SUPPLEMENTAL MATERIAL

CONTENTS

Supplemental Methods

Contributing cohorts

Contributing cohorts included Age, gene/environment susceptibility-Reykjavik (AGES-Reykjavik); Atherosclerosis Risk in Communities (ARIC); Chin-Shan Community Cardiovascular Cohort Study (CCCC); Cardiovascular Health Study (CHS); Costa Rica Study on Adults (CRS); Diet, Cancer, and Health (DCH) study; European Prospective Investigation into Cancer –Norfolk (EPIC-Norfolk); European Prospective Investigation into Cancer –Potsdam (EPIC-Potsdam); Framingham Heart Study (FHS); Health Professionals Follow-up Study (HPFS); the Hisayama study (HS); Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD); Melbourne Collaborative Cohort Study (MCCS); Multi-Ethnic Study of Atherosclerosis (MESA); Metabolic syndrome in men (METSIM); Monitoring Project on Risk Factors for Chronic Diseases (MORGEN); Monitoring Project on Cardiovascular Disease Risk Factors (MPCDRF); NHS: Nurses' Health Study; Northern Sweden Health and Disease Study (NSHDS); Physicians' Health Study (PHS); Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS); Singapore Chinese Health Study (SCHS); Scottish Heart Health Extended Cohort (SHHEC); 60-year-old Swedish men and women (60YO); Three City Study (3C Study); Uppsala Longitudinal Study of Adult Men (ULSAM); and Women's Health Initiative Memory Study (WHIMS). General cohort information and cohort-specific sources of support are presented in **Supplemental Tables 1-2**. Detailed information regarding study samples, fatty acid measurement, and genotyping methods in the contributing cohorts is given in the text below.

Detailed cohort information

AGES-Reykjavik: The Age, Gene/Environment Susceptibility Reykjavik Study

Physical activity assessment

Physical activity was assessed by self-report questionnaire. Participants were asked how often (never, rarely, occasionally, moderate, or high) they participated in moderate or vigorous physical activities in the past 12 months and in youth and midlife.

Measurement of fatty acid biomarker concentrations

Blood samples were collected at the AGES-Reykjavik baseline after an overnight fast and stored at -80C. Fatty acids were measured in plasma phospholipids at the Biomarker Laboratory, Fred Hutchinson Cancer Research Center. Plasma lipids were extracted by using the method of Folch et al.[1] Phospholipids were separated from other lipids by using one-dimensional thin-layer chromatography.[2] Fatty acid methyl esters were prepared by direct transesterification[3] and separated by using gas chromatography (Agilent Technologies 7890 Gas Chromatograph flame ionization detector; Supelco fused silica 100-m capillary column SP-2560; initially at 160°C for 16 min, ramped up at 3.08C/min to 240°C, and held for 15 min). The identification, precision, and accuracy were continuously evaluated by using both model mixtures of known fatty acid methyl esters and established in-house control pools. Fatty acids were expressed as the weight percentage of the total phospholipid fatty acids analyzed. The CV from pooled quality-control samples for LA, AA, ALA, EPA, DHA, and DPA were all 2.5%. CVs for other major fatty acids were 0.77% (palmitic), 0.47% (stearic), and 0.42% (oleic).

Ascertainment of fatal and non-fatal CVD events

Registries of vital status, cardiovascular disease and procedures, and hospital records with International Classification of Diseases (ICD, 9th edition and ICD-10th edition) were used to determine CVD events.

Definition of fatal and non-fatal CVD events

Total cardiovascular disease was defined as fatal or nonfatal myocardial infarction, coronary heart disease death, sudden cardiac death, or ischemic stroke. CVD mortality was defined as the composite of fatal MI, CHD death, sudden cardiac death, or fatal ischemic stroke. Total CHD was defined as fatal or nonfatal myocardial infarction, CHD death or sudden cardiac death.

Genotype ascertainment

Genotyping was conducted with the Illumina 370CNV BeadChip array. Genotype calling was carried out with Illumina BeadStudio. Exclusion on SNPs used for imputation: SNPs with call rate <97%, HWE p<1e-6, MAF <1%, Mishap p<1e-9, A/T and G/C SNPs, mismatches between Illumina, dbSNP and/or HapMap position. Exclusion on a per sample basis: sex mismatch, sample failure, genotype mismatch with reference panel. Imputation was done with MACH (version 1.0.16). The Imputation backbone was HapMap release 22 CEU (build 36).

ARIC: The Atherosclerosis Risk In Communities Study

General information

The Atherosclerosis Risk in Communities (ARIC) study is a multi-center, prospective study of atherosclerosis in a bi-racial population, including 15, 792 African American and Caucasian men and women aged 45-64 years. These adults were recruited from 4 US communities (Jackson, Mississippi, Minneapolis, MN; Washington County, MD; and Forsyth County, NC) in 1987-89. Plasma phospholipid and cholesterol ester fatty acids were measured in 3837participants living in Minneapolis, Minnesota.

Physical activity assessment

The Baecke Physical Activity assessed physical activity in study participants.

Measurement of fatty acid biomarker concentrations

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) as previously described.[4] 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 the percentage of total fatty acids.

Ascertainment and definition of fatal and non-fatal CVD events

Incident CVD consisted of incident coronary heart disease and stroke. A coronary heart disease event was defined as a validated definite or probable hospitalized myocardial infarction, a definite coronary heart disease death, an unrecognized myocardial infarction defined by ARIC electrocardiography reading, or coronary revascularization. A stroke event was defined as a validated definite or probable hospitalized ischemic or hemorrhagic stroke confirmed by imaging. CVD mortality were those who died from CVD. Incident CHD included fatal or nonfatal hospitalized myocardial infarction, fatal CHD, silent myocardial infarction identified by electrocardiography, or coronary revascularization. Incident ischemic stroke events included thrombotic or cardioembolic and were classified according to a computer algorithm, and an expert reviewer independently classified each eligible case using criteria adapted from the National Survey of Stroke; disagreements were adjudicated by a second expert physician.

Genotype ascertainment

In ARIC, genotyping was done using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, California). 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[5] and >8 SD along any of the first 10 principal components in EIGENSTRAT[6] 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%.

CCCC: The Chin-Shan Community Cardiovascular Cohort

Physical activity assessment

Data for physical activity was collected by Chin-Shan Community Cardiovascular Cohort Questionnaire and was defined as participants who had done exercise (the exercise that can make you sweat / at least lasting for 20 minutes) once per week or had a laborious job.

Measurement of fatty acid biomarker concentrations

In brief, 10-mL tubes of EDTA anti-coagulated blood were collected, refrigerated on-site, and forwarded to the core laboratory of the National Taiwan University Hospital within 3 h. The blood was centrifuged at 800g for 10 min, whereupon plasma was separated, dispensed into aliquots, and frozen at 70 C. All analyses of fatty acid content were performed by the same technician. After thawing the plasma, 0.5 mL samples were extracted and combined with 0.5 mL methanol followed by 1.0mL chloroform under a nitrogen atmosphere. The lipid extract was then filtered to remove proteins and methyl esters were separated and measured using a 5890 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a 30 m-FFAT WCOT glass capillary column (J & W Scientific, Folsom, CA) and a flame ionization detector. A total of 29 individual fatty acids were identified by comparing the retention times of peaks to the retention times of synthetic FA standards with known compositions (Supelco 37 Comp. FAME Mix, 47885-U; Bellefonte, PA, USA). The relative quantity of each FA (% of total FAs) was determined by integrating the area beneath the peak, and dividing the result by the total area for all FAs. The concentrations of saturated fatty acids, trans fat, monounsaturated fat, n-6 PUFA, DHA, and EPA were specified for further analysis.

Ascertainment and definition of fatal and non-fatal CVD events

In CCCC cohort, incident CVD events included coronary disease and stroke. Incident coronary heart disease included cases of nonfatal myocardial infarction, fatal coronary heart disease, and hospitalization due to percutaneous coronary intervention or coronary bypass surgery. Cases of stroke were ascertained by a sudden neurological deficit of vascular origin lasting longer than 24 h and supporting evidence from medical imaging devices. Data related to non-fatal ischemic events and cases of stroke were obtained from annual questionnaires and all cases were confirmed by neurologists and internists. Fatalities were attributed to coronary disease when hospital records listed myocardial infarction as the cause of death, when the death certificate listed coronary heart disease as the cause of death, or when coronary disease was the most plausible cause of death.

CHS: Cardiovascular Health Study

Physical activity assessment

In CHS, usual leisure-time activity was assessed at baseline (1989-90) and at 1992-93 using a modified Minnesota Leisure-Time Activities questionnaire. The questionnaire evaluated frequency and duration of 15 different activities during the prior 2 weeks, including gardening, mowing, raking, swimming, hiking, aerobics, tennis, jogging, racquetball, walking, golfing, bicycling, dancing, calisthenics, and riding an exercise cycle. Participant responses regarding types of activity, frequency, and duration were used to calculate weekly energy expenditure from leisure-time activity, expressed as kcal/wk.

Measurement of fatty acid biomarker concentrations

In CHS, blood was sampled after a 12-hr fast and stored at -70 °C before being shipped on dry ice from centralized long-term storage at -80C. Total lipids were extracted from plasma using Folch's method,[1] and phospholipid was separated from neutral lipid using 1-dimentional thin-layer chromatography. Fatty acid methyl esters were prepared using Lepage & Roy's method[3] and were analyzed using GC-FID. Interassay CVs: LA: 7.7%; AA: 0.81%; ALA: 3.1%; EPA: 2.1%; DPA: 1.5%; DHA: 1.6%.

Ascertainment of fatal and non-fatal CVD events

In CHS, incident CHD and stroke were identified from annual clinic visits, interim 6-month phone contacts, hospital records, and CMS and NDI data; with adjudication by centralized committees of physicians using standardized criteria based on data from participants, proxies, medical records, physician questionnaires, death certificates, medical examiner forms, CMS (HCFA) hospitalizations, NDI, and available diagnostic tests and consultations.[7, 8] Incident MI was classified using an algorithm which includes data on chest pain, cardiac enzyme measures, and ECGs. Suspected cardiac deaths not meeting criteria for definite MI were classified as CHD death if occurring within 72 h of chest pain or with antecedent history of CHD.

Potential strokes were centrally adjudicated using interviews, medical records, death certificates, medical examiner forms, CMS hospitalizations, and review of available CT or MRI scans.[9, 10] Stroke was defined as a neurological deficit of rapid onset lasting >24 h unless death supervened, or as a subarachnoid hemorrhage. Strokes were classified as ischemic if evidence of a focal brain deficit without evidence for primary hemorrhage; as hemorrhagic if bloody spinal fluid on lumbar puncture or evidence of blood in the subarachnoid space, ventricles, or parenchyma on cerebral imaging or at surgery or autopsy that did not appear consistent with hemorrhage into an infarction; or as unknown type if information was insufficient for classification. Adjudication decisions were typically unanimous for both stroke diagnosis and type.

All deaths were centrally adjudicated using available medical records, death certificates, and/or next of kin contacts. Underlying cause of death was classified into categories including CVD (and subtypes), cancer, lung disease, infection, trauma, etc.

Definition of fatal and non-fatal CVD events

In CHS, total CHD included nonfatal myocardial infarction (defined as chest pain with abnormal cardiac enzyme concentrations or serial electrocardiogram changes) and fatal CHD (defined as fatal MI or as fatal CHD events for which the participant had chest pain within 72 h of death or had a history of chronic CHD). Among the CHD deaths, fatal MI (SCD) was defined based on review by a cardiologist as a sudden pulse-less condition with a cardiac origin in a previously stable individual that occurred out of the hospital or in the emergency room.

Total CVD was defined as total CHD or ischemic stroke (fatal or non-fatal). CVD mortality was defined as fatal CHD or fatal ischemic stroke.

Genotype ascertainment

In CHS, genotyping was done using a high-density SNP marker platform (Illumina 370). Samples with call rates below 97% at genotyped markers were excluded. Imputation was conducted using BIMBAM software. SNPs for which Hardy Weinberg equilibrium resulted in $p<10\text{-}5$ were excluded from imputation.

CRS: The Costa Rica Study on Adults

Physical activity assessment

In the Costa Rica Study, physical activity was assessed using a previously validated questionnaire when the MI cases were in the step-down unit of the hospital.[11] Physical activity on controls was assessed at the same time. Activities were assigned metabolic equivalent (MET) values corresponding to their intensity as described in detail in Hastert et al.[12]

Measurement of fatty acid biomarker concentrations

Gas–liquid chromatography was used to quantify fatty acids from adipose tissue.[13] 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.[14] Peak retention times and area percentages of total fatty acids were analyzed with the ChemStation A.08.03 software (Agilent Technologies).[15] 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. CVs were 5.9% for AA and 2.6% for LA.

Ascertainment and definition of nonfatal myocardial infarction

Cases of first nonfatal acute MI were 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.

DCH: The Diet, Cancer and Health Study

Physical activity assessment

In DCH, physical activities (walking, cycling, house working, manual work, gardening and sports) were assessed by self-report at baseline (hours in summer and winter). A MET-score was calculated (METS/week).

Measurement of fatty acid biomarker concentrations

An adipose tissue biopsy was taken from the buttock of all participants using a luer lock system (Terumo, Terumo Corp, Tokyo, Japan) consisting of a needle, a venoject multi-sample luer adaptor, and an evacuated blood tube, according to the method of Beynen and Katan.[14] Samples were flushed with nitrogen and stored at −150 °C until analysis. When analyzed, biopsies were thawed, and approximately 3 mg of adipose tissue were removed to a glass and preheated at 50 °C for 10 min. Subsequently, the fat was dissolved in heptane at 50 °C, and fatty acids were transesterified by 2 mol/L KOH (Potassium hydroxide) in methanol at 50 °C for 2 min, according to IUPAC standard methods for analysis of oils, fats, and derivatives. The fatty acid composition was determined by gas chromatography using a Varian 3900 GC with a CP-8400 auto sampler (Varian, Middleburg, Netherlands) equipped with a flame ionization detector. Split injection mode, a CP-sil 88, 50 m×0.25 mm ID capillary column (Varian, Middleburg, Netherlands), temperature programming from 90 °C to 210 °C, and constant flow were used. Helium was used as carrier gas. Commercially available standards (Nu-chek-Prep, Inc., Minnesota, US) were used to identify the individual fatty acids. The AA and LA contents were expressed as percent of total fatty acids, and the interassay coefficient of variation (CV %) was 3.2% and 0.7%, respectively.

Ascertainment and definition of nonfatal myocardial infarction

The endpoint in this study was restricted to incident MI (unstable angina, stable angina and surgical procedures were not included), and the identification of cases has been described previously.[16] In brief, we included cases registered with an incident MI (International Classification of Disease (ICD) 8: 410-410.99 or ICD 10: I21.0-I21.9), identifying potential cases by linkage with the Danish National Patient Registry or the Danish Cause of Death Registry. Furthermore, we included participants with a sudden cardiac death diagnosis (ICD 8: 427.27 or ICD 10: I46.0-I46.9) if the cardiac arrest after validation was believed to be caused by an MI. From study baseline through 2003, potential cases were validated by review of medical records in accordance with the guidelines for use in epidemiology of the American Heart Association and the European Society of Cardiology.[17] From January 2004 until the end of follow-up in December 2009, and for participants whose medical records were not available for review in the initial period, we accepted all cases without further validation, provided they had a diagnosis of MI from a hospital ward, as these diagnoses have a positive predictive value above 90% in the Danish National Patient Registry.[17] All other potential cases were validated individually by assessment of the diagnoses and procedure codes of the involved participant in the National Patient Registry and the Causes of Death Registry.

EPIC-Norfolk: European Prospective Investigation into Cancer-Norfolk

Physical activity assessment

Validated physical activity index from leisure and work physical activity questionnaire.

Measurement of fatty acid biomarker concentrations

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 were retrieved from liquid nitrogen storage, thawed at room temperature and 20 μg of di-palmitoyl-D_{31}-phosphatidylcholine (Sigma, St. Louis, MO) internal standard was added to each 200 μl plasma sample. Following extraction of total lipids with chloroform/methanol, phospholipids were further purified by adsorption chromatography (LC-Si SPE, Supelco/Sigma, St. Louis, MO), transmethylated to fatty acid methyl esters and extracted with hexane. Analysis was carried out by gas chromatography with flame ionisation detection (220°C) using a 30 m x 0.32 mm x 0.2 um SP2340 fused silica capillary column (Supelco/Sigma, St. Louis, MO). Carrier gas was Helium at a constant flow of 1.3 ml/min. Samples of 0.5 μl were introduced onto the column via on-column injection. The column was held initially for 1 min at 65°C, then programmed at 5°C/min to 135°C, then at 2°C/min to 200°C, and finally at 10°C/min to 220°C. Run time was 60 min. Identification of individual fatty acid methyl esters was based on comparison with retention times of authentic standards (Sigma, St. Louis, MO). Plasma concentrations were measured by comparison of peak areas of individual fatty acids with the peak area of the palmitoyl-D_{31}-fatty acid methyl ester internal standard using individual calibration curves for each of the 22 fatty acid methyl esters measured. The chromatographic peak for palmitoyl-D_{31}-fatty acid methyl ester elutes about 1 minute earlier than non-labelled palmitoyl fatty acid methyl ester in a zone free of interference from other peaks. Each chromatogram was integrated automatically and checked for accuracy and specificity by a laboratory technician. Results were reported as mol% for each individual FA, taking into account the large difference in molecular mass between the shortest (myristic acid, 14:0) and longest (docosapentaenoic acid, 22:5n-3) chain FA measured. Analytical quality control was carried out by the daily use of standard quality control plasma samples. The CVs for the major fatty acids were between 3% and 13%.

Ascertainment of fatal and non-fatal CVD events

All participants are flagged for death certification with the National Health Service Central Register, UK with death certificates coded by nosologists according to the International Classification of Disease (ICD). Deaths due to coronary heart disease were defined using ICD9 410-414 or ICD10 I20-I25 as underlying cause of death; and deaths due to stroke, ICD9 430-438 or ICD10 I60-I69. Deaths due to cardiovascular diseases were defined as a death due to either coronary heart disease or stroke. The mortality data up to 30 June 2013 were collected.

For incidence data, the East Norfolk Health Authority database was used to identify all hospital contacts for participants using their National Health Service number. We used the ICD diagnostic codes listed to ascertain hospital episodes for coronary heart disease and stroke. Participants were identified as having an event during follow-up if they had a hospital admission and/or died with each episode as cause of death, with clinical validation through medical record inspection of a sample.[18] We ascertained the events up to December 2009.

Genotype ascertainment

In EPIC-Norfolk, genotyping was performed using the Affymetrix BioBank Axiom and ShapeIT v2.r790. Samples with call rates below 97% at genotyped markers were excluded. Imputation was conducted using IMPUTE 2.3.1 software (1000G phase 3 reference panel). SNPs for which Hardy Weinberg equilibrium resulted in $p<10\text{-}8$ were excluded from imputation.

EPIC-Potsdam: European Prospective Investigation into Cancer-Potsdam

Physical activity assessment

In EPIC-Potsdam, physical activity was assessed via self-reports made in a computer-guided interview. We considered sport activities and biking as leisure time physical activities, both calculated as the average time spent per week during the twelve months before baseline recruitment.

Measurement of fatty acid biomarker concentrations

Thirty milliliters of blood were taken from each participant during baseline examination and were centrifuged at 1000 g for 10 min at 4°C. Plasma, serum, red blood cells, and buffy coat were removed and stored at –80°C. The erythrocyte membrane FAs were analyzed at the Laboratory of the Dutch National Institute for Public Health and Environment between February and June 2008. Briefly, FA methyl esters (FAME) were separated on a GC-3900 gas chromatograph (Varian Inc., Middelburg, Netherlands) equipped with a 100 m x 0.25mm ID WCOT-fused silica capillary column and flame ionization detector with separation of FAME peaks based on mixed FAME standards (Sigma Aldrich, St Louis, USA). The Galaxie software version 1.9.3.2 (Varian Inc.) was used for quantification and identification of peaks. The FAs were expressed as the percentage of total FAs present in the chromatogram. Intraassay CVs (%): LA: 2.2, AA: 2.2, ALA: 10.2, EPA: 3.1, DPA: 2.2, DHA: 2.4

Ascertainment and definition of fatal and non-fatal CVD events

About every 2 years, information on incident diseases was collected. To identify potential CVD cases, several sources were used: self-report, death certificate or linkage with hospital information system. All identified potential CVD events

were ascertained by study physicians, in cooperation with the patients' attending physicians and hospitals, who provided a detailed medical verification of self-reports and death certificates by clinical records. Total CVD was defined as fatal or nonfatal myocardial infarction, sudden cardiac death or ischemic stroke. We considered data until the end of the fifth follow-up period (year 2009).

Genotype ascertainment
In EPIC-Potsdam, genotyping was performed by KBioscience using KASP SNP genotyping system. The mean SNP call rate was >95%. There was no significant departure from Hardy-Weinberg equilibrium for rs174546.

FHS: The Framingham Heart Study

Physical activity assessment
In FHS, self-reported values on typical activities and rest hours per day were gathered as part of the Physical Activity Questionnaire. Metabolic equivalents were computed using standard scoring approaches.

