Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Study Descriptions, Fatty Acid Ascertainment, and Genotyping in the Participating Cohorts.

Participants for the current analysis were drawn from 19 studies,including the Atherosclerosis Risk in Communities study (ARIC), the Cardiovascular Health Study (CHS), the Costa-Rican study, the European Prospective Investigation into Cancer (in Norfolk) (EPIC-Norfolk), European study on Antioxidants, Myocardial Infarction and Cancer (EURAMIC), Health Professionals Follow-up Study (HPFS), Invecchiare in Chianti (InCHIANTI), Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD), Melbourne Collaborative Cohort Study (MCCS), Multi-Ethnic Study of Atherosclerosis (MESA), Northern Sweden Health & Disease Study I & II (NSHDS I & II) Nurses' Health Study I (NHS 1), Physicians' Health Study (PHS), Scottish Heart Health Extended Cohort Study (SHHECS), Singapore Chinese Health Study (SCHS), Three City Study (3C Study) and the Uppsala Longitudinal Study of Adult Men (ULSAM 50 & 70).

The Atherosclerosis Risk in Communities Study

The ARIC study is a multi-center prospective investigation of atherosclerotic disease in a predominantly bi-racial population. White and African American men and women aged 45-64 years at baseline were recruited from 4 communities: Forsyth County, North Carolina; Jackson, Mississippi; suburban areas of Minneapolis, Minnesota; and Washington County, Maryland. A total of 15,792 individuals participated in the baseline examination in 1987-1989, with follow-up examinations in approximate 3-year intervals, during 1990-1992, 1993-1995, and 1996-1998. ARIC Study samples were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, California); for the current analysis only white participants were analyzed. Sample exclusion criteria included discordant with previous genotype data (n=83), genotypic and phenotypic sex mismatch (n=32), suspected first-degree relative of an included individual based on genotype data (n=297), genetic outlier as assessed by Identity by State (IBS) using PLINK and >8 SD along any of the first 10 principal components in EIGENSTRAT with 5 iterations (n=322). Autosomal SNPs were used for imputation after exclusion of SNPs with HWE deviation $p<5 \times 10^{-5}$, call rate <95%, or MAF<1%. Fatty acids were measured in EDTA plasma that had been frozen at -70˚C. Fatty acid assays were performed at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN) in Caucasians. Lipids were extracted with chloroform/methanol and separated by thin layer chromatography. Fatty acid methyl esters were prepared from the phospholipid fraction and separated by gas chromatography using an HP-5890 gas chromatograph (Hewlett- Packard, Palo Alto, CA) with a 100-m capillary Varian CP7420 column. We identified 29 fatty acids. The concentration of each fatty acid was expressed as to percentage of total fatty acids.

Costa-Rican Case-Control Study of Myocardial Infarction (Costa Rican adults)

Adults were recruited in a case–control study of diet and heart disease in Costa Rica. Participation was 98% for cases and 88% for controls. The cases were survivors of a first acute MI between 1994 and 2004. They were recruited from any of the six national hospitals in the metropolitan area of San José, the capital of Costa Rica. All cases were diagnosed by two independent cardiologists according to the World Health Organization criteria for MI which require typical symptoms plus either elevations in cardiac enzymes or typical electrocardiographic changes (Tunstall-Pedoe et al., 1994). Cases were ineligible if they (a) died during hospitalization, (b) were greater than or equal to 75 years on the day of their first acute MI, or (c) were physically or mentally unable to answer the questionnaire.

One free-living control subject was randomly selected to match the case for age (plus/minus 5 years), gender, and county of residence using information available at the Costa Rican National Census and Statistic Bureau. Control subjects were ineligible if they ever had an MI or were physically or mentally unable to answer the questionnaire. All subjects gave written informed consent on forms approved by the Human Subjects Committee of the Harvard School of Public Health and the Ethics Committee of the University of Costa Rica.

Gas–liquid chromatography was used to quantify fatty acids from adipose tissue (Baylin et al., 2002). Subcutaneous adipose biopsies were collected after an overnight fast using a modification of the Beynen and Katan method with a plastic syringe instead of a vacutainer (Beynen and Katan, 1985). Peak retention times and area percentages of total fatty acids were analyzed with the ChemStation A.08.03 software (Agilent Technologies; Truong et al., 2009). Samples were stored at −80°C for at most a year after collection and before fatty acid analysis. Control samples were run and no evidence of analyte instability was found. CV's were 6.4% for ALA, 20.3% for EPA, and 14.8% for DHA.

Genotyping of several candidate polymorphisms was performed at the University of Cincinnati using the SNPlex Genotyping System (Applied Biosystems). Data were collected, formatted, processed, and analyzed using the GeneMapper Analysis Software (Version 4.0), which assigned individual genotypes. Of the two SNPs examined in the present study, only one (rs968567) was genotyped in the Costa Rica Study; no proxies of the other SNP were genotyped either. Rs968567 was not found to be in violation of Hardy-Weinberg Equilibrium (P-value <10-5).

The Cardiovascular Health Study

The CHS is a population-based longitudinal study of risk factors for cardiovascular disease and stroke in adults 65 years of age or older, recruited at four field centers (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Pittsburgh, PA). Overall, 5201 predominantly Caucasian individuals were recruited

in 1989-1990 from random samples of Medicare eligibility lists, followed by an additional 687 African-Americans recruited in 1992-1993 (total n=5,888). The CHS genome-wide association study (GWAS), which had the primary aim of studying incident cardiovascular events, focused on 3980 CHS participants who were free of clinical cardiovascular disease at study baseline, consented to genetic testing, and had DNA available for genotyping. A total of 1,908 persons were excluded from the GWAS study sample due to the presence at study baseline of coronary heart disease, congestive heart failure, peripheral vascular disease, valvular heart disease, stroke, or transient ischemic attack. Fatty acids were measured on samples collected in the 3rd year of followup.

CHS Study samples were genotyped using the Illumina HumanCNV370-Duo BeadChip system. Because the other cohorts were predominantly of European descent, the African American participants were excluded from the genome-wide association analysis. Genotyping was successful in 3,291 Caucasian subjects. Participants were eligible for the present investigation if their genotyping was complete and they had available phenotype information. Samples with call rate <95% were excluded. A total of 306,655 autosomal SNPs were used in imputation after filtering out SNPs with HWE deviation $p \leq 1 \times 10^{-5}$, call frequency ≤97%, zero heterozygote frequency, missing from dbSNP, and >1 duplicate or Mendelian inconsistency.

Blood was drawn after a 12-hour fast and stored at -70^{o}C. Measurements were performed at the Fred Hutchinson Cancer Research Center, providing quantitative measurement of 42 fatty acids. Total lipids were extracted from plasma using methods of Folch, and phospholipids separated from neutral lipids by one-dimensional TLC. Fatty-acid-methyl-ester (FAME) samples were prepared by direct transesterification using methods of Lepage and separated using gas chromatography (Agilent5890 gas-chromatograph-FID-detector; Supelco fused-silica 100m capillary column SP-2560; initial 160°C 16 min, ramp 3.0°C/min to 240°C, hold 15 min). Identification, precision, and accuracy were continuously evaluated using model mixtures of known FAMEs and established in-house controls, with identification confirmed by GC-MS at USDA (Peoria, IL). CVs were <3% for most fatty acids.

EPIC-Norfolk

The European Prospective Investigation into Cancer (EPIC)-Norfolk is a prospective study of 25,639 men and women aged 40–79 years in Norfolk, UK similar in characteristics to UK general population samples, who participated in a baseline survey in 1993–1997. Participants completed a health and lifestyle questionnaire including data on medical history, smoking, alcohol intake, physical activity, social class, and education and attended a clinic for a health examination. EPIC-Norfolk participants were not asked to fast before giving blood. Blood samples were spun, separated into 0.5 ml fractions of serum and citrated plasma, placed in straws, sealed, and stored in liquid nitrogen.

Funding was obtained for blood FA analyses in 2003–2008. Selection of participants for analyses was based on a series of nested case control studies with incident cases of cancers and cardiovascular disease and up to four disease-free controls for each case. Citrated plasma straws retrieved from storage were thawed, di-palmitoyl-D31-phosphatidylcholine Sigma) internal standard was added to each plasma sample, total lipids extracted, and purified by adsorption chromatography (LC-Si SPE, Supelco/Sigma). Plasma phospholipids were analyzed by gas chromatography. Concentrations were measured by comparison of peak areas of individual FAs with the peak area of the internal standard using individual calibration curves. The SNPs evaluated in this project are not currently available in EPIC-Norfolk.

EURAMIC

The EURopean multicenter case-control study on Antioxidants, Myocardial Infarction and breast Cancer (EURAMIC) is comprised of men 70 years of age or younger, native residents speaking the official local language of 8 European countries or residents of Israel. The cases included men with a first acute myocardial infarction, confirmed by characteristic ECG changes and serum enzymes, hospitalized within 24 hours from the onset of symptoms. Cases were recruited from the coronary care units of participating hospitals. Controls were men without history of myocardial infarction, frequency-matched for age in 5-year intervals. In Finland, Israel, Germany, Scotland, and Switzerland, random sampling from local population registers was used for the selection of apparently healthy controls. Because of incomplete coverage or legal restrictions on the use of population registers, male controls were recruited in Russia and in the two Spanish centers from patients admitted to hospital for disorders not known to be associated with dietary factors. Where it was thought that low response rates from population-based samples would spoil the internal validity, control subjects were selected from the catchment area of the patient's general practitioner (The Netherlands) or by inviting friends and relatives of the patient (Norway). In The Netherlands, Russia, and Spain, recruitment methods were combined. Cases and controls were recruited concurrently during 1991 and 1992. Informed consent was obtained from study participants in accordance with the ethical standards of the responsible local committees on human experimentation. Information on smoking habits, history of hypertension, angina pectoris, and diabetes was collected for all subjects by standard questionnaires. Socioeconomic status, alcohol intake and family history of cardiovascular diseases were assessed through locally developed questionnaires. A nonfasting venous blood sample was drawn for cholesterol analysis. In cases, blood samples were drawn within 24 hours of hospital admission.

A subcutaneous adipose tissue specimen was taken from the buttock by needle aspiration as described by Beynen and Katan. In cases, the adipose tissue sample was taken within 7 days of hospital admission. Samples were immediately frozen on dry ice or liquid nitrogen and stored at −70°C in the original plastic

adaptors until analyzed. Adipose tissue and serum samples were transported on dry ice at −56°C to the analytical laboratories.

The fatty acid composition of adipose tissue was assayed centrally at the National Public Health Institute, Helsinki (Finland). Adipose tissue samples were saponified and acidified with HCl, and free fatty acids were extracted with hexane and methylated with acidic methanol. Fatty acid composition was determined by gas chromatography (HNU Nordion Oy, Finland, HRCG 412) with a 60-m-long SP-2380 column, an internal diameter of 0.32 mm, a phase layer 0.20 μm with a split injector. Helium was the carrier gas. Fatty acid peaks from C12:0 through C22:6 were identified by an SC-workstation (Sunicom Oy, Finland) in a temperature-programmed run. All fatty acids are expressed as proportion of total fatty acid peak area (%FA). Because of the very low levels of EPA in adipose tissue, EPA was below the detection limit of the chromatograph for most samples. Fish oil fatty acid levels were thus represented exclusively by DHA. The interassay coefficients of variation for ALA and DHA were 15% and 25%, respectively. Samples of cases and controls were analyzed simultaneously and blind to disease status.

HPFS

The Health Professionals Follow-Up Study (HPFS) is a prospective cohort study consisting of 51,529 U.S. male health professionals who were 40–75 years old at study inception in 1986. Medical history, lifestyle practices, and diet were assessed at baseline and updated every 2–4 years using self-administered questionnaires since study baseline. In 1993–1995, a total of 18,159 participants provided blood samples, which were centrifuged and aliquoted into cryotubes as plasma, buffy coat, and erythrocytes upon arrival. All cryotubes were stored in the vapor phase of liquid nitrogen freezers at a temperature ≤ −130°C. A nested case-control study of CHD was conducted among these participants who provided blood samples. Briefly, among those who were free of cardiovascular disease at blood draw, we prospectively identified incident CHD cases and selected one to two controls for each case using the risk-set sampling method from those who remained free of CHD events when the case was diagnosed. Cases and controls were matched on age (± 2 years), smoking status (never smoke, past smoker, current smoker: 1–14 cigarettes/day, 15+ cigarettes/day), and month of blood draw. Through 2008, a total of 460 CHD cases including 358 cases of nonfatal myocardial infarction (MI) and 102 cases of fatal CHD were identified and confirmed, and 894 controls were selected. All analyses were conducted for plasma and erythrocyte LIs separately.

Fatty acids were extracted from plasma and erythrocyte membranes using a hexane-isopropanol mixture and esterified with methanol and acetyl chloride. After esterification, the methanol and acetyl chloride were evaporated, and the fatty acid methyl esters were re-dissolved in isooctane. The methyl esters were analyzed using gas-liquid chromatography. Peak retention times and area percentages of total fatty acids were identified

by injecting known standards (Nu-Chek-Prep, Elysium, MN). A total of 35 plasma fatty acids and 36 erythrocyte membrane fatty acids were identified. The content of each fatty acid was expressed as a percentage of total fatty acids. Samples of matched case-control sets were handled identically and assayed in the same analytical run. Both technicians and laboratory personnel were blinded to case-control status of the samples. Laboratory control samples were run along with case-control samples. Coefficients of variation (CVs) of the assay were assessed by repeatedly analyzing quality-control samples. In plasma, CV's for ALA were 4%, EPA 7%, and DHA 10%; in erythrocytes, CV's for ALA were 12%, EPA 12%, and DHA 14%.

InCHIANTI

The Invecchiare in Chianti Study (InCHIANTI) study is a population-based epidemiological study performed in a sample of the population living in the Chianti region of Tuscany, Italy. 1616 residents were selected from the population registry of Greve in Chianti and Bagno a Ripoli. The participation rate was 90% (n=1453), and the subjects age ranged between 21 and 102 years.

The plasma fatty acids measurement method has been described previously (1). Briefly, blood samples were collected in the morning after a 12-hr overnight fast. Aliquots of plasma were immediately obtained and stored at -80 C. Fatty acid methyl esters (FAME) were prepared through transesterification using Lepage and Roy's method with modification Rodriguez-Palmero et al (2, 3). Separation of FAME was carried out on an HP-6890 gas chromatograph (Hewlett- Packard, Palo Alto, CA) with a 30-m fused silica column (HP-225; Hewlett-Packard). FAMEs were identified by comparison with pure standards (NU Chek Prep, Inc., Elysian, MA). For quantitative analysis of fatty acids as methyl esters, calibration curves for FAME (ranging from C14:0 to C24:1) were prepared by adding six increasing amounts of individual FAME standards to the same amount of internal standard (C17:0; 50xg). The correlation coefficients for the calibration curves of fatty acids were in all cases higher than 0.998 in the range of concentrations studied. Fatty acid concentration was expressed as a percentage of total fatty acids. The coefficient of variation for all fatty acids was on average 1.6% for intraassay and 3.3% for interassay.

1. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik JM. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. The Journal of clinical endocrinology and metabolism 2006; 91(2): 439-46

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

3. Rodriguez-Palmero M, Lopez-Sabater MC, Castellote-Bargallo AI, De la Torre-Boronat MC, Rivero-Urgell M. Comparison of two methods for the determination of fatty acid profiles in plasma and erythrocytes. J Chromatogr A 1998; 793(2): 435-40

KIHD

The KIHD study was designed to investigate risk factors for cardiovascular disease, atherosclerosis, and related outcomes in a population-based sample of men from eastern Finland (1). The baseline examinations were carried out in 1984-1989. A total of 2682 men who were 42, 48, 54 or 60 years old at baseline (82.9% of those eligible) were recruited.

Venous blood samples were collected between 8AM and 10AM in 1984-1989 after an overnight fast. Serum esterified and nonesterified fatty acids were determined from frozen samples in 1991-1992 with a NB-351 capillary column (HNU-Nordion, Helsinki, Finland) by a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Company, Avondale, Pa, USA, since 1999 Agilent Technologies Inc., USA) with a flame ionization detector after chloroform-methanol extraction and methylation for esterified fatty acids (2). Each analyte had an individual reference standard and the analytes were quantified with an internal standard method using eicosanarachidic acid C20H40O2. Results were obtained in μmol/L. The coefficient of variation (CV) for repeated measurements of major esterified fatty acids was ~5%. The esterified fatty acid concentrations were adjusted for serum LDL and HDL cholesterol and triglyceride concentrations. The CV for major nonesterified fatty acids was ~15%. No adjustment was conducted for nonesterified fatty acids. Fatty acid concentrations are expressed as the sum of esterified and nonesterified fatty acids.

1. Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. Ann Clin Res 1988;20:46-50

2. Laaksonen DE, Lakka TA, Lakka HM, Nyyssonen K, Rissanen T, Niskanen LK, Salonen JT. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabet Med 2002;19:456-464

Serial measures

In KIHD, serial measures were available in individuals without prevalent CHD (n~500); serial measures are also available for cholesterol esters in ULSAM.

KIHD: Spearman correlations between baseline, 4-year and 11-year serum fatty acid measurements:

	BL vs. 4 y	BL vs. 11 y	4 y vs. 11 y
EPA	0.51	0.36	0.45
DPA	0.55	0.41	0.30
DHA	0.59	0.51	0.56
ALA	0.33	0.38	0.40

Analysis includes men without CVD at the time of the 11-year follow-up measurements, n=496.

MCCS

The Melbourne Collaborative Cohort Study (MCCS) recruited 41,514 persons (17 049 men) between 1990 and 1994. Persons aged 40-69 y were invited. The Cancer Council Victoria's Human Research Ethics Committee approved the study. Subjects gave written consent to participate and for the investigators to obtain access to their medical records. Blood was collected from all participants into sodium-heparin evacuated tubes, centrifuged immediately (3000 rpm, 15 min, 20°C), portioned into aliquots, and stored in liquid nitrogen. The fatty acid analysis has been described in detail elsewhere. Briefly, samples were re-aliquotted on ice under red light conditions before being refrozen and transported to the laboratory of one of us (RG) in Adelaide. Total lipids were extracted from plasma, and the extracts were separated by thin-layer chromatography into phospholipids, triacylglycerol, and cholesterol esters on silica gel plates (silica gel 60H; Merck, Darmstadt, Germany). Phospholipid fatty acid methyl esters were separated and quantified with a Hewlett-Packard (Palo Alto, CA) 5880 gas-liquid chromatograph by using a capillary column equipped with flame ionization detection and the Hewlett-Packard Chem-Station data system.

The Multi-Ethnic Study of Atherosclerosis

The MESA Study is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. MESA researchers study a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84. Thirty-eight percent of the recruited participants are white, 28 percent African-American, 22 percent Hispanic, and 12 percent Asian, predominantly of Chinese descent, as well as 2,128 additional individuals from 594 families recruited through MESA Family by utilizing the existing MESA framework, yielding 3,026 sibling pairs divided between African Americans and Hispanic-Americans. Participants were recruited from six field centers across the United States: Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University and University of California - Los Angeles.

MESA participants included in this analysis gave informed consent (MESA Genetics Candidate Gene Evaluation Cohort). Plasma phospholipid fatty acids were measured at baseline in a subset of participants randomly selected from within each race/ethnicity stratum. Analyses were limited to a subset of 2856 participants with these fatty acid measurements. Fasting blood was drawn and serum and EDTA-anticoagulant tubes were collected and processed using a standardized protocol. The serum and plasma samples were aliquoted and stored at −70 °C until time of use. Phospholipid fatty acids were extracted from EDTA plasma using the method previously described by Cao et al.28 In brief, lipids were extracted from the plasma using a

chloroform/methanol extraction method, and the cholesterol esters, triglyceride, phospholipids and free fatty acids were separated by thin layer chromatography. Fatty acids from the phospholipids were derivatized to methyl esters and detected by gas chromatography flame ionization. The fatty acids detected were expressed as a percent of total fatty acids. The following representative correlation values were obtained from intra-laboratory quality control (n =20): EPA, 3.3%; DPA, 2.9% and DHA, 2.7%.

NSHDS I & NSHDS II

The Northern Sweden Health and Disease Study (NSHDS) consists of three subcohorts: theVästerbotten Intervention Programme (VIP), the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) Study, and the Mammography Screening Project (MSP). All subcohorts are population based. No data from the MSP is included in the present study, due to lack of questionnaire data. The Northern Sweden MONICA Study started in 1986. Around every fifth year, 2000 or 2500 inhabitants aged 25-74 years are randomly invited to participate (1). The VIP started in 1985 and was coordinated with the MONICA Study. The VIP is a long-term project intended for health promotion of the population of the county of Västerbotten (approximately 255,000 inhabitants). All individuals 40, 50, and 60 years of age in the county are invited for screening (2). The participants in the MONICA Study and the VIP were asked to complete a questionnaire concerning various lifestyle factors, including diet, and to donate a blood sample to be frozen for later research purposes. By December 1999, the VIP and the MONICA Study comprised 73,750 unique individuals (about 90 % from the VIP and 10 % from the MONICA Study). NSHDS1 consists of prospective myocardial infarction cases occurring until 30 September 1994, with two controls matched for sex, age, time of baseline and geographical region. NSHDS2 consists of prospective myocardial infarction cases occurring until 31 December 1999 (and not included in NSHDS1), with one or two controls, matched as for NSHDS1.

