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Supplementary appendix

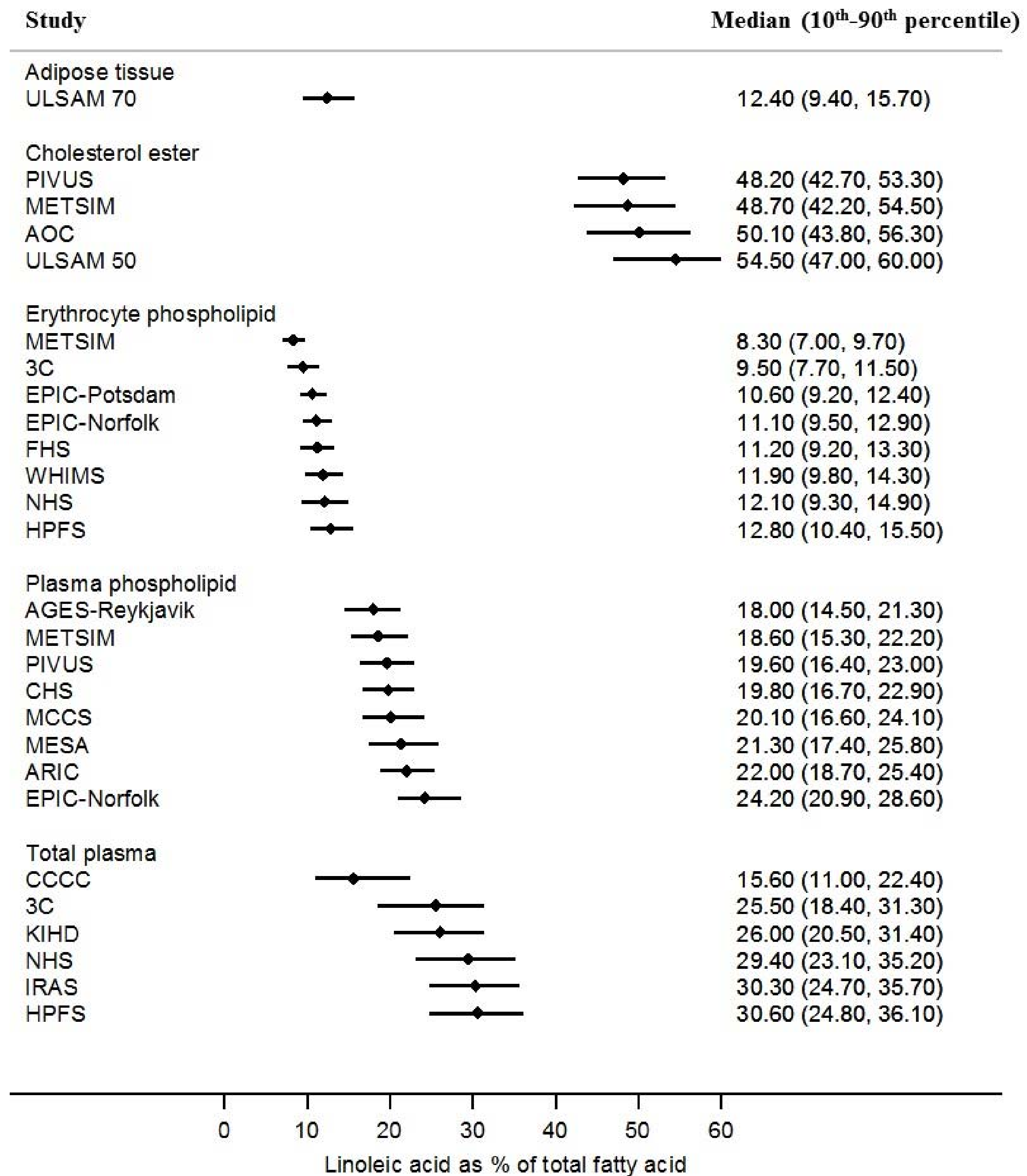
This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Wu J H Y, Marklund M, Imamura F, et al. Omega-6 fatty acid biomarkers and incident type 2 diabetes: pooled analysis of individual-level data for 39 740 adults from 20 prospective cohort studies. *Lancet Diabetes Endocrinol* 2017; published online Oct 11. http://dx.doi.org/10.1016/S2213-8587(17)30307-8.