Measurement of fatty acid biomarker concentrations
The fatty acid composition of RBC samples were analyzed by gas chromatography equipped with a SP 2560 capillary column after direct transesterification for 10 minutes in boron trifluoride/ methanol and hexane at 100 °C as previously described. This technique generates fatty acids primarily from erythrocyte glycerophospholipids. Erythrocytes were isolated from blood drawn after a 10–12 h fast and frozen at −80 °C immediately after collection. All fatty acids present at >1% abundance had CVs of ≤7%.

Ascertainment of fatal and non-fatal CVD events
CVD events were ascertained annually, with data through 2014 available for this analysis. All identified events were adjudicated by the FHS Endpoint review committee and represent a combination of both active and passive ascertainment. In particular, information about CVD events on follow-up was obtained with the aid of medical histories, physical examinations at the study clinic, hospitalization records, and communication with personal physicians.[19]

Definition of fatal and non-fatal CVD events
Total ischemic stroke was defined as cerebrovascular accident death, definite cerebrovascular accident, acquired brain injury, transient ischemic attack, cerebral embolism, intracerebral hemorrhage, subarachnoid hemorrhage, and other cerebrovascular accident. Total CHD was defined as myocardial infarction with or without diagnostic ECG, unrecognized myocaridal infarction (silent or not), angina pectoris (first episode only), Intermittent claudication (history and ECG) and death by CHD. CVD mortality was defined as death by CHD, cerebrovascular accident or other CVD. Total CVD is defined as any one of the three categories above (Total ischemic stroke, total CHD and CVD mortality).

Genotype ascertainment
Genotyping was conducted using the Affymetrix 500K SNP chip, with Imputation for markers with genotype call rates below 97% or small (<1x10-6) HWE p-values using Mach and the CEU HapMap dataset for reference.

HPFS: The Health Professionals Follow-Up Study

Physical activity assessment
In Health Professionals Follow-Up Study, physical activity was assessed using mailed questionnaires at baseline and every 2 years thereafter. Subjects were asked to report the average amount of time they spent per week on each of the following activities: walking, jogging, running, bicycling, calisthenics or use of a rowing machine, lap swimming, squash or racquetball, and tennis. They were also asked about their usual walking pace, specified as easy or casual (<2 miles/h), normal (2-2.9 miles/h), brisk (3-3.9 miles/h), or striding (≥4 miles/h). From this information, weekly energy expenditure in metabolic equivalent hours (MET-hours) was calculated.

Measurement of fatty acid biomarker concentrations
From April 1993 through August 1995, 18 225 blood samples from study participants were received, aliquoted and stored in liquid nitrogen. Blood samples were collected in three 10 ml liquid EDTA blood tubes, placed on ice packs stored in styrofoam containers, and returned to the laboratory by overnight courier. 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 LA were 2%, AA 5%, ALA 4%, and EPA 7%; in erythrocytes, CV's for LA were 9%, AA 10%, ALA 12%, and EPA 12%.

Ascertainment and definition of fatal and non-fatal CVD events

Participants with incident cardiovascular disease (defined as nonfatal myocardial infarction, fatal coronary heart disease, or stroke) were identified and asked (or relatives in cases of fatal events) for permission to review their medical records. Physicians who were unaware of other questionnaire information used standardized criteria to confirm and classify the events. Deaths were ascertained from relatives, postal authorities, and the National Death Index, and the cause of death was classified on the basis of medical records, death certificates, and autopsy findings. A diagnosis of myocardial infarction was confirmed on the basis of standardized criteria, which included typical symptoms plus either diagnostic electrocardiographic changes or elevated cardiac enzyme levels.[20, 21 21] Deaths were ascertained by contact with family members or through the National Death Index. Fatal heart disease was confirmed on the basis of medical records or autopsy reports or, if heart disease was listed as the cause of death, on the basis of the death certificates and evidence of previous heart disease in the records. Stroke was diagnosed according to standard criteria, consisting of a constellation of neurologic deficits of sudden or rapid onset that lasted at least 24 hours or until death.[22, 23] Stroke subtypes were also classified as previously described.[22, 23]

HS: The Hisayama Study

Physical activity assessment

In Hisayama Study, physical activity was defined as having regular exercise at least 3 times per week during leisure-time or not.

Measurement of fatty acid biomarker concentrations

Serum fatty acids levels were assayed by gas chromatography (SRL, Tokyo, Japan). Briefly, total lipids in plasma were extracted according to the Folch's procedure, followed by hydrolysis to free fatty acids. Free fatty acids were esterified with potassium methoxide/methanol and boron trifluorideemethanol. The methylated fatty acids were analyzed using GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with omegawax-250 capillary column (SUPELCO, Sigma Aldrich Japan, Tokyo, Japan). Reproducibility (i.e. the coefficient of variation) of the determination of serum EPA, DHA, and AA levels by this method was reported to be 4.4%, 2.3%, and 3.8%, respectively.[24]

Ascertainment and definition of fatal and non-fatal CVD events

In Hisayama Study, participants were followed up prospectively from the date of their comprehensive assessment by annual health examinations. For any subjects who did not undergo an annual examination in any year, or who moved out of town, their health status was checked by mail or telephone. We also established a daily monitoring system among the study team, local physicians, and the members of the town's health and welfare office. When a subject died, an autopsy was performed at the Department of Pathology of Kyushu University. Both fatal and nonfatal CVD events were identified by either.

The outcomes used in the analyses were total CHD, ischemic stroke, total cardiovascular disease and cardiovascular death. Total CHD comprised of fatal or nonfatal myocardial infarction, total cardiovascular disease of fatal or nonfatal myocardial infarction or ischemic stroke, coronary heart disease (CHD) death, and sudden cardiac death. CHD death or sudden cardiac death, and cardiovascular death of fatal MI, CHD death, sudden cardiac death, or fatal ischemic stroke. The definition of each component was as follows;

Ischemic stroke was diagnosed when a sudden onset of non-convulsive and focal neurological deficit persisted for more than 24 hours, and when morphological findings, including those from neuro-images and autopsy, showed evidence of brain infarction. Myocardial infarction comprised acute and silent myocardial infarctions. Acute myocardial infarction was diagnosed when a subject met at least two of the following criteria: (1) typical symptoms, including prolonged severe anterior chest pain; (2) abnormal cardiac enzymes more than twice the upper limit of the normal range; (3) evolving diagnostic electrocardiographic changes; and (4) morphological changes, including local asynergy of cardiac wall motion on electrocardiography, persistent perfusion defect on cardiac scintigraphy, or myocardial necrosis or scars ≥1 cm long accompanied by coronary atherosclerosis at autopsy. Silent myocardial infarction was defined as myocardial scarring without any historical indication of clinical symptoms or abnormal cardiac enzyme changes. CHD death was defined as death from cardiovascular causes (ICD10 coding in "I" category). Sudden cardiac death was defined when subjects died within 1 hour after the onset of acute illness and were free of severe illness.

Genotype ascertainment

Genomic DNA was extracted from whole blood using a QIAmp DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was dissolved in 20 μL Tris-HCl buffer (10 mmol/L, pH 8.0) containing 1 mmol/L EDTA and was stored at -80°C until use. Genotyping was performed by using of either the multiplex polymerase

chain reaction-based invader assay (Third Wave Technologies) 17 or TaqMan assay (Applied Biosystems) in a blinded manner to the clinical information of study subjects.

KIHD: The Kuopio Ischemic Heart Disease Risk Factor Study

Physical activity assessment

Physical activity was assessed using the 12-month leisure-time physical activity questionnaire. The checklist included the most common physical activities of middle-aged Finnish men, selected on the basis of a previous population study in Finland. For each activity performed, the subject was asked to record the frequency (number of sessions per month), average duration (hours and minutes per session), and intensity (scored as 0 for recreational activity, 1 for conditioning activity, 2 for brisk conditioning activity, 3 for competitive, strenuous exercise). A trained nurse checked and completed the questionnaire in an interview. The intensity of physical activity was expressed in metabolic units (MET or metabolic equivalents of oxygen consumption).

Measurement of fatty acid biomarker concentrations

Venous blood samples were collected between 8AM and 10AM in 1984-1989 after an overnight fast. Serum total fatty acids were determined from frozen samples 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. Serum was extracted with chloroform-methanol and fatty acids were methylated with methanol and sulphuric acid prior to gas chromatography. Each analyte had an individual reference standard and the analytes were quantified with an internal standard method using eicosane. Results for fatty acids were obtained in μmol/L and in the data analyses proportion of a fatty acid from the total fatty acids was used. The coefficient of variation (CV) for repeated measurements of fatty acids was 9.6% for linoleic acid and 9.2% for arachidonic acid.

Ascertainment of fatal and non-fatal CVD events

Deaths were ascertained by linkage to the national Causes of Death Register using the personal identification codes (social security number). Data on fatal and non-fatal coronary events were obtained by computer linkage to the national hospital discharge and death certificate registers. The diagnostic classification of events was based on symptoms, electrocardiographic (ECG) findings, cardiac enzyme elevations, autopsy findings (80%), and history of CHD together with the clinical and ECG findings of the paramedic staff. All the documents were cross-checked in detail by two physicians. Diagnostic information was collected from hospitals and classified using identical diagnostic criteria. Each suspected coronary event (ICD-9 codes 410–414 and ICD-10 codes I20–I25) was classified into a definite acute MI, a probable acute MI, a typical acute chest pain episode of more than 20 min indicating CHD, an ischemic cardiac arrest with successful resuscitation, or no acute coronary event by a physician using the original patient records. Acute coronary events that did not lead to death during the following 24 hours were considered as a non-fatal event. A death was determined SCD when it occurred either within 1 h after the onset of an abrupt change in symptoms or within 24 h after onset of symptoms when autopsy data did not reveal a noncardiac cause of sudden death. The deaths due to aortic aneurysm rupture, cardiac rupture or tamponade, and pulmonary embolism were not included as SCD.
Incident strokes between years 1984-1992 were observed through FINMONICA stroke register and between years 1993 and 2012 through computerized linkage to the national hospital discharge registry. The diagnosis of stroke was based on sudden onset of clinical signs or focal or global disturbance of cerebral function lasting 24 hours (except in the case of sudden death or if interrupted by surgical intervention) with no apparent cause other than a vascular origin. Each suspected stroke (International Classification of Diseases [ICD]-9 codes 430-439 and ICD-10 codes I60–I68 and G45–G46) was classified into 1) a definite stroke, 2) no stroke, or 3) an unclassifiable event. The FINMONICA stroke register data were annually rechecked with the data obtained from the computerized national hospital discharge and death registers. Definite strokes and unclassifiable events were included in the group of any stroke. Each definite stroke was classified into 1) an ischemic stroke (ICD-9 codes 433-434; ICD-10 code I63) or 2) a hemorrhagic stroke (ICD-9 codes 430-431; ICD-10 codes I60-I61). If the subject had multiple nonfatal strokes during follow-up, the first stroke was considered as the end point. CT was performed in 90% of the patients by 1993, and CT, MRI, and autopsy reached 100% by 1997.

Definition of fatal and non-fatal CVD events

CVD mortality was defined as fatal myocardial infarction, CHD death, sudden cardiac death, or fatal ischemic stroke. Incident ischemic stroke was defined as cerebral infarction (ICD-9 codes 430-431, ICD-10 code I63). Total CHD was defined as fatal or non-fatal myocardial infarction, CHD death or SCD. Total CVD was defined as fatal or nonfatal myocardial infarction, CHD death, sudden cardiac death, or ischemic stroke.

MCCS: The Melbourne Collaborative Cohort Study

Physical activity assessment

In MCCS, the physical activity score combines the frequencies (none, 1-2 times per week and ≥3 times per week) of 3 activity types: vigorous exercise (vig), non-vigorous exercise (nonvig) and walk for recreaction /exercise (walk). After recoding the frequency response as 0, 1.5 and 4, the score was calculated as 2vig + nonvig + walk according to Ainsworth et al.[25]

Measurement of fatty acid biomarker concentrations
The measurement of plasma phospholipid fatty acids in MCCS have been described in detail.[26] Briefly, total lipids were extracted from plasma with chloroform/methanol (2:1, by volume). Lipid extracts were separated into PL, triglyceride and CE classes by thin-layer chromatography (TLC). Phospholipid fractions were transesterified and the resulting fatty acid methyl esters (FAME) were extracted with n-heptane and transferred into gas chromatography vials. FAME were separated and quantified with a Hewlett-Packard 5880 gas liquid chromatograph using a capillary column equipped with flame ionization detection and Hewlett-Packard Chem-Station data system. FAME were identified by comparison of retention times to authentic lipid standards (NuChek Prep Inc.: Elysian, MN). The between batch coefficients of variation were between 1% and 12%.

Ascertainment of fatal CVD events
In MCCS, fatal events were identified via periodic linkage with the Victorian Registry of Births, Deaths and Marriages and the National Death Index. Deaths are notified to these official registries and participant identity is confirmed by thorough clerical review of names, sex, DOB, marital status, address and years of Australian residency. There are 8 deaths in the current sample informed by a third party and unconfirmed, for which date of deaths were estimated and no cause of death given. Non-fatal events are not available.

Definition of fatal CVD events
In MCCS, fatal myocardial infarction, fatal coronary heart disease, sudden cardiac death and fatal ischemic stroke were defined by the ICD9 and ICD10 classification per recorded on the death certificates (issued by the Victorian Registry of BDM and National Death Index). CVD mortality was defined as the composite of fatal myocardial infarction, fatal coronary heart disease, sudden cardiac death and fatal ischemic stroke. Fatal CHD was defined as fatal myocardial infarction, fatal coronary heart disease, or sudden cardiac death.

MESA: Multi-Ethnic Study of Atherosclerosis
Physical activity assessment
The MESA Typical Week Physical Activity Survey (TWPAS) was adapted from the Cross-Cultural Activity Participation Study and designed to measure weekly duration and frequency of various leisure physical activities. The survey has 28 items in categories of household chores, lawn/yard/garden/farm, care of children/ adults, transportation, leisure walking, dancing and sport activities, conditioning activities, leisure activities, and occupational and volunteer activities. Questions differentiated between light-, moderate-, and heavy-intensity activities. Minutes of activity were summed for each activity and multiplied by metabolic equivalent (MET) level.

Measurement of fatty acid biomarker concentrations
In MESA, blood was sampled after a 12-hr fast. Plasma phospholipid FAs (i.e., circulating ALA, LA, EPA, and DHA were measured using fasting blood samples and expressed as a percentage of total FAs. Details of sample shipping, repository, and processing, as well as extraction of plasma phospholipid FAs, are described elsewhere.[4, 27]

Ascertainment of fatal and non-fatal CVD events
In MESA, an interviewer contacted each participant by telephone to inquire about all interim hospital admissions, cardiovascular outpatient diagnoses, and deaths at 9-12 month intervals. Copies of all death certificates and medical records for all hospitalizations and outpatient cardiovascular diagnoses were requested. In the case of out-of-hospital cardiovascular deaths next-of-kin interviews were conducted. Hospital records were obtained for an estimated 98% of hospitalized cardiovascular events, and some information was available for 95% of outpatient diagnostic encounters. MESA coordinating center collated the abstracted or original end point hospital records suggesting cardiovascular events. Each record was evaluated by 2 cardiologists, cardiovascular epidemiologists, or neurologists for classification and assignment of incidence dates. If, after review and adjudication, disagreements persisted, a full Mortality and Morbidity Review Committee made the final classification.

Definition of fatal and non-fatal CVD events
Total CHD was defined as fatal or non-fatal myocardial infarction, resuscitated cardiac arrest, or CHD death; and ischemic stroke was defined as fatal or non-fatal ischemic stroke. Total CVD was defined as incident CHD or ischemic stroke and CVD mortality was defined as death due to myocardial infarction or ischemic stroke.

METSIM: Metabolic Syndrome In Men

Physical activity assessment

In METSIM, leisure time physical activity was assessed by self-report at baseline using 4 categories:
1st=a little or none
2nd= Physical exercise in context of other hobbies or physical exercise occasionally
3rd= Physical exercise <=2 times a week at least 30 min at a time
4th= Physical exercise regularly>=3 times a week at least 30 min at a time

Measurement of fatty acid biomarker concentrations

Phospholipids, cholesterol esters and triglycerides: Lipids were extracted from plasma sample with chloroform-methanol (2:1) and lipid fractions were separated with an aminopropyl column. FAs in lipid fractions were transmethylated with 14% borontrifluoride in methanol. Finally, FA methyl esters were analyzed by 7890A gas-chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a 25-m free FA phase column (Agilent Technologies). Cholesteryl nonadecanoate (Nu Chek Prep, Inc., Elysian, MA, USA), trinonadecanoin and phosphatidylcholine dinonadecanoyl (Larodan Fine Chemicals, Malmo, Sweden) served as internal standards.
The interassay (between runs) CVs for cholesteryl ester LA, AA, ALA, and EPA were 0.9, 1.6, 0.9, and 3.4 %, respectively. For plasma phospholipid LA, AA, ALA, and EPA interassay CVs were 1.4, 1.2, 1.4, and 2.4%, respectively.
Erythrocyte membranes: Erythrocytes were separated from EDTA-blood and then hemolyzed in the tris-HCl buffer (pH 7.6, 10 mmol/L). Fatty acid methyl esters were prepared by direct trans-esterification using acetyl chloride and analyzed by 7890A gas-chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a 25-m free FA phase column (Agilent Technologies). Pure standards (NU Chek Prep Inc) were used to identify FA methyl esters and to prepare calibration curves. Heptadecanoic acid methyl ester (17:0) served as an internal standard.
Interassay (between runs) CVs for erythrocyte LA, AA, ALA, and EPA were 3.21, 2.55, 3.21, and 4.22, respectively.

Ascertainment and definition of fatal and non-fatal CVD events

An incident cardiovascular event was defined as myocardial infarction, coronary heart disease death or fatal and non-fatal cerebral infarction which occurred after the baseline study. Cardiovascular events were defined according to internationally accepted criteria[28, 29] and verified from the hospital records.

MORGEN: Monitoring Project on Risk Factors for Chronic Diseases

MPCDRF: Monitoring Project on Cardiovascular Disease Risk Factors

Physical activity assessment

In MORGEN, physical activity was assessed with a validated physical activity questionnaire designed for the 'European Prospective Investigation into Cancer and nutrition' (EPIC) including questions on occupational and leisure time physical activity. The question on cycling (Ainsworth code: 02010, 7.0 METs) included cycling towards and from work and in leisure time as an example. For the MORGEN Study, the questionnaire was extended with two open-ended questions on type, frequency, and duration of sports (≥4 METs), and one open-ended question on other strenuous activities. Cycling and sports were classified as 'yes' or 'no'.
In MPCDRF, leisure time physical activity was assessed into the following four categories: 1. Little physical activity, 2. Physical activity during at least 4 hours a week, 3. Regular physical activity, 4. Regular strenuous physical activity.

Measurement of fatty acid biomarker concentrations

Participants donated non-fasting blood at baseline. EDTA-plasma of MPCDRF was stored at -30°C and EDTA-plasma of MORGEN was stored at -80°C until analyzed in 2010. Fatty acids were measured in plasma cholesteryl esters by gas chromatography. In short, to isolate cholesteryl esters, lipids from EDTA plasma were dissolved and separated by solid phase extraction silica columns (Chrompack, Middelburg, The Netherlands). The fatty acids were identified by comparison with known standards (Nu-chek prep, Inc. Elysian, MN, USA). Fatty acids were expressed as mass percentages of total fatty acid methyl esters (g/100 g). A quality control plasma pool was analyzed in duplicate in each run. Coefficients of variation of the controls (intra and inter assay combined) ranged between 3 and 3.5%. Laboratory technicians were blinded to the status of the samples. Cases and controls were randomly distributed over the runs.

Ascertainment of fatal and non-fatal CVD events

Vital status was checked through linkage with the national population register. Participants were followed for cause specific mortality through linkage with Statistics Netherlands. Information on nonfatal stroke was obtained from the national hospital discharge register. Participants were followed until event, death, date of loss-to-follow-up (predominantly because of emigration) or 1 January 2006, whichever came first.

Definition of fatal and non-fatal CVD events

Fatal CHD included fatal MI (I21, I22) and fatal cardiac arrest (CA; I46), according to the International Classification of Diseases (ICD-10, WHO). Incident ischemic stroke (I63, I65, and I66) according to the International Classification of Diseases (ICD-10, WHO). For hospital admissions and for causes of death coded until January 1, 1996, corresponding ICD-9 codes were used.