Relative levels of fatty acids were determined in plasma phospholipids. Fatty acids were separated by gas-liquid chromatography after separation of the lipids by thin-layer chromatography and transmethylation (3) at the Unit for Clinical Nutrition Research, Department of Public Health and Caring Science, Uppsala University, Sweden. The fatty acid methyl esters were separated on a 25 m wall-coated open-tubular glass capillary column coated with SLP OV-351 (Quadrex Corporation, New Haven, USA), with He as a carrier gas. A Hewlett-Packard (Avondale, PA,USA) system was used and the fatty acids were identified by comparing retention times with those of NuCheck Prep (Elysian, MN, USA) fatty acid methyl ester standards and PUFA mix no. 2 (Supelco, Bellefonte, PA, USA). The method imprecision (calculated as the CV for duplicate preparations and measurements) has been reported as <1 - 5.5 % (4).

1. Stegmayr B, Lundberg V, Asplund K. The events registration and survey procedures in the Northern Sweden

MONICA Project. Scand J Public Health Suppl 2003; 61:9-17

2. Norberg M, Wall S, Boman K, Weinehall L. The Vasterbotten Intervention Programme: background, design and implications. Glob Health Action 3: 10.3402/gha.v3i0.4643

3. Boberg et al. Platelet fatty acid composition in relation to fatty acid composition in plasma and to serum lipoprotein lipids in healthy subjects with special reference to the linoleic acid pathway. ClinSci 1985; 68: 581-7

4. Smedman AE et al. Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors. Am J ClinNutr 1999; 69: 22-9

NHS I

The current study was derived from a large prospective cohort of > 120,000 female registered nurses aged 30-55 years enrolled in 1976(1). Blood samples were provided by 32,826 women in 1989-90. Women who provided samples were very similar to those who did not provide samples in terms of age, BMI, parity, the percentage of current smokers, and duration of oral contraceptive use2. Blood samples were collected in sodium heparin, centrifuged immediately on arrival in the lab (1200 x g, 15 min, room temperature), and distributed in aliquots of plasma, RBCs and buffy coat fractions that were stored, after receipt, in liquid-nitrogen freezers from -130 °C to -196°C until analysis. Fatty acid concentrations were measured in stored total plasma and RBC samples by using gas-liquid chromatography. Detailed methods have been previously described (2,3). Concentrations of individual circulating fatty acids were expressed as a percentage of total fatty acids in plasma or RBC membranes. Technicians and laboratory personnel were unaware of all clinical information, including the disease status, of participants. To minimize laboratory drift, each case-control pair or triplet was shipped in the same batch and analyzed in the same run in a random sequence and under identical conditions. Blinded quality control samples were also included. Forty fatty acids were quantified using these methods. Intraassay CV's for plasma samples were 3% for ALA, 7% for EPA, 6% for DPA, 3% for DHA: for RBC, 11% for ALA, 12% for EPA, 5% for DPA, and 7% for DHA.

1. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. J Womens Health 1997; 6(1):49-62

2. Sun Q, Ma J, Campos H, Hu FB. Plasma and erythrocyte biomarkers of dairy fat intake and risk of ischemic heart disease. Am J ClinNutr 2007; 86(4):929-937

3. Baylin A, Kabagambe EK, Siles X, Campos H. Adipose tissue biomarkers of fatty acid intake. Am J ClinNutr. 2002;76(4):750-757

Three City (3C) Study

The Three-City Study (3C Study) is a population-based longitudinal study of the relation between vascular diseases and dementia in persons aged 65 years and older. A total of 9,294 participants (3,649 men and 5,645 women) were recruited from three French cities: Bordeaux (South-West), Dijon (North-East) and Montpellier (South-East).Fasting blood samples were collected at baseline to measure fatty acids. Details of the assessment of plasma fatty acids were described previously (1) . Briefly, plasma of fasting blood samples was used to extract total lipids, from which the composition of fatty acids were determined. Gas chromatograph (Trace, Thermoelectron, Cergy-Pontoise, France) with a 25-m Carbowax capillary column was applied to measure the level of plasma fatty acids, expressed as a percentage of total fatty acids.

1. Samieri C, Féart C, Letenneur L, Dartigues J, Pérès K, Auriacombe S, Peuchant E, Delcourt C, and Barberger-Gateau P. Low plasma eicosapentaenoic acid and depressive symptomatology are independent predictors of dementia risk. Am J ClinNutr 2008; 88(3): 714-21

PHS

Study participants are members of the Physicians Health Study (PHS) I and II who provided blood samples between 1995 and 2001. The PHS I is a completed randomized trial designed to study the effects of low-dose aspirin and beta-carotene on cardiovascular disease and cancer (1). The PHS II is a randomized (started in 1997) designed to study the effects of various vitamins on the risk of cardiovascular disease and cancer (2).

Using a prospective nested case-control design, we randomly selected 1,000 incident CHD cases among PHS participants who provided blood samples between 1995 and 2001. For each CHD case, we randomly selected one control among participants that were alive and free of CHD at the time of the index case diagnosis and matched on age at blood collection (within 1 year), year of birth (within 2 years), race, and time of blood collection (within 3 months). Each case was eligible to serve as a control before CHD diagnosis. Likewise, each control was eligible to later become a CHD case to assure that the control series is a representative sample of the study base that generated all the cases. Each study participant gave written informed consent, and the Brigham and Women’s Hospital Institutional Review Board approved the study protocol.

All investigators including laboratory personnel were blinded to the case status of all participants. RBC fatty acid profiles were quantified using an established gas chromatography method. Briefly, lipids were extracted from RBC membranes followed by saponification and methylation. The resultant fatty acid methyl esters were analyzed using an Autosystem XL gas chromatograph (Perkin Elmer, Boston MA) equipped with a 100m x 0.25mm i.d (film thickness 0.25μm) capillary column (SP-2560, Supelco). Peaks of interest were

identified by comparison with authentic fatty acid standards (Nu-Chek Prep, Inc. Elysian, MN) and expressed as molar percentage (mol %) proportions of total fatty acids. Interassay coefficients of variation were <4.5% for fatty acids present at levels >1 mol% and <7.1% for fatty acids present at levels <1 mol%.

1. [No authors listed] Final report on the aspirin component of the ongoing Physicians' Health Study. Steering Committee of the Physicians' Health Study Research Group. N Engl J Med 1989; 321:129-35

2. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J, Bubes V, Manson JE, Glynn RJ, Gaziano JM. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. JAMA. 2008; 300(18):2123-33. doi: 10.1001/jama.2008.600

SHHEC

The SHHEC is a prospective cohort study of men and women that included participants in the Scottish Heart Health Study (SHHECS), as well as the Scottish Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) project. Adipose tissue was extracted in the SHHS as well as two of the sub-surveys of the MONICA project – a 1986 MONICA study in north Glasgow and Edinburgh and a 1987 survey in Aryshire and Arran (all in Scotland). The SHHECS and the two additional surveys used the same clinical protocols and questionnaires to assess demographics, medical history, tobacco use, dietary intake, anthropometric measures, and blood pressure. Adipose tissue was taken during clinic visits from the outer upper arm using a 3mm skin biopsy punch and stored. Adipose tissue sample was available among 4391 subjects after excluding those with baseline CVD. Adipose tissue FA were measured by gas chromatography.

SCHS

We conducted a nested case-control study of coronary heart disease within the Singapore Chinese Health Study (SCHS), a prospective cohort of 63,257 Singaporeans of Chinese origin who were recruited between April 1993 and December 1998 1. At recruitment, subjects were interviewed in-person using a structured questionnaire which included socio-demographic information, medical history and life style characteristics. Blood was collected from 28,439 participants mostly between 2000 and 2005. During an average follow-up of 15 years, less than 1% of subjects were lost to follow-up. Cases had fatal coronary artery disease (CAD) or non-fatal myocardial infarction (MI) identified through the Singapore Registry of Births and Deaths and the Hospital Discharge Database respectively. For non-fatal cases, medical records were retrieved and reviewed by cardiologists and we only included those that had confirmed myocardial infarction using the Multi-Ethnic Study of Atherosclerosis criteria. Some additional cases were identified through the Singapore Myocardial Infarction database which uses similar procedures to confirm MI. For each case, a control was selected from SCHS participants who were alive and free of CAD at the time of diagnosis or death of the index case and were matched for sex, dialect group, year of birth, year of recruitment and date of blood collection.

The Institutional Review Board of the National University of Singapore has approved this study.

Nineteen plasma fatty acid biomarkers were measured using combined gas chromatography-tandem mass spectrometry (GC-MS/MS) on an Agilent 7890 GC system (Shanghai, China) equipped with a 7001B QQQ triple quadruple mass detector (PA, USA) and an auto sample injector. In brief, 60 μL of plasma sample and 0.5 mL of NaOH-methanol solution (0.5 mol/mL) with type I internal standard (30 μg/mL 2-methylhexadecanoic acid in MeOH) were added into a PTFE screw-capped Pyrex tube. The mixture was heated at 80°C for 10 min after vigorous shaking. After cooling down to room temperature, methylation was done by adding 0.5 mL BF3-MeOH reagent (14%, w/v) followed by heating at 80°C for 3 min. After cooling down to room temperature, 0.5 mL hexane and 0.2 mL NaCl saturated solution were added, after which the mixture was vortexed for 3 min and centrifuged for 5 min at 3000 rpm. The clear n-hexane top layer (0.2 mL) was transferred and dried under a stream of N2, and re-dissolved with 0.1 mL of type II internal standard solution (0.5 μg/mL ethyl nonadecanoate in hexane). One μl was injected into the GC/MS/MS for analysis. Using this method, fatty acids from both free and esterified (triglycerides, phospholipids, cholesterol esters) fractions were measured. The within batch CV% were 4.0 for ALA, 6.3 for EPA, and 5.0 for DHA. The between batch CV% were 13.0 for ALA, 16.8 for EPA, and 12.2 for DHA.

We used the IlluminaHumanOmniZhongHua-8 Bead Chip which is customized for Chinese populations for genotyping. Based on quality control procedures, we excluded samples with a call rate < 98%, samples with extreme heterozygosities (> mean ± 3 standard deviation, SD), samples with 1st degree relatedness, identified from identity-by-state analyses, such as monozygotic twins, full-sibling pairs and parent-offspring (only 1 sample from each pair was retained, prioritizing the cases and samples with higher call-rates) and samples with discordant ethnic membership from Singaporean Chinese ethnicity as identified by principal components analysis. Quality control procedures for SNPs excluded non-autosomal SNPs, SNPs with poor call-rates (< 95%), SNPs with significant deviations of Hardy-Weinberg Equilibrium (HWE) in controls (< 1x10-6) and SNPs with minor allele frequencies (MAF) < 0.01.

ULSAM 50 & 70

ULSAM is a unique, ongoing, longitudinal, epidemiologic study based on all available men, born between 1920 and 1924, in Uppsala County, Sweden. The DNA samples available for genotyping in the ULSAM project have been obtained and prepared in three different ways: The men who participated in ULSAM-70 revisited the clinic between 960108 and 960618 to leave a blood sample for DNA preparation (n = ~729). Whole peripheral blood was incubated with lysis buffer (NH_4Cl, $KHCO_3$ and EDTA) and centrifuged twice at 6 °C (supernatant discarded), before washed with a wash solution (NaCl, Tris, and EDTA) and centrifuged again. After discarding the supernatant, the pellet was dissolved in SET (Tris-HCl, EDTA and NaCl). SDS (10%) and Proteinase K (15 mg/ml) were added to the tube and after rigorous shaking the samples were incubated overnight at 37 °C. Saturated NaCl solution was added and the samples were again centrifuged at 6 °C. The supernatant was then transferred to a new tube and 75% EtOH was added to precipitate the DNA. When the DNA was dry, it was dissolved in TE-4 (Tris and EDTA, pH=7.5) and incubated overnight at 37°C. Those subjects who did not revisit the clinic during 1996, left a blood sample for DNA preparation at ULSAM-77 (n = ~368). These samples were sent to Eurona Medical, where DNA was prepared from EDTA blood with QIAamp DNA blood Maxi Kit (QIAGEN, Hilden, Germany). Muscle biopsies (n=49) were obtained from the subjects at ULSAM-70. The muscle biopsies (10-15 mg) were put in eppendorf tubes together with proteinase K and incubated over night at 60°C. The tubes were then centrifuged and the supernatant transferred to a new set of tubes and isopropanol was added to precipitate the DNA. The samples were centrifuged again and the supernatant discarded. The DNA was washed with cold EtOH (70%) and left to dry at room temperature 5-10 minutes. The pellet was dissolved in dd water.

For analysis of the fatty acid composition of the serum cholesterol esters, serum was extracted with a hexane-isopropanol solution (1+4) (Hara and Radin 1978). Cholesterol esters were separated from the extract by thin layer chromatography before inter-esterification (acidic methanol at 85°C, 2 h) (Stoffel et al 1959), and free cholesterol liberated in the reaction was removed by an aluminium oxide column to avoid contamination of the gas liquid chromatography column. The percentage composition of methylated fatty acids 14:0 to 22:6 was determined by gas chromatography (a 25 m NB-351 silica capillary column, i.d. 0.32 mm, phase layer 0.20 mm) with use of a flame ionisation detector and with helium as carrier gas. Every 25th sample was a serum control pool. The precision of the between-series analysis (n=35) varied from 2% (large peaks) to 10% (smaller peaks) and between successive gas chromatography runs (n=17). Intraassay CV: 0.2-5% depending on the fatty acid; interassay: 2-10% depending on the fatty acid. Intra-individual correlations of cholesterol esters in men at age 50 and 70: r=0.45 for linoleic, r=0.58 for arachidonic acid.

SNPs were genotyped using a 1536-plex Golden Gate Assay and the Bead Station genotyping system

from Illumina. The SNPs were selected from the dbSNP database (www.ncbi.nlm.nih.gov). All SNPs conferred to Hardy-Weinberg equilibrium according to a chi-square test ($p>0.05$).

ULSAM-70

Initially the adipose tissue fatty acid composition was analyzed in a random subsample of 318 men, whereas the fatty acid composition in serum cholesterol esters was analysed in 611 men. In December 2008, 535 new samples were analyzed for adipose tissue fatty acid composition. Four additional fatty acids were included in this analysis, namely lauric acid 12:0 (Z555), myristic acid 14:0 (Z556), pentadecanoic acid 15:0 (Z557) and heptadecanoic acid 17:0 (Z558). Subcutaneous adipose tissue was collected as described above. Prior to the fatty acid analysis the biopsy was weighed and homogenized.

The fatty acid compositions of the serum lipids and subcutaneous adipose tissue were analyzed using an extraction with chloroform in the presence of methanol, butylated hydroxytoluene, and NaH_2PO_4 overnight, and evaporated under nitrogen. The dry extracts were dissolved in a few drops of chloroform and applicated on thin liquid chromatography plates for separation of the lipids in a solvent system consisting of petroleum ether:diethylether:acetic acid (81:18:1, by volume) (Boberg 1966). The lipid fractions were visualized in UV light and scraped off separately. The lipid esters were trans methylated in warm, acidic environment overnight. The methyl esters were extracted with petroleum ether and deionised water, and the solvent was evaporated under nitrogen. The fatty acid methyl esters were dissolved in hexane and separated by gas-liquid chromatography (GLC). The Hewlett Packard GLC system used for the analyses was consisted a GC 5890, automatic sampler 7671A, integrator 3392A, and 25 m Quadrex Fused Silica capillary column OV-351, with helium as the carrying gas. The temperature program used during the separation of the fatty acid methyl esters was 130-220°C. The fatty acids were identified by comparison of the retention times of separation was controlled by Nu Check Prep GLC reference standard GLC-68A.

The amounts of fatty acids were given as the relative percentage of the sum of the fatty acids analyzed, namely 16:0, 16:1 n-7, 18:0, 18:1 n-9, 18:2 n-6, 18:3 n-6, 18:3 n-3, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-3, and 22:6 n-3.

Methods for Evaluating Interactions of rs174546 and rs968567 With Fatty Acid Biomarkers (ALA, EPA, DPA, DHA) & Outcomes (Total CHD, Nonfatal MI, Fatal CHD)

To assess interactions for rs174546 and rs968567, an additive genetic model, i.e. regression of phenotype on the number of reference alleles, or equivalently the imputed dosage for imputed genotypes, was used. Interaction terms for each SNP were constructed by creating a cross-product term of the omega-3 FA exposure of interest (continuous) by the SNP (ordinal; 0, 1, or 2 T alleles) and added to the fully adjusted model:

$S(x)=\exp(\beta\ n3\ +\ \beta\ SNP + \beta\ n3 \times SNP + covariates)$

For each SNP, the β coefficient and its robust standard error (SE) was recorded for the main effect of the omega-3 FA exposure, the interaction term, and the covariance matrix

Mean differences in fatty acid exposures for each value of # coded alleles were calculated for each cohort and meta-analyzed

$\hat{E}_0 = \beta_{ALA}$ (zero copies of T allele)

$\hat{E}_1 = \beta_{ALA} + \gamma_{int}$(one copy of T allele)

$\hat{E}_2 = \beta_{ALA} + 2\ \gamma_{int}$(two copies of T alleles)

(where β_{ALA} is the regression coefficient for one SD of the exposure; γ_{in} is coefficient for the interaction term, exposure*SNP)

In order to calculate SE of Ê1 and Ê2, we used the following:

Var $(\hat{E}_1) = Var(\beta_{ALA}) + Var(\gamma_{int}) + 2$ covariance $(\beta_{ALA}\gamma_{int})$
Var $(\hat{E}_2) = Var(\beta_{ALA}) + 4\ Var(\gamma_{int}) + 4$ covariance $(\beta_{ALA}\gamma_{int})$
and $Var = SE^2$

eTable 1. Ascertainment of Incident Nonfatal Myocardial Infarction (MI), Fatal Coronary Heart Disease (CHD), and Total CHD in 19 Participating Studies.

Study	Non-fatal MI			Fatal CHD			Total CHD		
	# cases	Median/ max follow-up (y)	Ascertainment/ definition	# cases	Median/ max follow-up (y)	Ascertainment/ definition	# cases	Median/ max follow-up (y)	Ascertainment/ definition
ARIC	NA	NA	NA	NA	NA	NA	500	22.4/25.1	Self-reported history of a physician-diagnosed heart attack, evidence of a prior myocardial infarction by electrocardiogram (ECG) or self-report of cardiovascular surgery orcoronary angioplasty.
Costa-Rican adults (cc)	1430	NA	Cases of first non-fatal acute MI were centrally ascertained by two independent cardiologists in the participating hospitals and deemed eligible if they met the World Health Organization criteria, survived hospitalization, were under 75 years of age on the day of their first MI, and able to answer the questionnaire	0	NA	NA	0	NA	NA
CHS	490	21.6 (max)	Exams, interviews, death or medical forms; events centrally adjudicated by morbidity and mortality committee/ Chest pain with abnormal cardiac enzyme concentrations or serial ECG changes	516	21.6 (max)	Fatal MI or as fatal CHD events for which the participant had chest pain within 72 h of death, or a history of chronic CHD	1163	21.6 (max)	Ischemic heart disease, fatal or nonfatal myocardial infarction
EPIC-Norfolk	708	12.9/16.1	East Norfolk Health Authority database/ ICD9 410–414 or ICD10 I22–I25 from hospital episodes	507	12.9/16.1	East Norfolk Health Authority database with clinical validation through medical record inspection of a sample / ICD9 410–414 or ICD10 I22–	1215	12.9/16.1	ICD9 410–414 or ICD10 I22–I25

