybean oil, reduces the risk of CHD.[5] Our findings evaluating in vivo levels of LA status across multiple global studies add strong support for the cardiovascular benefits of LA.

Although AA has long been considered an archetypical proinflammatory and prothrombotic fatty acid, growing evidence suggests that its effects may be more complex.[35] In the present investigation, AA levels were not associated with higher risk of CVD, and indeed in some analyses were associated with lower risk. These results

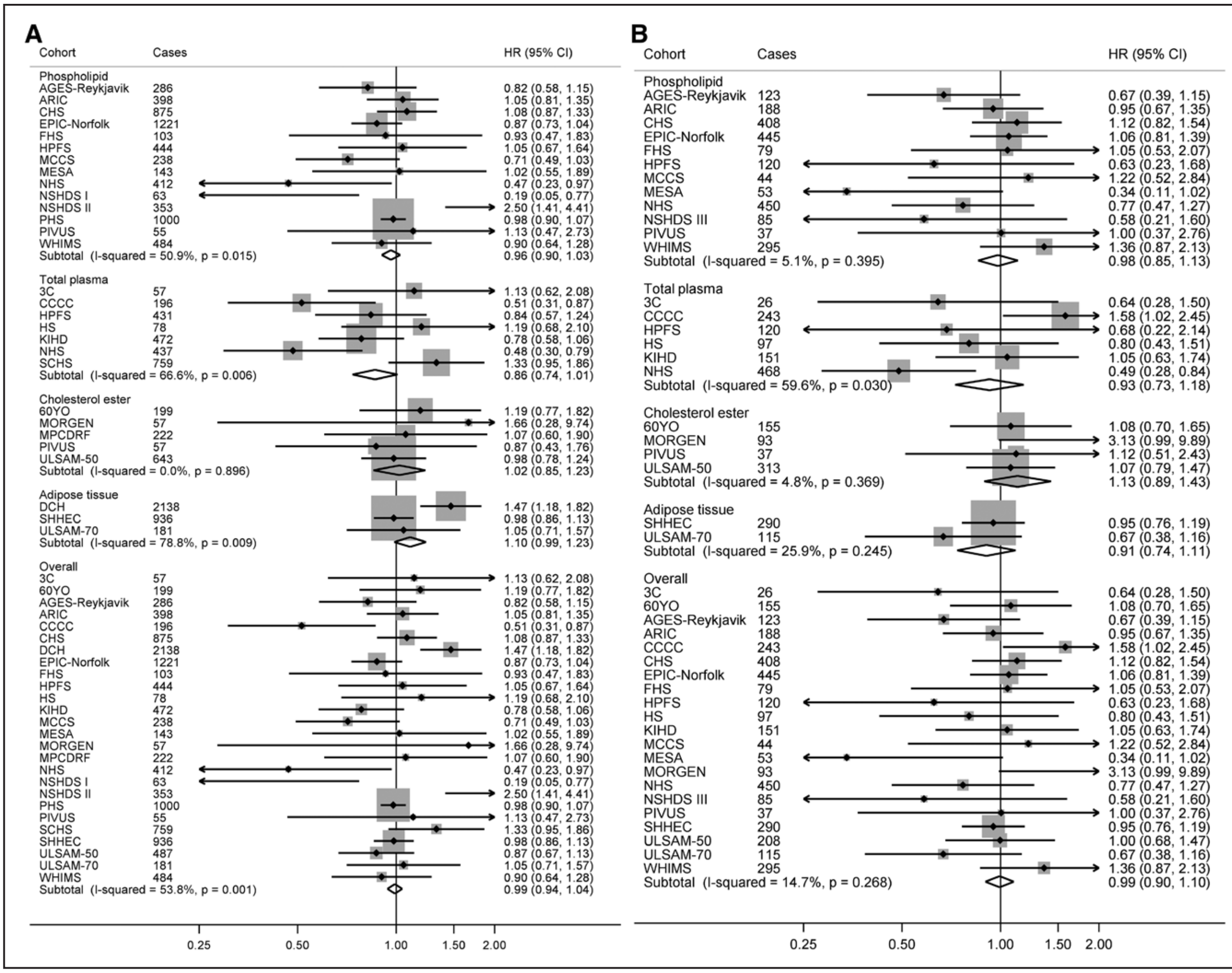

Figure 5. Circulating and tissue arachidonic acid (20:4n6) and incident coronary heart disease and ischemic stroke.
Associations of arachidonic acid with total coronary heart disease (**A**) and ischemic stroke (**B**) in pooled analysis of 30 prospective studies. Study-specific estimates for hazard ratio (HR) per interquintile range (ie, range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]) of biomarker linoleic acid were pooled based on the following order: (1) adipose tissue, (2) erythrocyte phospholipid, (3) plasma phospholipid, (4) cholesterol ester, and (5) total plasma. Study weights are indicated (gray squares) by individual biomarker compartment and overall. Study-specific analyses were conducted using models that included the following covariates: age (linear), sex (male/female), race (binary: white/non-white, or study-specific), field or clinical center if applicable (study-specific categories), body-mass index (linear), education (less than high school graduate, high school graduate, some college or vocational school, college graduate), smoking (current, former, or never; if former not assessed, then current or not current), physical activity (quintiles of metabolic equivalents per week; or if metabolic equivalents unavailable, quintiles of study-specific definitions of physical or leisure activity), alcohol intake (none, 1–6 drinks/wk, 1–2 drink/d, >2 drink/d [14 g alcohol=1 standard drink]), diabetes mellitus (yes or no; defined as treatment with oral hypoglycemic agents, insulin, or fasting plasma glucose >126 mg/dL), treated hypertension (yes or no; defined as hypertension drug use; or if unavailable, as diagnosed/history of hypertension according to study-specific definitions), treated hypercholesterolemia (yes or no; defined as lipid-lowering drug use; if unavailable, as diagnosed/history of hypercholesterolemia according to study-specific definitions), regular aspirin use (yes or no), biomarker concentrations of α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), sum of *trans*-18:1 fatty acids, and sum of *trans*-18:2 fatty acids (all linear; expressed as % total fatty acids). If data did not allow such categorization, study-specific categories were used. See Table 1 footnote for abbreviations of cohorts.