NHS: The Nurses' Health Study

Physical activity assessment

In Nurses' Health Study, the leisure-time physical activity was assessed in MET-hours per week beginning in 1986. The physical activity included walking or hiking outdoors; jogging (>10 minutes per mile); running (≤10 minutes per mile); bicycling (including stationary bike); swimming laps; tennis; calisthenics, aerobics, aerobic dance, or rowing machine; or squash or racquetball. Physical activity was reassessed in 1988, 1992, 1994, 1996, 1998, and 2000. The 1992 through 2000 questionnaires included other vigorous activities (e.g., lawn mowing) and lower-intensity exercise (e.g., yoga, stretching). Each activity on the questionnaire was assigned a metabolic equivalent task (MET) score based on the classification by Ainsworth et al.[25]

Measurement of fatty acid biomarker concentrations

In 1989 and 1990, interested women in the Nurses' Health Study were sent supplies needed to collect blood samples. The samples were sent back on ice by a prepaid overnight courier. Ninety-seven percent of the samples were received within 24 h of blood drawing. Immediately on arrival, the samples were centrifuged (1200 × g for 15 min at room temperature) and then divided into aliquots of plasma, erythrocytes, and buffy coat fractions. These aliquots were stored in liquid nitrogen freezers at −130 °C or colder until analysis in 2000 and 2002. The methods used to collect and store blood samples have been proven reliable. Fatty acids in serum phospholipids stored at −80 °C for 7–12 y showed minimal degradation over time. The blood samples were stored at a much lower temperature (−130 °C), which was intended to minimize any influences on fatty acid concentrations caused by the long-term storage.

Fatty acid concentrations were determined by gas-liquid chromatography. The methods were described elsewhere.[30] Within-run CV percentages were assessed by repeatedly analyzing pooled samples. The CV percentages of the most abundant fatty acids in plasma were generally lower than those in erythrocytes, although they were all reasonably low. For example, the CV percentage for linoleic acid (18:2n–6), 1.8% (plasma) compared with 2.8% (erythrocytes); and for DHA, 3.4% (plasma) compared with 7.2% (erythrocytes). The CV percentages of trans fatty acids, for which the concentrations were relatively low, were higher than those of the more abundant fatty acids. The average CV percentage of 18:1 trans isomers was 8.0% for plasma and 7.6% for erythrocytes and of 18:2 trans isomers was 6.9% for plasma and 10.0% for erythrocyte.[31] The within-run CV for EPA was 8.5% (plasma) and 16.7% (erythrocytes); and for α-linolenic acid (ALA), it was 6.5% for plasma and 6.5% for erythrocytes.[32]

Ascertainment and definition of fatal and non-fatal CVD events

Nurses with incident cardiovascular disease (defined as nonfatal myocardial infarction, fatal coronary heart disease, or stroke) were identified and asked (or relatives in cases of fatal events) for permission to review their medical records. Physicians who were unaware of other questionnaire information used standardized criteria to confirm and classify the events. Deaths were ascertained from relatives, postal authorities, and the National Death Index, and the cause of death was classified on the basis of medical records, death certificates, and autopsy findings. A diagnosis of myocardial infarction was confirmed on the basis of standardized criteria, which included typical symptoms plus either diagnostic electrocardiographic changes or elevated cardiac enzyme levels.[20, 21] Deaths were ascertained by contact with family members or through the National Death Index. Fatal heart disease was confirmed on the basis of medical records or autopsy reports or, if heart disease was listed as the cause of death, on the basis of the death certificates and evidence of previous heart disease in the records. Stroke was diagnosed according to standard criteria, consisting of a constellation of neurologic deficits of sudden or rapid onset that lasted at least 24 hours or until death.[22, 23] Stroke subtypes were also classified as previously described.[22, 23]

NSHDS: The Northern Sweden Health and Disease Study

Physical activity assessment

In VIP, occupational and leisure time physically activity are assessed with a validated self-administered questionnaire. Occupational physical activity can be categorized as 1) sedentary or standing, 2) light but partly physically active, 3) light and physically active, 4) sometimes physically straining, or 5) physically straining most of the time. Leisure time physical activity during the past three months can be categorized as exercising 1) never, 2) occasionally, 3) 1-2 times/week, 4) 2-3 times/week, or 5) >3 times/week.

In MONICA, occupational physical activity during the past 12 months is asked for with the answer alternatives 1) I am retired, 2) sedentary, 3) light but partly physically active, 4) active and sometimes physically straining, 5) heavy work. Leisure time physical activity during the past 12 months is asked for with the answer alternatives 1) hardly ever, 2) mostly

sedentary, sometimes a walk or similar, 3) light physical activity at least two hours a week, 4) more strenuous physical activity (like jogging, tennis, swimming, badminton, gymnastics etc.) 1-2 hours a week, 5) more strenuous physically activity at least three hours a week, 6) heavy physical activity several times a week (like running, skiing, soccer, swimming). The MONICA-survey questionnaire from 1986 is deviating on these questions. The question "how often do you exercise?" is asked, with the answer alternatives 1) never, 2) 1-2 times/month, 3) once/week, 4) 2-3 times/week, and 5) 4 times/week or more. Concerning occupational physical activity the question "Is your work physically heavy?" is asked, with the answer alternatives 1) yes, often, 2) yes, sometimes, 3) no, seldom, or 4) no, almost never.

Measurement of fatty acid biomarker concentrations
Fatty acids were separated by gas-liquid chromatography after separation of the lipids by thin-layer chromatography and transmethylation[33] 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 relative amounts of the fatty acids were expressed as a percentage of all fatty acids analyzed.
The method imprecision (calculated as the CV for duplicate preparations and measurements) has been reported as <1 - 5.5 %.[34]

Ascertainment and definition of fatal and non-fatal CVD events
MI cases were identified by screening for MI events in hospital medical records, primary care journals and death certificates according to the WHO MONICA criteria.[35] A non-fatal MI case had to be classified as a definite infarction to be included. A definite infarction was required to meet at least one of the following criteria: Typical serial ECG progression (defined by the Minnesota codes); at least one measurement of elevated cardiac markers to more than twice the upper limit of normal combined with abnormal ECG and typical symptoms; or at least one measurement of elevated cardiac markers to more than twice the upper limit of normal combined with atypical symptoms and an ECG progression labeled probable. The diagnoses for fatal MI cases were based on autopsies or confirmed by medical records as being caused by coronary heart disease. An acute stroke case was defined as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 hours (unless interrupted by surgery or death) with no apparent cause other than a vascular origin". All transient ischemic attacks, silent brain infarction (events without clinical signs), stroke caused by trauma, subarachnoid hemorrhage and acute stroke with concomitant brain tumor or severe blood disease were excluded based on WHO criteria.[35]

PHS: Physicians' Health Study
Physical activity assessment
In PHS, exercise was obtained through self- reported at baseline and follow up questionnaires.

Measurement of fatty acid biomarker concentrations
RBC fatty acids were quantified using an established gas chromatography method that was previously.[36] 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%.

Ascertainment of fatal and non-fatal CVD events
In PHS, events were ascertained using annual follow up questionnaire and validated by an endpoint committee through review of medical records. Confirmed death is based on the medical record and/or death certificate.

Definition of fatal and non-fatal CVD events
In this study, Total CHD was defined as fatal or nonfatal myocardial infarction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft. All events were adjudicated by an endpoint committee based on the review of medical records.[37]

PIVUS: The Prospective Investigation of the Vasculature in Uppsala Seniors
Physical activity assessment
Physical activity was assessed in two questions regarding time spent in moderate and vigorous activity each week:
1. How many times per week do you devote yourself to moderate exercise for at least 30 minutes (e.g. walking, cycling, playing golf, gardening etc.)?
2. How many times per week do you devote yourself to vigorous exercise for at least 30 minutes (e.g., running, swimming, playing tennis or football, etc.)?

Time spent at each activity level was summarized to hours and multiplied with an assigned metabolic equivalent (MET) value, 4 for moderate and 8 for vigorous physical activity. Remaining time was multiplied by a MET value of 1.5 (e.g. sitting, reading, eating etc.). MET values were derived with inspiration from Ainsworth et al. (145). Total MET hours per week were summarized and divided by 7 days and then by 24 hours to provide an individual PAL. The approximated PAL ranged from 1.5 to 1.7. Regular physical activity as defined in background statistics was defined as ≥3.5 h per week hard or moderate physical activity.

Measurement of fatty acid biomarker concentrations
Fatty acid composition in cholesterol esters and phospholipids were measured by gas chromatography. Serum (0.5 mL) was mixed with 2.5 mL methanol, 5 mL chloroform (with 0.005% added butylated hydroxytoluene, BHT) and 7.5 mL NaH2PO4 (0.2 mol/l) and stored in 4°C over night for lipid extraction. The chloroform phase was then removed with a syringe and evaporated to dryness on a 30°C heating block using nitrogen gas. The lipid residue was dissolved in chloroform and the lipid fractions were separated by thin-layer chromatography (TLC); the adsorbent containing POPOP as fluorescent agent. The TLC-plates were eluted at room temperature with the solvent system petroleum ether/diethyl ether/acetic acid (81:18:1 by volume). The lipid fractions were visualized in UV light and the spots containing cholesterol esters and phospholipids were scraped off into vials and methylated at 60°C overnight after addition of 2 mL H2SO4 (5%) in methanol. The fatty acid methyl esters were extracted into 3 mL petroleum ether (0.005% BHT) after addition of 1.5 mL distilled water. The phases were separated after thorough mixing and centrifugation at 1500g for 10 minutes. The petroleum ether phase was pipetted off and the solvent was evaporated under nitrogen gas on a 30°C heating block. The fatty acid methyl esters were dissolved in 120 µL hexane and placed in vials. The fatty acid methyl esters were separated by gas-liquid chromatography on a 30-m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, USA) with helium gas as a carrier gas. An Agilent Technologies system consisting of model GLC 6890N, autosampler 7683 and Agilent ChemStation was used. The temperature was programmed to 150-260° C. The fatty acids were identified by comparing each peak´s retention time with fatty acid methyl ester standards Nu Check Prep (Elysian, MN, USA). Fatty acids are presented as the relative sum of the fatty acids analyzed in each compartment.

Ascertainment of fatal and non-fatal CVD events
During the follow-up period, fatal and non-fatal CVD events were collected and validated by an experienced clinician using medical records, the Swedish in-hospital registry and the Swedish cause of death registry.

Definition of fatal and non-fatal CVD events
Incident CHD was defined as fatal or nonfatal myocardial infarction (ICD-10 code I21) or hospitalization due to percutaneous coronary intervention. Incident ischemic stroke was defined as fatal and non-fatal ischemic stroke (ICD-10 code I63). Total CVD was defined as incident CHD or ischemic stroke and CVD mortality was defined as death due to myocardial infarction or ischemic stroke.

Genotype ascertainment
Genotyping was performed using the Illumina OmniExpress and Illumina Metabochip in PIVUS. Sample quality control (QC) was first performed for the OmniExpress or Omni2.5 chip, and for individuals that passed this QC, the QC for Metabochip was also performed. The quality-controlled data of OmniExpress or Omni2.5 and Metabochip was merged. General sample exclusion criteria included: 1) genotype call rate <95%; 2) heterozygosity >3 SD; 3) gender discordance; 4) duplicated samples; 5) identity-by-descent match; and 6) ethnic outliers. General SNP exclusion criteria of genotyped data before imputation included: 1) monomorphic SNPs; 2) Hardy-Weinberg equilibrium (HWE) P-value<1×10-6; 3) genotype call rate<0.99 (SNPs with minor allele frequency [MAF] <5%) or <0.95 (SNPs with MAF≥5%); 4) MAF<1%. Out of 982 samples, 958 passed QC for the OmniExpress, and out of those, 949 passed QC on the Metabochip. Further, 645,318 out of 733,202 OmniExpress SNPs, and 123,771 out of 185,801 Metabochip SNPs passed QC. Imputation was performed for the quality-controlled genotype data of each cohort in IMPUTE v.2.2.2 using haplotypes from the 1000 Genomes, March 2012 release (multi-ethnic panel on NCBI build 37 [b37]).

SCHS: The Singapore Chinese Health Study

Physical activity assessment
The interviewer-administered baseline questionnaire asked about the number of hours per week in the past year spend on strenuous sports, vigorous work, and moderate activity providing 8 answer categories ranging from ‘never’ to ’31 hours or more’. For each type of activity a list of examples was provided.

Measurement of fatty acid biomarker concentrations
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 5.31% for LA and 4.44% for AA. The between batch CV were 6.80% for LA and 7.91% for AA.

Ascertainment and Definition of fatal and non-fatal CVD events
Cases of incident non-fatal myocardial infarction (MI) were identified through the Hospital Discharge Database. Medical records were retrieved and reviewed by cardiologists and we only included those cases 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 based on WHO criteria to confirm MI. Fatal coronary heart disease (CHD) was identified through the Singapore Registry of Births and Deaths and defined as having ICD-9 code 410-414 stated as the primary cause of death. Total CHD was defined as non-fatal MI or fatal CHD.

Genotype ascertainment
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 ± 3standard 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.

SHHEC: The Scottish Heart Health Extended Cohort

Fat biopsy participants
Fat biopsies were obtained early in the program from March 1985 to December 1986, therefore omitting some Scottish Heart Health Study populations and the repeat MONICA surveys. The age range for that was 25-64.

Composition of the SHHEC study
The sampling frame for SHHEC surveys was the National Health Service register of patients in each district, which should have been comprehensive, although it was not completely so, for example omitting vagrants of no fixed address. It did not record race or ethnicity and was sampled randomly. Any possible ethnic or racial underrepresentation in SHHEC could therefore have resulted from differential willingness of minority groups to participate as volunteers. Place of birth was recorded in the SHHEC questionnaire, not ethnicity nor color, and these items were unavailable in the sampling frame so there is no way of checking for representativeness. The population mix of Scotland has become more mixed and multi-ethnic since 1985-6, but it always was so, from centuries of seafaring, empire, and accepting refugees and economic migrants. Response rates in the early surveys averaged 74% overall.

Physical activity assessment
The physical activity questionnaire used early in SHHEC was found to be simplistic with some misleading results. It was not convertible to METS and not analyzed for this study

Medication
The beginnings of SHHEC in 1984-5 antedated the revolution in primary and secondary prevention of cardiovascular disease that followed. Specific questions on daily aspirin and lipid lowering drugs came in later surveys. Prescribed medication, self-reported in those having fat biopsies, after excluding those with prior cardiovascular disease, showed only 4 participants on aspirin for any reason (respondents may have omitted self-medication) and none at all on lipid-lowering drugs.

Measurement of fatty acid biomarker concentrations
The method of obtaining fat biopsies for the SHHEC study was designed to combine speed, practicability and participant compliance, and necessitated written informed consent. Understandably a proportion refused as it involved being stabbed over the triceps muscle without local anesthetic with a skin biopsy punch, to obtain a sample of underlying fat. One attempt only was allowed per person and sometimes no fat was obtained, but the technique produced over four thousand specimens. Fatty tissue was dissected off the skin in the biopsy specimen obtained from the punch, and was

stored in saline and refrigerated for transport to the laboratory, where it was stored at -40°C pending processing. The method and resultant findings have been reported.[38-42]

In the laboratory, total lipid was extracted from frozen homogenized adipose tissue biopsy specimens (5mg) in an isopropranol/heptane mixture (4:1 v/v). Neutral lipids were partitioned from phospholipids and free fatty acid into heptane under alkaline conditions. The extract was dried and redissolved in toluene. Fatty acid methyl esters were obtained by transesterification with sodium methoxide in dry methanol (50C for 15min).The fatty acid methyl esters were separated on a 1.5 m x 4 mm glass column packed with 10% SP-2330 on 100/120 mesh Chromosorb WAW (Supelco) installed in a Hewlett-Packard 5890A gas chromatograph. Eluting peaks were detected using flame ionisation. The fatty acid methyl esters were identified by comparison of retention times with those of standard mixtures. From the repeated analysis of a post-mortem sample of adipose tissue, the coefficients of variation for the estimation of individual fatty acids were found to range from 2% for the major peaks to 22% for the trace constituents (i.e., those peaks comprising less than 1% of total fatty acids). Individual fatty acids were quantified as a percentage of the total amount of fatty acids present in the sample.

The technology and discrimination of fatty acids were therefore those available in the mid-1980s.

Ascertainment of fatal and non-fatal CVD events
Subjects who gave written permission were followed up through death registrations and the national record linkage database, up to the end of 2009.

Definition of fatal and non-fatal CVD events
CVD mortality was defined as deaths attributed to a cardiovascular cause [International Classification of Diseases (ICD) 9 codes 390–459, ICD 10 codes I00-I99]. Total CVD was defined as deaths attributed to a cardiovascular cause [International Classification of Diseases (ICD) 9 codes 390–459, ICD 10 codes I00-I99]; plus any hospital discharge diagnosis post-recruitment of CHD (ICD 9 410-414, ICD 10 I20-I25) or cerebrovascular disease (ICD 9 430– 4, 436-8, ICD10, I60-I69); or surgical codes for coronary artery bypass graft or percutaneous coronary angioplasty. Incident ischemic stroke was not available (validating test results were not available and ICD coding changed unaccountably during one hospital admission from the specific to another, sometimes to non-specific) Analyses were therefore based on total cerebrovascular disease (less transient ischemic attacks) which was defined as deaths or any hospital discharge diagnosis post-recruitment attributed to cerebrovascular disease, excluding transient ischemic attacks (ICD 9 430–4, 436-8, ICD10, I60-I69). Total CHD was defined as deaths attributed to CHD, or any hospital discharge diagnosis post-recruitment of CHD (ICD 9 410-414, ICD 10 I20-I25); or surgical codes for coronary artery bypass graft or percutaneous coronary angioplasty.

60YO: 60-year-old Swedish men and women
Physical activity assessment
Physical activity in leisure-time during the past year was asked for in the baseline questionnaire and the participants classified themselves into one of four groups: 1) low physical activity, a sedentary lifestyle with less than 2 h of light PA/week (i.e. walking, cycling); 2) light physical activity (generally without sweating) at least 2 h/week (i.e. walking, gardening, fishing, table tennis, bowling);3) moderate physical activity, regular activity once or twice a week, at least 30 min each time (i.e. jogging, swimming, tennis, badminton); and 4) high physical activity, intensive regular activity more than twice a week, at least 30 min each time (i.e. running, swimming, tennis, badminton, aerobics or other strain exercise).We created four categories, inactive, light active moderately active and intense active respectively from the possible alternative questions presented above.

Measurement of fatty acid biomarker concentrations
Fatty acid composition in serum CE was measured by gas chromatography. In brief, serum CE were methylated, extracted in petroleum ether, evaporated, and redissolved in hexane before analysis by gas chromatography with flame ionization detection. Thirteen different FA were quantified (14:0-22:6n3) and the proportion of each was expressed as a percentage of all measured FA. Fatty acid composition in one serum sample was repeatedly analyzed in duplicates in all batches for quality control and the intra- and interassay coefficient of variations were ≤0.24 and ≤2.49 %, respectively, for the fatty acids utilized for statistical analyses (EPA, AA, LA, and ALA).

Ascertainment of fatal and non-fatal CVD events
In the 60YO, CVD events were identified using the Hospital Care Register and the Cause of death register in Sweden. All participants were followed regarding incident of CVD and death up to 31st December 2012. During the follow-up (median 14.5 y), 306 men and 185 women suffered from their first CVD event Participants that had a prior event of CVD at the baseline were excluded by analysis.

Definition of fatal and non-fatal CVD events

Total CVD incidence included fatal and non-fatal myocardial infarction, sudden cardiac death or ischemic stroke and death for coronary heart disease; international classification of Disease 10th revision (ICD-10) codes: I20, I21, I25, I46, and I63. Total CHD incidence included: fatal and non-fatal myocardial infarction, sudden cardiac death, or death for coronary heart disease; international classification of Disease 10th revision (ICD-10) codes: I20, I21, I25, I46, and I63. CVD mortality included the composite of fatal myocardial infarction, death for coronary heart disease and sudden cardiac death. Ischemic stroke included fatal and non-fatal ischemic stroke; international classification of Disease 10th revision (ICD-10) codes I63.

3C: The Three-City Study

Physical activitiy assessment

In the 3C study, regular exercise was defined as engaging in vigorous physical activity (e.g., running, swimming, playing tennis) ≥ 1 hour /week, or having at least one hour of leisure time physical activity (e.g., walking, housing, hunting) per day.

Measurement of fatty acid biomarker concentrations

In the 3C study, fasting blood samples were collected at the baseline visit into heparinized evacuated tubes and centrifuged at 1000 _ g for 10 min. Total lipids were extracted from plasma with 5 mL of hexane/isopropanol (3:2, by vol). The plasma fatty acid composition was determined from 2 mL of the lipid extract after transformation into isopropyl esters. Separation of isopropyl esters was made on a gas chromatograph (Trace, Thermoelectron, France) using a 25-m Carbowax capillary column (internal diameter: 0.32 mm). Column conditions were 180 °C for 5 min, increasing by 7.5 °C/min to 220 °C for 30 min. The injector was set at 60 °C and the flame ionization detector at 250 °C. Helium was used as the carrier gas (flow rate: 2 mL/min). The peaks were identified by comparison with reference fatty acid esters (Sigma, St Louis, MO), and peak areas were measured with an automatic integrator (DP700; Fisons Instruments, France). The results for each fatty acid were expressed as a percentage of total fatty acids.