						I25 as underlying cause of death			
EURAMIC	633	Incident cases	Patients with a first acute myocardial infarction (ICD9 410), confirmed by characteristic electrocardiographic changes and elevated enzyme levels who had been hospitalized within 24 hrs after the onset of symptoms to the coronary care units of participating hospitals	0	NA	NA	0	NA	NA
HPFS - total plasma*	367	4.0/15.0	Self-report followed by medical record/physician review; confirmed if WHO criteria met (symptoms + ECG or elevated enzymes)	123	4.0/15.0	Next of kin reports or National Death Register followed by hospital, autopsy or death records; confirmed if CHD previously reported + no other probable cause of death reported	466	4.0/15.0	Total of non-fatal MI and fatal CHD
HFFS - RBC*	371	4.0/15.0	Self-report followed by medical record/physician review; confirmed if WHO criteria met (symptoms + ECG or elevated enzymes)	108	4.0/15.0	Next of kin reports or National Death Register followed by hospital, autopsy or death records; confirmed if CHD previously reported + no other probable cause of death reported	479	4.0/15.0	Total of non-fatal MI and fatal CHD
InCHIANTI	NA	NA	NA	NA	NA	NA	115	9.0/10	All recorded CHD and MI
KIHD	364	22.9/27.8	National Death Register; ICD-9 codes/ Acute coronary event not leading to death	168	23.7/27.8	ICD-9 codes, SCD: death within 1hr of change in symptoms, or 24h if autopsy did not reveal non-cardiac cause	452	22.9/27.8	Fatal or non-fatal acute MI, CHD death or SCD
Melbourne Collaborative Cohort Study	733	11.8/16.2	Self-reported MI, bypass, angina, angioplasty	391	9.7/22.1	ICD-9 410-414 , ICD-10 120-125	1124	9.7/22.1	Total of non-fatal MI and fatal CHD
MESA	70	8.5/10.9	Centrally adjudicated as definite, probable, or absent MI, based on combinations of symptoms, ECG, and cardiac biomarker levels. In most cases, definite or probable MI required	47	8.5/10.9	Definite fatal CHD required a documented MI within the previous 28 days, chest pain within the 72 hours before death, or a history of CHD, and required the absence of a	94	8.5/10.9	MI, resuscitated cardiac arrest, or CHD death

			either abnormal cardiac biomarkers (2 times upper limits of normal) regardless of pain or ECG findings; evolving Q waves regardless of pain or biomarker findings; or a combination of chest pain, and ST-T evolution or new LBBB, and biomarker levels 1-2 times upper limits of normal			known non-atherosclerotic or non-cardiac cause of death. If the definite fatal CHD criteria were not met, possible fatal CHD could be assigned with an underlying cause of death consistent with fatal CHD and required the absence of a known non-atherosclerotic or non-cardiac cause of death			
Northern Sweden Health & Disease Study (cc study 1)	63	not given	Medical records using WHO MONICA criteria/ Definite infarction based on ECG progression, or ECG + elevated enzymes + symptoms		not given	Autopsies or medical records listing CHD cause		1.3/4.3	Total of non-fatal MI and fatal CHD
Northern Sweden Health & Disease Study (cc study 2)	273	not given	Medical records using WHO MONICA criteria/ Definite infarction based on ECG progression, or ECG + elevated enzymes + symptoms	80	not given	Autopsies or medical records listing CHD cause	353	3.7/10.8	Total of non-fatal MI and fatal CHD
NHS-I (cc)-total plasma	380 (355 for RBC)	16.0 (max)	Self-report followed by medical record/physician review; confirmed if WHO criteria met (symptoms + ECG or elevated enzymes)	57	16.0 (max)	Next of kin reports or National Death Register followed by hospital, autopsy or death records; confirmed if CHD previously reported + no other probable cause of death reported	437 (409 for RBC)	16.0 (max)	Total of non-fatal MI and fatal CHD
PHS	249	NA	Self-report followed by medical record/physician review	167	NA	Self-report adjudicated by an endpoint committee. The diagnosis of myocardial infarction was confirmed by using World Health Organization criteria and revascularization procedures were confirmed by hospital records. Total of Fatal MI, coronary death, and sudden death (n=41)	416	NA	Self-report adjudicated by an endpoint committee. The diagnosis of myocardial infarction was confirmed by using World Health Organization criteria Total of non-fatal MI, fatal MI, coronary death, and sudden death

SHHEC	755	23.4/24.8	Subjects providing written permission were followed through national record linkage database to 2009/any hospital discharge diagnosis post-recruitment of CHD (ICD 9 410-414, ICD 10 I20-I25) or surgical codes for bypass graft or percutaneous angioplasty	143	23.4/24.8	Subjects providing written permission were followed through death registration and the national record linkage database to 2009/any hospital discharge diagnosis post-recruitment of CHD (ICD 9 410-414, ICD 10 I20-I25) or surgical codes for bypass graft or percutaneous angioplasty	898	23.4/24.8	Total of non-fatal MI and fatal CHD
SCHS	467	4.7/14.4	Acute myocardial infarction was identified through linkage with the Singapore Hospital Discharge Database (ICD9-410) and confirmation was based on medical record review by a cardiologist using the MESA criteria. In addition, some confirmed cases of myocardial infarction were identified through linkage with the Singapore Myocardial Infarction Registry	292	4.6/11.3	Deaths classified by first cause of death ICD9 410-414 with use of the Singapore National Death Registry	759	4.6/14.4	Total of non-fatal MI and fatal CHD
Three City Study	NA	NA	Self-report followed by medical record/hospitalization reports and physician/family interview	NA	NA	Deaths classified by ICD-10 codes I210–I219, I251–I259, I461 and R960.	57	6.6/8.5	Stable and unstable angina pectoris, coronary balloon dilatation or artery bypass, non-fatal MI and death due to CHD
ULSAM 50	372	30/42	Acute myocardial infarction (ICD-8code 410, ICD-9 code 410, or ICD-10 code I21) with use of Swedish Hospital Discharge Register data	275	32/42	Deaths classified by ICD codes 410-414, 429.2 (9^{th} revision) or I20-25, I46 (10^{th} edition) with use of the Swedish Cause of Death Register	647	30/42	Total of non-fatal MI and fatal CHD
ULSAM 70	86	14/20	Acute myocardial infarction (ICD-8code 410, ICD-9 code 410, or ICD-10 code I21) with use of Swedish Hospital Discharge Register data	74	15/20	Deaths classified by ICD codes 410-414, 429.2 (9^{th} revision) or I20-25, I46 (10^{th} edition) with use of the Swedish Cause of Death Register	181	14/20	Total of non-fatal MI and fatal CHD

eTable 2. Baseline Descriptives for α-Linolenic Acid (ALA; 18:3n-3), Docosapentaenoic Acid (DPA; 22:5n-3), EicosapentaenoicAcid (EPA; 20:5n-3), and Docosahexaenoic Acid (DHA; 22:6n-3) Biomarkers in Participants ≥18 Years With Measured Fatty Acid Biomarker Data.*

Study	Country	Biomarker type	Year measured	Total # FA measured	Fatty acid	n	Mean ± SD †	Median †	Minimum †	Maximum †
ARIC	United States	Plasma phospholipid	1987-1989	29	ALA (18:3n-3)	3793	0.14 ± 0.05	0.14	0.02	0.39
					DPA (22:5n-3)	3793	0.90 ± 0.17	0.89	0.05	1.73
					EPA (20:5n-3)	3793	0.57 ± 0.32	0.51	0	7.70
					DHA (22:6n-3)	3793	2.82 ± 0.89	2.66	0	8.88
Costa-Rican adults (cc study)	Costa Rica	Adipose tissue	1995-2004	58	ALA (18:3n-3)	3370	0.64 ± 0.21	0.60	0.06	1.76
					DPA (22:5n-3)	3370	0.18 ± 0.05	0.18	0	0.53
					EPA (20:5n-3)	3352	0.04 ± 0.02	0.04	0	0.12
					DHA (22:6n-3)	3370	0.14 ± 0.05	0.14	0	0.98
CHS	United States	Plasma phospholipid	1992-1993	42	ALA (18:3n-3)	3941	0.14 ± 0.05	0.14	0.05	0.48
					DPA (22:5n-3)	3941	0.83 ± 0.17	0.82	0.11	1.74
					EPA (20:5n-3)	3941	0.59 ± 0.37	0.51	0.11	8.52
					DHA (22:6n-3)	3941	3.05 ± 0.98	2.89	0.78	8.34
EPIC-Norfolk	UK	Plasma phospholipid	2001-2004	22	ALA (18:3n-3)	7356	0.23 ± 0.09	0.22	0.03	1.06
					DPA (22:5n-3)	7384	1.41 ± 0.38	1.37	0.28	3.85
					EPA (20:5n-3)	7384	1.28 ± 0.83	1.07	0.15	9.64
					DHA (22:6n-3)	7384	5.15 ± 1.63	4.89	1.24	15.35
EURAMIC	Finland, Norway, Scotland, Ireland, Germany, Switzerland, Spain, Israel, Russia	Adipose tissue	1991-1992	not given	ALA (18:3n-3)	1326	0.77 ± 0.37	0.72	0	2.37
					DPA (22:5n-3)	1326	0.22 ± 0.11	0.22	0	1.67
					EPA (20:5n-3)	NA	NA	NA	NA	NA
					DHA (22:6n-3)	1326	0.23 ± 0.16	0.21	0	1.24
HPFS	United States	Total plasma	1994	42	ALA (18:3n-3)	1291	0.61 ± 0.24	0.55	0.07	2.43
					DPA (22:5n-3)	1291	0.51 ± 0.14	0.50	0.20	1.19
					EPA (20:5n-3)	1291	0.63 ± 0.45	0.53	0.07	5.51
					DHA (22:6n-3)	1291	1.72 ± 0.76	1.58	0.37	5.30
		RBC	1994	42	ALA (18:3n-3)	1342	0.21 ± 0.22	0.17	0.04	4.33
					DPA (22:5n-3)	1342	1.97 ± 0.39	1.98	0.64	3.33
					EPA (20:5n-3)	1342	0.51 ± 0.29	0.44	0.10	3.17
					DHA (22:6n-3)	1342	3.68 ± 1.20	3.55	0.90	8.37
InCHIANTI	Italy	Total plasma	1998-2000	20	ALA (18:3n-3)	839	0.45 ± 0.24	0.39	0.03	1.71
					DPA (22:5n-3)	n/a				
					EPA (20:5n-3)	839	0.63 ± 0.22	0.59	0.19	3.50
					DHA (22:6n-3)	839	2.32 ± 0.77	2.25	0.35	5.78

KIHD	Finland	Total plasma	1991-1992	14	ALA (18:3n-3)	1837	0.74 ± 0.24	0.71	0.24	2.80
					DPA (22:5n-3)	1837	0.55 ± 0.10	0.54	0.24	1.19
					EPA (20:5n-3)	1837	1.66 ± 0.90	1.46	0.23	8.67
					DHA (22:6n-3)	1837	2.46 ± 0.73	2.36	0.91	6.58
Melbourne Collaborative Cohort Study	Australia	Plasma phospholipid	1990-1994	57	ALA (18:3n-3)	5266	0.17 ± 0.08	0.15	0.02	0.94
					DPA (22:5n-3)	5279	1.22 ± 0.24	1.21	0.49	2.33
					EPA (20:5n-3)	5279	1.07 ± 0.50	0.97	0.01	6.12
					DHA (22:6n-3)	5279	4.03 ± 1.08	3.91	1.11	12.27
MESA	United States	Plasma phospholipid	2000-2002	28	ALA (18:3n-3)	2856	0.18 ± 0.08	0.16	0.03	2.54
					DPA (22:5n-3)	2856	0.96 ± 0.89	0.93	0.37	2.42
					EPA (20:5n-3)	2856	0.96 ± 0.23	0.70	0.09	14.46
					DHA (22:6n-3)	2856	4.19 ± 0.73	3.99	1.18	10.41
Northern Sweden Health & Disease Study I	Sweden	Plasma phospholipid	1995	15	ALA (18:3n-3)	183	0.21 ± 0.10	0.21	0.09	0.60
					DPA (22:5n-3)	183	1.07 ± 0.18	1.06	0.63	2.00
					EPA (20:5n-3)	183	1.46± 0.63	1.29	0.04	3.99
					DHA (22:6n-3)	183	4.69 ± 1.10	4.47	2.60	8.43
Northern Sweden Health & Disease Study II	Sweden	Plasma phospholipid	2007	16	ALA (18:3n-3)	759	0.21 ± 0.08	0.20	0.06	0.60
					DPA (22:5n-3)	759	1.15 ± 0.20	1.12	0.55	2.09
					EPA (20:5n-3)	759	1.51 ± 0.63	1.37	0.41	5.44
					DHA (22:6n-3)	759	4.67 ± 1.07	4.54	2.12	8.55
NHS I §	United States	Total plasma	1989-1990	40	ALA (18:3n-3)	603	0.42 ± 0.18	0.42	0.02	1.56
					DPA (22:5n-3)	603	0.69 ± 0.60	0.43	0.16	2.66
					EPA (20:5n-3)	417	0.48 ± 0.23	0.44	0.07	1.47
					DHA (22:6n-3)	603	1.88 ± 1.14	1.48	0.33	6.86
		RBC	1989-1990	40	ALA (18:3n-3)	574	0.16 ± 0.05	0.15	0.03	0.39
					DPA (22:5n-3)	574	1.70 ± 0.33	1.67	0.87	2.82
					EPA (20:5n-3)	380	0.66 ± 0.64	0.43	0.13	3.77
					DHA (22:6n-3)	574	3.37 ± 1.05	3.20	0.87	8.55
PHS	United States	RBC	1995-2000	33	ALA (18:3n-3)	2000	0.18 ± 0.07	0.17	0.05	0.70
					DPA (22:5n-3)	2000	1.87 ± 0.48	1.91	0.20	5.05
					EPA (20:5n-3)	2000	0.58 ± 0.34	0.50	0.05	2.95
					DHA (22:6n-3)	2000	3.56 ± 1.33	3.47	0.30	11.76
SHHEC	Scotland	Adipose tissue	1984-1986	12	DPA (22:5n-3)	4391	0.26 ± 0.09	0.25	0.03	1.42
					DHA (22:6n-3)	4391	0.19 ± 0.09	0.18	0.02	1.37
SCHS	Singapore	Total plasma	1994-2005	19	ALA (18:3n-3)	1555	0.34 ± 0.28	0.28	0.09	5.95
					DPA (22:5n-3)	NA	NA	NA	NA	NA
					EPA (20:5n-3)	1555	0.48 ± 0.35	0.40	0.09	4.80
					DHA (22:6n-3)	1555	2.34 ± 1.35	1.95	0.24	12.20
Three City Study	France	Total plasma	1999-2000	12	ALA (18:3n-3)	1416	0.4 ± 0.2	0.2	0.1	3.0
					DPA (22:5n-3)	1416	1.0 ± 0.6	0.9	0.1	5.1

					EPA (20:5n-3)	1416	0.5 ± 0.2	0.5	0.2	4.0
					DHA (22:6n-3)	1416	2.4 ± 2.8	2.3	0.2	5.9
ULSAM 50	Sweden	Cholesterol esters	1970	12	ALA (18:3n-3)	2001	0.66 ± 0.16	0.64	0.23	1.77
					DPA (22:5n-3)	NA	NA	NA	NA	NA
					EPA (20:5n-3)	2001	1.35 ± 0.63	1.25	0.19	6.44
					DHA (22:6n-3)	2001	0.70 ± 0.21	0.68	0.20	2.57
ULSAM 70	Sweden	Adipose tissue	1990	17	ALA (18:3n-3)	752	1.04 ± 0.23	1.02	0.40	2.39
					DPA (22:5n-3)	722	0.28 ± 0.09	0.27	0.08	0.72
					EPA (20:5n-3)	666	0.15 ± 0.06	0.14	0.05	0.63
					DHA (22:6n-3)	728	0.34 ± 0.14	0.31	0.07	1.00

*Fasting status was 100% for ARIC, study of Costa-Rican adults, CHS, KIHD, MESA, NSHDS I, NSHDS II, 3C Study, ULSAM 50 and ULSAM 70; 71% for NHS I; 66% for MCCS. Participants were not fasting in EPIC-Norfolk at time of biomarker sampling. Fasting status was not specified for EURAMIC, HPFS, InCHIANTI, PHS, SHHEC, SCHS.

†Values are % total fatty acids (FA).

§Values for controls only.

eTable 3. Median Values (% of Total Fatty Acids) for Quintiles 1 and 5 of Biomarker α-Linolenic Acid (ALA; 18:3n-3), DocosapentaenoicAcid (DPA; 22:5n-3), EicosapentaenoicAcid (EPA; 20:5n-3), Docosahexaenoic Acid (DHA; 22:6n-3), and the Sum of EPA, DPA and DHA.*

Biomarker	Study	# FA measured	Quintile	Median for each quintile (% of total fatty acids)				
				ALA	EPA	DPA	DHA	EPA + DPA + DHA
Plasma phospholipid	ARIC	29	Q1	0.09	0.31	0.69	1.87	3.15
			Q5	0.21	0.84	1.12	3.94	5.59
	CHS	46	Q1	0.09	0.29	0.61	1.91	3.10
			Q5	0.22	1.08	1.08	4.57	6.46
	EPIC-Norfolk	22	Q1	0.14	0.57	0.97	3.32	5.24
			Q5	0.34	2.51	1.89	7.27	10.97
	MCCS	57	Q1	0.09	0.58	0.91	2.80	4.66
			Q5	0.27	1.66	1.59	5.42	8.42
	MESA	28	Q1	0.10	0.36	0.70	2.38	3.91
			Q5	0.27	1.78	1.23	6.27	9.27
	NSHDS II	16	Q1	0.13	0.90	0.91	3.37	5.61
			Q5	0.31	2.31	1.39	6.06	9.41
Erythrocyte phospholipid	NHS I	40	Q1	0.09	0.29	0.61	1.91	3.10
			Q5	0.22	1.08	1.08	4.57	6.46
	PHS	not given	Q1	0.12	0.27	1.36	2.07	4.04
			Q5	0.25	1.07	2.46	5.72	9.09
Total plasma	HPFS	not given	Q1	0.36	0.28	0.36	0.98	1.76
			Q5	0.88	1.11	0.68	2.73	4.35
	InCHIANTI	20	Q1	0.24	0.40	n/a	1.32	1.80
			Q5	0.83	0.92	n/a	3.44	4.24
	KIHD	14	Q1	0.47	0.88	0.44	1.64	3.11
			Q5	1.04	2.65	0.68	3.36	6.49
	NHS I	46	Q1	0.16	0.25	0.29	0.90	1.51
			Q5	0.64	0.79	1.86	3.77	6.14
	Three City Study	57	Q1	0.2	0.4	n/a	1.4	2.7
			Q5	0.6	1.7	n/a	3.4	5.9
	SCHS	19	Q1	0.18	0.26	n/a	1.11	1.40
			Q5	0.55	0.74	n/a	4.21	4.87
Cholesterol ester	ULSAM 50	12	Q1	0.48	0.70	n/a	0.47	1.22
			Q5	0.87	2.14	n/a	0.97	3.06

Adipose tissue	Costa-Rican adults	58	Q1	0.39	0.01	0.12	0.09	0.25
			Q5	0.92	0.07	0.25	0.21	0.51
	EURAMIC	not given	Q1	0.38	n/a	0.12	0.08	0.22
			Q5	1.31	n/a	0.35	0.42	0.77
	SHHEC	12	Q1	n/a	n/a	0.16	0.10	0.28
			Q5	n/a	n/a	0.37	0.30	0.66
	ULSAM 70	17	Q1	0.76	0.09	0.19	0.19	0.51
			Q5	1.32	0.23	0.40	0.52	1.11

*Median values of quintile 1 and 5 represent the 10^{th} and 90^{th} percentiles of exposure, respectively, in each study. Cohort medians were used for prospective studies; medians for controls were used for case-control studies. NSHDS I did not contribute categorical data to the analysis.

eTable 4. Descriptives for Continuous Covariates in Participants ≥18 Years With Measured Fatty Acid Biomarker Data.*

Study	Age (years)		Body mass index (kg/m^2)		Linoleic acid (LA) (18:2n-6)		Arachidonic acid (AA) (20:4n-6)		Total trans fatty acids	
	n	Mean ± SD (min - max)	n	Mean ± SD (min - max)	n	Mean ± SD (min - max)	n	Mean ± SD (min - max)	n	Mean ± SD (min - max)
ARIC	3793	54.5 ± 6.0 (44.0 - 66.0)	3793	26.3 ± 4.9 (14.4 - 56.3)	3793	22.0 ± 2.7 (9.0 - 32.4)	3793	11.4 ± 2.0 (5.3 - 20.0)	n/a	n/a
Costa-Rican adults	2878	58.6 ± 11.0 (18.0 - 82.0)	2878	26.2 ± 4.2 (13.5 - 49.6)	2878	15.3 ± 3.9 (5.4 - 30.9)	2878	0.5 ± 0.1 (0.1 - 1.1)	2878	3.5 ± 1.1 (1.0 - 27.9)
CHS	3941	74.4 ± 5.3 (65.0 - 97.0)	3941	26.6 ± 4.6 (14.7 - 53.2)	3941	19.7 ± 2.5 (10.6 - 28.9)	3941	11.1 ± 1.9 (5.0 - 18.9)	3941	2.22 ± 0.73 (0.1 - 4.89)
EPIC-Norfolk	5105	62.7 ± 8.4 39.0 - 78.0	5105	26.4 ± 3.6 16.3 - 49.1	5105	24.4 ± 3.4 (10.8 - 38.0)	5105	9.5 ± 1.9 (4.1 - 17.9)	5105	n/a
EURAMIC	1326	53.9 ± 9.2 (26 - 71)	1311	26.2 ± 3.6 (17.3 - 56.7)	1326	13.8 ± 4.8 (4.7 - 32.5)	1326	0.41 ± 0.17 (0 - 2.55)	1326	1.54 ± 0.90 (0 - 5.51)
HPFS-total plasma	1291	64 ± 9.0 (46 - 80)	1291	25.8 ± 3.7 (16 - 43.7)	1291	30.4 ± 4.4 (10.3-43.1)	1291	7.20 ±1.75 (2.3-13.1)	1291	2.23 ±1.17 (0.43 - 10.09)
HPFS-RBC	1342	64 ± 9.0 (46 - 80)	1342	25.8 ± 3.7 (16 - 43.7)	1342	13.2 ± 3.0 (6.4-37.0)	1342	13.0 ± 1.78 (4.04-17.61)	1342	1.71 ± 0.66 (0.45-6.29)
InCHIANTI	839	65.7 ± 15.4 (21-94)	839	27.0 ± 4.0 (18.0 - 46.6)	839	25.2 ± 3.9 (6.8-37.0)	838	8.1 ± 1.9 (2.9-15.9)	n/a	n/a
KIHD	1837	52.4 ± 5.4 (42.0 - 61.3)	1829	26.7 ± 3.5 (18.8 - 48.6)	1837	26.6 ± 4.4 (10.3 - 41.3)	1837	4.8 ± 1.0 (1.4 - 9.2)	n/a	n/a
Melbourne Collaborative Cohort Study	5479	56.1 ± 8.7 (36.0 - 75.3)	5475	27.3 ± 4.6 (15.4 - 53.0)	5278	20.0 ± 3.0 (9.7 - 31.2)	5279	10.4 ± 1.8 (5.0 - 18.1)	5279	1.0 ± 0.4 (0.1 - 4.1)
MESA	2856	61.5 ± 10.1 (44.0 - 84.0)	2856	27.9 ± 5.5 (15.4 - 54.5)	2856	21.4 ± 4.3 (11.4 - 36.1)	2856	12.0 ± 2.6 (3.6 - 21.2)	2856	0.2 ± 0.73 (0.05 - 0.68) (18:2)
Northern Sweden Health & Disease Study I	183	53.7 ± 7.6 (29.0 - 63.0)	183	26.2 ± 4.0 (19.0 - 42.9)	183	21.7 ± 2.3 (16.1 - 29.6)	183	8.3 ± 1.3 (5.4 - 12.1)	183	1.5 ± 0.5 (0.4 - 3.2) (18:1)
Northern Sweden Health & Disease Study II	759	53.6 ± 7.6 (30.0 - 77.0)	759	26.4 ± 3.9 (18.7 - 49.4)	759	21.3 ± 2.6 (14.2 - 30.3)	759	8.8 ± 1.3 (1.7 - 13.0)	n/a	n/a
NHS-I - total plasma**	603	59.9 ± 6.4 (42.9 - 69.6)	590	25.1 ± 4.3 (17.2 - 44.4)	603	26.9 ± 7.7 (1.4 - 41.3)	603	8.4 ± 3.5 (0.1 - 16.5)	603	2.3 ± 1.2 (0.7 - 12.2)
NHS-I - RBC**	574	59.9 ± 6.4 (42.9 - 69.6)	560	25.1 ± 4.3 (17.2 - 44.4)	574	13.2 ± 1.9 (7.7 - 19.3)	574	13.6 ± 1.7 (7.5 - 17.9)	574	2.0 ± 0.8 (0.7 - 5.0)