do not provide support for adverse cardiovascular effects of AA. Although AA is the precursor to potentially proinflammatory leukotrienes, it is also the main precursor to key anti-inflammatory metabolites, such as epoxyeicosatrienoic acids and prostaglandin E_2, and other mediators that actively resolve inflammation, such as lipoxin A_4, as well.[35] It also gives rise to prostacyclin, a potent antiaggregatory and vasodilatory molecule.[36] These complex biological effects preclude simplistic inference on the health effects of AA metabolites and further support the importance of the empirical assessment of relationships with clinical events, such as in our investigation.

Overall, our findings provide little support for the hypothesis that LA or AA, the major n-6 PUFA, may increase CVD risk. We also identified little evidence for any interaction between n-6 and n-3 PUFA levels, consistent with previous reviews of dietary data.[1] n-6 PUFA may also have additional metabolic benefits. For example, a recent pooled analysis from FORCE identified a strong inverse association of circulating and adipose tissue LA levels and the incidence of type 2 diabetes mellitus, with no significant associations for AA.[25] Taken together with results of randomized controlled feeding trials of blood lipids, glucose-insulin homeostasis, and

other metabolic risk factors; prospective cohort studies of self-reported consumption; and (older, methodologically limited) clinical trials of LA-rich plant oils, our novel findings do not support recommendations of some[10] to reduce n-6 PUFA consumption or reduce the n-6:n-3 ratio (as opposed to increasing n-3 intake). Rather, the findings from the present study, together with the previous research summarized above, support the independent cardioprotective benefits of LA.

Our results provide important evidence that helps inform clinical and population recommendations. Dietary guidelines from several organizations, including the American Heart Association, recommend increased consumption of n-6 PUFA to prevent CVD.[7] However, some researchers[9,10,37] and other national guidelines[4] currently recommend the avoidance of n-6 PUFA and reductions from current intake levels. Furthermore, current trends in oil production are leading to increased use of high-oleic, LA-depleted seed oils,[38] which can increase the risk of insufficient PUFA consumption in population subgroups. Our findings, combined with previous evidence from metabolic feeding trials, support the cardiovascular benefits of LA and a need to harmonize international guidelines and priorities for oilseed production and use.

A unique strength of our investigation was the ability to assess associations across distinct lipid compartments across which LA (AA) levels intercorrelate to varying degrees (eg, r=0.4–0.9),[26,39,40] suggesting that each compartment reflects partly differing metabolic and physiological influences. Yet, our findings were generally concordant across compartments, providing support for common or similar biological effects of these n-6 fatty acids across these compartments.