Ascertainment of fatal and non-fatal CVD events

In the 3C study, history of stroke and hospitalizations for a CHD event was assessed at baseline during a face-to-face interview conducted by trained psychologists and nurses, and occurrence of hospitalizations for a CHD event was recorded at each follow-up examination performed two, four, six and eight years after the baseline visit, either during a face-to-face interview or by self-questionnaire. At each of these visits, the participants were asked if they had had a stroke history or stroke symptoms, and if they had been hospitalized. In those who screened positively for stroke, further medical data was collected, including emergency medical service and hospitalization reports, neuro-imaging reports, interview with the patient's physician or the family. According to the diagnostic criterion of the World Health Organization, a stroke was defined as a new focal neurological deficit of sudden or rapid onset, of presumed vascular origin, that lasted 24 h or more, or leading to death. A specific validation committee composed of neurologists reviewed all available information and confirmed or not the diagnosis of stroke and its subtype (ischemic or hemorrhagic). Patients with transient ischemic attacks were not included. Fatal events were classified according to the 10th revision of the International Classification of Diseases. In those who reported a possible CHD event, further medical data was collected, including emergency medical service and hospitalization reports, and interview with the patient's physician or the family.

Definition of fatal and non-fatal CVD events

Fatal CHD was defined as ICD-10 codes I210–I219, I251–I259, I461 and R960. Total CHD events included stable and unstable angina pectoris, coronary balloon dilatation or artery bypass, non-fatal MI and death due to CHD. Total CVD was defined as ischemic heart disease, fatal or nonfatal myocardial infarction, and fatal or non-fatal ischemic stroke. CVD mortality was defined as deaths classified by ICD-10 codes I210–I219, I251–I259, I461 and R960.

Genotype ascertainment

In 3C study, genotyping was done using a high-density SNP marker platform (Illumina 610). Samples with call rates below 95% at genotyped markers were excluded. Imputation was conducted using IMPUTE software. SNPs for which Hardy Weinberg equilibrium resulted in $p<10\text{-}6$ were excluded from imputation.

ULSAM: The Uppsala Longitudinal Study of Adult Men

Physical activity assessment

The leisure time physical activity was assessed by questionnaire, and participants were classified according to the duration and vigorousness of their physical activity.

Measurement of fatty acid biomarker concentrations

For analysis of the fatty acid composition of the serum cholesterol esters in ULSAM-50, serum was extracted with a hexane-isopropanol solution (1+4).[43] Cholesterol esters were separated from the extract by thin layer chromatography before inter-esterification (acidic methanol at 85°C, 2 h),[44] and free cholesterol liberated in the reaction was removed by an aluminum 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.
Initially the adipose tissue fatty acid composition was analysed in a random subsample of 318 men. In December 2008, 535 new samples were analysed for adipose tissue fatty acid composition. Four additional fatty acids were included in this analysis, namely lauric acid 12:0, myristic acid 14:0, pentadecanoic acid 15:0 and heptadecanoic acid 17:0.
In ULSAM-70, subcutaneous adipose tissue was collected with biopsy as described previously.[14, 45] The subject lay face down and the biopsy was taken with a needle coupled to a vacuum tube from the upper, outer quadrant of the buttocks. The sample was collected in the connector between the needle and the tube, and stored at -70°C in the connector for some weeks until analysis. Prior to the fatty acid analysis the biopsy was weighed and homogenized.
The fatty acid composition of subcutaneous adipose tissue was analysed according to Boberg et al[33] as previously described.[46] Adipose tissue (5–25 mg) was extracted in 2.5 ml methanol and 5.0 ml chloroform (containing 0.005% [wt/vol.] butylated hydroxytoluene [BHT] as an antioxidant) and 7.5 ml 0.2 mol/l sodium dihydrogen phosphate (NaH2PO4) was added and the extract was left at 4°C overnight. The chloroform phase was evaporated to dryness under nitrogen and the lipid esters were transmethylated at 60°C overnight after addition of 2 ml of 1 mol/l H2SO4 in methanol. The methyl esters were extracted into 3 ml petroleum benzine containing 0.005% (wt/vol.) BHT after adding 1.5 ml of distilled water. The phases were separated after thorough mixing and centrifugation at 1,500×g for 10 min. The petroleum benzine phase was pipetted off and the solvent was evaporated under nitrogen. The methyl esters were then redissolved in Uvasol grade hexane (Merck, Darmstadt, Germany). The fatty acid methyl esters were separated by gas–liquid chromatography (GLC) on a 30 m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, Franklin, MA, USA), with helium as carrier gas. An Agilent Technologies (Santa Clara, CA, USA) system consisting of a model GLC 6890N, autosampler 7683 and an Agilent ChemStation was used. The temperature was programmed to 150–260°C. The fatty acids were identified by comparing each peak's retention time with fatty acid methyl ester standard Nu Check Prep (Elysian, MN, USA). For the first 319 samples, a different column was used (25 m Quadrex [Woodbridge, CT, USA] fused silica capillary column OV-351) and the temperature was lower (130–220°C), as fewer fatty acids were included in the analysis; methodology was otherwise analogous, with similar validity.

Ascertainment of fatal and non-fatal CVD events
The Swedish Hospital Discharge Register and the Swedish Cause of Death Register were used to ascertain incident cases of fatal and non-fatal CVD

Definition of fatal and non-fatal CVD events
Total CHD was defined as non-fatal myocardial infarction (ICD-10: I21; ICD-9 & ICD-8: 410), CHD death (ICD-10: I20-I22; ICD-9 & ICD-8: 410-411, 413), or sudden cardiac arrest (ICD-10: I46.1; ICD-9: 427.5; ICD-8: 427.27). Ischemic stroke was defined as fatal or non-fatal ischemic stroke (ICD-10: I63.0-I63.5, I63.8-I63.9; ICD-9: 433-434; ICD-8: 432-434). Total CVD was defined as Total CVD or ischemic stroke. CVD mortality was defined as CHD death fatal ischemic stroke, or sudden cardiac arrest.

Genotype ascertainment
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 January and June, 1996, to leave a blood sample for DNA preparation (n = ~729). Whole peripheral blood was incubated with lysis buffer (NH4Cl, KHCO3 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. The SNP rs174547 was genotyped in March 2010 using Cardio-Metabo Chip from Illumina. The call rate was 99.75% and the accuracy was 100%.

WHIMS: The Women's Health Initiative Memory Study

Physical activity assessment

In WHIMS, self-reported information on sedentary behavior and usual physical activity at baseline was assessed with standard conversions to METs.

Measurement of fatty acid biomarker concentrations

The fatty acid composition of RBC samples were analyzed by gas chromatography equipped with a SP 2560 capillary column after direct transesterification for 10 minutes in boron trifluoride/ methanol and hexane at 100 C as previously described. This technique generates fatty acids primarily from RBC glycerophospholipids. During the aliquoting phase, the RBC samples were stored improperly at -20°C for a period of approximately 2 weeks, causing oxidative degeneration of the PUFAs before measurement. The original FA levels were estimated with multiple imputations using independent data on fatty acid degradation and length of time the samples were exposed to -20°C.[47] All fatty acids present at >1% abundance had CVs of ≤6.5%.

Ascertainment of fatal and non-fatal CVD events

The majority of CVD events were ascertained through a central adjudication process across the entire study, this included the time of the main study and the first extension time period (2005-2010). The second extension time period (2010-2015) used self-report for CVD events. Ischemic Stroke was adjudicated for CT and OS participants through Ext1. In Ext2 it is only adjudicated for the Medical Record Cohort. Clinical MI and death were adjudicated for CT and OS ppts through Ext1. In Ext2 they are only adjudicated for the Medical Record Cohort. Definite Silent MI was only adjudicated for CT participants during the core WHI study.

Definition of fatal and non-fatal CVD events

Total ischemic stroke was defined as a stroke where the diagnosis question indicates an ischemic stroke. Strokes from cause of death, Form 121 and early versions of 132 were not able to determine hemorrhagic/Ischemic classification. Total ischemic stroke includes inpatient strokes for the core WHI study and inpatient and outpatient strokes after the core study closeout. Total CHD was defined as the first occurrence of clinical MI, definite silent MI or a death due to definite CHD or possible CHD. CVD mortality was defined as death by cebrovascular, definite CHD or possible CHD. Total CVD is defined as any one of the three categories above (total ischemic stroke, total CHD and CVD mortality).

Genotype ascertainment

Genotyping was conducted using the Human Omni Express Exome-8v1_B, with imputation using the 1000 genomes reference panel.

Supplemental Table 1. General information of contributing cohorts	
Study	**General information**
AGES-Reykjavik	The AGES-Reykjavik Study is a sample of 5,764 men and women who were survivors from an established single center population based cohort; the Reykjavik Study, begun in 1967 to study heart disease. The AGES-Reykjavik Study was designed to examine risk factors, including genetic susceptibility and gene/environment interaction, in relation to disease and disability in old age. At study baseline (2002–2006), participants were aged 66–96 years. Fatty acids were measured in an ancillary case-cohort study of fracture. Participants were those without a history of osteoporotic fracture and incident osteoporotic fracture during follow-up (cases) and the cohort were those who had no implanted devices or severe kidney disease and had consented to link their data to the fracture registry in Iceland.[48]
ARIC	The Atherosclerosis Risk in Communities (ARIC) study is a multi-center, prospective study of atherosclerosis in a bi-racial population, including 15, 792 African American and Caucasian men and women aged 45-64 years. These adults were recruited from 4 US communities (Jackson, Mississippi, Minneapolis, MN; Washington County, MD; and Forsyth County, NC) in 1987-89. Plasma phospholipid and cholesterol ester fatty acids were measured in 3837participants living in Minneapolis, Minnesota
CCCC	The Chin-Shan Community Cardiovascular Cohort study has begun in 1990, following 1703 men and 1899 women aged 35 years old and above, homogenous in Chinese ethnicity, in Northern Taiwan for the study of cardiovascular diseases. The cohort was assembled from the registry data of the bureaucracy and the participants were recruited by house-to-house visits. The study was approved by the IRB in the National Taiwan University Hospital. Participants received baseline health examination at the community health center. We excluded persons with a baseline history of cardiovascular disease and missing metabolic syndrome status. A total of 3515 subjects were included in this study. We recruited the subjects by volunteer basis and respondent rates were up to 83%. In the survey, all of the study participants were individually interviewed from a structured questionnaire, for the information on socio-demographic characteristics, physical activity, smoking, alcohol drinking habits, dietary characteristics, personal and family histories of diseases and hospitalizations. With informed consent, the participants underwent physical examinations and laboratory tests. The examiners undertook training in the questionnaire collections and measures. Blood samples utilized for biomarker fatty acid measurements were collected at three time points, i.e., 1992-1993, 1994-1995, and 1999-2000.
CHS	CHS is a community-based cohort established by the NHLBI. Medicare eligibility lists from 4 US communities (Sacramento County, CA; Washington County, MD; Forsyth County, NC; and Allegheny County, PA) were used to randomly select and enroll 5201 men and women in 1989-1990; an additional black subcohort of 687 individuals were recruited and enrolled in 1992-1993. Inclusion criteria: Adults 65yrs or older, non-institutionalized, expected to remain in current community for >3yrs, not under active hospice care or cancer treatment.
CRS	The Costa Rica Study (total n=4547) is a population-based case-control study of nonfatal myocardial infarction (MI). Eligible case subjects were diagnosed as survivors of an acute MI for the first time by two independent cardiologists at any of the three recruiting hospitals in the catchment area, which comprised 18 counties in Costa Rica.[49] Exclusion criteria for cases included: death during hospitalization, age of 75 years or greater at the time of MI, or being physically or mentally unable to answer the questionnaire. Controls were randomly selected using the national census data and 1:1 matched to cases on age group (+/-5 years), sex, and area of residence. Exclusion criteria for controls included history of a prior MI and being unable to answer the questionnaire. After excluding participants with missing covariates and rematching cases to controls, we have included 1687 cases and 1687 controls in the presented analysis.
DCH	The Diet, Cancer and Health (DCH) Study is a Danish prospective cohort establish between 1993 and 1997 consisting of 57,053 men and women. A case-control study nested within the DCH was conducted. After appropriate exclusions, the study included 2,138 incident MI cases and 3,011 randomly selected (sex stratified) sub-cohort participants. At inclusion, participants filled in a questionnaire concerning lifestyle and medical history. Inclusion criteria: Age 50 to 64 years, born in Denmark, living in the greater Copenhagen or Aarhus area, and with no previous cancer diagnosis registered in the Danish Cancer Registry.
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.
EPIC-Potsdam	The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study is part of the multi-centre prospective cohort study EPIC. In Potsdam, Germany, 27,548 subjects (16,644 women aged mainly 35-65 years and 10,904 men aged mainly 40-65 years) from the general population were recruited between 1994 and 1998. We randomly selected 2,500 individuals from all participants of the EPIC-Potsdam study population who provided blood samples (*n*=26,444) for a subcohort. For the present analysis, we have excluded participants with prevalent CVD (n=182), unclear disease status (n=16) as well as participants with implausible fatty acid values (n=1,704).
FHS	The Framingham Heart Study (FHS) Offspring sample is a population based longitudinal study of families living in Framingham, Massachusetts. The offspring study was initiated in 1971 and consisted of a sample of 5,124 individuals, offspring of the original cohort and their spouses.
HS	The Hisayama Study is an ongoing, population-based prospective cohort study of cardiovascular disease and its risk factors in the town of Hisayama, a suburb in the metropolitan in Japan. In 2002 and 2003, a total of 3328 residents, aged 40 years or older (77.6% of the total population in this age group) underwent the annual health examination. After excluding 30 subjects who did not consent to participate in the study, 190 subjects with a history of cardiovascular disease, and 5 subjects without available data on serum fatty acid levels, the remaining 3103 participants were enrolled in the study.
HPFS	The Health Professionals Follow-Up Study (HPFS) began in 1986. The purpose of the study is to evaluate a series of hypotheses about men's health relating nutritional factors to the incidence of serious illnesses, such as cancer, heart disease, and other vascular diseases. This all-male study is designed to complement the all-female Nurses' Health Study, which examines similar hypotheses. The HPFS is sponsored by the Harvard School of Public Health and is funded by the National Cancer Institute. In the beginning, Walter Willett, Principal Investigator, Meir Stampfer, and colleagues enlisted 51,529 men in health professions aged 40 to 75 years at baseline in 1986 to participate in the study. This group is composed of 29,683 dentists, 4,185 pharmacists, 3,745 optometrists, 2,220 osteopath physicians, 1,600 podiatrists, and 10,098 veterinarians. Among the study participants are 531 African-Americans and 877 Asian-Americans. Every two years, members of the study receive questionnaires with questions about diseases and health-related topics like smoking, physical activity, and medications taken. The questionnaires that ask detailed dietary information are administered in four-year intervals. Between 1993 and 1995, a blood sample was requested from all subjects and returned from 18 225 participants.
KIHD	The KIHD study was designed to investigate risk factors for CVD, atherosclerosis, and related outcomes in a population-based, randomly selected sample of men from eastern Finland. 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 in two cohorts. The first cohort consisted of 1166 men who were 54 years old, enrolled in 1984-1986, and the second cohort included 1516 men who were 42, 48, 54 or 60 years old, enrolled in 1986-1989.
MCCS	The MCCS was designed to be a prospective cohort study. Between 1990 and 1994, 41 514 volunteers, (24 469 women and 17 045 men) aged 40-69 y were recruited. Participants were recruited from electoral rolls, advertisements, and community announcements. About 24% of the cohort are southern European migrants to Australia who were deliberately over-sampled to extend the range of lifestyle exposures and to increase genetic variation. Inclusion criteria: age 40-69 y, Melbourne residents of any ethnic origin.
MESA	MESA is an NHLBI funded study of the characteristics of subclinical cardiovascular disease 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 at baseline men and women aged 45-84. Thirty-eight percent of the recruited participants are white, 28% African-American, 22% Hispanic, and 12% Asian, predominantly of Chinese descent. 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.

METSIM	The population-based METSIM study includes 10 197 Finnish men, aged from 45 to 73 y at the baseline study (2005-2010) and who were living in Kuopio or surrounding communities and were willing to participate in the study. Plasma FAs were measured in a random sample of 1364 men of the entire cohort. Of them, data related to CVD was available in 1353 participants.
MORGEN & MPCDRF	The Monitoring Project on Risk Factors for Chronic Diseases (MORGEN Study) is a population-based cohort study of over 22,654 men and women, aged 20-65 years, from three cities in the Netherlands (Amsterdam, Doetinchem and Maastricht). The MORGEN Study is part of the European Prospective Investigation into Cancer and Nutrition study Information on diet, lifestyle, and cardiovascular risk factors was collected and blood was drawn at baseline (1993-1997). Participants are followed for fatal and nonfatal cardiovascular disease endpoints. The Monitoring Project on Cardiovascular Disease Risk Factors (MPCDRF) is a Dutch population-based cohort with similar data as the MORGEN Study (see above), but with longer follow-up for fatal endpoints. In MPCDRF, baseline (1987-1991) and follow-up data were collected in ~36,000 subjects aged 20-59 years. Baseline blood samples and information on lifestyle, and cardiovascular risk factors were collected in 35,475 subjects aged 20–59 years during 1987–1991 in MPCDRF and in 22,654 subjects aged 20–65 years during 1993–1997 in the MORGEN Study.[50] For 7,754 participants who participated in both cohorts, we used the more recent MORGEN data. In addition, we excluded participants who did not provide informed consent for vital status follow-up and participants with a history of myocardial infarction (MI) or stroke at baseline, resulting in 26,987 participants in MPCDRF and 21,335 participants in MORGEN. Fatal CHD was assessed in MPCDRF and MORGEN,[51] while total stroke was evaluated only in MORGEN.[52] For each case, one control from the same cohort was selected based on incidence density sampling. Controls were selected from those persons under study who survived at least as long as the index case. A person was eligible to serve as a control for multiple cases at a given moment in time and could serve both as control and case. Cases were individually matched to controls on age (+/−0.5y), gender, and date of entry in the cohort (+/−0.5y). For fatal CHD, plasma was available for 222 case-control pairs of MPCDRF and 57 pairs of MORGEN and. In MPCDRF, five participants were selected as a control twice and four participants served both as a control and as a case. In MORGEN, 1 participant was selected both as case and control. For total stroke, plasma was available for 179 case-control pairs. Five participants were selected as a control twice. One participant served both as a control and as a case.
NHS	The Nurses' Health Study was established by Dr. Frank Speizer in 1976 with funding from the National Institutes of Health. The primary motivation in starting the NHS was to investigate the potential long term consequences of the use of oral contraceptives, a potent drug that was being prescribed to hundreds of millions of normal women. Married registered nurses who were aged 30 to 55 in 1976, who lived in the 11 most populous states and whose nursing boards agreed to supply the study with their members' names and addresses were enrolled in the cohort if they responded to our baseline questionnaire. The original states were California, Connecticut, Florida, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, Pennsylvania and Texas. Approximately 122,000 nurses out of the 170,000 mailed responded. Every two years cohort members receive a follow-up questionnaire with questions about diseases and health-related topics including smoking, hormone use and menopausal status. In 1980, the first food frequency questionnaire was collected. Subsequent diet questionnaires were collected in 1984, 1986 and every four years since. At the request of some of the nurses and with the addition of investigators to the research team interested in quality of life issues, question related to quality-of-life were added in 1992 and repeated every four years. Because certain aspects of diet cannot be measured by questionnaire, particularly minerals that become incorporated in food from the soil in which it is grown, the nurses submitted 68,000 sets of toenail samples between the 1982 and 1984 questionnaires. Similarly, to identify potential biomarkers, such as hormone levels and genetic markers, 33,000 blood samples were collected in 1989-90 followed by second samples from 18,700 of these participants in 2000-01. These samples are stored and used in case/control analyses.
NSHDS	The Northern Sweden Health and Disease Study (NSHDS) consists of three subcohorts: the Västerbotten Intervention Program (VIP) cohort, the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) cohort, and the Mammary (mammography) Screening Program. The VIP and MONICA cohorts have been used for cardiovascular and diabetes studies, and these are therefore further described here. The VIP is a long-term project intended for health promotion of the population of the county of Västerbotten (approximately 254,000 inhabitants). Individuals 40, 50, and 60 years of age in the county are invited for screening. They are 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 September 2017, the VIP cohort comprised 156,300 sampling occasions from 105,700 unique individuals who were 40, 50 and 60 years old at baseline. Screenings within the MONICA cohort, with a similar design as VIP (VIP was designed after MONICA), have been performed about every fifth year since 1986. Until 2014, 15,300 random blood samples from 11,800 individuals from the counties of Västerbotten and Norrbotten, ranging in age from 25 to 74 had been included. Both cohorts are population-based. Within NSHDS, prospective case-control cohorts on MI (FIA) and stroke (Castro) have been formed. Cases and controls were matched for sex, age, sampling year and geographical area. Fatty acids were analyzed in plasma phospholipids for the following prospective case-control cohorts: 1. FIA 1 (NSHDS I): 64 cases with MI and 119 controls 2. FIA 2 (NSHDS II): 353 cases with MI and 406 controls 3. Castro 1 (NSHDS III):: 107 stroke cases and 210 controls (86 with ischemic stroke and 169 controls)
PHS	PHS consists of two completed randomized trials in nearly 30,000 male physicians to assess the effects of aspirin, beta-carotene and vitamins on the incidence of CVD and cancers.[53, 54] In an ancillary study of PHS, we measured red blood cell fatty acids on 1000 pairs using a risk set sampling design among PHS participants who provided blood sample between 1995 and 2001 and were alive and free of CHD. Detailed description of the ancillary study has been published.[55]
PIVUS	The PIVUS study started in 2001 with the primary aim to investigate the predictive power of different measurements of endothelial function and arterial compliance in a random sample of 1000 subjects aged 70 living in the community of Uppsala. As secondary aims, the study also included measurements of cardiac function and structure by ultrasound and MRI, evaluation of atherosclerosis by ultrasound and MRI, 7 day food intake recordings, detailed ECG analysis, cardiovascular autonomic function, body composition by DXA, DNA analysis and lung function, as well as a number of biochemical markers. The inclusion of subjects in the study was completed in June 2004. In March 2006 a reinvestigation of the cohort at the age of 75 was started. The major measurements performed at age 70 will be repeated at age 75. In addition, we will focus on infarcts in the brain and heart, so both of these items will be investigated by brain and myocardial MRI in all subjects. Also cognitive tests are included in the reinvestigation that was completed in September 2009. Of the initial 1016 subjects, 52 had died during the 5 years and 827 attended the re-examination at age 75. In the spring of 2011 we started the 80-year reinvestigation of the cohort. This round will continue until the summer of 2014 and we expect that 500-600 of the original sample will attend. We measure the traditional CV risk factors, 2 cognitive function tests, ultrasound of the carotid arteries and the heart, a DXA scan and an MRI scan of the brain and abdominal region.
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. 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. All participants of the nested case-control study were free of coronary heart disease and stroke at the time of blood collection. Cases were fatal coronary artery disease (CAD) or non-fatal myocardial infarction (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.
SHHEC	The completed Scottish Heart Health Extended Cohort (SHHEC) study is a population-based, randomly-sampled prospective cohort, which includes both The Scottish Heart Health Study[38] and the Scottish surveys in the World Health Organization Multinational MONItoring of Trends and Determinants in CArdiovascular Disease (MONICA) Project,[39] sharing a common protocol approved by relevant research ethics committees. Men and women aged 40-59 were recruited in 1984-1987 across 23 districts of Scotland for participation in The Scottish Heart Health Study. The Scottish MONICA Project recruited adults aged 25-64 from Edinburgh in 1986 and north Glasgow in 1986, 1989, 1992, (25-76) and 1995. Participants completed health questionnaires, a physical examination, and had non-fasting venous blood drawn at recruitment, and gave written consent to being followed-up through their medical records. They were