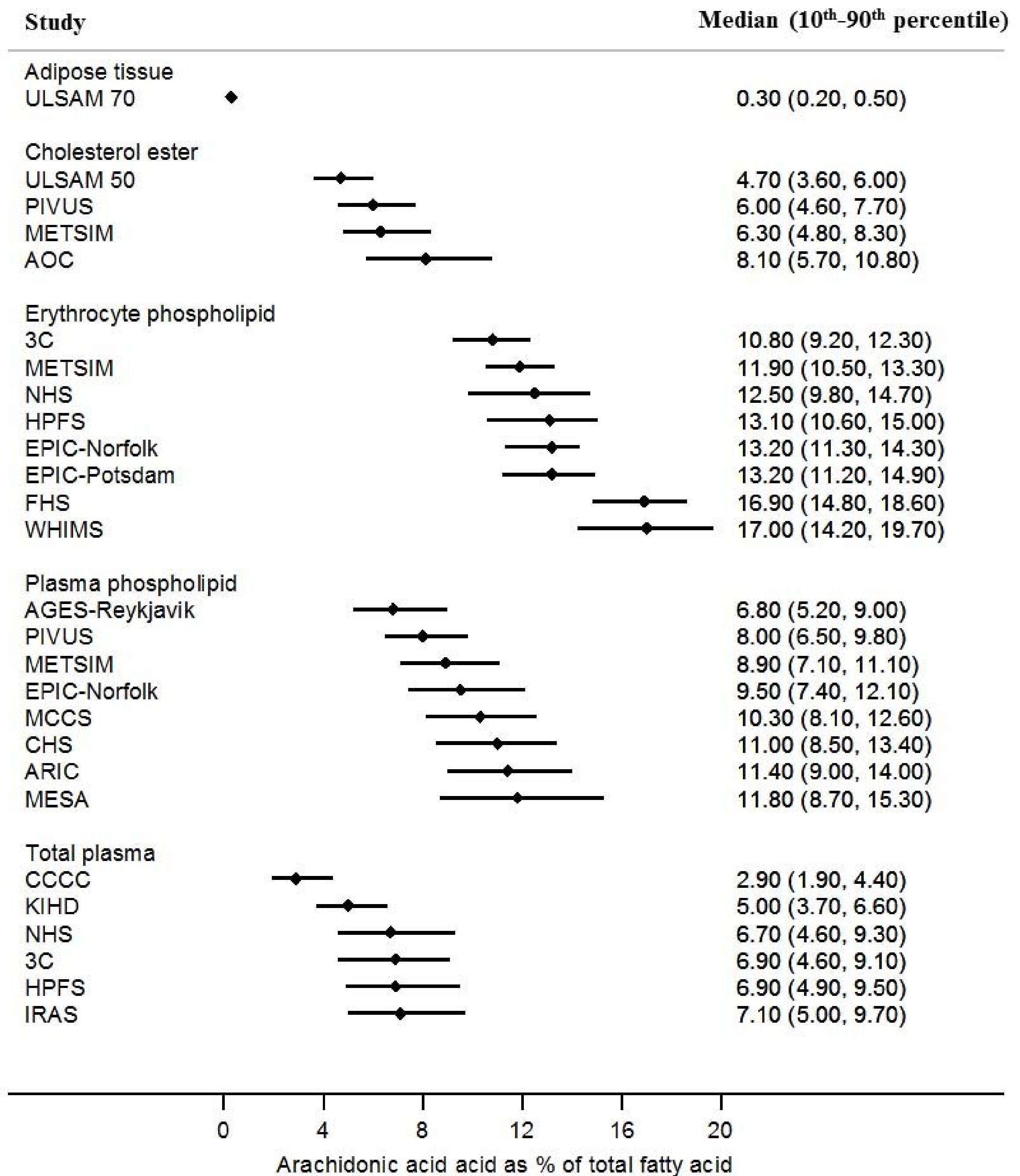
Supplementary Figures

eFigure 1 Level of (A) linoleic and (B) arachidonic acids across different biomarker compartments measured in the 20 contributing cohorts. Fatty acid concentrations were calculated as percent of total fatty acids, and indicated as median (circles) and interquintile range (lines extending from the circles), respectively.