PHS	832	71.0 ± 9.4 (50.6- 92.0)	832	25.8 ± 3.6 (17.9 -46.1)	832	12.7 ± 1.7 (6.2 -20.7)	832	12.8 ± 2.2 (2.8 -18.9)	832	2.2 ± 0.6 (0.9 -5.1)
SHHEC	4391	48.7 ± 7.3 (25.0 - 65.0)	4391	25.6 ± 4.0 (15.4 - 56.2)	4391	9.2 ± 2.6 (3.3 - 27.6)	4391	0.6 ± 0.2 (0.2 - 4.3)	n/a	n/a
SCHS	1555	66.2 ± 7.8 (47.0 - 83.0)	1555	23.0 ± 3.0 (14.2 - 41.6)	1555	36.3 ± 4.6 (20.3 - 51.1)	1555	7.6 ± 1.7 (2.2 - 15.2)	1555	0.06 ± 0.08 (0 - 2.18)
Three City Study	1416	75 ± 5 (66 - 98)	1383	26 ± 4 (14 - 45)	1416	24.9 ± 5.4 (4.1 - 40.8)	1416	6.7 ± 1.9 (1.0 - 16.1)	n/a	n/a
ULSAM 50	1992	49.7 ± 0.59 (48.6 - 51.1)	1992	25.0 ± 3.2 (15.1 - 42.1)	1992	54.0 ± 5.2 (27.2 - 68.0)	1992	4.77 ± 0.94 (2.03-8.87)	n/a	n/a
ULSAM 70	763	71.0 ± 0.62 (69.4 - 73.6)	759	26.4 ± 3.5 (15.1 - 42.1)	763	12.6 ± 2.8 (5.6 - 30.0)	763	0.3 ± 0.1 (0.0-0.7)	n/a	n/a

*Fatty acid values are % total fatty acids.

**Values are provided for controls only.

eTable 5. Frequencies (%) for Categorical Covariates and Physical Activity Assessment in Participants ≥18 Years With Measured Fatty Acid Biomarker Data.

Study	Sex (male)	Race	Smoking	Alcohol	Physical activity assessment	Education	Drug-treated hypertension	Drug-treated high cholesterol	Prevalent CHD, diabetes, stroke*	Regular aspirin use
ARIC	48.0	100 (Caucasian)	22.8 (current) 40.3 (former) 36.9 (never)	grams/week	Questionnaire measuring two index scores: leisure activity with and without sports	6.4 (<HS) 34.6 (=HS) 59.0 (>HS)	17.0	n/a	4.1 (CHD) 7.2 (diabetes) 1.5 (stroke/ TIA)	n/a
Costa-Rican adults	72.8	100 (Hispanic)	30.0 (current) 70.0 (not currently)	60.8 (none) 22.9 (1-6 dr/wk) 9.6 (1-2 dr/d) 6.6 (≥2 dr/d)	Questionnaire measuring frequency and time on several occupational and leisure time activities during the past year	5.7 55.1 24.8 14.4	34.5	29.0	19.4 (diabetes); no stroke/CHD	17.7 (more than 1/wk)
CHS	40.0	87.2 (Caucasian) 12.3 (Black) 0.5 (Other)	10.9 (current) 44.1 (former) 45.0 (never)	44.2 (none) 17.4 (<1 dr/wk) 16.2 (1-3 dr/wk) 22.2 (≥3 dr/wk)	Walking habits, Minnesota Leisure-Time Activities questionnaire, exercise intensity	31.1 (<HS) 35.5 (=HS) 33.4 (>HS)	50.3	5.9	20.8 (CHD) 14.3 (diabetes) 3.9 (stroke-excludes TIA)	37.4
EPIC-Norfolk	49.4	99.8 (Caucasian)	10.3 (current) 43.8 (former) 44.9 (never)	14.7 (none) 51.2 (<1 dr/day) 18.3 (1-2 dr/day) 14.8 (≥3 dr/day)	Validated physical activity index from leisure and work physical activity questionnaire	51.1 (<HS) 38.0 (=HS) 10.8 (>HS)	19.4	1.9	0 (CHD) 0 (diabetes) 0 (stroke)	7.4
EURAMIC	100	100 (Caucasian)	17.6 (current) 32.8 (former) 49.6 (never)	grams/wk	n/a	n/a	31.8 (history of hypertension)	n/a	0 (CHD) 5.7 (diabetes) 0 (stroke)	n/a

HPFS - total plasma	100	94 (Caucasian)	40.3 (never) 46.3 (former) 8.6 (current) 4.7 (missing)	24.0 (none) 43.8 (1-6 dr/wk) 17.0 (1-2 dr/d) 14.1 (≥2 dr/d) 1.0 (missing)	Validated questionnaires; leisure-time & exercise assessed every 2y; METs recorded	n/a	32.2 (history of hypertension)	43.7 (history of high cholesterol)	0 (CHD) 5.5 (diabetes) 0 (stroke)	23.2
HPFS - RBC	100	95 (Caucasian)	41.1 (never) 45.8 (former) 8.9 (current) 4.3 (missing)	24.0 (none) 44.0 (1-6 dr/wk) 17.0 (1-2 dr/d) 14.1 (≥2 dr/d) 1.0 (missing)	Validated questionnaires; leisure-time & exercise assessed every 2y; METs recorded	n/a	32.3 (history of hypertension)	43.5 (history of high cholesterol)	0 (CHD) 5.7 (diabetes) 0 (stroke)	23.2
InCHIANTI	44.7	100 (Caucasian)	55.2 (never) 24.6 (former) 20.3 (current)	grams/day	Validated physical activity index from leisure and work physical activity questionnaire	years	27.4	4.89	0 (CHD) 10.1 (diabetes) 2.5 (stroke)	n/a
KIHD	100	100 (Caucasian)	29.8 (current) 34.2 (former) 36.0 (never)	13.0 (none) 64.8 (<1 dr/day) 13.6 (1-2 dr/day) 8.5 (≥3 dr/day)	Leisure-time survey; Frequency, duration, intensity (METs) recorded	69.7 (<HS) 20.5 (=HS) 9.7 (>HS)	14.2	0.2	0 (CHD) 4.6 (diabetes) 0 (stroke)	4.8
Melbourne Collaborative Cohort Study	48.9	100 (Caucasian)	12.8 (current) 33.5 (former) 53.7 (never)	32.1 (none) 29.4 (<1 dr/day) 21.7 (1-2 dr/day) 16.7 (≥3 dr/day)	Baseline & follow-up frequency of walking and activity; modified IPAQ questionnaire	58.1 (<HS) 17.8 (=HS) 24.1 (>HS)	25.5	not given	7.6 (CHD) 4.5 (diabetes) 1.8 (stroke)	not given
MESA	46.7	25.4 (Caucasian) 24.7 (Black) 25.0 (Chinese) 24.9 (Hispanic)	13.7 (current) 31.8 (former) 54.5 (never)	2.1 (none) 97.8 (1-6 dr/wk) <1% (>6 dr/wk)	Frequency of leisure activity and sports/ exercise; METs recorded	21.8 (<HS) 18.0 (=HS) 60.3 (>HS)	32.0	15.6	0 (CHD) 11.8 (diabetes) 0 (stroke)	17.4
Northern Sweden	79.2	Caucasian (‘vast	29.5 (current) 21.0 (former)	intakes/day of beer, wine, or	Questionnaire based, with	7.7 (>HS)	not given	not given	0 (CHD) 3.6 (diabetes)	not

Health & Disease Study I		majority')	49.4 (never)	spirits available	differences between VIP and MONICA subcohorts				0 (stroke)	given
Northern Sweden Health & Disease Study II	75.9	Caucasian ('vast majority')	29.8 (current) 25.3 (former) 44.9 (never)	intakes/day of beer, wine, or spirits available	Questionnaire based, with differences between VIP and MONICA subcohorts	13.4 (>HS)	not given	not given	0 (CHD) 7.1 (diabetes) 0 (stroke)	not given
NHS-I- total plasma**	0	99.3 (Caucasian)	26.7 (current) 36.8 (former) 36.3 (never) 0.2 (missing)	36.5 (none) 42.8 (<1 dr/day) 11.9 (1-2 dr/day) 5.3 (≥3 dr/day) 3.5 (missing)	Validated questionnaires; leisure-time & exercise assessed every 2y; METs recorded	100 (>HS)	17.3	28.9	0 (CHD) 3.7 (diabetes) 0 (stroke)	42.9 (none) 24.3 (1-4 d/mo) 32.8 (≥5 d/mo)
NHS-I- RBC**	0	99.3 (Caucasian)	26.3 (current) 36.6 (former) 36.9 (never) 0.2 (missing)	36.4 (none) 43.2 (<1 dr/day) 11.7 (1-2 dr/day) 5.2 (≥3 dr/day) 3.5 (missing)	Validated questionnaires; leisure-time & exercise every 2y; METs recorded	100 (>HS)	17.4	29.1	0 (CHD) 3.7 (diabetes) 0 (stroke)	43.9 (none) 23.5 (1-4 d/mo) 32.6 (≥5 d/mo)
PHS	100	94.0 (White/non hispanic)	3.9 (current) 46.8 (former) 49.4 (never)	28.1 (<1/week) 21.3 (1-4/week) 19.1 (5-4/week) 31.5 (7/week)	Self-reported questionnaires; Frequency of vigorous exercise	100 (>HS)	31.3 (history of hypertension)	28.6 (history of high cholesterol)	8.2	38.5 (random-ized to aspirin)
SHHEC	52.3	Caucasian ('vast majority')	44.6 (current) 55.4 (not current)	25.3 (none) 35.5 (1-6 dr/wk) 22.5 (1-2 dr/day) 16.7 (≥2 dr/day)	Not validated	2.0 (<HS) 67.7 (=HS) 30.3 (>HS)	7.7	Unknown, but rare	0 (CHD) 1.1 (diabetes) 0 (stroke/TIA)	n/a
SCHS	64.5	100 (Chinese)	28.6 (current) 15.7 (former) 55.8 (never)	77.6 (none) 18.1 (1-6 dr/wk) 2.4 (1-2 dr/day) 1.9 (≥2 dr/day)	Interviewer-administered questionnaire on moderate and vigorous	73.0 (<HS) 21.4 (=HS) 5.7 (>HS)	36.0	n/a	0 (CHD) 26.8 (diabetes) 0 (stroke)	n/a

					activity					
Three City Study	39.4	n/a	5.0 (current) 30.4 (former) 65.6 (never)	24.1 (none) 20.9 (<1 dr/day) 40.0 (1-2 dr/day) 15.0 (≥3 dr/day)	Questionnaire based; vigorous exercise and leisure time activity	62.1 (<HS) 20.8 (=HS) 17.1 (>HS)	56.0	31.1	14.3 (CHD) 10.2 (diabetes) 3.6 (stroke)	19.5
ULSAM 50	100	Caucasian	51.2 (current) 23.4 (former) 25.4 (never)	n/a	Questionnaire based; leisure-time physical activity categories	63.0 (<HS) 26.4 (=HS) 10.5 (>HS) 0.15 (missing)	4.0	15.2	0 (CHD/ stroke) 1.46 (diabetes)	not given
ULSAM 70	100	Caucasian	20.3 (current) 49.0 (former) 29.6 (never)	30.6 (<1/week) 50.1 (1-6 dr/wk) 13.7 (1-2 dr/day) 1.4 (>2 dr/day) 7.9 (missing)	Questionnaire based; leisure-time physical activity categories	56.4 (<HS) 30.0 (=HS) 13.6 (>HS)	31.6	7.2	0 (CHD/ stroke) 14.4 (diabetes)	not given

*Participants with prevalent CHD and stroke were excluded from analyses.

**Values are provided for controls only.

eTable 6. Description of Fish Oil Supplement Use at Time of Biomarker Sampling.

Study	Fish oil supplement use
ARIC	Not given, but FA were measured in 1987-1989, before use was common
CHS	4% taking fish oil supplements. Sensitivity analyses show that users are at higher risk of CHD events, and no inverse associations between exposures and outcomes were observed among supplement users. However, users comprise only 17 cases, and inclusion of such few individuals in the main analysis does not affect effect estimates for CHS
Costa Rican adults	None reported (0%)
EPIC-Norfolk	33% taking fish oil supplements
EURAMIC	Not assessed, but FA were measured in 1991-1992, before use was common
HPFS	2% taking fish oil supplements
InCHIANTI	Not given
KIHD	None reported (0%)
MCCS	Not given
MESA	Not assessed at time of FA biomarker sampling
NSHDS I	Not assessed
NSHDS II	Not assessed
NHS I	In 1990, fish oil supplementation among CHD cases=2.5% and among CHD controls=1.7%
PHS	Not given
SCHS	Not assessed
SHHEC	Not assessed, but FA were measured in 1984-1986, before use was common
Three City (3C) Study	<10 participants taking fish oil supplements
ULSAM 50	Not assessed, but FA were measured in 1970, before use was common
ULSAM 70	Not assessed, but FA were measured in 1990, before use was common

eTable 7. Relative Risks of Total CHD, Nonfatal MI, and Fatal CHD by Quintile of α-Linolenic Acid (ALA; 18:3n3), Eicosapentaenoic Acid (EPA; 20:5n3), Docosapentaenoic Acid (DPA; 22:5n3), and Docosahexaenoic Acid (DHA; 22:6n3), Using Random Effects Weights.[*]

Exposure	Quintile	Total CHD		Nonfatal MI		Fatal CHD	
ALA		I^2	*RR (95% CI)*	I^2	*RR (95% CI)*	I^2	*RR (95% CI)*
	1		1.0 (ref)		1.0 (ref)		1.0 (ref)
	2	17	1.00 (0.91-1.11)	48	0.96 (0.81-1.14)	51	1.00 (0.80-1.21)
	3	19	1.03 (0.93-1.14)	59	1.00 (0.82-1.22)	66	0.90 (0.69-1.17)
	4	40	1.01 (0.89-1.15)	65	0.94 (0.75-1.18)	77	0.94 (0.69-1.28)
	5	37	1.00 (0.88-1.14)	67	0.91 (0.71-1.17)	49	0.82 (0.64-1.06)
EPA							
	1		1.0 (ref)		1.0 (ref)		1.0 (ref)
	2	0	1.00 (0.92-1.09)	0	0.90 (0.80-1.01)	67	1.05 (0.80-1.40)
	3	0	0.95 (0.87-1.04)	27	0.85 (0.73-0.98)	68	1.10 (0.82-1.48)
	4	0	0.92 (0.84-1.01)	21	0.85 (0.73-0.98)	0	0.84 (0.72-0.97)
	5	45	0.89 (0.77-1.03)	57	0.71 (0.56-0.90)	47	0.92 (0.72-1.19)
DPA							
	1		1.0 (ref)		1.0 (ref)		1.0 (ref)
	2	0	0.98 (0.90-1.07)	0	0.94 (0.85-1.04)	31	1.03 (0.84-1.27)
	3	0	0.96 (0.88-1.05)	34	0.92 (0.81-1.08)	0	0.87 (0.75-1.02)
	4	4	0.92 (0.84-1.02)	0	0.89 (0.80-1.00)	28	0.95 (0.77-1.18)
	5	0	0.93 (0.85-1.02)	0	0.90 (0.80-1.01)	0	0.76 (0.65-0.90)
DHA							
	1		1.0 (ref)		1.0 (ref)		1.0 (ref)
	2	0	0.95 (0.88-1.03)	0	0.91 (0.83-1.01)	33	0.99 (0.83-1.19)
	3	18	0.97 (0.88-1.07)	0	0.93 (0.84-1.03)	25	0.89 (0.75-1.06)
	4	0	0.90 (0.83-0.98)	0	0.87 (0.79-0.97)	0	0.90 (0.79-1.03)
	5	20	0.91 (0.82-1.02)	0	0.87 (0.78-0.97)	0	0.77 (0.64-0.89)

[*]Quintile estimates were pooled using random effects meta-analysis.

**eTable 7 (continued). Relative Risks of Total CHD, Nonfatal MI, and Fatal CHD by Quintile of α-Linolenic Acid (ALA; 18:3n3), Eicosapentaenoic Acid (EPA; 20:5n3), Docosapentaenoic Acid (DPA; 22:5n3), and Docosahexaenoic Acid (DHA; 22:6n3), Using Inverse-Variance Weights.*

Exposure	Quintile	Total CHD		Nonfatal MI		Fatal CHD	
ALA		I^2	*RR (95% CI)*	I^2	*RR (95% CI)*	I^2	*RR (95% CI)*
	1		1.0 (ref)		1.0 (ref)		1.0 (ref)
	2	17	0.99 (0.91-1.08)	48	0.91 (0.81-1.01)	51	1.01 (0.88-1.15)
	3	19	1.01 (0.93-1.10)	59	0.94 (0.84-1.05)	66	0.89 (0.78-1.01)
	4	40	0.97 (0.89-1.06)	65	0.84 (0.74-0.94)	77	0.80 (0.70-0.91)
	5	37	0.95 (0.87-1.04)	67	0.83 (0.73-0.94)	49	0.82 (0.71-0.96)
EPA							
	1		1.0 (ref)		1.0 (ref)		1.0 (ref)
	2	0	0.99 (0.92-1.09)	0	0.90 (0.80-1.01)	69	0.99 (0.86-1.13)
	3	0	0.95 (0.87-1.04)	22	0.85 (0.76-0.96)	68	1.03 (0.90-1.19)
	4	0	0.92 (0.84-1.01)	22	0.86 (0.76-0.97)	0	0.84 (0.72-0.97)
	5	45	0.90 (0.82-0.99)	63	0.77 (0.67-0.87)	47	0.87 (0.74-1.02)
DPA							
	1		1.0 (ref)		1.0 (ref)		1.0 (ref)
	2	0	0.98 (0.90-1.07)	0	0.94 (0.85-1.04)	31	0.99 (0.85-1.15)
	3	0	0.97 (0.88-1.06)	28	0.94 (0.85-1.05)	0	0.88 (0.75-1.02)
	4	4	0.92 (0.84-1.01)	0	0.89 (0.80-0.99)	28	0.92 (0.79-1.08)
	5	0	0.92 (0.84-1.02)	0	0.90 (0.80-1.01)	0	0.76 (0.65-0.90)
DHA							
	1		1.0 (ref)		1.0 (ref)		1.0 (ref)
	2	0	0.95 (0.88-1.03)	0	0.91 (0.83-1.01)	33	0.95 (0.83-1.08)
	3	18	0.96 (0.88-1.03)	0	0.93 (0.85-1.03)	25	0.87 (0.76-0.99)
	4	0	0.90 (0.83-0.98)	0	0.87 (0.79-0.97)	0	0.90 (0.79-1.04)
	5	20	0.91 (0.83-0.99)	0	0.87 (0.79-0.97)	0	0.77 (0.66-0.89)

* Quintile estimates were pooled using inverse-variance meta-analysis.

eTable 8. Relative Risk (95% CI) of Incident Total CHD, Nonfatal MI, and Fatal CHD by ω-3 Fatty Acid Biomarker, According to Prespecified Potential Sources of Heterogeneity[1]