The inverse association of LA levels with ischemic stroke was more pronounced in T-allele carriers of rs174547, a polymorphism in *FADS1* associated with higher fatty acid desaturase activities[27,41] and *FADS1* expression.[42] Although located in *FADS1,* rs174547 is also in strong linkage disequilibrium with polymorphisms in *FADS2* (encoding the LA-desaturating FADS2) and has emerged as the main genetic determinant of circulating LA and AA in a recent genome-wide association study.[27] The T-allele has been linked to several metabolic traits including higher cholesterol (total, low-density lipoprotein, and high-density lipoprotein)[43] and fasting glucose,[44] but also lower triglycerides[43] and heart rate.[45] The pleiotropy of the *FADS* cluster and the specificity for ischemic stroke rather than all CVD end points complicates the interpretation of the observed gene-LA interaction, which should therefore be viewed cautiously. Yet, one could also speculate that carriers of the major T-allele derive greater benefits from the established low-density lipoprotein–lowering effects of dietary LA and thus have accentuated health benefits, a ripe area for further investigation.

Few previous meta-analyses of LA and AA levels in CVD have been performed. In one analysis of 10 published studies with 28 000 participants and 3800 events, LA was not significantly associated with coronary events, whereas AA was associated with a 17% reduction in risk.[18] In a meta-analysis of acute myocardial infarction and coronary syndromes including many retrospective case-control studies, circulating and adipose tissue LA levels were inversely associated with the risk of CHD events, whereas overall associations for AA were null.[17] Our investigation considerably extends these previous results by focusing on prospective studies, performing new individual-level study-specific analyses using a standardized and harmonized analysis protocol, including a much larger number of participants and events, and evaluating several major CVD outcomes. It is important to note that our consortium also greatly minimizes publication bias by incorporating new (unpublished) findings from all available studies, rather than pooling only previous published results.

Other strengths include the use of in vivo n-6 PUFA levels, which complement self-reported dietary estimates, reduce errors from memory, and allow assessment of biologically relevant in vivo levels, especially important for AA. Outcomes in nearly all studies were defined by centralized adjudication processes or validated registries rather than from self-report alone, reducing the potential for missed or misclassified end points. Inclusion of cohorts from 13 countries across several continents enhances generalizability. The large numbers of participants and events allowed us to explore several potential effect modifiers and the shape of the associations.

Potential limitations deserve attention. For certain compartments, such as adipose tissue, few studies were available. Most individuals were of European descent, lowering statistical power for evaluating other races/ethnicities. Despite extensive efforts to harmonize study-specific methods, some dissimilarities remained between cohorts in outcome definitions (see Expanded Methods in the online-only Data Supplement) and covariate categorization (Table V in the online-only Data Supplement). Although such variety and unmeasured background population characteristics may increase generalizability, these may also have contributed to the moderate between-study heterogeneity observed for some exposure-outcome relationships. Fatty acids were measured once at baseline, and changes over time could lead to misclassification, which would attenuate the associations. However, reasonable temporal reproducibility has been reported for LA and AA concentrations over time.[46] Because few studies evaluated multiple compartments, and because cholesterol esters were only assessed by studies from Northern Europe, we were hampered in drawing any conclusions of true

predictive differences between lipid fractions. Although fatty acid analytical methods were not standardized across studies, the use of a quintile-based statistical approach minimizes this concern. We did not adjust for non–fatty acid dietary factors, but pooling results across multiple cohorts with different population characteristics increases the validity of the findings. Although all studies consistently adjusted for other major CVD risk factors, we cannot exclude residual confounding attributable to unmeasured or imprecisely measured covariates. However, the concordance of the present observed associations with other lines of evidence on cardiovascular benefits of LA[1,5,6,32] provide biological plausibility for our findings. We did not evaluate the associations after exclusion of early cases. However, such sensitivity did not produce results substantially different from the main findings in our previous pooling projects[24,25] and in cohort-specific analyses,[23] suggesting that the observed associations are not likely attributable to reverse causation.

In summary, based on pooled individual-level analyses of prospective studies, circulating and adipose tissue biomarker concentrations of LA were inversely associated with CVD, whereas AA was not associated with higher CVD risk. Together with previous research, these results support the CVD benefits of LA.

ARTICLE INFORMATION

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