(A) Linoleic acid

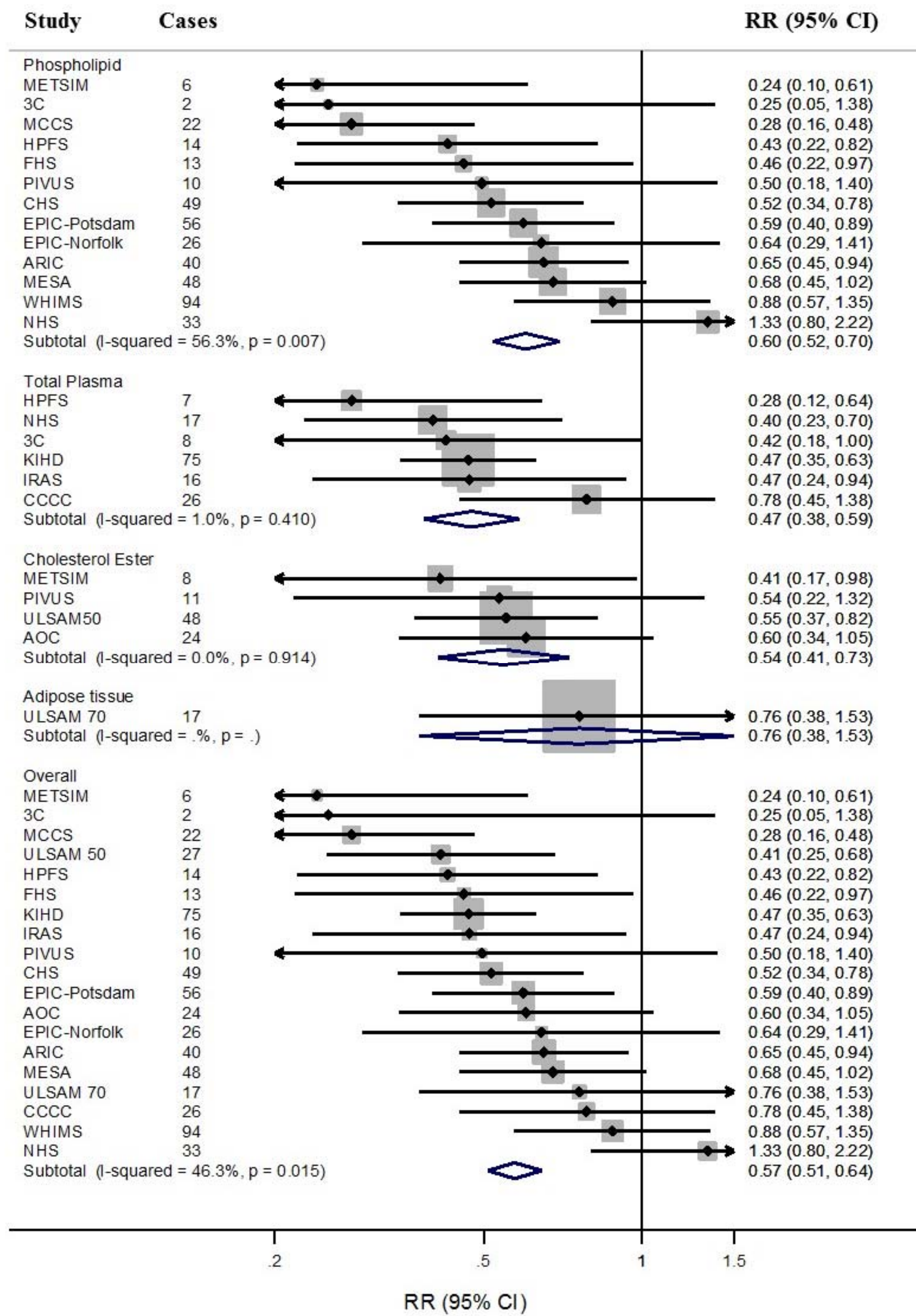


(B) Arachidonic acid

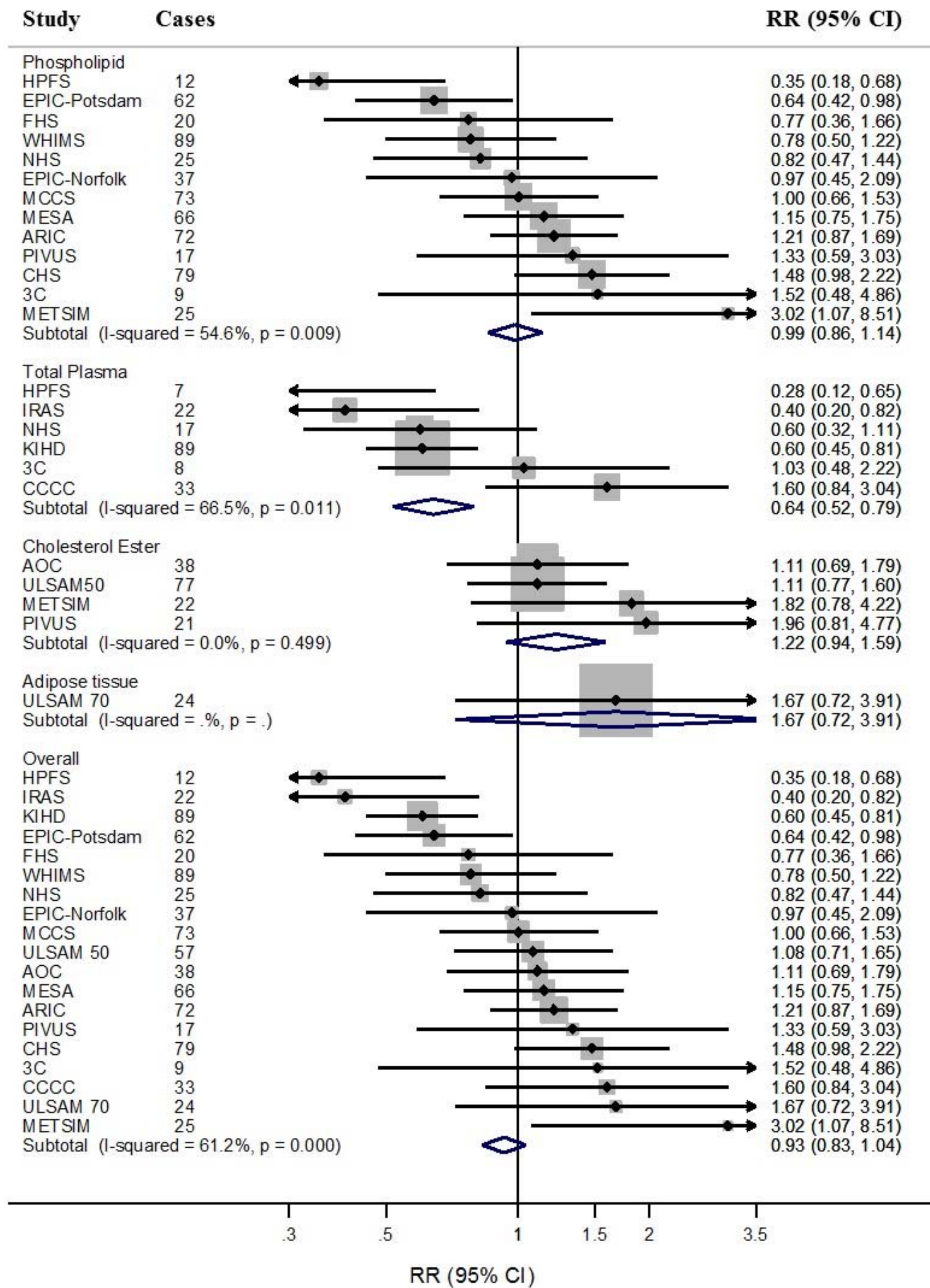


eFigure 2. Pooled relative risks (RR) of type 2 diabetes mellitus (T2D) for the highest vs. the lowest quintile n-6 PUFA. Association was assessed in multivariable models for each cohort, and results pooled using inverse-variance weighted meta-analysis. If multiple biomarkers were available within a study, one was chosen for the overall analysis with order of preference based on the biomarker that may best reflect long-term dietary intake, i.e. adipose tissue > phospholipid > total plasma > cholesterol ester. Similarly, erythrocyte phospholipid was preferred over plasma phospholipid if both were available from a cohort. AGES-Reykjavik was excluded from these analyses due to the limited number of incident T2D cases.