	ALA			EPA		
Sources	Total CHD	Nonfatal MI	Fatal CHD	Total CHD	Nonfatal MI	Fatal CHD
Age (y)						
<60	1.05(0.89-1.22)	0.99(0.90-1.10)	1.08(0.86-1.37)	1.03(0.95-1.11)	1.01(0.92-1.11)	1.02(0.88-1.17)
≥60	0.92(0.83-1.02)	0.92(0.85-1.02)	0.88(0.83-0.96)	0.89(0.80-1.00)	1.04(0.95-1.14)	0.88(0.80-0.97)
$P_{(heterogeneity)}$[2]	0.76	0.46	0.32	0.28	0.79	0.17
Sex						
Males	0.92(0.81-1.04)	0.95(0.83-1.09)	0.86(0.78-0.94)	1.01(0.95-1.08)	1.04(0.97-1.12)	0.92(0.84-1.01)
Females	0.97(0.82-1.15)	0.98(0.87-1.11)	0.94(0.81-1.08)	0.92(0.82-1.03)	1.01(0.91-1.13)	0.90(0.74-1.09)
$P_{(heterogeneity)}$	0.60	0.82	0.40	0.47	0.86	0.84
Race						
Caucasian	0.94(0.87-1.01)	0.97(0.91-1.03)	0.87(0.81-0.95)	0.99(0.94-1.04)	1.04(0.98-1.10)	0.91(0.84-0.99)
Black	0.71(0.24-1.98)	0.68(0.18-2.60)	0.57(0.12-2.32)	0.78(0.40-1.55)	0.66(0.17-2.56)	0.52(0.13-1.99)
Hispanic	0.85(0.65-1.12)	1.19(1.03-1.35)	1.09(0.70-1.66)	0.71(0.23-2.25)	0.52(0.11-2.31)	0.19(0.01-3.36)
Chinese	0.83(0.52-1.33)	0.83(0.46-1.48)	0.70(0.26-1.87)	0.41(0.22-0.60)	0.46(0.22-0.71)	0.33(0.13-0.85)
$P_{(heterogeneity)}$	All > 0.28	All > 0.07	All > 0.22	0.02 (Chinese)	0.01 (Chinese)	All > 0.06
LA (%TFA)						
<median	0.91(0.80-1.02)	0.86(0.79-0.95)	0.92(0.84-1.01)	1.03(0.98-1.08)	1.03(0.97-1.10)	0.85(0.70-1.02)
≥median	1.25(0.84-1.87)	1.02(0.92-1.13)	0.98(0.88-1.12)	0.89(0.79-1.02)	0.98(0.89-1.08)	1.10(0.89-1.23)
$P_{(heterogeneity)}$	0.44	0.26	0.59	0.22	0.92	0.31
AA (%TFA)						
<median	0.99(0.86-1.16)	0.95(0.88-1.02)	0.98(0.91-1.06)	1.02(0.96-1.08)	1.04(0.97-1.12)	0.89(0.69-1.14)
≥median	0.97(0.90-1.05)	0.96(0.83-1.12)	0.83(0.75-0.93)	0.90(0.79-1.04)	1.01(0.93-1.09)	0.98(0.89-1.08)
$P_{(heterogeneity)}$	0.83	0.93	0.09	0.28	0.75	0.56
Diabetes						
Yes	1.00(0.76-1.28)	0.96(0.64-1.34)	0.80(0.60-1.08)	1.19(1.01-1.37)	0.95(0.87-1.34)	0.80(0.60-1.08)
No	0.97(0.93-1.02)	0.95(0.89-1.00)	0.95(0.83-1.09)	0.93(0.97-1.12)	1.03(0.97-1.09)	0.97(0.91-1.04)
$P_{(heterogeneity)}$	0.77	0.95	0.34	0.55	0.55	0.20
Statin use						
Yes	1.17(0.41-3.32)	2.53(0.41-15.8)	0.11(0.01-325)	0.82(0.48-1.40)	0.59(0.19-1.85)	1.08(0.93-1.26)
No	0.95(0.89-1.01)	0.95(0.86-1.06)	0.88(0.81-0.97)	1.00(0.94-1.06)	1.03(0.97-1.10)	0.93(0.84-1.02)
$P_{(heterogeneity)}$	0.70	0.79	0.78	0.60	0.58	0.65

Aspirin use						
Yes	0.80(0.67-0.95)	0.82(0.65-1.04)	0.92(0.83-1.00)	0.89(0.76-1.04)	0.96(0.78-1.18)	0.92(0.74-1.18)
No	0.98(0.92-1.04)	0.98(0.91-1.05)	0.91(0.84-0.98)	1.02(0.97-1.08)	1.05(0.99-1.12)	1.05(0.99-1.12)
$P_{(heterogeneity)}$	0.12	0.21	0.90	0.33	0.49	0.42
LC-PUFA (%TFA)	1.01(0.94-1.06)	0.94(0.83-1.07)	0.98(0.88-1.09)	n/a	n/a	n/a
<median	0.95(0.88-1.02)	0.92(0.85-1.00)	0.87(0.80-0.95)	n/a	n/a	n/a
≥median	0.53	0.82	0.52			
$P_{(heterogeneity)}$						
Baseline year						
<2000	1.03(0.97-1.09)	0.94(0.77-1.13)	0.95(0.82-1.09)	0.95(0.86-1.04)	0.91(0.80-1.03)	0.89(0.76-1.05)
>2000	0.96(0.89-1.03)	0.95(0.87-1.04)	0.87(0.82-0.93)	0.85(0.60-1.20)	0.85(0.62-1.15)	0.88(0.81-0.98)
$P_{(heterogeneity)}$	0.47	0.78	0.30	0.32	0.64	0.80

	DPA			DHA		
Sources	Total CHD	Nonfatal MI	Fatal CHD	Total CHD	Nonfatal MI	Fatal CHD
Age (y)						
<60	0.93(0.86-1.02)	0.95(0.86-1.06)	0.92(0.79-1.07)	0.96(0.87-1.06)	0.93(0.85-1.03)	0.92(0.79-1.06)
≥60	0.94(0.88-1.01)	1.00(0.92-1.10)	0.87(0.80-0.95)	0.92(0.86-0.98)	0.97(0.89-1.06)	0.88(0.80-0.96)
$P_{(heterogeneity)}$[2]	0.94	0.52	0.55	0.55	0.68	0.67
Sex						
Males	0.95(0.88-1.02)	0.97(0.90-1.05)	0.91(0.80-1.02)	0.96(0.88-1.05)	0.96(0.89-1.04)	0.91(0.84-0.99)
Females	0.90(0.80-1.01)	0.96(0.84-1.11)	0.86(0.73-1.02)	0.92(0.84-1.01)	0.95(0.85-1.06)	0.84(0.72-0.98)
$P_{(heterogeneity)}$	0.63	0.90	0.42	0.57	0.77	0.52
Race						
Caucasian	0.94(0.89-0.99)	0.98(0.91-1.04)	0.89(0.83-0.96)	0.93(0.89-0.98)	0.96(0.90-1.02)	0.88(0.82-0.95)
Black	1.13(0.36-3.54)	0.97(0.12-8.03)	0.57(0.14-2.30)	0.83(0.68-1.02)	0.86(0.61-1.22)	0.77(0.55-1.09)
Hispanic	0.51(0.06-4.33)	0.25(0.02-2.70)	0.10(0.01-3.49)	1.14(0.79-1.65)	1.08(0.71-1.66)	1.32(0.77-2.26)
Chinese	0.10(0.01-3.91)	0.03(0.01-2.82)	0.17(0.01-14.3)	0.81(0.72-0.91)	0.85(0.75-0.98)	0.71(0.55-0.92)
$P_{(heterogeneity)}$	All > 0.26	All > 0.22	All > 0.28	All > 0.46	All > 0.50	All > 0.44
LA (%TFA)						
<median	0.94(0.88-0.99)	1.00(0.93-1.08)	0.83(0.76-0.91)	0.94(0.89-1.00)	0.96(0.89-1.04)	0.92(0.83-1.00)
≥median	0.95(0.88-1.03)	0.94(0.84-1.05)	1.02(0.91-1.14)	0.95(0.88-1.03)	0.94(0.85-1.03)	0.90(0.82-0.99)
$P_{(heterogeneity)}$	0.90	0.47	0.05	0.88	0.76	0.88

AA (%TFA)						
<median	0.90(0.70-1.12)	0.96(0.87-1.06)	0.86(0.77-0.96)	0.97(0.92-1.04)	0.97(0.91-1.03)	0.95(0.87-1.03)
≥median	0.97(0.88-1.06)	1.03(0.95-1.12)	0.92(0.83-1.02)	0.98(0.93-1.05)	0.95(0.89-1.01)	0.88(0.82-0.94)
$P_{(heterogeneity)}$	0.52	0.82	0.54	0.92	0.87	0.35
Diabetes						
Yes	0.93(0.76-1.15)	0.98(0.88-1.11)	0.85(0.68-1.06)	0.98(0.84-1.16)	1.00(0.82-1.24)	0.94(0.77-1.16)
No	0.96(0.91-1.01)	1.00(0.93-1.08)	0.90(0.83-0.98)	0.95(0.90-1.00)	0.96(0.91-1.02)	0.87(0.80-1.05)
$P_{(heterogeneity)}$	0.59	0.82	0.68	0.76	0.89	0.76
Statin use						
Yes	0.82(0.48-1.40)	0.36(0.05-2.66)	0.25(0.02-3.00)	0.87(0.71-1.06)	0.72(0.48-1.07)	1.12(0.81-1.56)
No	0.94(0.89-1.00)	0.99(0.92-1.05)	0.86(0.79-0.94)	0.94(0.89-1.00)	0.98(0.91-1.05)	0.85(0.78-0.93)
$P_{(heterogeneity)}$	0.52	0.55	0.40	0.52	0.36	0.38
Aspirin use						
Yes	0.85(0.72-1.00)	1.03(0.81-1.31)	0.74(0.61-0.90)	0.84(0.70-0.99)	0.85(0.66-1.09)	1.02(0.84-1.23)
No	0.95(0.90-1.01)	0.98(0.91-1.05)	0.90(0.83-0.98)	0.95(0.89-1.00)	0.97(0.92-1.05)	0.87(0.80-0.95)
$P_{(heterogeneity)}$	0.30	0.66	0.25	0.28	0.25	0.23
LC-PUFA						
(%TFA)	n/a	n/a	n/a	n/a	n/a	n/a
<median	n/a	n/a	n/a	n/a	n/a	n/a
≥median						
$P_{(heterogeneity)}$						
Baseline year						
<2000	0.95(0.90-1.01)	0.97(0.92-1.03)	0.94(0.86-1.02)	0.97(0.92-1.02)	0.96(0.90-1.02)	0.91(0.84-0.99)
>2000	0.91(0.79-1.04)	0.89(0.70-1.14)	0.84(0.67-1.06)	0.92(0.86-0.97)	0.95(0.89-1.03)	0.86(0.78-0.94)
$P_{(heterogeneity)}$	0.64	0.40	0.28	0.45	0.84	0.40

[1]Relative risks and 95% CI (per 1SD unit increment) for each study were pooled using random effects meta-analysis. Study-specific median subgroup estimates were used, unless otherwise indicated.

[2]*P*-heterogeneity for a given exposure-outcome pairing was obtained using meta-regression of the potential effect modifier (dichotomous) in models including all subgroup risk estimates for the given effect modifier. For sources of heterogeneity with ≥3 subgroups, p-heterogeneity from meta-regression was obtained for each indicator category relative to a chosen reference category (the first listed category).

eTable 9. Genotype Ascertainment for Studies Contributing to SNP Analysis.

Study	CHS	ULSAM 50 & 70	Three City (3C) Study	Costa-Rican study	MESA	SCHS
Array type	Illumina 370 CNV Beadchip Platform	Illumina GoldenGate assay	Illumina Human610-Quad BeadChips	SNPlex genotyping system	Affy 6.0	Illumina Omni Zhong Hua-8 Bead
Genotype calling	Beadstudio	BeadStudio-v3.2	BeadStudio	Genemapper	Birdseed monomorphic SNPs observed heterozygosity > 53%, missing rate > 5%, all unresolved gender mismatches, cryptic duplicates, and call rate < 95%	Beadstudio v2011.1 and genotyping module v1.9.4.
QC filters for genotyped SNPs used for imputation	Call rate <96% HWE p value <10^-5	No imputation	No imputation	n/a	95%	Call rate <95%
Imputation software	BIMBAM	No imputation	No imputation	n/a	IMPUTE version 2.2.2	HWE p value <10^-6
Imputation backbone	phased CEU haplotypes, HapMap release 22 (build 36)	No imputation	No imputation	n/a	1,000 Genomes haplotypes release: -1,000 Genomes Phase I integrated variant set (NCBI build 37 / hg19)	MAF < 0.01
Filtering of imputed genotypes?	none	No imputation	No imputation	n/a	none	Impute v2.2
SNP: rs174546						
Minor allele frequency (MAF)	0.32	0.35 (ULSAM 50) 0.36 (ULSAM 70)	0.2951	not genotyped	0.33	HapMap r22 (build 36) JPT+CHB

Coded allele	T	T	T		T	n/a
Imputation quality (1= same as genotyped)	1	No imputation	No imputation		0.999	0.34
SNP: rs968567						
Minor allele frequency (MAF)	0.17	0.15 (ULSAM 50) 0.13 (ULSAM 70)	0.1361	0.08	0.17	T
Coded allele	T	A	T		T	n/a (genotyped)
Imputation quality (1= same as genotyped)	0.98	No imputation	No imputation	1	0.97	Not available; no variation was observed at this SNP

eTable 10. Interaction of ω-3 Fatty Acid Biomarkers With Selected SNPs in *FADS* Genes for Incident Total CHD, Nonfatal MI, and Fatal CHD.*

		ALA			EPA			DPA			DHA		
SNP	# T	Total CHD	Nonfatal MI	Fatal CHD	Total CHD	Nonfatal MI	Fatal CHD	Total CHD	Nonfatal MI	Fatal CHD	Total CHD	Nonfatal MI	Fatal CHD
174-546	0	0.95 (0.80-1.12)	0.89 (0.71-1.12)	1.03 (0.80-1.34)	1.14 (0.99-1.30)	1.02 (0.84-1.24)	1.16 (0.93-1.46)	1.05 (0.81-1.36)	1.20 (0.85-1.70)	0.65 (0.43-0.97)	1.13 (0.92-1.27)	1.19 (1.01-1.80)	1.05 (0.86-1.29)
	1	0.99 (0.89-1.10)	1.01 (0.82-1.26)	1.02 (0.86-1.21)	1.09 (0.99-1.20)	0.82 (0.67-1.01)	1.13 (0.94-1.36)	1.07 (0.89-1.29)	0.80 (0.53-1.20)	0.92 (0.68-1.23)	1.11 (1.01-1.22)	1.00 (0.84-1.18)	1.07 (0.90-1.27)
	2	1.04 (0.83-1.30)	1.13 (0.76-1.70)	1.00 (0.73-1.37)	0.98 (0.80-1.21)	0.70 (0.48-1.01)	1.05 (0.76-1.45)	1.08 (0.80-1.44)	0.53 (0.25-1.13)	1.30 (0.85-1.97)	1.10 (0.92-1.32)	0.84 (0.61-1.16)	1.07 (0.80-1.42)
968-567	0	1.01 (0.89-1.15)	0.90 (0.71-1.13)	1.00 (0.82-1.23)	1.10 (0.98-1.24)	0.96 (0.79-1.16)	1.21 (1.00-1.47)	1.05 (0.82-1.35)	1.05 (0.70-1.59)	0.76 (0.51-1.12)	1.10 (0.99-1.23)	1.13 (0.96-1.34)	1.14 (0.94-1.38)
	1	0.88 (0.73-1.05)	0.95 (0.72-1.26)	0.92 (0.73-1.26)	1.05 (0.89-1.24)	0.81 (0.59-1.11)	1.00 (0.75-1.33)	1.05 (0.84-1.31)	0.80 (0.44-1.46)	1.00 (0.63-1.60)	1.12 (0.98-1.27)	0.95 (0.73-1.24)	1.02 (0.79-1.33)
	2	0.76 (0.54-1.09)	0.74 (0.48-1.15)	0.91 (0.53-1.57)	1.00 (0.72-1.40)	0.62 (0.34-1.15)	0.81 (0.47-1.39)	1.03 (0.65-1.64)	0.67 (0.24-1.92)	1.30 (0.54-3.12)	1.14 (0.87-1.50)	0.85 (0.52-1.41)	0.89 (0.53-1.48)

*Data are pooled RR ± 95% CI, representing the RR change per each additional T allele of SNPs rs174546 and rs968567 for each exposure-outcome pairing. Interaction terms for each SNP were constructed by creating a cross-product term of the omega-3 fatty acid exposure of interest (continuous) by the SNP (ordinal; 0, 1, or 2 T alleles) and added to the fully adjusted model: $S(x)=\exp(\beta\ n3\ +\ \beta\ SNP + \beta\ n3 \times SNP + covariates)$; for more details, see Appendix (pg 38). Interaction terms were pooled using random effects meta-analysis.

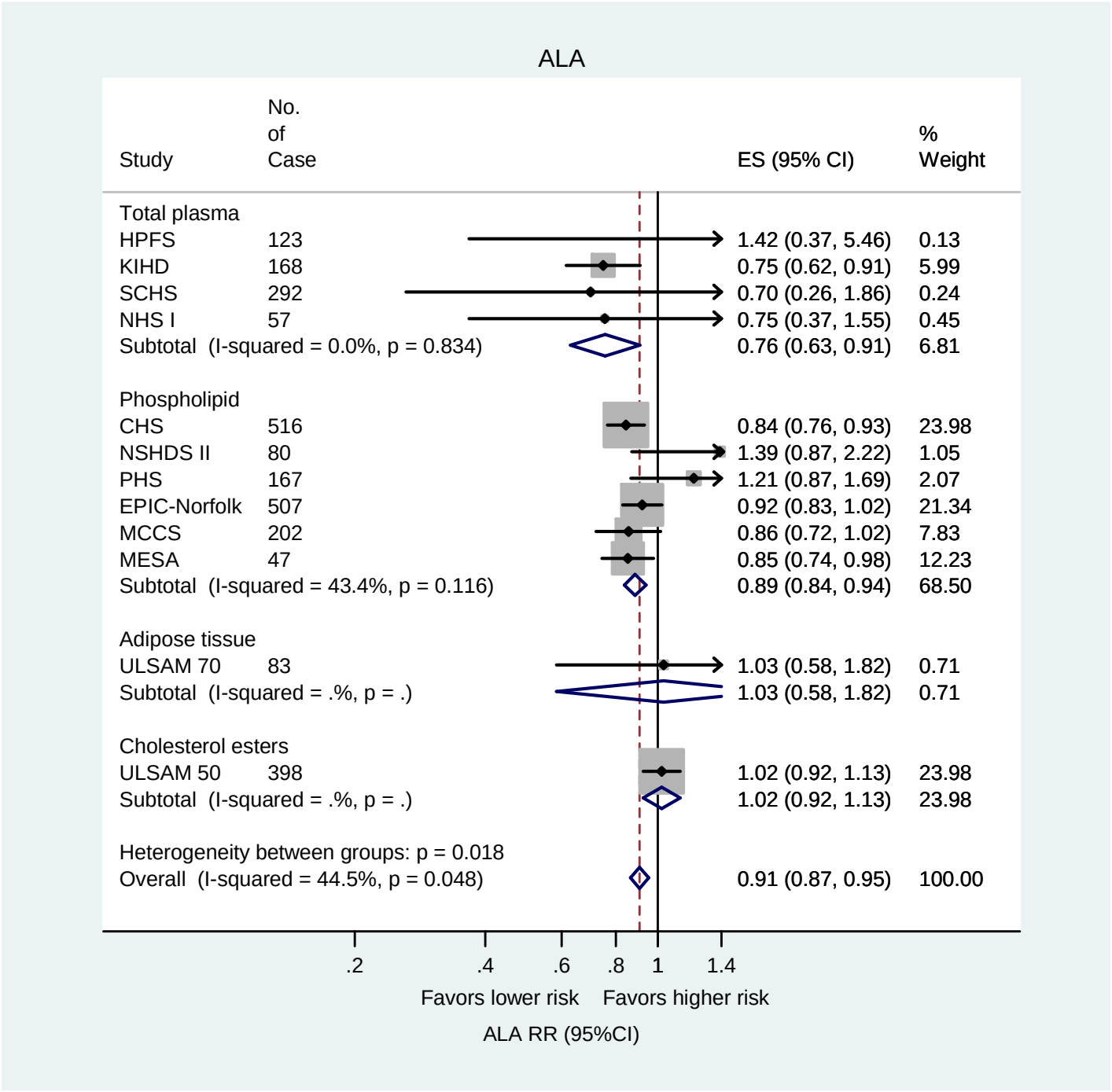
eTable 11. Sensitivity Analyses for the Association of ω-3 Fatty Acid Biomarkers (RR [95% CI] per 1-SD Increase) and Incidence of Total CHD, Nonfatal MI, and Fatal CHD.