	flagged on the National Health Service Register for deaths, and matched with the Scottish record linkage system both for deaths and hospital admissions, most recently in early 2010.
60YO	The 60-year-old Swedish men and women (60YO) is a population-based cohort. Every third man and woman living in Stockholm County, Sweden who was born between 1 July 1937 and 31 June 1938 (60 years old) was invited to participate in a screening of cardiovascular disease risk factors between August 1997 and March 1999. Of these (5,460), 4,232 (78% response rate), 2,039 men and 2,193 women, agreed to participate. At the baseline, the participants underwent a physical examination that included anthropometric measurements, blood pressure was also measured and blood samples were drawn after overnight fasting. The participants also completed an extensive questionnaire that included providing information about their disease history, health status, medication therapy, lifestyle, and nutritional habits. Inclusion criteria: 60 years old men and women residing in Stockholm County between August 1997 and March 1999.
3C Study	The Three-City (3C) study is an ongoing multicenter prospective cohort study of vascular risk factors for dementia which started in 1999-2000 and included 9,294 community dwellers in three French cities: Bordeaux (n=2,104), Dijon (n=4,931) and Montpellier (n=2,259). Individuals living in one of these cities, aged 65 years and over and not institutionalized were eligible for recruitment into the 3C study. The protocol of the 3C study has been approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research of the Kremlin-Bicêtre University Hospital (Paris). All participants gave their written informed consent. The baseline data collection included socio-demographic and lifestyle characteristics, symptoms and complaints, main chronic conditions, medication use, neuropsychological testing, clinical examination including blood pressure measurement, electrocardiogram (ECG) and blood sampling. Total plasma fatty acids were measured at baseline from fasting blood samples among 1,416 individuals from the Bordeaux centre.
ULSAM	The Uppsala Longitudinal Study of Adult Men ULSAM is a community-based cohort of men living in Uppsala county, Sweden. The origin of this longitudinal study was the "Uppsala Primary Preventive Study", carried out between September 1970 and September 1973. The study comprised all men living in the County of Uppsala born between 1920 and 1924 selected from the register of County Council. All men (n=2841) were invited for the investigation, 81.7% (n=2322) participated. The mean age at this baseline examination was 49.6 (SD +/- 0.6), hence this starting cohort was referred to as ULSAM-50. After this baseline examination, all men were invited to participate in follow-up investigations at the ages 70, 82 and 88. Between the age 50 and 70, 422 had died and 219 had moved out of the Uppsala region. Of the 1681 men invited, 460 did not participate in this follow up, leaving 1221 men who participated (response rate of 73%) aged around 70 (ULSAM-70). The men were invited by a letter, which also explained the aim of the examination. They received the letter 7-10 days prior to the examination. Those born at the beginning of the year were called first. Six individuals were called every weekday except for the vacation period in Sweden between June 25 and August 15. A second invitation letter was sent at the end of the examination of each age class to those who had not come after the first invitation. The screening examination program included a medical questionnaire and interview, blood and urine sampling, blood pressure and anthropometric measurements, intravenous glucose tolerance test, ECG recording, chest X-ray and pure tone audiometry. At the baseline exam (ULSAM-50), fatty acid composition was assessed in serum cholesterol, whereas at the second exam 20 years later (ULSAM-70), fatty acids were measured in both cholesterol esters and adipose tissue.
WHIMS	The Women's Health Initiative Memory Study (WHIMS) examined the effects of postmenopausal hormone therapy on cognitive function in women aged 65-80 years. Recruitment began in 1996.

Supplemental Table 2. Cohort sources of support	
Study	**Funding**
AGES-Reykjavik	Office of Dietary Supplements, NIH contract N01-AG012100, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament).
ARIC	The Atherosclerosis Risk in Communities study was performed as a collaborative study supported by contracts HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, HHSN268201100012C, R01HL087641, R01HL59367, and R01HL086694 from the National Heart, Lung, and Blood Institute; contract U01HG004402 from the National Human Genome Research Institute; and contract HHSN268200625226C from the National Institutes of Health. We thank the staff and participants of the Atherosclerosis Risk in Communities study for their important contributions. Infrastructure was partly supported by grant UL1RR025005, a component of the National Institutes of Health and National Institutes of Health Roadmap for Medical Research.
CCCC	This research was partly supported by Ministry of Science and Technology, Taiwan (MOST 103-2314-B-002 -135 -MY3, NSC 102-2314-B-002 -080 -MY2, and NSC 100-2314-B-002 -113 -MY3).
CHS	The Cardiovascular Health Study (CHS) was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants U01HL080295 and U01HL130114 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.
CRS	The Costa Rica Study on adults was supported by grant R01HL081549 from the National Institutes of Health.
DCH	Not reported.
EPIC-Norfolk	EPIC-Norfolk was funded by grants from Medical Research Council and Cancer Research UK. Drs Imamura, Forouhi and Wareham acknowledge support from the core Medical Research Council Epidemiology Unit Programmes (MC_UU_12015/1 and MC_UU_12015/5).
EPIC-Potsdam	Supported by the German Federal Ministry of Science (01 EA 9401) and the European Union (SOC 95201408 05F02) for the recruitment phase of the EPIC-Potsdam Study. The follow-up of the EPIC-Potsdam Study was supported by the German Cancer Aid (70-2488-Ha I) and the European Community (SOC 98200769 05F02). The present study was also supported by a grant from the German Research Foundation (DFG, SCHU 1516/5-1).
FHS	The Framingham Heart Study is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with Boston University (Contract No. N01-HC-25195). This manuscript was not prepared in collaboration with investigators of the Framingham Heart Study and does not necessarily reflect the opinions or conclusions of the Framingham Heart Study or the NHLBI.
HPFS	The Health Professionals Follow-up Study (HPFS) was supported by grants UM1 CA167552, R01 HL35464, AA11181, HL35464, CA55075, HL60712, and P30DK046200 from the National Institutes of Health.
HS	This study was supported in part by Grants-in-Aid for Scientific Research (A) (16H02644) and (C) (15K09267, 15K08738, 15K09835, 16K09244, 17K09114, 17K09113, and 17K01853) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the Japan Agency for Medical Research and Development (dk0207025).
KIHD	The Kuopio Ischaemic Heart Disease Risk Factor Study was supported by grants 41471 and 1041086 from the Academy of Finland, Helsinki, Finland.
MCCS	The Melbourne Collaborative Cohort Study recruitment was funded by VicHealth and Cancer Council Victoria and was further supported by grants 209057, 251553, and 504711 from Australia's National Health and Medical Research Council and by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry and the Australian Institute of Health and Welfare, including the National Death Index and the Australian Cancer Database.
MESA	The Multi-Ethnic Study of Atherosclerosis (MESA) and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute in collaboration with MESA investigators. Support for MESA is provided by contracts N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-001079, and UL1-TR- 000040 from the National Heart, Lung, and Blood Institute.

METSIM	The METSIM Study was funded by the grants from The European Union, the Academy of Finland, and the Juselius Foundation.
MORGEN & MPCDRF	The Monitoring Project on Risk Factors for Chronic Diseases (MORGEN Project) and the Monitoring Project on Cardiovascular Disease Risk Factors (MPCDRF) were financially supported by the Ministry of Health, Welfare and Sport of the Netherlands, the National Institute for Public Health and the Environment, Bilthoven, The Netherlands, and the Europe Against Cancer Program of the European Union. An unrestricted grant from the Alpro Foundation, Belgium covered the costs of the fatty acid measurements.
NHS	The Nurses Health Study was supported by research grants UM1 CA186107, R01 CA49449, R01 HL034594, P01CA87969, P30DK046200, R01HL034594, and R01HL088521 from the National Institutes of Health.
NSHDS	The Northern Sweden Health and Disease Studies I-III were supported by the Swedish Cancer Society and the Swedish Research Council.
PHS	The current study is supported by R21HL088081. PHS is funded by NIH CA-34944, CA-40360, CA-097193, HL-26490 and HL-3459.
PIVUS	The study was supported by Uppsala University Hospital; The Swedish Research Council for Health, Working Life and Welfare; The Swedish Research Council (K2015-54X-22081-04-3); and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (2016-01639)
SCHS	The Singapore Chinese Health Study was supported by grant NMRC 1270/2010 from the Singapore National Medical Research Council and grants R01CA 144034 and UM1 CA182876 from the National Institutes of Health. W-P Koh is supported by the National Medical Research Council, Singapore (NMRC/CSA/0055/2013).
SHHEC	The Scottish Heart Health Extended Cohort study was funded by the Scottish Health Department Chief Scientist Organization, British Heart Foundation, and FP Fleming Trust.
60YO	This study was supported by Stockholm County Council, Swedish Heart and Lung-Foundation, Swedish Research Council.
3C Study	The Three-City study was conducted under a partnership agreement between the Institut National de la Santé et de la Recherche Médicale, the University Bordeaux 2 Victor Segalen, and Sanofi. The Fondation pour la Recherche Médicale funded the preparation and initiation of the study. The Three-City study was also supported by the Caisse Nationale Maladie des Travailleurs Salariés, Direction Générale de la Santé, MGEN, Institut de la Longévité, Conseils Régionaux d'Aquitaine et Bourgogne, Fondation de France, Ministry of Research–Institut National de la Santé et de la Recherche Médicale Programme Cohortes et collections de données biologiques, grant COGINUT ANR-06-PNRA-005 from the Agence Nationale de la Recherche, grant FCS 2009-2012 from the Fondation Plan Alzheimer, and the Caisse Nationale pour la Solidarité et l'Autonomie. Dr Samieri was sponsored by a grant from the Fondation Plan Alzheimer.
ULSAM	The Uppsala Longitudinal Studies of Adult Men 50 and 70 were funded by the Swedish Research Council for Health, Working Life and Welfare; Uppsala City Council; and The Swedish Research Council (K2015-54X-22081-04-3); and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (2016-01639).
WHIMS	The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C." The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at: http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf.

Supplemental Table 3. Invited studies not participating in the analysis

Study	Country	Biomarker compartment	Design*	Reference
Declined to participate				
Coronary Artery Risk Development in Young Adults (CARDIA)	USA	Plasma phospholipids	PC	Friedman et al., 1988[56]
Norwegian case-control study	Norway	Adipose tissue	RCC	Pedersen et al., 2000[57]
Nutrition and Health of Aging Population in China Study (NHAPC)	China	Erythrocyte phospholipids	PC	Zhu et al., 2013[58]
Ineligible for inclusion†				
European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE)	Finland	Cholesterol ester	PC	Erkillä et al., 2003[59]
The Alpha Omega Cohort (AOC)	Netherlands	Cholesterol ester	PC	Sijtsma et al., 2015[60]
Failed to respond				
European community multicenter study on antioxidants, myocardial Infarction, and breast cancer (EURAMIC)	Finland, Germany, Israel, Netherlands, Russia, United Kingdom, Spain, Switzerland	Adipose tissue	RCC	Guallar et al., 1999[61]
Invecchiare in Chianti (InCHIANTI)	Italy	Total plasma	PC	Ferrucci et al., 2006[62]

*PC, prospective cohort; RCC, retrospective case-control. †Included only individuals with history of coronary artery disease (EUROASPIRE) or myocardial infarction (AOC).

Supplemental Table 4. Descriptives for continuous covariates in participants with measured fatty acid (FA) biomarker data*

	Age (years)		Body mass index (kg/m2)		Alpha-linolenic acid (18:3n3)		Eicosapentaenoic acid (20:5n3)		Trans-18:1 FA† (18:1*t*)		Trans-18:2 FA† (18:2*t*)	
Study†	**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)**	**N**	**Mean±SD (min-max)**
AGES-Reykjavik	1195	76.6±5.7 (67-97)	1195	27.1±4.3 (13.6-46.6)	1195	0.23±0.08 (0.09-0.75)	1195	2.89±1.66 (0.55-10.5)	1195	0.87±0.27 (0.25-2.23)	1195	0.16±0.03 (0.09-0.40)
ARIC	3749	63.9±5.6 (44-65)	3749	27.0±4.6 (15.9-51.1)	3749	0.14±0.05 (0.02-0.39)	3749	0.56±0.29 (0.00-5.19)	n/a	n/a	n/a	n/a
CCCC	1820	60.6±10.5 (35-94)	1820	23.3±3.3 (13.9-43.3)	1838	0.45±0.18 (0.03-2.63)	1838	0.38±0.25 (0.06-5.90)	1838	2.56±0.69 (0.82-9.30)	1838	4.98±1.31 (1.55-11.8)
CHS	2907	72.1±5.1 (65-95)	2907	26.7±4.7 (13.6-53.3)	2907	0.15±0.05 (0.04-0.47)	2907	0.59±0.38 (0.11-8.52)	2907	1.76±0.64 (0.18-7.58)	2907	0.26±0.08 (0.09-1.07)
CRS	3374	58.3±11.1 (18-82)	3374	26.2±4.2 (13.5-49.6)	3374	0.64±0.21 (0.06-1.76)	3374	0.04±0.02 (0.00-0.21)	3374	1.45±0.57 (0.24-13.69)	3374	1.13±0.36 (0.40-3.75)
DCH	5149	57.2±4.45 (50-66)	5149	26.6±4.0 (16.3-52.6)	5149	0.85±0.17 (0.31-1.69)	5149	0.10±0.05 (0.01-0.83)	5149	1.49±0.40 (0.00-3.43)	5149	0.24±0.06 (0.09-0.49)
EPIC-Norfolk	7016	63.1±8.4 (40-78)	7016	26.5±3.8 (15.2-49.1)	7016	0.23±0.09 (0.03-1.06)	7016	1.27±0.82 (0.15-9.63)	7016	0.15±0.08 (0.00-0.87)	7016	0.24±0.03 (0.11-0.40)
EPIC-Potsdam	1704	49.5±8.8 (20-67)	1704	26.0±4.3 (16.4-54.8)	1704	0.16±0.08 (0.06-2.30)	1704	0.82±0.30 (0.08-2.60)	1704	0.53±0.14 (0.20-2.10)	n/a	n/a
FHS	2500	65.6±8.8 (40-90)	2493	28.2±5.4 (13.8-56.3)	2500	0.19±0.10 (0.04-2.07)	2500	0.73±0.46 (0.17-6.61)	2500	1.64±0.54 (0.34-4.72)	2500	0.25±0.08 (0.07-1.03)
HPFS												
- erythrocyte	1560	65.3±8.5 (48-82)	1492	25.8±3.3 (16.0-43.3)	1563	0.20±0.20 (0.04-4.33)	1563	0.50±0.28 (0.10-3.17)	1563	1.58±0.67 (0.33-5.86)	1563	0.22±0.09 (0.06-1.08)
- total plasma	1508	65.2±8.6 (48-82)	1545	25.8±3.3 (16.0-43.3)	1510	0.62±0.25 (0.07-2.43)	1510	0.61±0.44 (0.07-5.51)	1510	1.76±0.97 (0.18-9.02)	1510	0.47±0.22 (0.02-2.55)
HS	3103	60.9±12.5 (40-99)	3103	23.1±3.4 (12.4-46.0)	3103	0.7±0.2 (0.2-2.5)	3103	2.3±1.3 (0.3-12.6)	n/a	n/a	n/a	n/a
KIHD	1837	52.4±5.4 (42-61)	1829	26.7±3.5 (18.8-48.6)	1837	0.74±0.24 (0.24-2.80)	1837	1.66±0.90 (0.23-8.67)	n/a	n/a	n/a	n/a
MCCS	6265	56.3±8.6 (36-75)	6262	27.2±4.5 (15.4-51.7)	6250	0.17±0.08 (0.02-1.13)	6265	1.06±0.49 (0.01-6.12)	6245	0.80±0.33 (0.07-3.71)	6246	0.10±0.04 (0.00-0.34)
MESA	6282	62.1±10.2 (44-84)	6282	28.3±5.5 (15.4-61.9)	2722	0.18±0.08 (0.03-2.54)	2722	0.96±0.89 (0.09-14.5)	2722	1.27±0.66 (0.10-5.14)	2722	0.20±0.08 (0.05-0.68)
METSIM												
- cholesterol ester	1353	55.0±5.6 (45-68)	1353	26.5±3.5 (17.4-48.1)	1353	1.02±0.28 (0.37-2.85)	1353	2.43±1.17 (0.40-9.71)	n/a	n/a	n/a	n/a
- erythrocyte	1353	55.0±5.6 (45-68)	1353	26.5±3.5 (17.4-48.1)	1353	0.19±0.05 (0.07-0.70)	1353	1.53±0.60 (0.37-4.87)	n/a	n/a	n/a	n/a
- phospholipid	1353	55.0±5.6 (45-68)	1353	26.5±3.5 (17.4-48.1)	1353	0.32±0.12 (0.04-0.89)	1353	2.29±1.14 (0.47-10.3)	n/a	n/a	n/a	n/a

MORGEN (*CHD*)	57	51.8±7.2 (33-64)	57	26.2±3.8 (20.3-39.7)	57	0.51±0.14 (0.24-0.86)	57	0.89±0.57 (0.33-3.15)	n/a	n/a	n/a	n/a
MORGEN (*Stroke*)	179	50.0±9.5 (23-65)	179	25.9±4.3 (16.1-46.7)	179	0.52±0.15 (0.18-0.97)	179	0.75±0.45 (0.14-3.87)	n/a	n/a	n/a	n/a
MPCDRF	222	50.5±7.5 (27-59)	222	25.9±3.9 (18.6-40.1)	222	0.38±0.14 (0.00-0.81)	222	0.59±0.42 (0.00-3.30)	n/a	n/a	n/a	n/a
NHS												
-erythrocyte	1883	60.4±6.3 (42-70)	1880	25.6±4.8 (15.5-57.1)	1726	0.48±0.17 (0.00-2.54)	1600	0.50±0.45 (0.05-3.77)	1883	1.94±0.77 (0.48-7.49)	1970	0.93±0.87 (0.16-12.81)
-total plasma	1970	60.4±6.3 (43-70)	1970	25.6±4.8 (15.5-57.1)	1635	0.14±0.06 (0.00-1.37)	1687	0.46±0.27 (0.00-3.65)	1478	2.31±1.20 (0.00-11.3)	1883	0.38±0.13 (0.14-1.65)
NSHDS I	183	53.7±7.6 (29-63)	183	26.2±4.0 (19.0-42.9)	183	0.21±0.10 (0.09-0.60)	183	1.46±0.63 (0.40-3.99)	183	1.49±0.53 (0.39-3.18)	n/a	n/a
NSHDS II	759	53.6±7.6 (30-77)	759	26.4±3.9 (18.7-49.4)	759	0.21±0.08 (0.06-0.60)	759	1.51±0.63 (0.41-5.44)	n/a	n/a	n/a	n/a
NSHDS III	317	55.0±7.8 (30-70)	312	26.7±4.1 (15.9-43.0)	317	0.39±0.12 (0.02-0.98)	317	1.43±0.56 (0.44-4.46)	317	1.31±0.50 (0.06-2.76)	n/a	n/a
PHS	2000	68.7±8.7 (50.4-92.0)	2000	25.7±3.3 (18.4-47.3)	2000	0.18±0.07 (0.05-0.70)	2000	0.58±0.34 (0.05-2.95)	2000	1.20±0.39 (0.29-3.54)	2000	0.19±0.39 (0.00-1.15)
PIVUS												
-phospholipid	835	70.2±0.2 (70-71)	835	26.9±4.3 (16.6-49.8)	835	0.06±0.03 (0.00-0.12)	835	2.23±1.11 (0.64-8.79)	n/a	n/a	n/a	n/a
-cholesterol ester	835	70.1±0.2 (70-71)	835	26.9±4.3 (16.6-49.8)	835	0.97±0.23 (0.46-2.08)	835	2.35±1.14 (0.59-8.11)	n/a	n/a	n/a	n/a
SCHS	1555	66.2±7.8 (47-83)	1555	23.0±3.0 (14.2-41.6)	1555	0.34±0.28 (0.09-5.95)	1555	0.48±0.35 (0.09-4.81)	1555	0.06±0.08 (0.00-2.18)	n/a	n/a
SHHEC	4391	48.7±7.3 (25-65)	4391	25.6±4.0 (15.4-56.2)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
60YO	4232	60.3±0.1 (60-61)	4232	26.8±4.3 (15.4-51.8)	4150	0.88±0.20 (0.24-2.28)	4150	2.07±0.99 (0.28-13.91)	n/a	n/a	n/a	n/a
3C study	1416	75.0±5.0 (66-98)	1383	26.0±4.0 (14.0-45.0)	1416	0.40±0.20 (0.10-6.00)	1416	1.00±0.60 (0.10-5.10)	n/a	n/a	n/a	n/a
ULSAM 50	1992	49.6±0.6 (49-51)	1992	25.0±3.2 (15.1-42.1)	1992	0.66±0.16 (0.23-1.77)	1992	1.35±0.63 (0.19-6.44)	n/a	n/a	n/a	n/a
ULSAM 70	763	71.0±0.6 (69-74)	759	26.4±3.5 (18.1-46.3)	763	1.04±0.23 (0.40-2.39)	763	0.14±0.07 (0.00-0.63)	n/a	n/a	n/a	n/a
WHIMS	5263	70.0±3.8 (63-79)	5263	28.2±5.5 (13.8-66.3)	5263	0.17±0.08 (0.02-1.26)	5263	0.71±0.39 (0.11-0.72)	5263	2.09±0.66 (0.00-7.24)	5263	0.43±0.18 (0.08-9.22)

*FA, fatty acid. See Table 1 footnote for abbreviations of cohorts. †Sum of quantified isomers.