(A) Linoleic acid

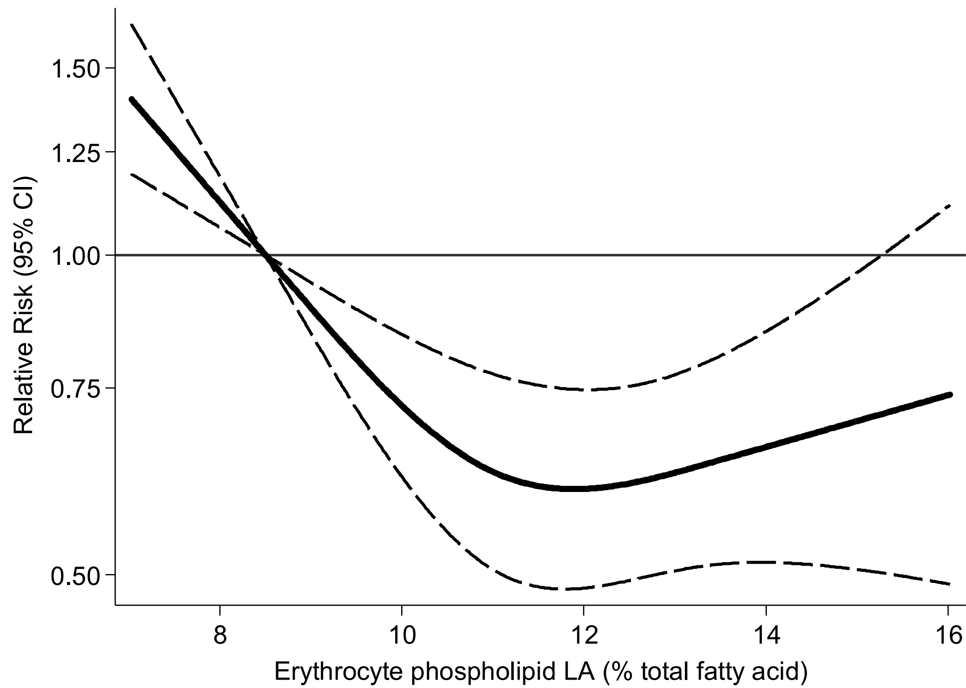


(B) Arachidonic acid

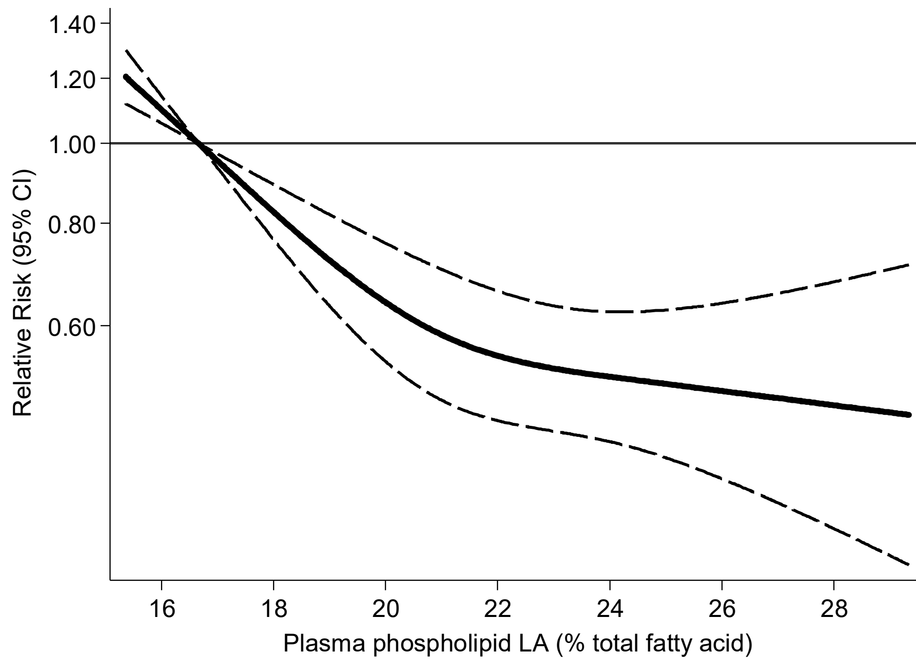


eFigure 3 Relationship between linoleic acid level and risk of type 2 diabetes evaluated using restricted cubic splines. Associations were evaluated using multivariate meta-analysis with 3-knot restricted cubic splines, for each of the biomarker compartments. The reference value was set at the 10th percentile of each fatty acid-biomarker exposure. The 95% confidence interval is depicted as the area between the dashed lines. Non-linear associations were examined for all biomarker compartments except adipose tissue due to only having 1 study that measured fatty acid in this compartment. Our spline analyses excluded any evidence for increased risk of T2D with highest biomarker LA levels in plasma phospholipid, plasma, and cholesterol esters, and for increased risk at all but the very highest erythrocyte levels.

(A) Erythrocyte phospholipid, 8 cohorts.
P-linearity<0.001
P-nonlinearity = 0.005