	ALA			EPA			DPA			DHA		
Analysis	Total CHD	Nonfatal MI	Fatal CHD	Total CHD	Nonfatal MI	Fatal CHD	Total CHD	Nonfatal MI	Fatal CHD	Total CHD	Nonfatal MI	Fatal CHD
Main analysis	1.00 (0.95-1.05)	0.95 (0.87-1.05)	0.91 (0.84-0.98)	0.94 (0.87-1.02)	0.92 (0.83-1.01)	0.91 (0.82-1.00)	0.94 (0.90-0.99)	0.97 (0.93-1.02)	0.90 (0.85-0.96)	0.95 (0.91-1.00)	0.96 (0.92-1.01)	0.90 (0.84-0.96)
Exclude cases ≤2yr baseline*	1.01 (0.95-1.07)	0.96 (0.87-1.06)	0.91 (0.84-0.99)	0.93 (0.86-1.02)	0.93 (0.84-1.03)	0.91 (0.80-1.02)	0.95 (0.90-1.00)	0.98 (0.91-1.01)	0.91 (0.85-0.97)	0.95 (0.90-1.01)	0.96 (0.91-1.02)	0.90 (0.84-0.97)
Censor at yr 6 of follow-up**	0.99 (0.94-1.05)	0.98 (0.89-1.07)	0.91 (0.84-0.99)	0.93 (0.86-1.02)	0.92 (0.82-1.02)	0.91 (0.82-1.00)	0.96 (0.89-1.03)	0.95 (0.89-1.02)	0.91 (0.85-0.98)	0.93 (0.89-0.99)	0.95 (0.92-0.99)	0.90 (0.83-0.95)
Prospective studies only	1.00 (0.95-1.05)	0.98 (0.90-1.06)	0.91 (0.84-0.98)	0.94 (0.87-1.02)	0.93 (0.84-1.03)	0.91 (0.82-1.00)	0.94 (0.90-0.99)	0.97 (0.91-1.00)	0.90 (0.85-0.96)	0.95 (0.91-1.00)	0.96 (0.92-1.00)	0.90 (0.84-0.96)
Exclude self-report events	0.97 (0.92-1.02)	0.93 (0.85-1.03)	0.90 (0.83-0.97)	0.92 (0.83-1.01)	0.92 (0.82-1.02)	0.89 (0.78-1.01)	0.95 (0.90-1.00)	0.98 (0.93-1.03)	0.88 (0.82-0.94)	0.94 (0.89-0.99)	0.96 (0.91-1.00)	0.88 (0.82-0.95)

*Cases identified in first 2 years after biomarker sampling were excluded in main (continuous) models to minimize the effect of reverse causation due to a pre-existing health condition

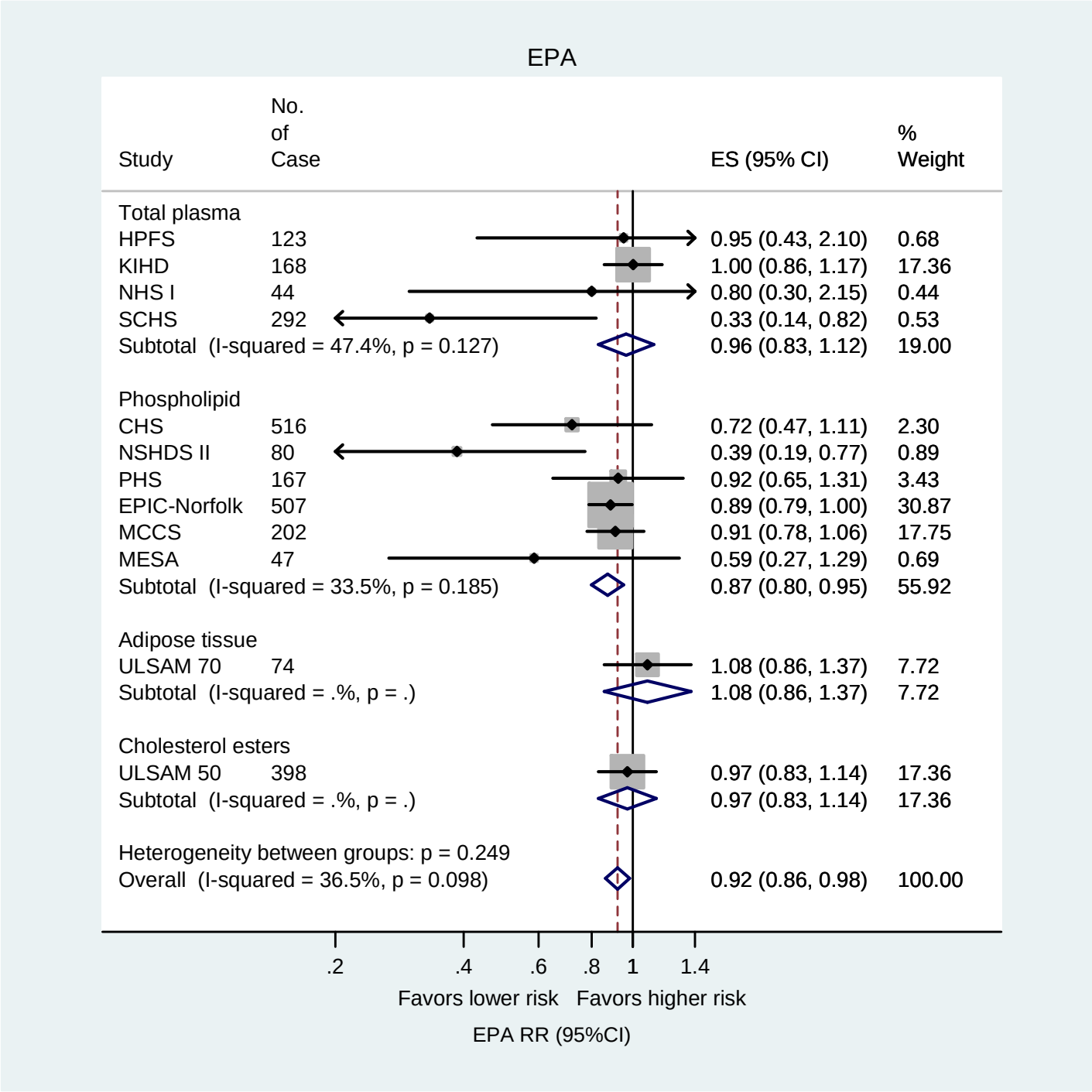
**Participants were censored in main (continuous) models at the first 6 years of follow-up to minimize exposure misclassification due to within-person variation over time.

eFigure 1. Relative Risk of Fatal CHD per 1-SD Increase in Biomarker ALA, EPA, DPA, and DHA.



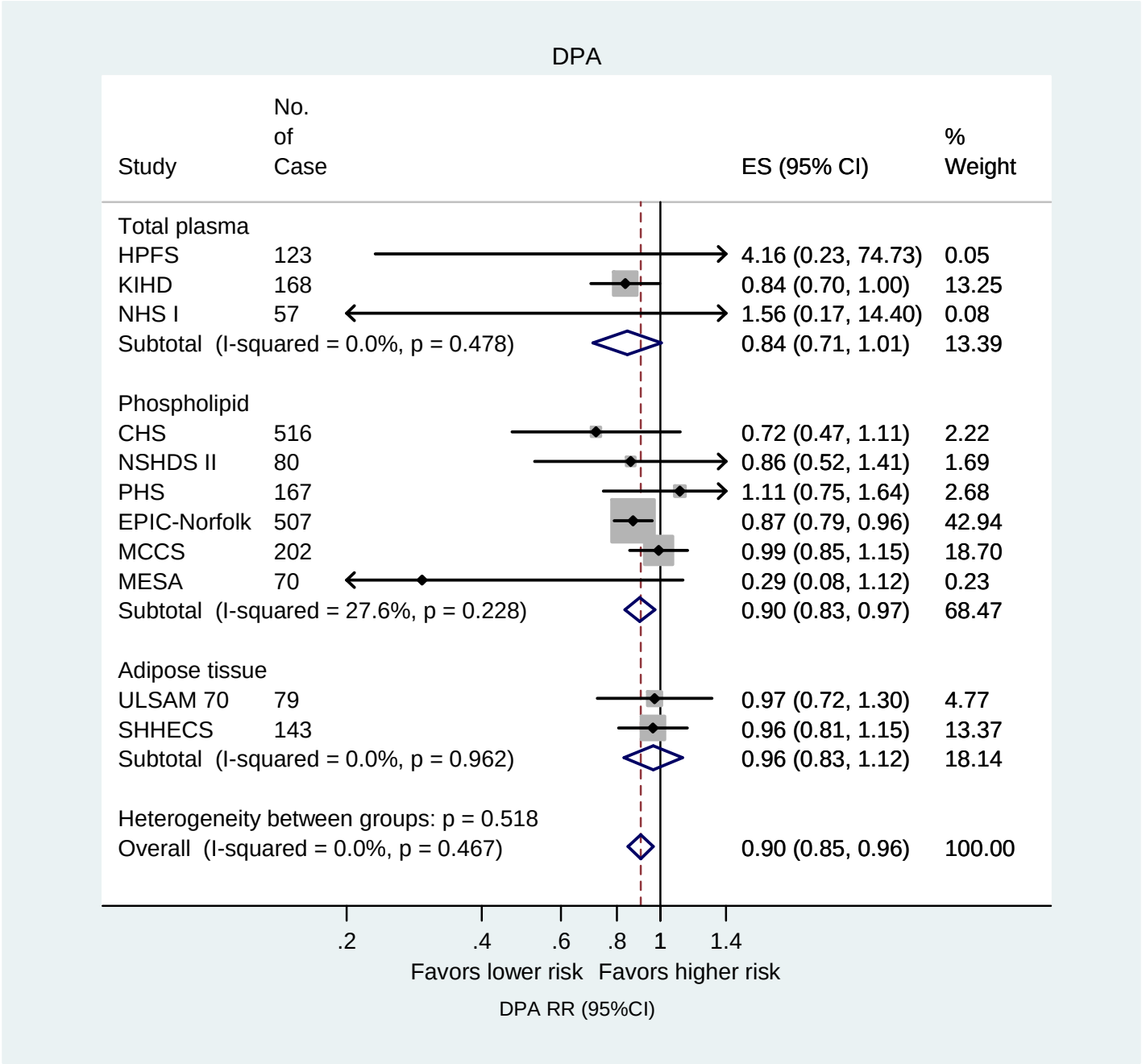
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 1. (continued)



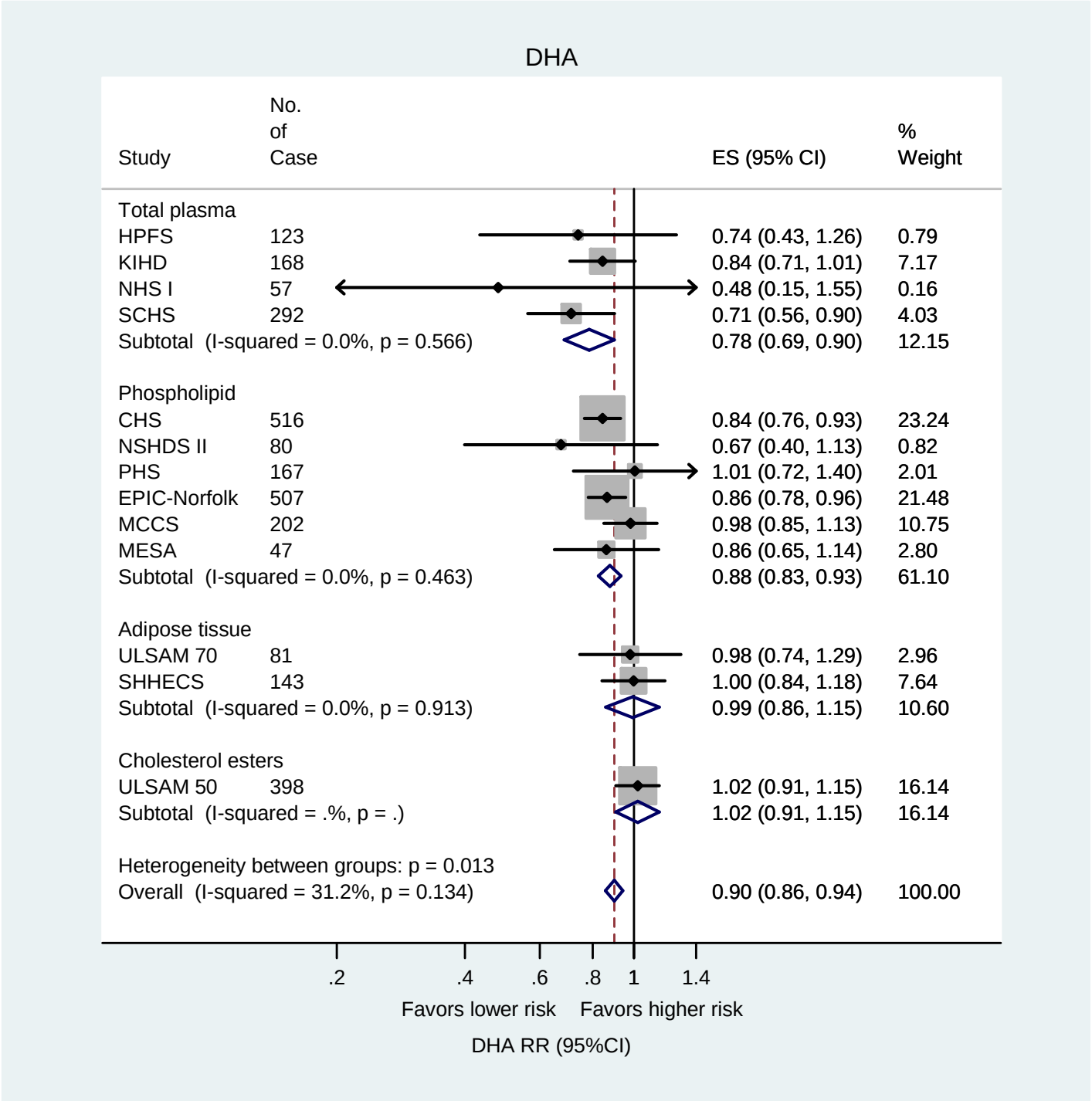
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 1. (continued)



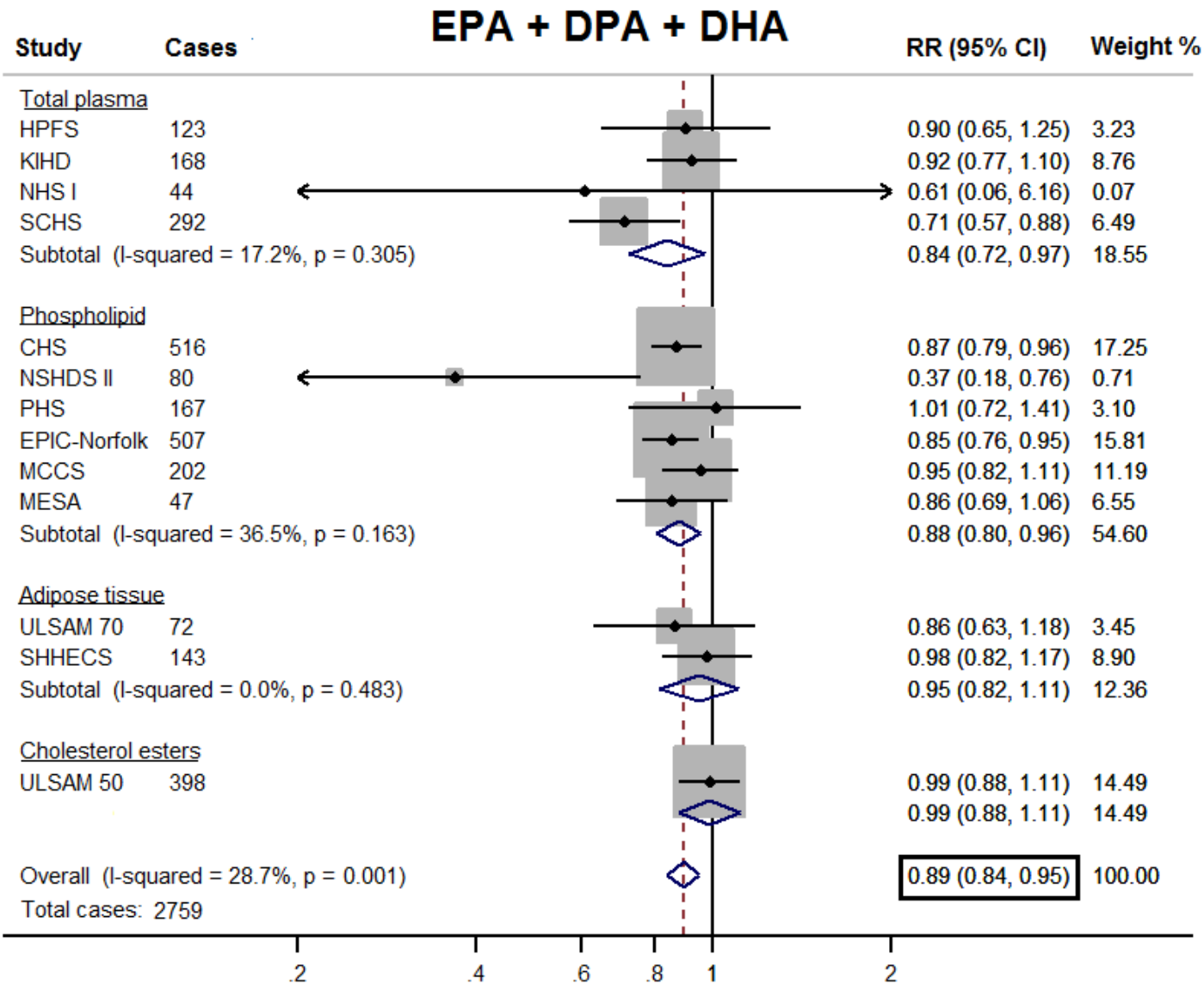
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 1. (continued)



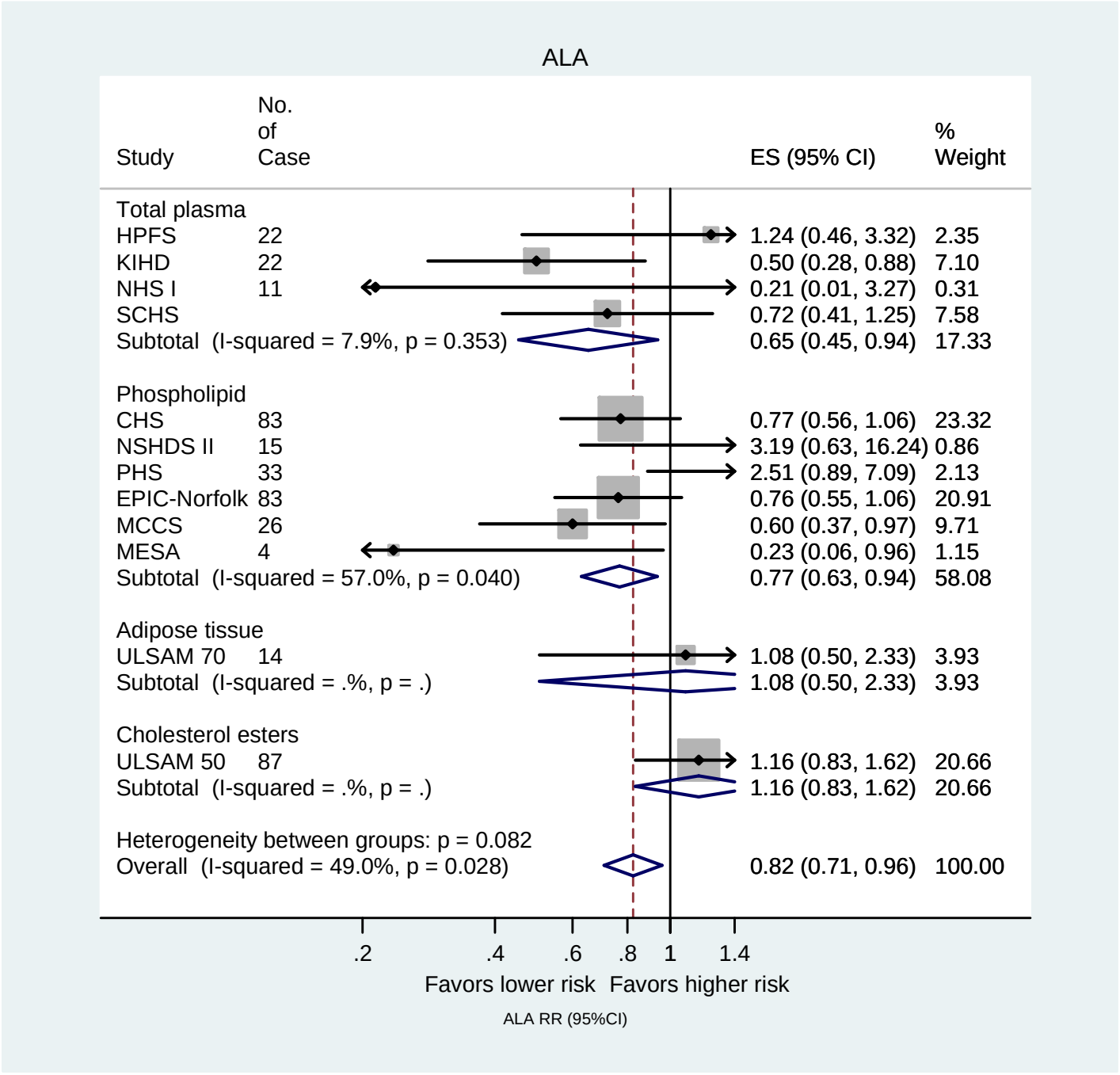
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 2. Relative Risk (RR) of Fatal CHD per 1-SD Increase in Biomarker EPA, DPA, and DHA.