Supplemental Table 5. Frequencies (%) for categorical covariates in participants with measured fatty acid biomarker data.

Study*	Sex (male)	Race	Smoking	Alcohol	Education†	Prevalent diseases‡	Regular aspirin use
AGES-Reykjavik	39	100 (Caucasian)	45.4 (Current) 42.5 (Former) 12.1 (Never)	35.6 (None) 60.4 (<1-6 dr/wk) 2.92 (1-2 dr/d) 1.12 (≥2 dr/d)	24.1 (<HS) 48.5 (=HS) 27.4 (>HS)	Treated HTN: 79 Treated HCL: 14 Diabetes: 24	26
ARIC	48	100 (Caucasian)	22.8 (Current) 40.4 (Former) 36.8 (Never)	42.3 (None) 4.86 (<1-6 dr/wk) 29.3 (1-2 dr/d) 23.6 (≥2 dr/d)	6.32 (<HS) 34.7 (=HS) 59.0 (>HS)	Treated HTN: 25 Treated HCL: 3 Diabetes: 7	n/a
CCCC	55	100 (Asian)	43.5 (Smoker) 56.5 (Non-smoker)	67.5 (Abstainer) 32.5 (Drinker)	95.8 (<9 y) 4.2 (≥9 y)	Treated HTN: 37 Treated HCL:: n/a Diabetes: 16	n/a
CHS	36	87 (Caucasian) 13 (Other)	9.2 (Current) 43.3 (Former) 47.4 (Never)	53.8 (None) 33.3 (<1-6 dr/wk) 6.1 (1-2 dr/d) 6.8 (≥2 dr/d)	25.3 (<HS) 28.4 (=HS) 46.3 (>HS)	Treated HTN: 17 Treated HCL: 6 Diabetes: 13	30
CRS	73	100 (Hispanic)	30 (Current) 34 (Former) 36 (Never)	61 (None) 23 (<1-6 dr/wk) 10 (1-2 dr/d) 7 (≥2 dr/d)	6 (<HS) 55 (=HS) 39 (>HS)	Treated HTN: 35 Treated HCL: 29 Diabetes: 19	18
DCH	61	N/A	42.5 (Current) 28.1 (Former) 29.4 (Never)	3 (None) 42 (<1-6 dr/wk) 26 (1-2 dr/d) 30 (≥2 dr/d)	36.5 (<HS) 39.4 (=HS) 24.0 (>HS)	Treated HTN: 23 Treated HCL: 16 Diabetes: 3	25
EPIC-Norfolk	49	100 (Caucasian)	11 (Current) 45.6 (Former) 43.3 (Never)	29.2 (None) 47.7 (<1-6 dr/wk) 14.7 (1-2 dr/d) 8.4 (≥2 dr/d)	3.4 (<HS) 33.7 (=HS) 63.0 (>HS)	Treated HTN: 24 Treated HCL: 1 Diabetes: 4	8
EPIC-Potsdam	37	100 (Caucasian)	22.0 (Current) 31.7 (Former) 46.4 (Never)	2.9 (None) 61.9 (<1-6 dr/wk) 20.7 (1-2 dr/d) 14.6 (≥2 dr/d)	2.9 (<HS) 26.2 (=HS) 70.9 (>HS)	Treated HTN: 15 Treated HCL: 3 Diabetes: 4	n/a
FHS	43	100 (Caucasian)	9.5 (Smoker) 90.5 (Non-smoker)	24.8 (None) 48.7 (<1-6 dr/wk) 20.6 (1-2 dr/d) 5.9 (≥2 dr/d)	n/a	Treated HTN: 44 Treated HCL: 38 Diabetes: 13	40
HPFS	100	98 (Caucasian) 2 (Black) <1 (Asian) <1 (Other)	8.4/8.6 (Current) 49.4/48.6 (Former) 42.2/42.8 (Never)	25(None) 44 (<1-6 dr/wk) 17 (1-2 dr/d) 14 (≥2 dr/d)	100 (>HS)	Treated HTN: 25 Treated HCL: 26/27 Diabetes: 5	38

HS	42	100 (Asian)	22.1 (Smoker) 77.9 (Non-smoker)	44 (None) 30 (<1-6 dr/wk) 9 (1-2 dr/d) 17 (≥2 dr/d)	n/a	Treated HTN: 23 Treated HCL: 9 Diabetes: 81	2.4
KIHD	100	100 (Caucasian)	29.8 (Current) 34.2 (Former) 36.0 (Never)	13.0 (None) 64.8 (<1-6 dr/wk) 13.6 (1-2 dr/d) 8.5 (≥2 dr/d)	54.2 (<HS) 2.1 (=HS) 43.7 (>HS)	Treated HTN: 14 Treated HCL: 0 Diabetes: 5	5
MCCS	46	100 (Caucasian)	12.0 (Current) 32.3 (Former) 55.6 (Never)	42.9 (None) 29.3 (<1-6 dr/wk) 14.6 (1-2 dr/d) 13.2 (≥2 dr/d)	57.8 (<HS) 10.5 (=HS) 31.8 (>HS)	Treated HTN: 18 Treated HCL: n/a Diabetes: 6	14
MESA	47	39 (Caucasian) 26 (Black) 23 (Hispanic)	13.1 (Current) 36.3 (Former) 50.6 (Never)	34.3 (None) 42.0 (<1-6 dr/wk) 15.0 (1-2 dr/d) 8.9 (≥2 dr/d)	18.2 (<HS) 18.3 (=HS) 63.5 (>HS)	Treated HTN: 37 Treated HCL: 16 Diabetes: 11	23
METSIM	100	100 (Caucasian)	18.2 (Current) 38.1 (Former) 43.7 (Never)	26.2 (None) 35.0 (<1-6 dr/wk) 22.5 (1-2 dr/d) 16.3 (≥2 dr/d)	n/a	Treated HTN: 13 Treated HCL: 14 Diabetes: 5	1
MORGEN (*CHD*)	79	100 (Caucasian)	26.3 (Smoker) 73.7 (Non-smoker)	33.3 (None) 14.0 (<1-6 dr/wk) 31.6 (1-2 dr/d) 21.1 (≥2 dr/d)	54.4 (<HS) 28.1 (=HS) 17.5 (>HS)	Treated HTN: 11 Treated HCL: 0 Diabetes: 0	n/a
MORGEN (*Stroke*)	53	98 (Caucasian) 2 (Other)	25.7 (Smoker) 74.3 (Non-smoker)	1.1 (None) 40.0 (<1-6 dr/wk) 21.2 (1-2 dr/d) 20.1 (≥2 dr/d)	54.2 (<HS) 24.0 (=HS) 21.8 (>HS)	Treated HTN: 8 Treated HCL: 3 Diabetes: 1	n/a
NHS	0	95 (Caucasian) 4 (Black) <1 (Asian) <1 (Other)	21-22[§] (Current) 36-38[§] (Former) 40-41[§] (Never)	41 (None) 46 (<1-6 dr/wk) 8 (1-2 dr/d) 5 (≥2 dr/d)	100 (>HS)	Treated HTN: 25 Treated HCL: 36 Diabetes: 6	49
MPCDRF	70	97 (Caucasian) 3 (Other)	37.8 (Smoker) 62.2 (Non-smoker)	34.2 (None) 18.9 (<1-6 dr/wk) 22.1 (1-2 dr/d) 24.8 (≥2 dr/d)	71.6 (<HS) 13.5 (=HS) 14.4 (>HS)	Treated HTN: 9 Treated HCL: 1 Diabetes: 3	n/a
NSHDS I	79	100 (Caucasian)	29.5 (Current) 21.0 (Former) 49.4 (Never)	27 (None) 29 (≤0.5 dr/wk) 18 (≤1.0 dr/wk) 27 (>1.0 dr/wk)	57 (<HS) 34 (=HS) 9 (>HS)	Treated HTN: 57 Treated HCL: 83 Diabetes: 4	n/a
NSHDS II	76	100 (Caucasian)	29.8 (Current) 25.3 (Former) 44.8 (Never)	28 (<0.05 dr/wk) 25 (≤0.50 dr/wk) 27 (≤1.05 dr/wk) 21 (>1.05 dr/wk)	47 (<HS) 39 (=HS) 13 (>HS)	Treated HTN: 54 Treated HCL: 84 Diabetes: 7	n/a

NSHDS III	61	100 (Caucasian)	19 (Current) 27 (Former) 54 (Never)	27 (none) 24 (≤0.21 dr/wk) 29 (≤1.19 dr/wk) 21 (>1.19 dr/d)	53 (<HS) 36 (=HS) 11 (>HS)	Treated HTN: 20 Treated HCL: 87 Diabetes: 9	n/a
PHS	100	98 (Caucasian) 2 (Other)	3 (Current) 48 (Former) 50 (Never)	34 (<1 dr/wk) 31 (1-4 dr/wk) 28 (5-7 dr/wk) 7 (>7 dr/wk)	100 (>HS)	Treated HTN: 45 Treated HCL: 26 Diabetes: 9	n/a
PIVUS	47	100 (Caucasian)	10.0 (Current) 40.0 (Former) 50.0 (Never)	33 (None) 51 (<1-6 dr/wk) 13 (1-2 dr/d) 3 (≥2 dr/d)	55 (<HS) 18 (=HS) 26 (>HS)	Treated HTN: 28 Treated HCL: 11 Diabetes: 11	11
SCHS	65	100 (Asian)	36.5 (Current) 15.7 (Former) 28.6 (Never)	77.6 (None) 18.1 (<1-6 dr/wk) 2.4 (1-2 dr/d) 1.9 (≥2 dr/d)	73.0 (<HS) 21.4 (=HS) 5.6 (>HS)	Treated HTN: 36 Treated HCL: n/a Diabetes: 27	n/a
SHHEC[‖]	52	Race not recorded[#]	44.6 (Smoker) 55.4 (Non-smoker)	25.3 (None) 41.4 (<1-6 dr/wk) 16.6(1-2 dr/d) 16.7 (≥2 dr/d)	2.0 (<HS) 67.7 (=HS) 30.3 (>HS)	Treated HTN: 5.6 Treated HCL: 0.0% Diabetes mellitus: 1.1	0.1
60YO	48	100 (Caucasian)	21.5 (Current) 38.6 (Former) 39.8 (Never)	7.9 (None) 60.2 (<1-6 dr/wk) 18.0 (1-2 dr/d) 13.9 (≥2 dr/d)	59.1 (<HS) 13.2 (=HS) 27.6 (>HS)	Treated HTN: 20 Treated HCL: 5 Diabetes: 7	6
3C	39	100 (Caucasian)	5.0 (Current) 30.4 (Former) 64.6 (Never)	24.1 (None) 20.9 (<1-6 dr/wk) 40.0 (1-2 dr/d) 15.0 (≥2 dr/d)	62.1 (<HS) 20.8 (=HS) 17.1 (>HS)	Treated HTN: 56 Treated HCL: 31 Diabetes: 10	20
ULSAM 50	100	100 (Caucasian)	51.2 (Current) 23.4 (Former) 25.4 (Never)	n/a	63.0 (<HS) 26.4 (=HS) 10.6 (>HS)	Treated HTN: 15 Treated HCL: 4 Diabetes: 1	n/a
ULSAM 70	100	100 (Caucasian)	20.5 (Current) 49.5 (Former) 29.9 (Never)	7.7 (None) 29.9 (<1-6 dr/wk) 49.0 (1-2 dr/d) 13.4 (≥2 dr/d)	56.4 (<HS) 30.0 (=HS) 13.6 (>HS)	Treated HTN: 32 Treated HCL: 7 Diabetes: 14	n/a
WHIMS	0	88 (Caucasian) 6 (Black) 2 (Hispanic) 2 (Asian) 2 (Other)	7.0 (Current) 37.8 (Former) 54.8 (Never)	44.3 (None) 42.8 (<1-6 dr/wk) 8.3 (1-2 dr/d) 4.6 (≥2 dr/d)	7.1 (<HS) 22.5 (=HS) 70.5 (>HS)	Treated HTN: 27 Treated HCL: 15 Diabetes: 5	23

[*]See Table 1 footnote for abbreviations of cohorts. [†]HS, High school or equivalent. [‡]Prevalent treated hypertension (HTN) was defined as hypertension drug use; or if unavailable, as diagnosed/history of hypertension. Prevalent treated hypercholesterolemia (HCL) was defined as lipid-lowering drug use; or if unavailable, as diagnosed/history of hypercholesterolemia. Prevalent diabetes mellitus was defined as treatment with oral hypoglycemic agents, insulin, or fasting plasma glucose >126 mg/dL. [§]In NHS, proportions of current, former, and never smokers were different in analyses using total plasma compared to erythrocyte phospholipids. [‖]See cohort description in appendix for further explanations. [#]96% UK birth, 1% born outside Europe.

Supplemental Table 6. Baseline proportions for linoleic acid (LA; 18:2n6) and arachidonic acid (AA; 20:4n6) biomarkers in participants with measured fatty acid (FA) biomarker data.[*]

	FA measured[†]	Participants	LA, % of total fatty acids				AA, % of total fatty acids				Analytical CV, %[‡]	
Study	**(n)**	**(n)**	**Mean ± SD**	**Median**	**Min**	**Max**	**Mean ± SD**	**Median**	**Min**	**Max**	**LA**	**AA**
Plasma phospholipid												
AGES-Reykjavik	41	1 195	17.7±2.9	17.8	7.38	27.3	6.8±1.41	6.7	3.4	13.1	2.5	2.5
ARIC	29	3 749	22.0±2.7	22.0	8.95	32.4	11.5±1.95	11.4	5.3	20.0	n/a[§]	n/a[§]
CHS	45	2 907	19.7±2.5	19.6	11.4	28.8	11.1±2.00	11.1	5.0	18.9	7.7	0.8
EPIC-Norfolk	27	7 016	24.3±3.4	24.2	10.8	40.2	9.5±1.88	9.4	3.8	17.9	0.9	4.0
MCCS	55	6 265	20.1±3.0	20.0	9.7	31.2	10.4±1.78	10.3	5.0	18.2	1-12	1-12
MESA	29	2 722	21.5±3.4	21.3	11.4	36.1	12.0±2.60	11.8	3.6	22.2	<10	<10
METSIM	22	1 353	18.6±2.7	18.6	9.6	28.5	9.0±1.62	8.9	4.7	16.8	1.4	1.2
NSHDS I	15	183	21.7±2.3	21.7	16.1	29.6	8.3±1.27	8.3	5.4	12.1	≤5.5	≤5.5
NSHDS II	16	759	21.3±2.6	21.0	14.2	30.3	8.8±1.30	8.8	1.7	13.0	≤5.5	≤5.5
NSHDS III	17	317	21.2±2.4	21.2	13.9	28.2	8.3±1.18	8.2	4.9	11.9	≤5.5	≤5.5
PIVUS	16	835	19.7±2.6	19.6	10.7	28.6	8.0±1.27	7.9	4.1	13.9	<10	<10
Erythrocyte phospholipid												
EPIC-Potsdam	32	1 704	10.8±1.3	10.7	5.5	16	13.1±1.7	13.3	2.5	18.5	2.2	2.2
FHS	22	2 500	11.3±1.7	11.2	5.7	19.8	16.8±1.6	16.8	9.0	23.2	≤7.0	≤7.0
HPFS	42	1 563	13.0±3.0	12.8	5.9	37.0	12.8±1.9	13.1	4.0	17.6	9.0	10
METSIM	20	1 353	8.3±1.1	8.2	4.0	12.9	11.9±1.1	12.0	8.1	15.1	3.2	2.6
NHS	40	1 883	12.3±2.3	12.3	3.1	25.5	12.7±2.1	12.9	3.4	17.9	10	10
PHS	n/a[§]	2 000	12.7±1.7	12.6	6.2	21.4	12.7±2.3	13.6	2.2	20.1	<4.5	<4.5
WHIMS	22	5 263	12.0±1.8	11.9	5.6	30.0	17.0±2.2	17.0	6.3	26.0	≤6.5	≤6.5
Total plasma												
CCCC	29	1 838	15.6±4.4	15.1	2.8	30.8	3.1±1.0	2.9	0.1	8.0	6.5	4.3
HPFS	50	1 510	30.3±4.4	30.5	10.4	43.1	7.0±1.8	6.9	1.8	13.1	2.0	5.0
HS	24	3 103	27.0±4.5	27.3	9.3	41.6	5.0±1.0	5.0	1.7	9.5	n/a[§]	3.8
KIHD	14	1 837	26.6±4.4	26.7	10.3	41.3	4.8±1.0	4.8	1.4	9.2	9.6	9.2
NHS	50	1 970	29.1±4.9	29.5	0.0	46.0	6.9±1.8	6.8	0.0	13.1	7.0	10
SCHS	19	1 555	36.3±4.6	36.2	20.3	51.1	7.6±1.7	7.5	2.2	15.2	6.8	7.9
3C	12	1 416	24.9±5.4	25.3	4.1	40.8	6.7±1.9	6.8	1.0	16.1	0.7	0.02
Cholesterol esters												
60YO	13	4 150	48.4±4.2	48.8	26.4	63.9	6.33±1.17	6.2	3.1	13.3	≤2.5	≤2.5
METSIM	13	1 353	48.4±5.0	48.6	20.3	62.4	6.43±1.41	6.3	2.7	13.9	0.9	1.6
MORGEN (*CHD*)	37	57[‖]	54.4±4.8	54	44.5	64.7	6.40±1.50	6.0	3.3	10.9	3.0-3.5	3.0-3.5
MORGEN (*Stroke*)	37	179[‖]	55.2±5.3	55.4	41.7	71.9	6.50±1.60	6.6	2.7	11.3	3.0-3.5	3.0-3.5
MPCDRF	37	222[‖]	43.8±6.3	43.8	23.1	63.8	3.90±1.20	3.8	1.0	8.8	3.0-3.5	3.0-3.5
PIVUS	14	846	49.0±4.2	49.3	34.4	61.7	6.09±1.21	6.0	2.8	11.7	<10	<10
ULSAM 50	12	1 992	54.0±5.2	54.5	27.2	68	4.77±0.94	4.7	2.0	8.9	2.0-10	2.0-10
Adipose tissue												
CRS	35	3 374	15.4±3.8	15.3	5.4	30.9	0.48±0.14	0.47	0.10	1.06	5.9	2.6

DCH	32	5 149	n/a[§]	n/a[§]	n/a[§]	n/a[§]	0.38±0.10	0.36	0.12	1.31	n/a[§]	0.7
SHHEC	12	4 391	9.2±2.6	8.8	3.3	27.6	0.63±0.17	0.61	0.19	4.30	2-22	2-22
ULSAM 70	13	763	12.6±2.8	12.4	5.6	30.0	0.35±0.10	0.37	0.00	0.73	<10	<10

[*]AA, arachidonic acid; CHD, coronary heart disease; CVD, cardiovascular disease; FA, fatty acid; LA, linoleic acid. See Table 1 footnote for abbreviations of cohorts. [†]Total number of individual fatty acids measured in the biomarker compartment. [‡]Coefficient of variation as a measure of precision of fatty acid assessment. [§]Not reported. [‖]Values are only shown for controls.