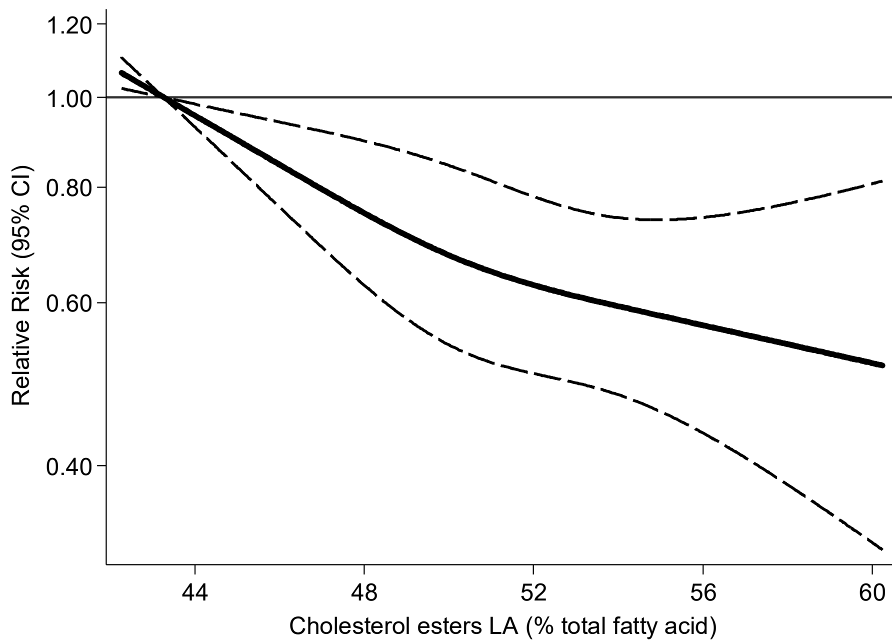


(B) Plasma phospholipid, 7 cohorts.
P-linearity<0.001,
P-nonlinearity=0.03



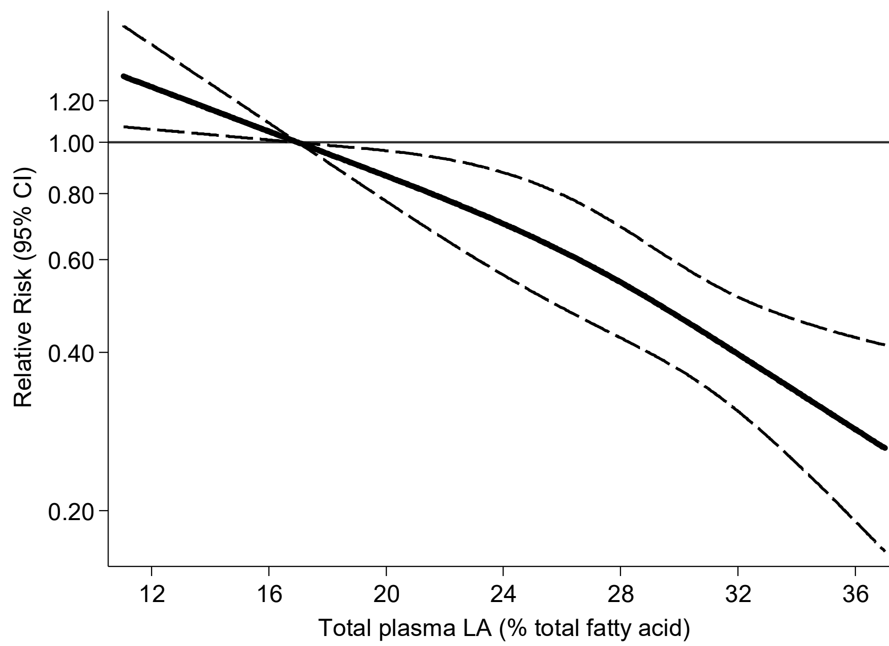
(C) Cholesterol Esters, 4 cohorts

P-linearity<0.001
P-nonlinearity=0.40



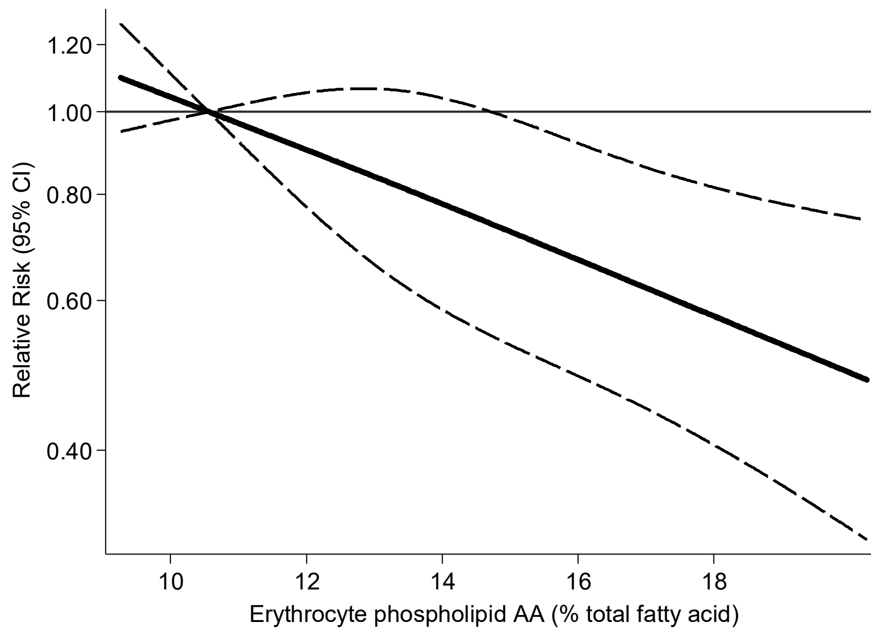
(D) Total plasma, 6 cohorts

P-linearity<0.001
P-nonlinearity=0.46

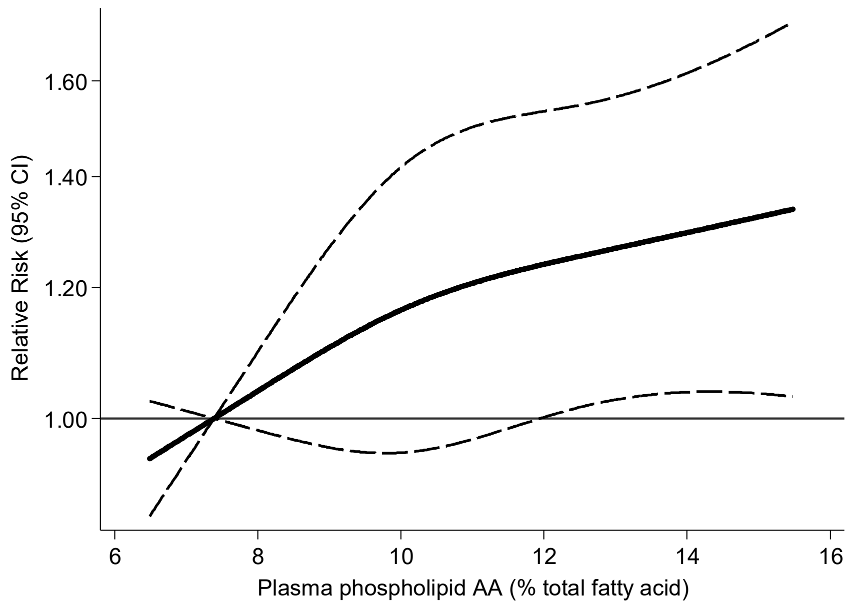


eFigure 4 Relationship between arachidonic acid level and risk of type 2 diabetes evaluated using restricted cubic splines. Associations were evaluated using multivariate meta-analysis with 3-knot restricted cubic splines, for each of the biomarker compartments. The reference value was set at the 10th percentile of each fatty acid-biomarker exposure. The 95% confidence interval is depicted as the area between the dashed lines.