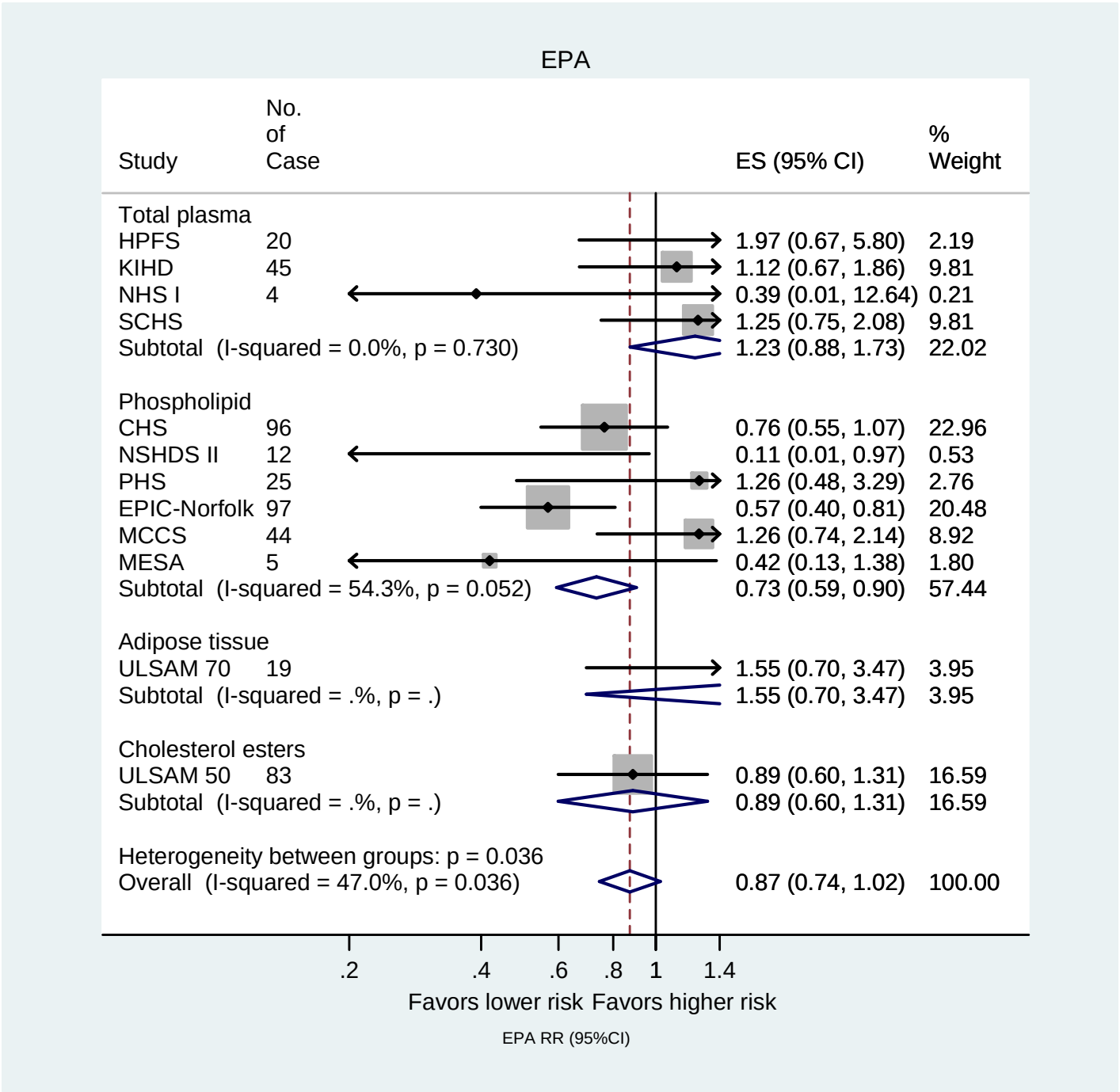
Continuous estimates were pooled using random effects meta-analysis.

eFigure 3. Relative Risk of Fatal CHD in Highest vs. Lowest Quintiles of Biomarker ALA, EPA, DPA, and DHA



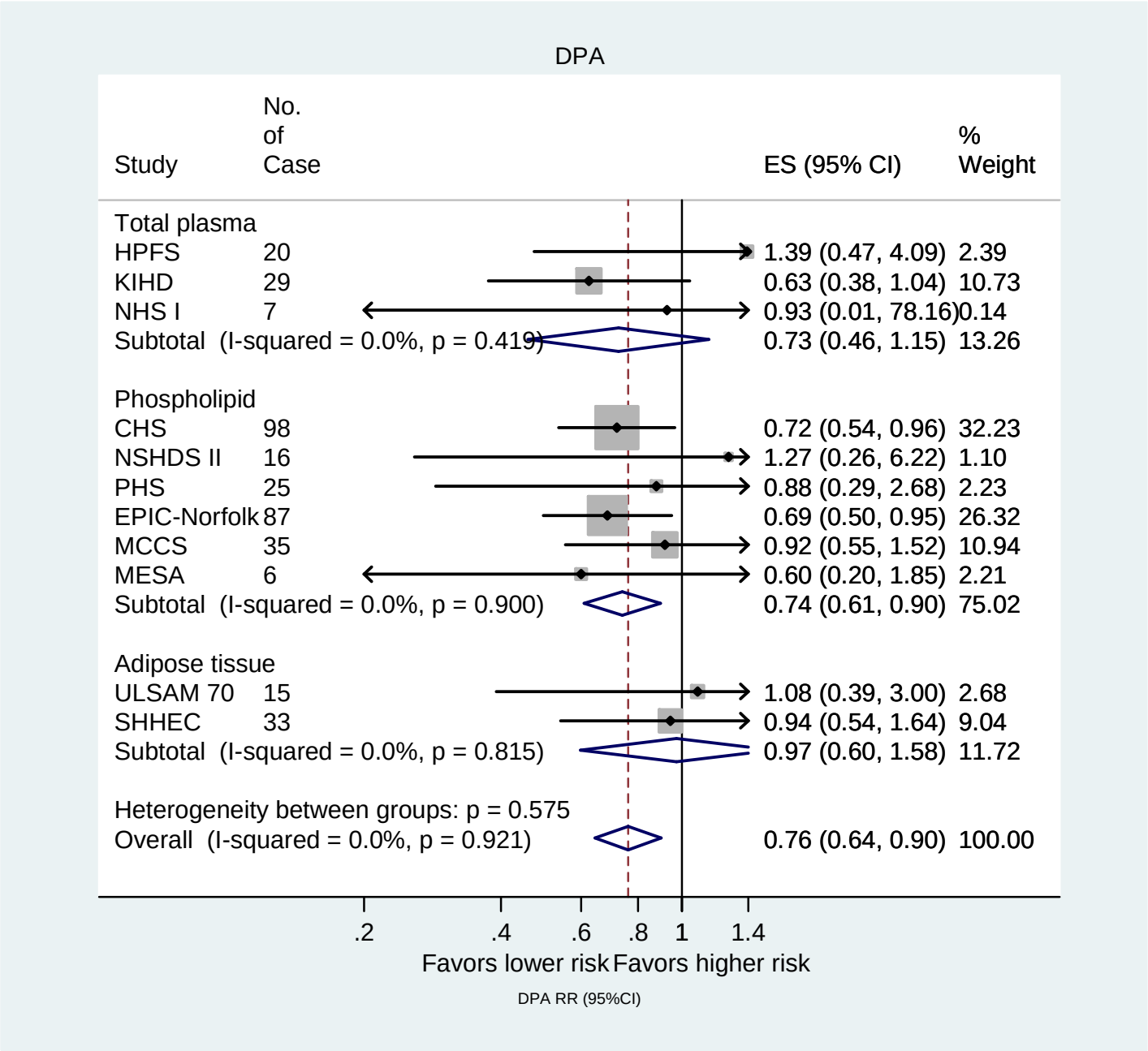
Quintile estimates were pooled using inverse-variance meta-analysis.

eFigure 3. (continued)



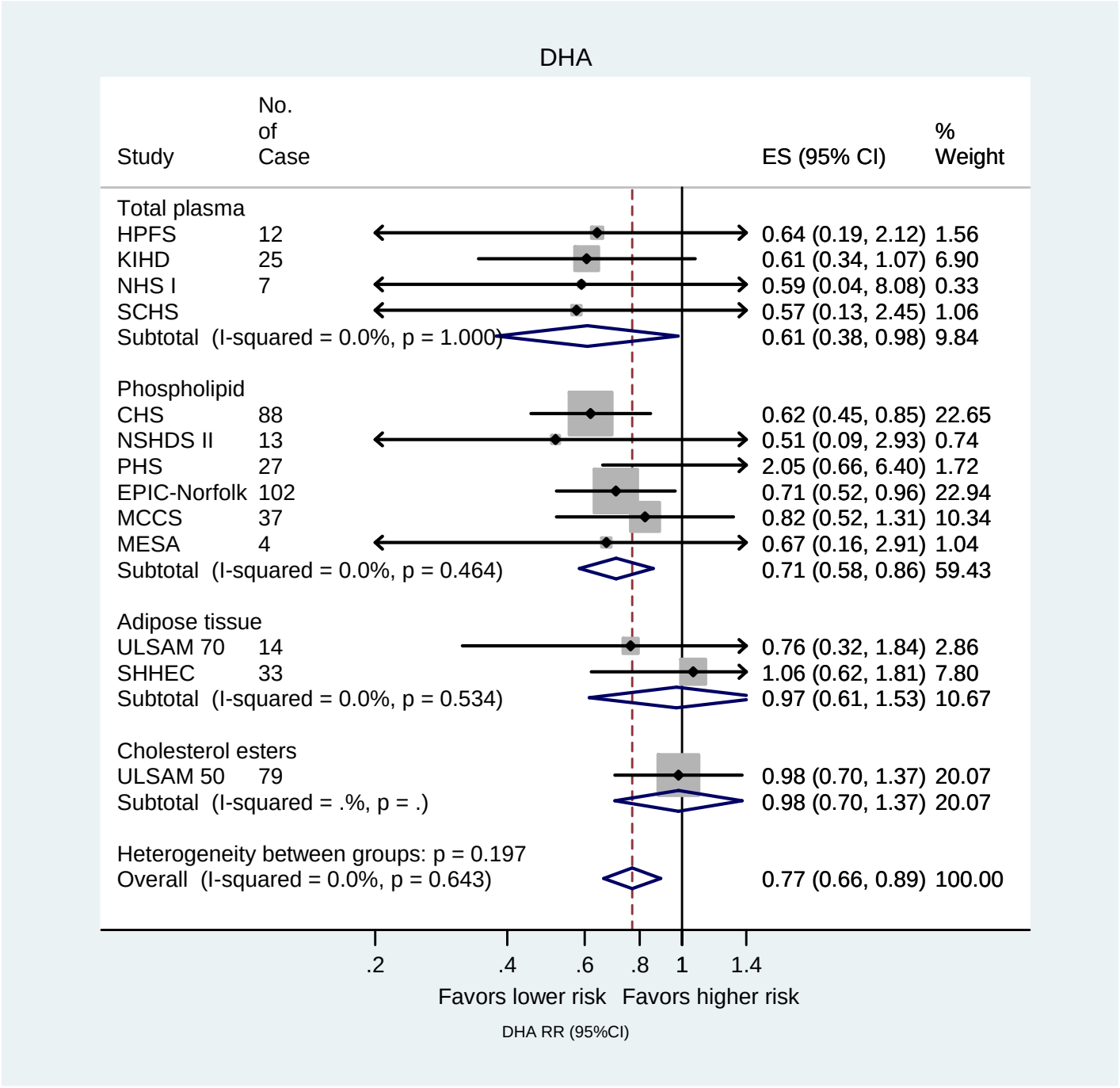
Quintile estimates were pooled using inverse-variance meta-analysis.

eFigure 3. (continued)



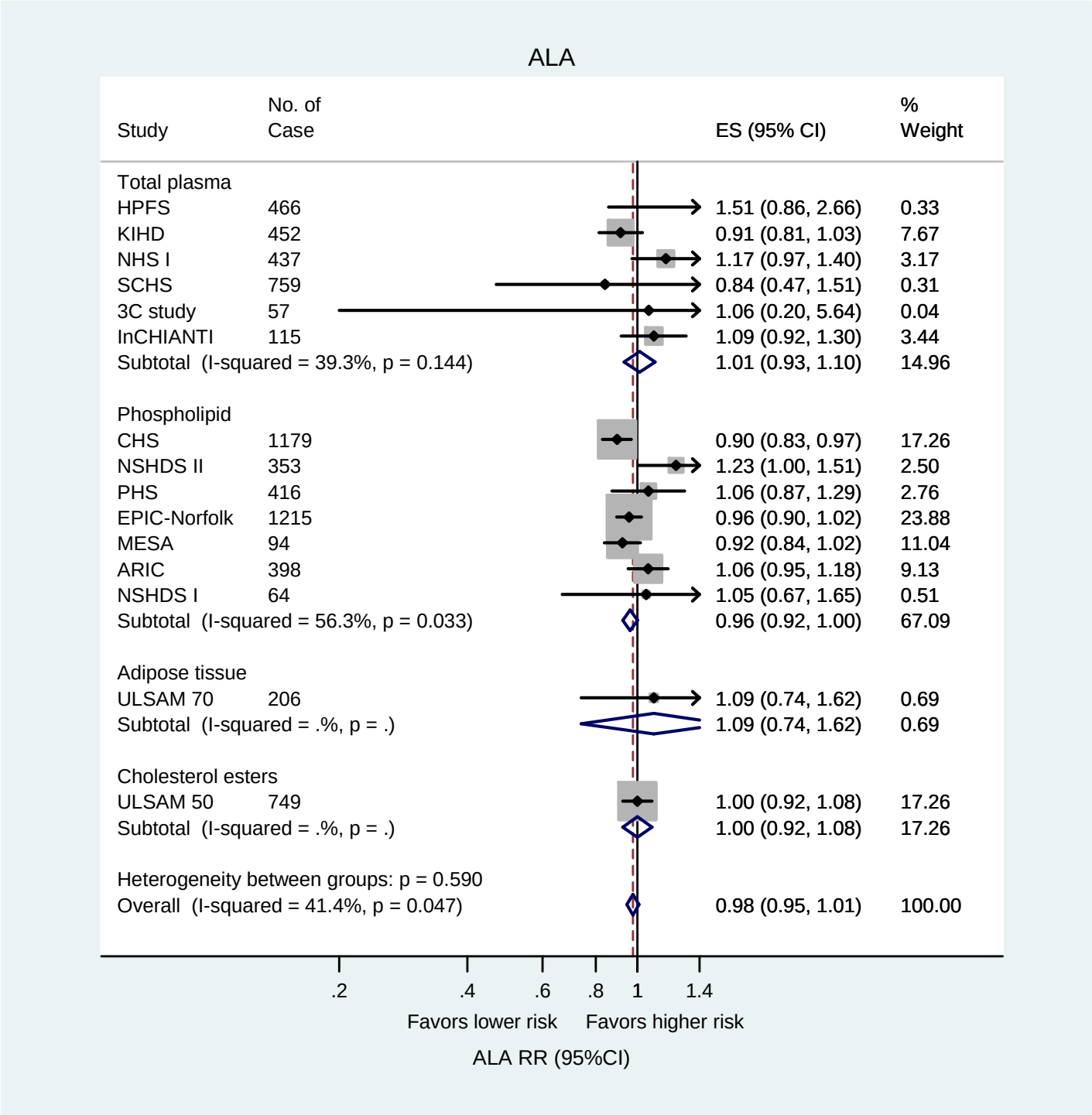
Quintile estimates were pooled using inverse-variance meta-analysis.

eFigure 3. (continued)



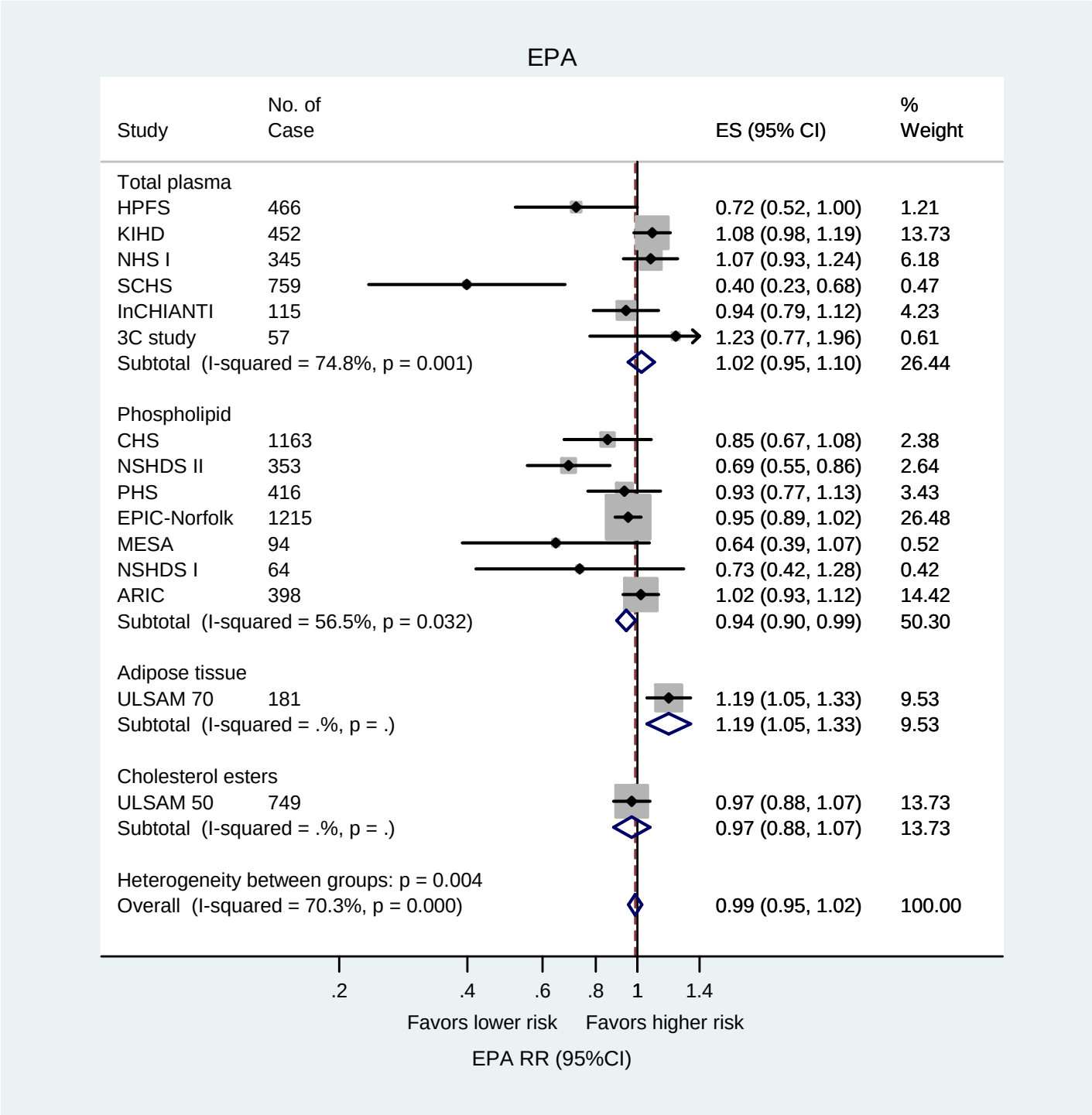
Quintile estimates were pooled using inverse-variance meta-analysis.

eFigure 4. Relative Risk of Total CHD per 1-SD Increase in Biomarker ALA, EPA, DPA, and DHA.