Supplemental Table 7. Number of cases of incident cardiovascular disease (CVD), CVD mortality, incident coronary heart disease (CHD), and incident ischemic stroke in 31 participating studies. [*,†]

	Total CVD		CVD mortality		Total CHD		Ischemic stroke	
Cohort	**Cases**	**Median/max follow-up (y)**	**Cases**	**Median/max follow-up (y)**	**Cases**	**Median/max follow-up (y)**	**Cases**	**Median/max follow-up (y)**
AGES-Reykjavik	369	10.0/13.3	162	10.0/12.3	286	10.3/13.3	123	10.7/13.3
ARIC	547	22.6/25.1	289	22.7/25.1	398	22.6/25.1	188	22.7/25.1
CCCC	421	11.0/22.8	306	14.1/14.9	196	12.0/22.2	243[‡]	10.4/22.8
CHS	1299	11.0/22.1	832	13.0/22.1	875	11.6/22.1	408	12.0/22.1
CRS	n/a	n/a	n/a	n/a	1687	n/a	n/a	n/a
DCH	n/a	n/a	n/a	n/a	2138	13.6/16.1	n/a	n/a
EPIC-Norfolk	1526	11.9/16.1	951	17.6/20.8	1221	12.8/16.1	445[‡]	13.1/16.1
EPIC-Potsdam	50	10.4/13.2	n/a	n/a	n/a	n/a	n/a	n/a
FHS	196	6.4/9.3	37	7.3/9.3	103	6.4/9.3	79	6.4/8.8
HPFS								
-erythrocyte	564	12.6/17.4	n/a	n/a	444	13.8/17.4	120[‡]	8.7/17.4
-total plasma	551	12.6/17.4	n/a	n/a	431	13.8/17.4	120[‡]	8.7/17.4
HS	222	10.2/10.4	98	10.2/10.4	78	10.2/10.4	97	10.2/10.4
KIHD	562	18.4/27.2	267	24.6/28.8	472	23.7/28.8	151	18.9/22.8
MCCS	282[d]	7.1/18.5	282	7.1/18.5	238[d]	6.7/18.5	44[§]	8.0/18.0
MESA	202	8.4/10.9	208	8.6/10.9	143	8.5/10.9	53	8.5/10.9
METSIM	36	6.8/9.2	n/a	n/a	n/a	n/a	n/a	n/a
MORGEN	n/a	n/a	n/a	n/a	57[d]	10.2/13.7	93	10.5/13.0
MPCDRF	n/a	n/a	n/a	n/a	222[d]	23.7/28.8	n/a	n/a
NHS								
-erythrocyte	862	15.0/22.7	n/a	n/a	412	15.9/22.7	450[‡]	14.5/22.5
-total plasma	905	15.0/22.7	n/a	n/a	437	15.9/22.7	468[‡]	14.5/22.5
NSHDS I	n/a	n/a	n/a	n/a	64	not reported	n/a	n/a
NSHDS II	n/a	n/a	n/a	n/a	353	3.7/10.8	n/a	n/a
NSHDS III	n/a	n/a	n/a	n/a	n/a	n/a	85	2.5/6.5
PHS	n/a	n/a	n/a	n/a	1000	not reported	n/a	n/a
PIVUS	91	10.0/10.9	6	10.0/10.9	57	10.0/10.9	37	10.0/10.9
SCHS	n/a	n/a	n/a	n/a	759	4.6/14.4	n/a	n/a
SHHEC	1157	23.3/24.8	308	23.6/24.8	936	23.4/24.8	290[‡]	23.5/24.8
60YO	351	14.5/15.9	69	14.5/15.9	199	14.5/15.9	155	14.5/15.9
3C Study	81	6.6/9.8	8	6.8/9.8	57	6.6/8.5	26	6.6/9.8
ULSAM 50	822	27.9/41.7	285	31.9/41.7	643	29.4/41.7	313	30.2/41.7
ULSAM 70	255	12.9/20.2	110	15.0/20.2	181	14.0/20.2	115	14.2/20.2
WHI	795	15.5/20.2	290	15.5/20.2	484	15.5/20.2	295	15.5/20.2

[*]CHD, coronary heart disease; CVD, cardiovascular disease. See Table 1 footnote for abbreviations of cohorts. [†]See detailed cohort descriptions in the Supplemental Methods for further information on outcome ascertainment and definition. [‡]Total stroke. [§]Only fatal events.

Supplemental Table 8. Summary of hazard ratios of total CVD, CVD mortality, total CHD and ischemic stroke by quintile of linoleic acid (LA; 18:2n6) from pooled analysis.[*, †]

		Total CVD		CVD Mortality		Total CHD		Ischemic stroke	
Biomarker	**Quintile**	**HR (95%CI)**	**I^2 (%)**	**HR (95%CI)**	**I^2 (%)**	**HR (95%CI)**	**I^2 (%)**	**HR (95%CI)**	**I^2 (%)**
Phospholipid	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	1.00 (0.91-1.09)	0	0.81 (0.72-0.92)	0	1.00 (0.91-1.11)	22	1.04 (0.89-1.22)	13
	3	1.04 (0.95-1.14)	0	0.91 (0.81-1.03)	0	1.02 (0.92-1.14)	41	1.03 (0.87-1.21)	20
	4	0.97 (0.88-1.07)	15	0.85 (0.75-0.97)	0	0.99 (0.89-1.11)	46	0.92 (0.77-1.09)	0
	5	1.04 (0.94-1.15)	16	0.89 (0.77-1.02)	0	1.03 (0.92-1.16)	63	0.98 (0.81-1.18)	0
Total plasma	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	0.95 (0.83-1.09)	38	0.86 (0.69-1.08)	0	0.92 (0.78-1.07)	0	0.98 (0.78-1.22)	28
	3	0.95 (0.82-1.10)	0	0.78 (0.61-1.00)	0	0.89 (0.76-1.05)	0	0.87 (0.68-1.12)	0
	4	0.90 (0.77-1.05)	44	0.87 (0.68-1.13)	0	0.83 (0.70-0.99)	39	1.04 (0.81-1.34)	0
	5	0.99 (0.84-1.17)	63	0.81 (0.61-1.09)	0	0.91 (0.76-1.09)	28	0.98 (0.74-1.29)	23
Cholesterol ester	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	0.96 (0.81-1.14)	0	0.85 (0.65-1.13)	0	0.88 (0.72-1.08)	0	1.12 (0.86-1.47)	0
	3	0.89 (0.74-1.07)	0	0.70 (0.52-0.94)	0	0.85 (0.69-1.05)	0	0.98 (0.74-1.30)	0
	4	0.78 (0.65-0.95)	0	0.06 (0.05-0.09)	100	0.81 (0.65-1.00)	0	0.94 (0.70-1.27)	32
	5	0.79 (0.64-0.96)	0	0.64 (0.46-0.89)	0	0.81 (0.64-1.02)	0	0.84 (0.61-1.14)	0
Adipose tissue	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	0.92 (0.78-1.07)	0	0.70 (0.53-0.93)	0	0.92 (0.77-1.10)	0	0.85 (0.63-1.14)	0
	3	0.85 (0.72-1.01)	54	0.72 (0.54-0.97)	0	0.80 (0.66-0.98)	0	0.91 (0.67-1.24)	57
	4	0.86 (0.72-1.02)	0	0.66 (0.48-0.90)	0	0.87 (0.72-1.06)	0	0.84 (0.60-1.16)	16
	5	0.81 (0.68-0.98)	0	0.53 (0.38-0.75)	0	0.82 (0.67-1.01)	4	0.72 (0.51-1.02)	77
Overall	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	0.97 (0.91-1.03)	0	0.82 (0.75-0.90)	0	0.95 (0.88-1.02)	0	1.03 (0.92-1.15)	0
	3	0.96 (0.90-1.02)	0	0.83 (0.75-0.91)	0	0.92 (0.86-1.00)	23	0.96 (0.85-1.07)	2
	4	0.91 (0.84-0.97)	21	0.80 (0.73-0.89)	0	0.90 (0.84-0.98)	40	0.92 (0.81-1.04)	0
	5	0.94 (0.87-1.01)	50	0.77 (0.69-0.86)	21	0.92 (0.85-1.00)	54	0.90 (0.79-1.02)	3

[*]CHD, coronary heart disease; CVD, cardiovascular disease. [†]Categorical estimates were pooled using inverse-variance weighted meta-analysis.

Supplemental Table 9. Summary of hazard ratios of total CVD, CVD mortality, total CHD and ischemic stroke by quintile of arachidonic acid (AA; 20:4n6) from pooled analysis.[*, †]

		Total CVD		CVD Mortality		Total CHD		Ischemic stroke	
Biomarker	**Quintile**	**HR (95%CI)**	**I^2 (%)**	**HR (95%CI)**	**I^2 (%)**	**HR (95%CI)**	**I^2 (%)**	**HR (95%CI)**	**I^2 (%)**
Phospholipid	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	1.00 (1.00-1.00)	0	0.70 (0.62-0.79)	100	1.02 (0.92-1.13)	0	1.02 (0.87-1.19)	0
	3	0.98 (0.90-1.08)	0	1.00 (0.88-1.13)	0	0.92 (0.82-1.02)	29	0.98 (0.83-1.14)	0
	4	0.95 (0.87-1.04)	0	0.83 (0.73-0.95)	99	0.97 (0.87-1.08)	39	0.98 (0.83-1.15)	6
	5	0.96 (0.87-1.05)	0	0.91 (0.79-1.04)	0	0.91 (0.81-1.02)	54	0.99 (0.83-1.17)	11
Total plasma	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	0.94 (0.82-1.08)	8	0.81 (0.64-1.04)	0	0.89 (0.76-1.05)	48	1.15 (0.93-1.44)	0
	3	0.84 (0.72-0.97)	0	0.84 (0.66-1.08)	0	0.88 (0.74-1.03)	63	0.93 (0.73-1.18)	0
	4	0.83 (0.71-0.97)	0	0.83 (0.63-1.08)	0	0.82 (0.69-0.98)	49	0.94 (0.73-1.22)	4
	5	0.79 (0.67-0.93)	44	0.77 (0.56-1.04)	0	0.83 (0.69-1.00)	68	0.88 (0.65-1.17)	9
Cholesterol ester	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	0.93 (0.78-1.11)	49	1.01 (0.74-1.37)	0	0.90 (0.74-1.09)	0	0.93 (0.70-1.22)	54
	3	1.05 (0.88-1.25)	0	1.35 (1.01-1.81)	0	0.99 (0.82-1.20)	0	1.16 (0.89-1.53)	6
	4	0.93 (0.77-1.12)	16	0.39 (0.28-0.53)	100	0.90 (0.73-1.09)	40	1.18 (0.89-1.55)	0
	5	1.01 (0.84-1.22)	0	0.99 (0.72-1.36)	0	0.96 (0.79-1.18)	0	1.14 (0.86-1.50)	0
Adipose tissue	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	0.98 (0.83-1.16)	3	1.02 (0.74-1.40)	0	1.15 (1.00-1.32)	45	0.83 (0.60-1.14)	0
	3	0.97 (0.82-1.15)	0	0.96 (0.69-1.32)	0	1.13 (0.98-1.29)	67	0.95 (0.70-1.29)	0
	4	0.92 (0.78-1.10)	0	1.07 (0.78-1.46)	0	1.15 (0.99-1.33)	82	0.92 (0.67-1.26)	55
	5	1.01 (0.85-1.19)	0	1.00 (0.73-1.38)	0	1.24 (1.07-1.44)	80	0.84 (0.61-1.16)	0
Overall	1	Reference	-	Reference	-	Reference	-	Reference	-
	2	0.94 (0.88-1.00)	0	0.78 (0.70-0.85)	100	1.00 (0.93-1.07)	22	0.98 (0.88-1.10)	0
	3	0.94 (0.88-1.00)	0	1.02 (0.92-1.12)	0	0.97 (0.90-1.04)	48	0.99 (0.88-1.10)	0
	4	0.91 (0.85-0.98)	0	0.87 (0.79-0.96)	98	0.96 (0.89-1.03)	57	1.00 (0.89-1.13)	0
	5	0.92 (0.86-0.99)	0	0.91 (0.82-1.01)	0	0.99 (0.92-1.07)	60	0.99 (0.88-1.12)	0

[*]CHD, coronary heart disease; CVD, cardiovascular disease; [†]Categorical estimates were pooled using inverse-variance weighted meta-analysis.

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Supplemental Table 10. Hazard ratio (95% CI) of total CVD, CVD mortality, total CHD and ischemic stroke by n-6 fatty acid biomarker (per interquintile range) according to prespecified potential sources of heterogeneity.[*] [†]

Fatty acid	Sources of heterogeneity		n max studies[‡]	n max participants[‡]	Total CVD (n=10 477)	CVD mortality (n=4 508)	Total CHD (n=11 857)	Ischemic stroke (n=3 705)
LA	Age	<60 y	15	n/a	0.84 (0.76-0.93)	0.56 (0.47-0.67)	0.87 (0.78-0.96)	0.72 (0.59-0.88)
		≥60 y	20	n/a	0.99 (0.91-1.07)	0.84 (0.75-0.95)	0.99 (0.91-1.07)	0.96 (0.84-1.10)
		$P_{heterogeneity}$[§]			0.12	0.20	0.27	0.35
	Sex	Males	20	30 555	0.91 (0.84-0.99)	0.73 (0.64-0.82)	0.95 (0.88-1.02)	0.82 (0.70-0.96)
		Females	17	30 529	1.01 (0.91-1.11)	0.87 (0.74-1.02)	0.95 (0.84-1.07)	0.97 (0.83-1.14)
		$P_{heterogeneity}$			0.36	0.20	0.80	0.33
	Race	Caucasian	18	45 343	0.94 (0.88-1.01)	0.78 (0.71-0.87)	0.94 (0.87-1.01)	0.90 (0.80-1.01)
		Black	2	1 023	1.00 (0.38-2.62)	0.70 (0.26-1.87)	0.90 (0.27-3.04)	0.66 (0.12-3.65)
		Asian	4	6 496	0.52 (0.35-0.76)	0.46 (0.27-0.78)	0.74 (0.56-0.97)	0.61 (0.35-1.06)
		Hispanic	1	626	1.83 (0.60-5.63)	1.53 (0.44-5.37)	3.21 (0.88-11.7)	2.65 (0.29-23.84)
		$P_{heterogeneity}$			all ≥0.08	all ≥0.16	all ≥0.41	all ≥0.29
	ALA	<median	21	28 173	0.86 (0.78-0.96)	0.79 (0.68-0.92)	0.91 (0.82-1.02)	0.85 (0.71-1.01)
		≥median	21	28 173	1.03 (0.93-1.14)	0.79 (0.68-0.92)	0.96 (0.86-1.08)	0.96 (0.81-1.15)
		$P_{heterogeneity}$			0.13	0.99	0.52	0.29
	EPA	<median	21	28 173	0.96 (0.87-1.06)	0.82 (0.72-0.94)	0.98 (0.88-1.09)	1.02 (0.87-1.21)
		≥median	21	28 173	0.95 (0.86-1.06)	0.75 (0.64-0.87)	0.97 (0.87-1.09)	0.84 (0.70-1.01)
		$P_{heterogeneity}$			0.98	0.61	0.95	0.14
	Diabetes	yes	16	6 385	1.07 (0.94-1.22)	0.97 (0.83-1.14)	1.11 (0.96-1.29)	0.99 (0.80-1.23)
		no	20	51 300	0.93 (0.86-1.00)	0.70 (0.61-0.79)	0.92 (0.84-1.00)	0.89 (0.77-1.02)
		$P_{heterogeneity}$			0.19	0.01	0.03	0.51
	Treated	yes	12	3 958	1.23 (0.96-1.58)	1.71 (1.09-2.69)	1.20 (0.89-1.64)	1.53 (0.94-2.47)
	hypercholesterolemia	no	14	28 761	0.92 (0.84-1.01)	0.72 (0.63-0.82)	0.90 (0.80-1.00)	0.89 (0.76-1.04)
		$P_{heterogeneity}$			0.05	0.20	0.09	0.14
	Aspirin	yes	14	8 370	0.90 (0.79-1.02)	0.76 (0.63-0.90)	0.92 (0.79-1.07)	0.85 (0.67-1.09)
	use	no	15	36 658	1.04 (0.94-1.16)	0.84 (0.72-0.98)	1.04 (0.91-1.17)	0.98 (0.82-1.17)
		$P_{heterogeneity}$			0.43	0.50	0.57	0.41
	Baseline	<2000	19	49 280	0.93 (0.87-0.99)	0.77 (0.70-0.86)	0.93 (0.87-1.00)	0.87 (0.77-0.98)
	year	≥2000	7	13 412	0.98 (0.82-1.18)	0.81 (0.61-1.07)	1.03 (0.85-1.23)	0.96 (0.71-1.31)
		$P_{heterogeneity}$			0.97	0.57	0.41	0.55
AA	Age	<60 y	16	n/a	0.96 (0.88-1.04)	0.92 (0.79-1.06)	0.99 (0.91-1.08)	0.96 (0.83-1.11)
		≥60 y	21	n/a	0.97 (0.90-1.04)	0.93 (0.83-1.04)	0.99 (0.93-1.06)	1.04 (0.92-1.18)
		$P_{heterogeneity}$			0.91	0.88	0.93	0.46
	Sex	Males	21	33803	0.97 (0.90-1.04)	0.93 (0.83-1.04)	1.00 (0.94-1.06)	1.05 (0.92-1.19)
		Females	18	32211	0.93 (0.84-1.01)	0.99 (0.87-1.14)	1.01 (0.90-1.12)	0.97 (0.84-1.13)
		$P_{heterogeneity}$			0.66	0.30	0.73	0.56

	Race	Caucasian	18	45343	0.98 (0.93-1.04)	0.93 (0.85-1.03)	0.98 (0.91-1.04)	1.01 (0.91-1.11)
		Black	2	1023	0.82 (0.38-1.77)	0.50 (0.21-1.20)	0.94 (0.33-2.71)	0.57 (0.11-2.99)
		Asian	4	6496	0.99 (0.72-1.36)	1.15 (0.78-1.70)	1.02 (0.77-1.34)	1.71 (1.14-2.56)
		Hispanic	1	626	1.34 (0.51-3.48)	1.16 (0.42-3.22)	1.46 (0.47-4.51)	0.24 (0.03-2.17)
		$P_{heterogeneity}$			all ≥0.54	all ≥0.25	all ≥0.53	0.02 (Asian)
	ALA	<median	22	30 816	0.92 (0.84-1.01)	0.82 (0.72-0.93)	1.03 (0.95-1.12)	0.89 (0.76-1.05)
		≥median	22	30 816	0.96 (0.88-1.04)	1.04 (0.90-1.20)	0.94 (0.86-1.03)	1.20 (1.02-1.42)
		$P_{heterogeneity}$			0.57	0.04	0.70	0.02
	EPA	<median	22	30 816	0.92 (0.84-1.01)	0.89 (0.77-1.02)	0.98 (0.90-1.08)	1.03 (0.89-1.20)
		≥median	22	30 816	0.92 (0.84-1.01)	0.96 (0.83-1.10)	1.00 (0.91-1.09)	1.06 (0.89-1.26)
		$P_{heterogeneity}$			0.99	0.69	0.72	0.84
	Diabetes	yes	17	6556	0.81 (0.72-0.91)	0.91 (0.79-1.06)	0.86 (0.75-0.99)	1.02 (0.84-1.23)
		no	20	54622	0.99 (0.94-1.06)	0.98 (0.87-1.09)	1.06 (0.99-1.14)	0.99 (0.88-1.11)
		$P_{heterogeneity}$			0.24	0.72	0.05	0.84
	Treated	yes	12	3 958	0.96 (0.76-1.19)	0.60 (0.40-0.90)	0.96 (0.73-1.28)	1.18 (0.75-1.85)
	hypercholesterolemia	no	14	28 761	0.94 (0.86-1.04)	0.92 (0.80-1.05)	0.95 (0.85-1.07)	1.00 (0.85-1.17)
		$P_{heterogeneity}$			0.86	0.44	0.92	0.51
	Aspirin	yes	14	8 370	0.96 (0.86-1.08)	0.73 (0.63-0.86)	1.01 (0.89-1.15)	1.05 (0.84-1.31)
	use	no	15	36 658	0.97 (0.88-1.07)	0.93 (0.79-1.09)	0.99 (0.89-1.10)	1.07 (0.90-1.27)
		$P_{heterogeneity}$			0.75	0.31	0.94	0.90
	Baseline	<2000	20	54 566	0.95 (0.90-1.01)	0.95 (0.87-1.04)	0.99 (0.93-1.04)	1.02 (0.92-1.14)
	year	≥2000	7	13 412	0.96 (0.79-1.17)	0.79 (0.60-1.06)	1.05 (0.87-1.28)	0.76 (0.55-1.04)
		$P_{heterogeneity}$			0.94	0.35	0.64	0.10

*AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; CHD, coronary heart disease; CVD, cardiovascular disease; LA, linoleic acid. ‡Interquintile range was defined as the range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]). ‡Number of studies and participants included in each strata varied depending on outcome assessed. §Statistical differences between strata were explored by meta-regression, with Bonferroni correction for 10 total comparisons (corrected alpha=0.005) including genotype for each primary exposure (LA, AA) and outcome relationship given absence of prespecified hypotheses on sources of heterogeneity.