(A) Erythrocyte phospholipid, 8 cohorts.
P-linearity=0.001
P-nonlinearity = 0.95

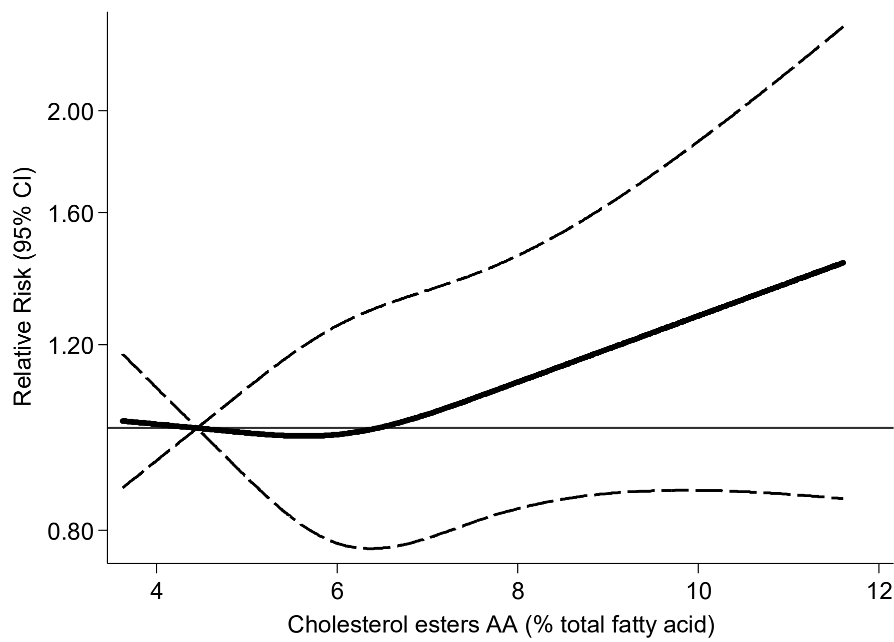


(B) Plasma phospholipid, 7 cohorts.
P-linearity=0.03
P-nonlinearity=0.54



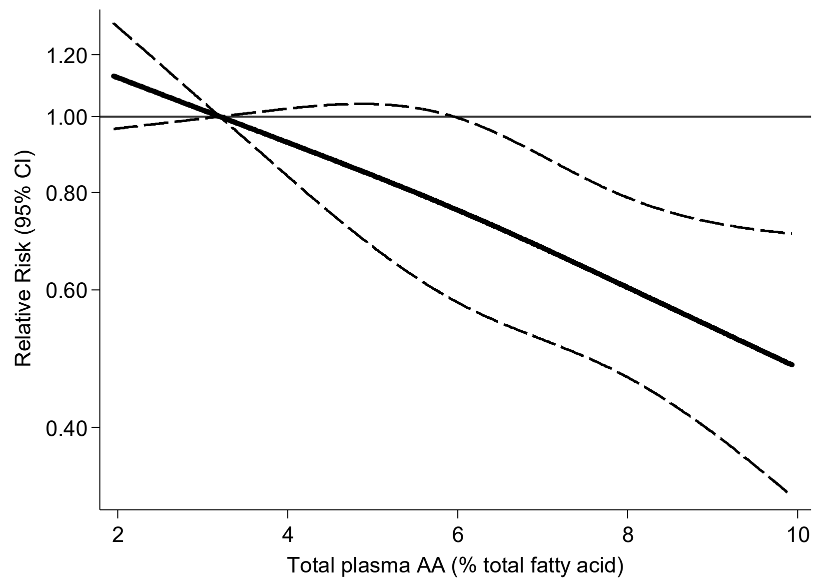
(C) Cholesterol Esters, 4 cohorts

P-linearity=0.23
P-nonlinearity=0.47



(D) Total plasma, 6 cohorts

P-linearity<0.001
P-nonlinearity=0.85



Supplementary Tables

eTable 1. Baseline Concentration of linoleic acid (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6) for 20 participating cohort studies