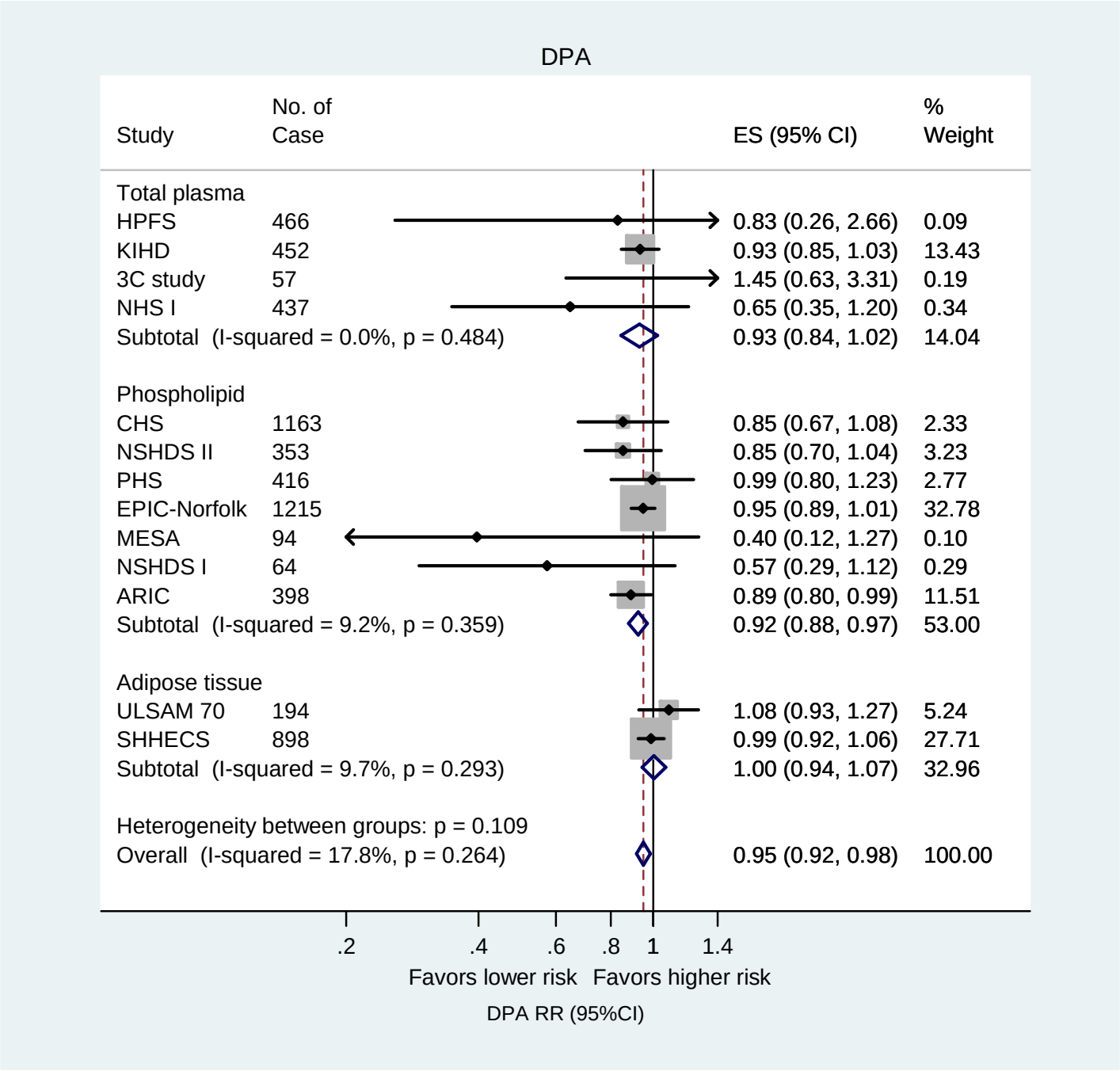
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 4. (continued)



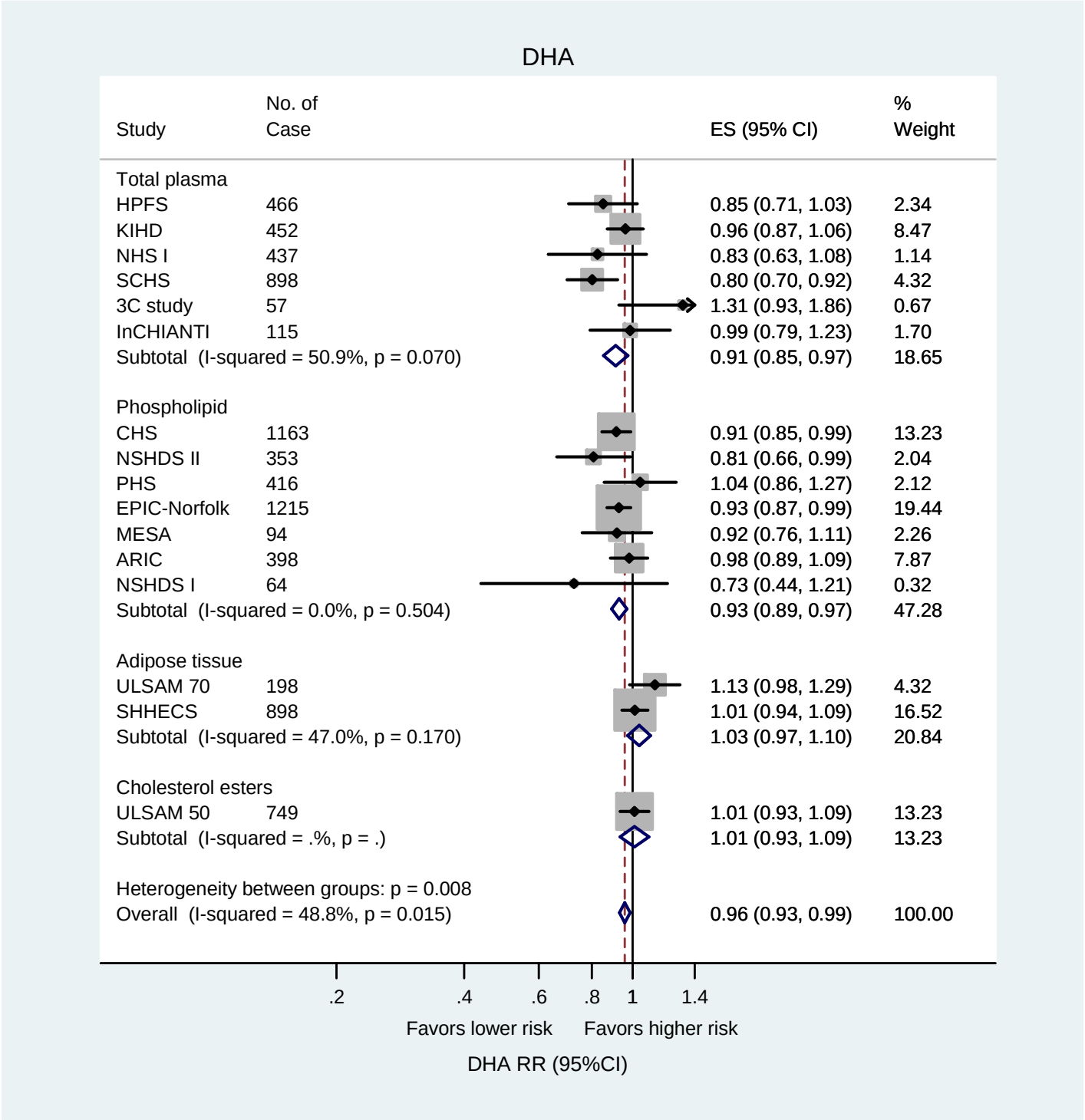
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 4. (continued)



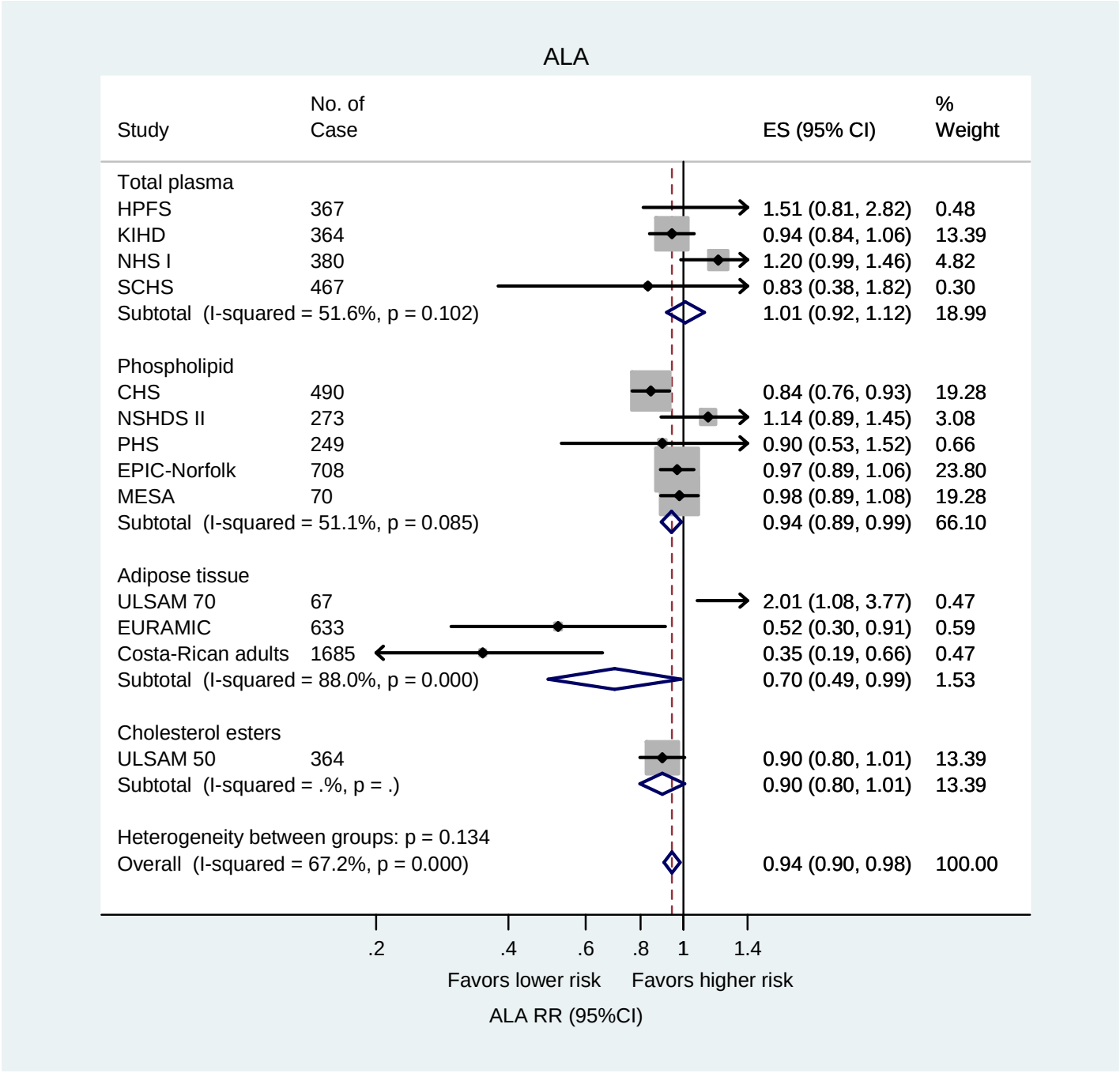
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 4. (continued)



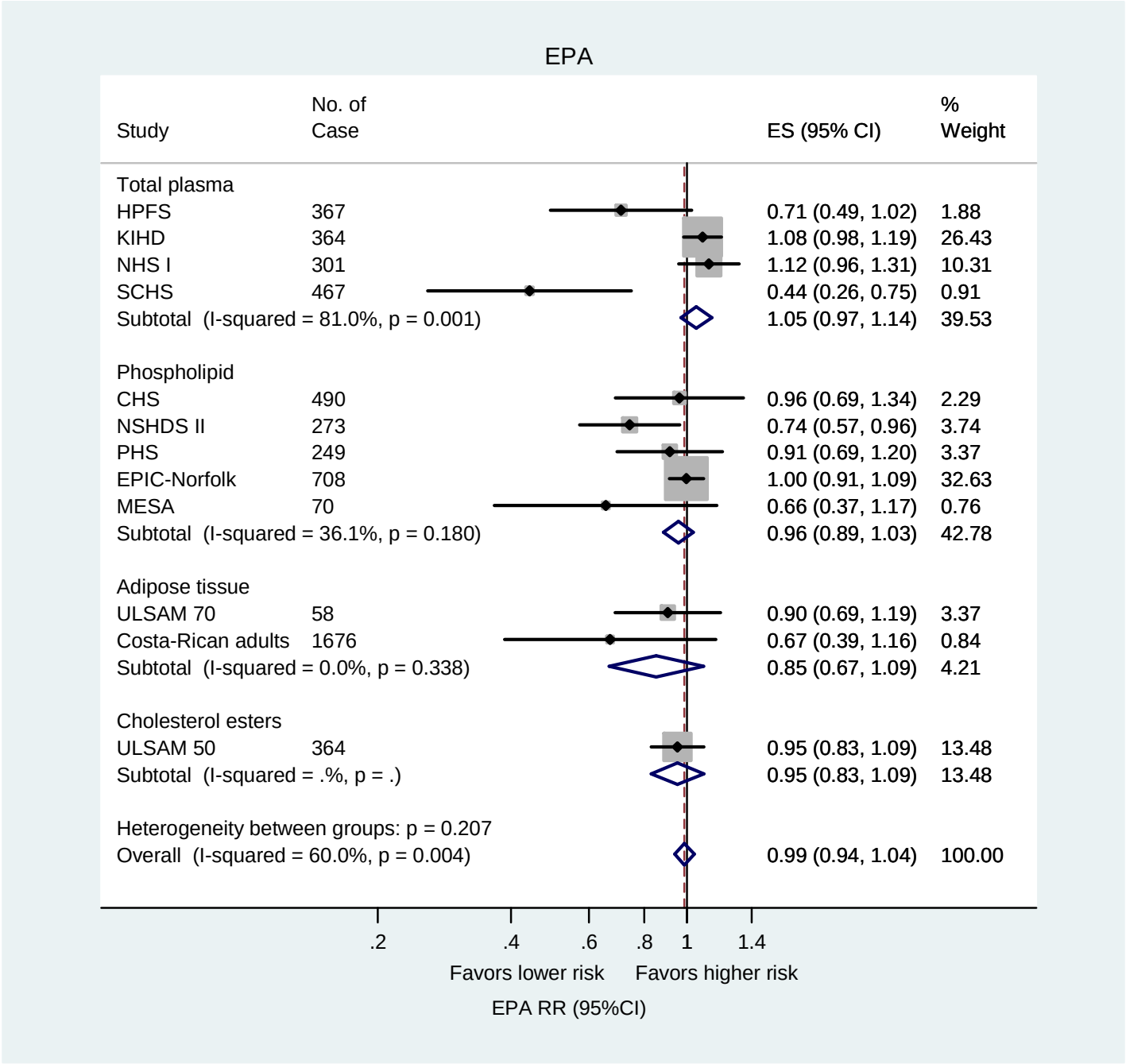
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 5. Relative Risk of Nonfatal MI per 1-SD Increase in Biomarker ALA, EPA, DPA, and DHA.



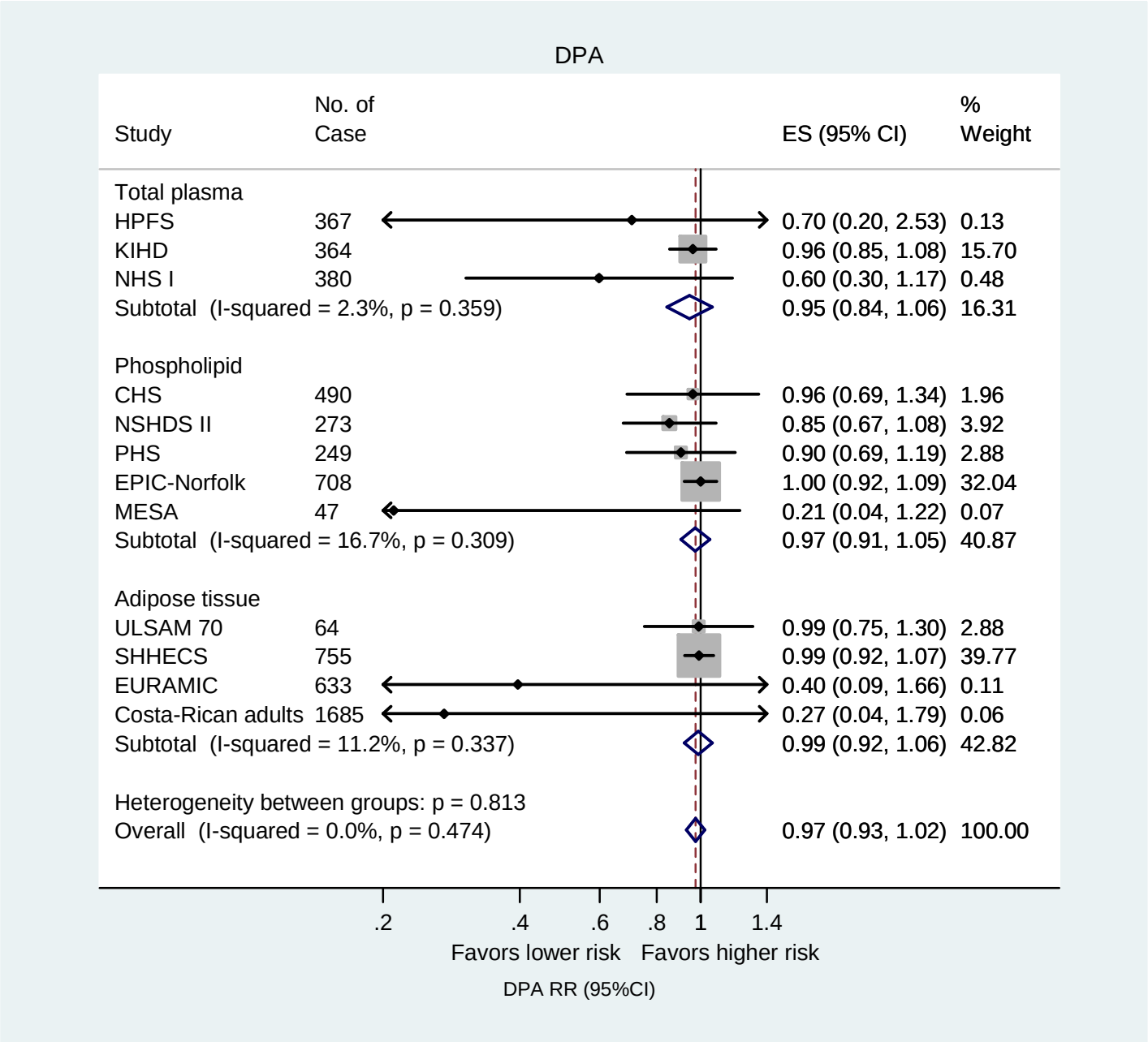
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 5. (continued)



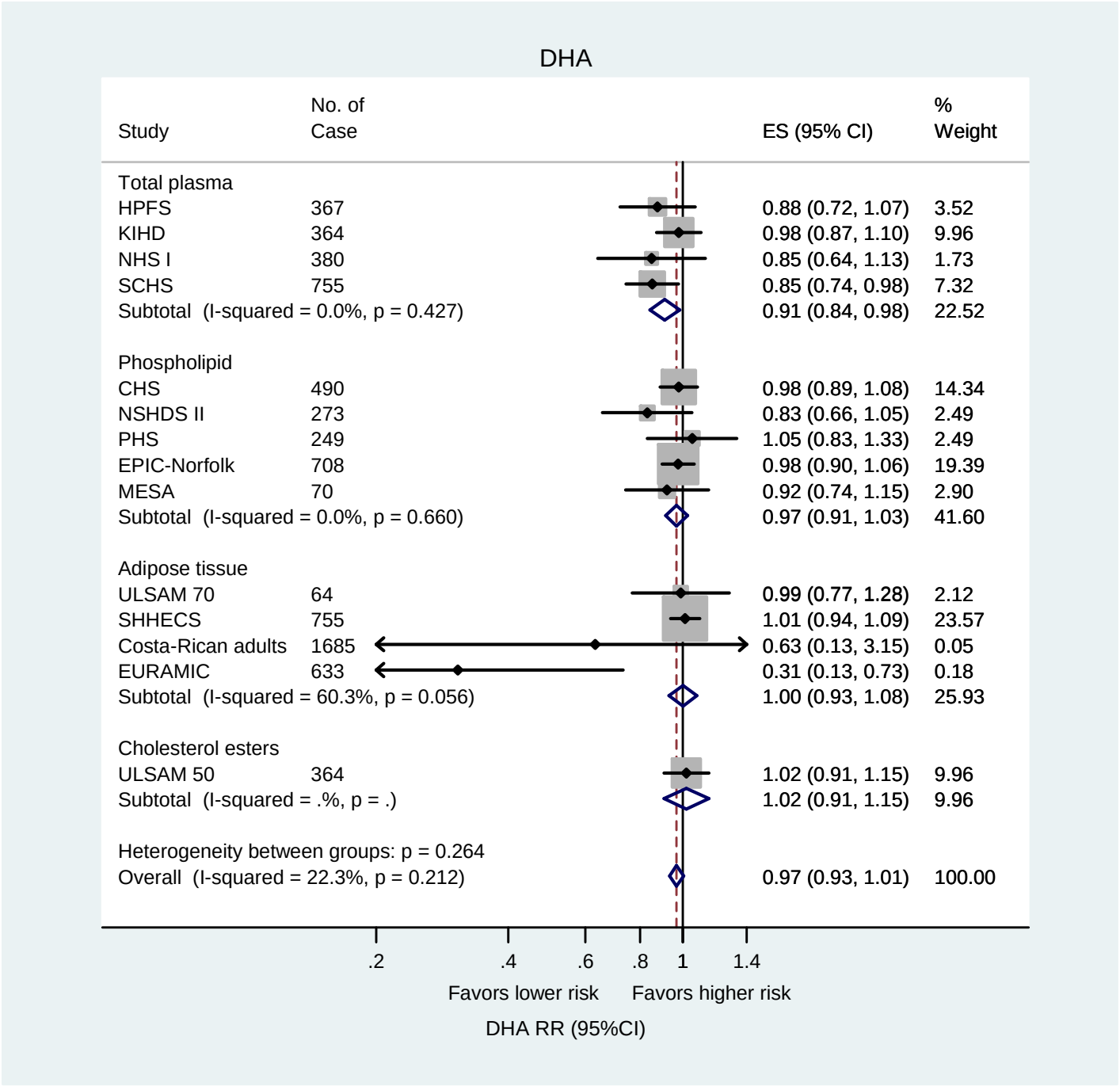
Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 5. (continued)



Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 5. (continued)



Continuous estimates were pooled using inverse-variance meta-analysis.

eFigure 6. Dose-Response Relations Between Biomarker α-Linolenic Acid (ALA; 18:3n3), Eicosapentaenoic Acid (EPA; 20:5n3), Docosapentaenoic Acid (DPA; 22:5n3), and Docosahexaenoic Acid (DHA; 22:6n3) and Relative Risks of Total CHD, Nonfatal MI, and Fatal CHD.

Relations were evaluated using multivariate random effects meta-analysis with 3-knot restricted cubic splines, for total plasma and phospholipid biomarker compartments. Dose-response curves showing statistically significant linear or non-linear relations are shown. The reference value was set at the 50th percentile of each fatty acid-biomarker exposure. The 95% confidence interval is depicted in the shaded regions.