Supplemental Table 11. Genotype Ascertainment for Studies Contributing to SNP Analysis*

Study	SNP†	N	Coded allele	Coded allele frequency	Imputation quality	Evaluated outcomes
AGES-Reykjavik	rs174547	702	T	0.60	Genotyped	All
ARIC	rs174547	3 248	T	0.66	Genotyped	All
CHS	rs174547	2 561	T	0.70	1.0	All
EPIC-Norfolk	rs174547	2 401	T	0.66	1.0	All
EPIC-Potsdam	rs174546	1 667	C	0.67	Genotyped	Total CVD
FHS	rs174547	2 316	T	0.66	Genotyped	All
HS	rs174547	1 926	T	0.59	Genotyped	All
METSIM	rs174550	1 338	T	0.58	Genotyped	Total CVD
PIVUS	rs174546	828	C	0.67	Genotyped	All
SCHS	rs174546	1 450	C	0.33	Genotyped	Total CHD
3C Study	rs174546	1 039	C	0.70	Genotyped	All
ULSAM 50	rs174547	1 043	T	0.65	Genotyped	All
ULSAM 70	rs174547	727	T	0.65	Genotyped	All
WHIMS	rs174547	3 304	T	0.65	1.0	All

*SNP, single nucleotide polymorphism. See Table 1 footnote for abbreviations of cohorts. †rs174547 was used in the analysis when available, otherwise SNPs in high linkage disequilibrium ($r^2 \geq 0.93$) with rs174547 were used as proxies.

Supplemental Table 12. Interaction of linoleic acid (LA) and arachidonic acid (AA)biomarkers with rs174547 in the FADS1 gene for total CVD, CVD mortality, total CHD, and ischemic stroke per interquintile range of n-6PUFA biomarker. *†

Fatty acid	Outcome	Studies	Hazard ratio (95% CI) per interquintile range of n-6PUFA biomarker			$P_{interaction}$
Genotype at rs174547 (estimated genotype frequency) ‡			CC (12-14%)	CT (45%)	TT (41-43%)	
LA	Total CVD	13	1.17 (0.97-1.41)	1.00 (0.88-1.13)	0.89 (0.76-1.05)	0.047
	CVD mortality	11	1.12 (0.85-1.48)	0.85 (0.70-1.03)	0.71 (0.55-0.90)	0. 55
	Total CHD	12	1.00 (0.81-1.24)	0.92 (0.80-1.05)	0.96 (0.81-1.13)	0.77
	Ischemic stroke	11	1.45 (1.07-1.96)	1.03 (0.84-1.27)	0.80 (0.61-1.03)	0.002
AA	Total CVD	13	0.99 (0.80-1.21)	0.93 (0.86-1.06)	0.79 (0.67-0.94)	0.47
	CVD mortality	11	0.93 (0.69-1.26)	0.95 (0.77-1.17)	0.94 (0.74-1.20)	0.74
	Total CHD	12	1.01 (0.79-1.28)	1.02 (0.88-1.18)	0.90 (0.76-1.06)	0.51
	Ischemic stroke	11	1.13 (0.81-1.58)	0.95 (0.76-1.18)	0.83 (0.63-1.11)	0.43

*AA, arachidonic acid; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; FADS, fatty acid desaturase; LA, linoleic acid.

†Interquintile range was defined as the range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]). ‡Min-max (depending on outcome) genotype frequency estimated separately in each cohort from allele frequency (eTable 8), assuming Hardy-Weinberg equilibrium and full linkage disequilibrium between rs174547 and the proxies, rs174546 and rs174550.

Supplemental Table 13. Association of linoleic acid (LA) and arachidonic acid (AA) biomarkers (HR [95% CI]) per interquintile range or per % of total fatty acids and total CVD, CVD mortality, total CHD, and ischemic stroke.[*] [†]

				Interquintile range, % of total FA	Risk per interquintile range			Risk per % of total FA	
FA	**Biomarker**	**Outcome**	**n studies**	**median (min-max)**	**HR (95% CI)**	***P***		**HR (95% CI)**	***P***
LA	Phospholipid								
	-plasma	Total CVD	8	6.98 (6.36-8.73)	1.05 (0.95-1.16)	0.38		1.00 (0.99-1.02)	0.52
		CVD Mortality	7	7.10 (6.36-8.73)	0.89 (0.79-1.01)	0.074		0.98 (0.97-1.00)	0.074
		Total CHD	9	6.74 (6.10-8.73)	1.04 (0.93-1.16)	0.53		1.00 (0.99-1.02)	0.61
		Ischemic stroke	8	6.92 (5.90-8.73)	0.96 (0.81-1.14)	0.66		0.99 (0.97-1.02)	0.58
	-erythrocyte	Total CVD	6	4.28 (2.63-5.60)	0.90 (0.77-1.05)	0.17		0.98 (0.95-1.02)	0.33
		CVD Mortality	2	4.28 (4.06-4.50)	0.82 (0.57-1.20)	0.31		0.96 (0.88-1.04)	0.31
		Total CHD	5	4.50 (4.06-5.15)	0.99 (0.87-1.11)	0.81		1.00 (0.97-1.02)	0.80
		Ischemic stroke	4	4.86 (4.06-5.61)	0.92 (0.71-1.19)	0.53		0.98 (0.93-1.04)	0.59
	Total plasma	Total CVD	6	11.4 (11.1-12.9)	0.90 (0.78-1.03)	0.12		0.99 (0.98-1.00)	0.12
		CVD Mortality	4	11.4 (11.1-12.9)	0.66 (0.50-0.86)	0.002		0.96 (0.94-0.99)	0.002
		Total CHD	7	11.6 (11.1-12.9)	0.86 (0.74-1.00)	0.053		0.99 (0.97-1.00)	0.058
		Ischemic stroke	6	11.4 (11.1-12.9)	0.84 (0.66-1.06)	0.15		0.99 (0.97-1.01)	0.15
	Cholesterol ester	Total CVD	4	11.6 (10.3-12.9)	0.74 (0.63-0.88)	<0.001		0.98 (0.96-0.99)	<0.001
		CVD Mortality	3	10.8 (10.3-12.9)	0.56 (0.43-0.73)	<0.001		0.95 (0.93-0.97)	<0.001
		Total CHD	5	12.9 (10.3-16.8)	0.78 (0.65-0.94)	0.009		0.98 (0.97-1.00)	0.009
		Ischemic stroke	4	11.9 (10.3-13.0)	0.67 (0.51-0.88)	0.004		0.97 (0.94-0.99)	0.004
	Adipose tissue	Total CVD	2	6.19 (6.10-6.27)	0.87 (0.75-1.01)	0.073		0.98 (0.96-1.00)	0.075
		CVD Mortality	2	6.19 (6.10-6.27)	0.60 (0.44-0.82)	0.001		0.92 (0.88-0.97)	0.001
		Total CHD	2	6.19 (6.10-6.27)	0.88 (0.74-1.03)	0.11		0.98 (0.95-1.00)	0.11
		Ischemic stroke	2	6.19 (6.10-6.27)	0.87 (0.65-1.15)	0.32		0.98 (0.93-1.02)	0.34
AA	Phospholipid								
	-plasma	Total CVD	8	4.66 (3.04-6.71)	0.95 (0.87-1.04)	0.28		0.99 (0.97-1.01)	0.32
		CVD Mortality	7	4.81 (3.04-6.71)	0.93 (0.82-1.04)	0.20		0.99 (0.96-1.01)	0.28
		Total CHD	9	4.52 (3.04-6.71)	0.96 (0.86-1.06)	0.43		0.99 (0.97-1.01)	0.42
		Ischemic stroke	8	4.66 (3.04-6.71)	0.98 (0.83-1.14)	0.76		1.00 (0.97-1.03)	1.00
	-erythrocyte	Total CVD	6	4.28 (2.81-5.55)	0.93 (0.79-1.10)	0.41		0.99 (0.95-1.02)	0.40
		CVD Mortality	2	4.75 (3.94-5.55)	1.06 (0.70-1.61)	0.79		1.01 (0.94-1.09)	0.78
		Total CHD	5	4.80 (3.94-5.55)	0.97 (0.89-1.05)	0.43		0.99 (0.98-1.01)	0.42
		Ischemic stroke	4	5.08 (3.94-5.55)	1.01 (0.76-1.34)	0.96		1.00 (0.95-1.06)	0.92
	Total plasma	Total CVD	6	3.50 (2.43-4.73)	0.81 (0.70-0.94)	0.005		0.94 (0.90-0.97)	0.001
		CVD Mortality	4	2.55 (2.43-4.40)	0.85 (0.66-1.09)	0.20		0.92 (0.84-1.02)	0.12
		Total CHD	7	4.38 (2.43-4.66)	0.86 (0.74-1.01)	0.065		0.97 (0.93-1.01)	0.13
		Ischemic stroke	6	3.50 (2.43-4.76)	0.93 (0.73-1.18)	0.53		0.95 (0.88-1.02)	0.13
	Cholesterol ester	Total CVD	4	2.86 (2.38-3.52)	1.03 (0.88-1.20)	0.69		1.01 (0.95-1.08)	0.67
		CVD Mortality	3	2.85 (2.38-2.93)	0.99 (0.76-1.29)	0.95		1.00 (0.90-1.11)	0.95
		Total CHD	5	2.88 (2.38-3.60)	1.02 (0.85-1.23)	0.80		1.01 (0.94-1.09)	0.76
		Ischemic stroke	4	2.87 (2.38-4.10)	1.13 (0.89-1.43)	0.32		1.06 (0.97-1.15)	0.23

			n	Interquintile range, % of total FA	Risk per interquintile range			Risk per % of total FA	
FA	**Biomarker**	**Outcome**	**studies**	**median (min-max)**	**HR (95% CI)**	***P***		**HR (95% CI)**	***P***
AA	Adipose tissue	Total CVD	2	0.31 (0.26-0.37)	0.98 (0.87-1.10)	0.69		0.95 (0.69-1.31)	0.74
		CVD Mortality	2	0.31 (0.26-0.37)	1.02 (0.84-1.23)	0.86		1.06 (0.62-1.79)	0.84
		Total CHD	3	0.26 (0.25-0.37)	1.10 (0.98-1.23)	0.093		1.23 (0.88-1.73)	0.22
		Ischemic stroke	2	0.31 (0.26-0.37)	0.91 (0.74-1.11)	0.34		0.79 (0.44-1.40)	0.42

*CHD, coronary heart disease; CVD, cardiovascular disease; HR, hazard ratio. †Interquintile range was defined as the range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]).

Supplemental Table 14. Sensitivity analyses for the association of linoleic acid (LA) and arachidonic acid (AA) biomarkers (HR [95% CI]) per interquintile range and total CVD, CVD mortality, total CHD, and ischemic stroke. *†

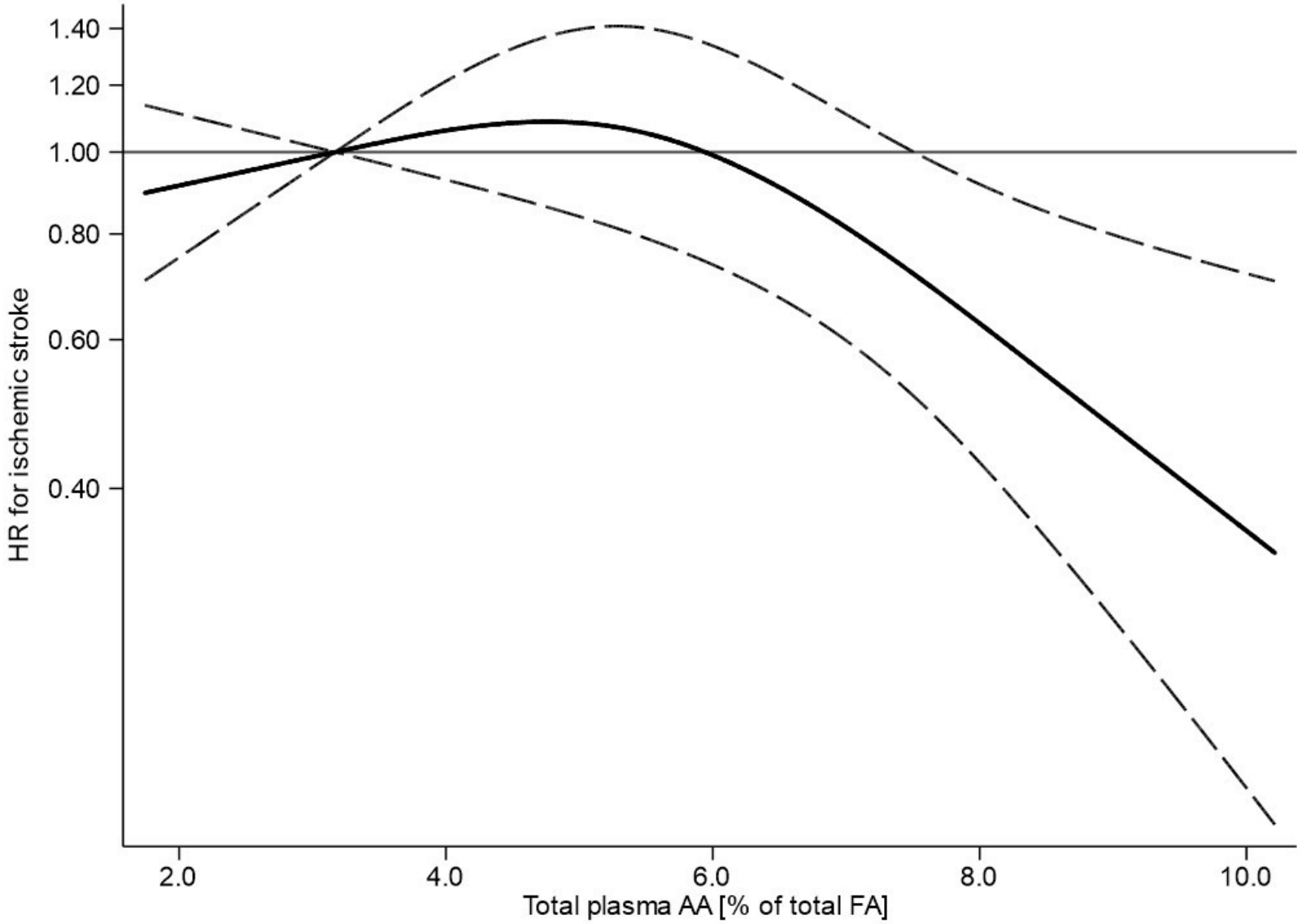
Fatty acid	Analysis	Total CVD	CVD mortality	Total CHD	Ischemic stroke
LA	Main analysis‡	0.93 (0.88-0.99)	0.78 (0.70-0.85)	0.94 (0.88-1.00)	0.88 (0.79-0.98)
	Alternative biomarker compartment priority§	0.92 (0.86-0.98)	0.77 (0.70-0.85)	0.94 (0.88-1.00)	0.87 (0.78-0.97)
	Censor at 10 y of follow-up	0.97 (0.89-1.05)	0.87 (0.76-1.01)	0.93 (0.84-1.02)	0.94 (0.79-1.11)
	Excluding studies (n=3) with only fatal events‖	0.94 (0.88-1.00)	n/a	0.94 (0.89-1.01)	0.89 (0.79-0.99)
	Including retrospective studies (n=1) #	n/a	n/a	0.95 (0.85-1.03)	n/a
AA	Main analysis‡	0.95 (0.90-1.01)	0.94 (0.86-1.02)	0.99 (0.94-1.04)	0.99 (0.90-1.10)
	Alternative biomarker compartment priority§	0.94 (0.89-1.00)	0.94 (0.86-1.03)	0.98 (0.93-1.03)	0.98 (0.89-1.09)
	Censor at 10 y of follow-up	0.91 (0.84-0.99)	0.82 (0.72-0.94)	1.02 (0.93-1.11)	0.99 (0.85-1.14)
	Excluding studies (n=3) with only fatal events‖	0.96 (0.90-1.02)	n/a	1.00 (0.94-1.05)	0.99 (0.90-1.10)
	Including retrospective studies (n=1) #	n/a	n/a	1.02 (0.97-1.08)	n/a

*CHD, coronary heart disease; CVD, cardiovascular disease; HR, hazard ratio. †Interquintile range was defined as the range between the midpoint of the bottom quintile [10th percentile] and the top quintile [90th percentile]). ‡Main analysis was conducted using inverse-variance weighted meta-analysis. §For studies evaluating multiple biomarker compartments, results from alternative fractions (i.e., total plasma for NHS and HPFS, and cholesterol ester for PIVUS and METSIM) were utilized in place of results from phospholipids. ‖Three studies included only fatal events and were excluded from this sensitivity analysis of associations with total CVD, total CHD, and ischemic stroke: MCCS (evaluated all outcomes), MORGEN and MPCDRF (both evaluated fatal CHD). #No retrospective studies assessing incident CVD, CVD mortality, or incident ischemic stroke were available.

Supplemental Table 15. Overview of participating cohorts with published correlations of self-reported intake and circulating or adipose tissue biomarker levels of linoleic acid (LA) and arachidonic acid (AA).[*]

Cohort[†]	N	Dietary assessment (unit)	Biomarker[‡]	Correlation coefficient		Adjustments	Reference
				LA	AA		
ARIC	3570	FFQ (% of total FA)	Plasma phospholipids	0.22[§] 0.26[‖]	N/A N/A	None	63
ARIC	3570	FFQ (% of total FA)	Cholesterol esters	0.28[§] 0.32[‖]	N/A N/A	None	63
CRS	196	FFQ (% of total FA)	Whole blood	0.43	0.05	Age, sex, and BMI	64
CRS	196	FFQ (% of total FA)	Total plasma	0.41	0.12	Age, sex, and BMI	64
CRS	196	FFQ (% of total FA)	Adipose tissue	0.52	0.11	Age, sex, and BMI	64
CRS	503	FFQ (energy-adjusted g/d)	Adipose tissue	0.58	0.04	None	13
HPFS	118	FFQ (% of total FA)	Adipose tissue	0.37	N/A	None	65
KIHD	2002	4-day food record (energy-adjusted g/d)	Total serum	0.51	N/A	None	
MCCS	4439	FFQ (% of total FA)	Plasma phospholipid	0.20[§] 0.58[‖]	0.03[§#] 0.06[‖]	None	26
MESA	2837	FFQ (g/d)	Plasma phospholipid	0.13	0.05	Age, sex, race/ethnicity, and energy intake	66
NHS	306	FFQ (% of total FA)	Total plasma	0.24	0.00[#]	None	31
NHS	306	FFQ (% of total FA)	Total plasma	0.25	-0.01[#]	Age, BMI, weight, smoking status, postmenopausal status, postmenopausal hormone use, period of blood assay, and fasting status.	31
NHS	306	FFQ (% of total FA)	Erythrocyte phospholipids	0.19	-0.06[#]	None	31
NHS	306	FFQ (% of total FA)	Erythrocyte phospholipids	0.24	-0.04[#]	Age, BMI, weight, smoking status, postmenopausal status, postmenopausal hormone use, period of blood assay, and fasting status.	31
NHS	140	FFQ (% of total FA)	Adipose tissue	0.35 0.23 0.37	-0.03[#**] -0.07[#††] -0.07[#‡‡]	None	67
ULSAM-70	433 ~229	7-day food diary (% of total FA)	Cholesterol esters	0.26 0.26[§§]	0.12 0.09[#§§]	None	68
ULSAM-70	789 ~418	7-day food diary (% of total FA)	Adipose tissue	0.37 0.42[§§]	0.08 0.10[§§]	None	68
WHIMS	648	FFQ (g/d)	Erythrocyte phospholipids	0.09	0.08[#]	None	69

[*]AA, arachidonic acid; FA, fatty acids; FFQ, food frequency questionnaire; LA, linoleic acid. [†]Table 1 footnote for abbreviations of cohorts [‡]All biomarkers were expressed as % of total fatty acids (FA). [§]Crude correlation coefficient. [‖]Correlation coefficient corrected for within-person variation in dietary intake and biomarker levels. [#]Non-significant correlation. [**]FFQ completed 12-18 months before biomarker sampling. [††]FFQ completed 0-6 months after biomarker sampling. [‡‡]Dietary estimate calculated as average from two FFQ completed 18 months apart (12-18 months before and 0-6 months after biomarkers sampling, respectively). [§§]Adequate reporters (defined as individuals not underreporting dietary intake according to the Goldberg cutoff as modified by Black).



Supplemental Figure 1. Dose-response relations between total plasma arachidonic acid (AA; 20:4n6) and hazard ratio (HR) of ischemic stroke.

Linear and non-linear associations were assessed using restricted cubic splines (knots at 10th, 50th, and 90th percentiles of total plasma AA). P for linear trend=0.36; P for nonlinearity=0.039.

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