Study	Country	Biomarker type	Year measured	Total number of fatty acids measured	Fatty acid	n	Mean ± SD[a]	Median[a]	Minimum[a]	Maximum[a]	Correlation between compartments
AGES-Reykjavik	Iceland	Plasma phospholipid	2002-2006	41	**LA**	753	17·9 ± 2·8	18·0	7·4	27·3	-
					AA	753	6·9 ± 1·6	6·8	3·8	16·5	
AOC	Netherlands	Cholesterol esters	2002-2006	38	**LA**	2888	50·1 ± 4·9	50·1	30·0	67·6	-
					AA	2888	8·2 ± 2·0	8·1	2·8	15·9	
ARIC	United States	Plasma phospholipid	1987-1989	29	**LA**	3494	22·0 ± 2·7	22·0	9·0	32·4	-
					AA	3494	11·5 ± 1·9	11·4	5·3	20·0	
CCCC	Taiwan	Total plasma	1992-1993	29	**LA**	616	16·1 ± 4·4	15·6	2·8	27·6	-
					AA	616	3·1 ± 1·0	2·9	0·1	6·9	
CHS	United States	Plasma phospholipid	1992	45	**LA**	3179	19·8 ± 2·5	19·8	10·6	28·8	-
					AA	3179	11·0 ± 1·9	11·0	5·0	18·3	
EPIC-Norfolk	England	Erythrocyte phospholipid	1993-1997	33	**LA**	383	11·0 ± 1·3	10·9	7·3	14·7	LA: 0·79
					AA	383	12·9 ± 1·4	13·1	4·0	16·5	AA: 0·63
		Plasma phospholipid		27	**LA**	383	23·9 ± 3·2	23·7	15·4	33·6	
					AA	383	9·5 ± 1·9	9·3	4·9	17·9	
EPIC-Potsdam	Germany	Erythrocyte phospholipid	1994-1998	32	**LA**	1724	10·8 ± 1·3	10·7	5·5	16·0	-
					AA	1724	13·1 ± 1·7	13·3	2·5	18·5	
FHS	United States	Erythrocyte phospholipid	2005-2008	22	**LA**	1913	11·2 ± 1·7	11·2	5·7	19·6	-
					AA	1913	16·8 ± 1·6	16·9	9·7	21·0	
HPFS	United States	Erythrocyte phospholipid	1994	42	**LA**	1545	12·9 ± 2·9	12·8	5·9	36·5	LA: 0·38
					AA	1545	12·8 ± 1·9	13·1	4·0	17·6	AA: 0·48
		Total plasma	1994	42	**LA**	1497	30·3 ± 4·4	30·6	10·3	43·1	
					AA	1497	7·0 ± 1·8	6·9	1·8	13·1	
IRAS	United States	Total plasma	1992-1994	34	**LA**	719	30·2 ± 4·2	30·3	16·6	41·3	-
					AA	719	7·3 ± 1·8	7·1	2·5	13·4	
KIHD	Finland	Serum	1991-1992 (men) 2003 (women)	14	**LA**	3145	26·0 ± 4·4	26·0	10·1	41·4	-
					AA	3145	5·1 ± 1·2	5·0	1·4	11·2	
MCCS	Australia	Plasma phospholipid	1990-1994	55	**LA**	4046	20·1 ± 2·9	20·1	9·7	31·2	-
					AA	4046	10·4 ± 1·8	10·3	5·0	18·2	
MESA	United States	Plasma phospholipid	2000-2002	27	**LA**	2230	21·5 ± 3·3	21·3	11·4	36·1	-
					AA	2230	11·9 ± 2·6	11·8	3·6	20·5	
METSIM	Finland	Erythrocyte phospholipid	2006-2010	20	**LA**	1302	8·3 ± 1·0	8·3	5·4	12·9	LA: 0·76
					AA	1302	11·9 ± 1·1	11·9	8·1	15·1	(Erythrocyte PL and CE)
		Cholesterol esters		13	**LA**	1302	48·6 ± 4·9	48·7	20·4	62·4	LA: 0·78
					AA	1302	6·4 ± 1·4	6·3	2·7	11·5	(Erythrocyte PL and plasma PL)
		Plasma phospholipid		22	**LA**	1302	18·7 ± 2·6	18·7	11·1	28·5	LA: 0·82

					AA	1302	9·0 ± 1·6	8·9	4·7	15·0	(CE and plasma PL) AA: 0·58 (Erythrocyte PL and CE) AA: 0·65 (Erythrocyte PL and plasma PL) AA: 0·92 (CE and plasma PL)
NHS	United States	Erythrocyte phospholipid	1990	42	**LA** **AA**	1500 1500	12·1 ± 2·3 12·3 ± 2·0	12·1 12·5	3·1 3·4	22·5 17·5	LA: 0·42 AA:0·51
		Total plasma	1990	42	**LA** **AA**	1595 1595	29·0 ± 4·9 6·8 ± 1·8	29·4 6·7	0 0	46·0 13·1	
PIVUS	Sweden	Cholesterol ester	2001-2004	14	**LA** **AA**	822 822	48·2 ± 4·1 6·1 ± 1·3	48·3 6·0	33·0 2·8	61·1 11·7	LA: 0·84 AA:0·91
		Plasma phospholipid		16	**LA** **AA**	861 861	19·6 ± 2·5 8·1 ± 1·3	19·6 8·0	10·7 4·1	28·6 13·6	
3C	France	Erythrocyte phospholipid	1999-2000	35	**LA** **AA**	574 574	9·6 ± 1·5 10·7 ± 1·6	9·5 10·8	4·0 1·3	14·4 14·4	LA: 0·59 AA:0·53
		Total plasma		12	**LA** **AA**	1220 1220	25·0 ± 5·4 6·8 ± 1·9	25·5 6·9	4·1 1·0	40·8 14·0	
ULSAM-50	Sweden	Cholesterol esters	1970-1973	12	**LA** **AA**	1891 1891	54·0 ± 5·2 4·8 ± 0·9	54·5 4·7	27·2 2·0	68·0 8·9	-
ULSAM-70	Sweden	Adipose tissue	1991-1995	17	**LA** **AA**	738 738	12·6 ± 2·8 0·4 ± 0·1	12·4 0·3	5·6 0·1	30·0 0·8	-
WHIMS	United States	Erythrocyte phospholipid	1996	22	**LA** **AA**	5799 5799	12·0 ± 1·8 17·0 ± 2·2	11·9 17·0	5·1 6·3	22·7 26·0	-

[a]Fatty acid values are % of total fatty acids, because these compositional differences are more relevant to differences in diet and endogenous metabolism than absolute levels, which are influenced by other factors such as overall circulating triglyceride levels

AGES-Reykjavik: Age, Gene/Environment Susceptibility Study (Reykjavik); AOC: Alpha Omega Cohort; ARIC: Atherosclerosis Risk in Communities Study; CCCC, Chin-Shan Community Cardiovascular study; CHS: Cardiovascular Health Study; EPIC-Norfolk: European Prospective Investigation into Cancer (Norfolk); EPIC-Potsdam: European Prospective Investigation into Cancer (Potsdam); FHS: Framingham Heart Study; HPFS: Health Professionals Follow-up Study; IRAS: Insulin Resistance Atherosclerosis Study; KIHD: Kuopio Ischaemic Heart Disease Risk Factor Study; MCCS: Melbourne Collaborative Cohort Study; MESA: Multi-Ethnic Study of Atherosclerosis; METSIM: Metabolic Syndrome in Men Study; NHS: Nurses' Health Study; PIVUS: Prospective Investigation of the Vasculature in Uppsala Seniors; 3C Study: Three City Study; ULSAM 50 & 70: Uppsala Longitudinal Study of Adult Men; WHIMS: Women's health initiative memory study.

eTable 2. Ascertainment of incident type 2 diabetes and number of cases for 20 participating cohort studies

Study	Number of cases	Median/Maximum follow up (yr)	Ascertainment/definition of T2D
AGES-Reykjavik	28	5·2/7·8	Participants were seen in clinic ~5 years after baseline. Prevalent diabetes at the clinic visit were determined from self-reported diabetes, diabetes medication use or fasting plasma glucose ≥7.0mmol/L. Time to incident diabetes was defined as the median time between baseline and follow-up visit.
AOC	154	3·4/4·8	Incident diabetes during the trial phase was defined as either a self-reported physician diagnosis or use of antidiabetic medication (based on telephone interviews at 12 and 24 months or examinations at 20 months (midterm examination; n=800) or 40 months (final examination).
ARIC	304	9 (max)	Participants were enrolled and measurements taken at baseline 1987-1989 and at 3 subsequent exams (3 years apart). Medication use was assessed at each study exam. Fasting glucose was measured at the study baseline (1987–1989) and in subsequent visits 1990–1992, 1993–1995, and 1996-98. Type 2 diabetes was defined as a single measure of fasting glucose concentration ≥7.0mmol/L, or new use of an insulin or oral hypoglycemic medication, or self-report of physician diagnosis.
CCCC	128	6/8·1	Participants were enrolled and measurements taken at baseline (1992-1993) and at 2 subsequent exams (1994-1995 and 1999-2000). Type 2 diabetes was defined as fasting glucose levels ≥ 7.0mmol/L or with use of hypoglycemic medication.
CHS	284	10·6/18	Participants were followed by means of annual study clinic examinations with interim phone contacts for 10 y and telephone contacts every 6 mo thereafter. Medication use was assessed annually. Fasting glucose was measured at the study baseline (1992–1993) and in 1996–1997, 1998–1999, and 2005–2006; nonfasting glucose was measured in 1994–1995. Type 2 diabetes was defined as a single measure of fasting glucose concentration ≥7.0mmol/L, nonfasting or 2-h postchallenge glucose concentration ≥11.1mmol/L, or new use of an insulin or oral hypoglycemic medication.
EPIC-Norfolk	199	5·8/12·1	Record linkage was used to trace each participant for diabetes diagnosis, including linkage with general practice diabetes registers, hospital outpatient diabetes registers, and hospital admissions information for diabetes. In addition, diabetes-related deaths were flagged by linkage to the National Death Registry (The Office for National Statistics, Newport, United Kingdom).
EPIC-Potsdam	488	6·2/10·1	Identified during follow-up (every 2-3 years) via self-reports of a diabetes diagnosis, diabetes-relevant medication, or dietary treatment due to diabetes. All incident cases were verified by questionnaires mailed to the diagnosing physician asking about the date and type of diagnosis, diagnostic tests, and treatment of diabetes. Only cases with a physician diagnosis of type 2 diabetes (ICD10: E11) and a diagnosis date after the baseline examination were considered as confirmed incident cases of type 2 diabetes.
FHS	98	5·8/9·0	Incident diabetes was defined as fasting glucose concentration ≥ 7.0mmol/L, HBA1C ≥ 6·5 or new use of insulin or oral hypoglycemic medication ascertained during follow up examinations.
HPFS	113	14·8/17·6	Incident cases of diabetes are identified by self-reports on mail questionnaires and confirmed by supplementary information collected about the diagnosis using the following criteria from the National Diabetes Data Group (NDDG) up until 1998: (1) manifestation of classic symptoms such as excessive thirst, polyuria, weight loss and hunger, in conjunction with elevated fasting glucose ≥7.8mmol/L or non-fasting glucose levels ≥11.1mmol/L (2) asymptomatic but elevated plasma glucose in two separate occasions or abnormal glucose tolerance test results and (3) receiving any hypoglycemic treatment for diabetes. After 1998 a fasting glucose concentration ≥7.0mmol/L was adopted per the new diagnostic criteria of the American Diabetes Association (ADA). Medical records were obtained for a subset of the subjects diagnosed with diabetes to validate the information obtained by the supplemental questionnaire. This supplemental questionnaire has been validated as a confirmation tool for diabetes diagnosis with high reliability (>98% of cases confirmed for those who provided records).
IRAS	146	5 (max)	Participants were followed up after a period of 5 years from study baseline 1992 – 1994 in 1997 – 1999. Fasting glucose, 2 – hour post challenge glucose, glycated hemoglobin, use of insulin or oral hypoglycemic medication was ascertained at that time by trained technicians. Type 2 diabetes was defined as 1) fasting glucose concentration ≥ 7.0mmol/L, or 2) 2-hour post oral glucose concentration ≥ 11.1mmol/L, or 3) new use of an insulin or oral hypoglycemic medication, or 4) Fasting or non-fasting HbA1C concentration ≥ 6·5%

KIHD	595	18·2/26·8	Type 2 diabetes was defined as a self-reported physician diagnosis and/or fasting plasma glucose ≥ 7.0mmol/L or 2-hour oral glucose tolerance test plasma glucose ≥11.1mmol/L at re-examination rounds 4, 11 and 20 years after the baseline, and by record linkage to the national hospital discharge registry, and to the Social Insurance Institution of Finland register for reimbursement of medicine expenses used for T2D for the entire study period until the end of the follow-up in Dec 31, 2010.
MCCS	336	NA	Approximately 4 y after baseline, the participants completed a mailed, self-administered questionnaire that covered diagnosis of diabetes
MESA	297	9·3/11·2	Participants were followed by means of bi-annual study clinic examinations with yearly interim phone contacts for 10 y Fasting glucose was measured at the study baseline (2000-2002) and in 2002-2004,2004-2005, 2005-2007, and 2010–2011. Diabetes was defined as fasting plasma glucose ≥7.0mmol/L.
METSIM	71	5·5/7·9	Type 2 diabetes diagnosis was based on an oral glucose tolerance test (fasting plasma glucose ≥7.0mmol/L and/or 2 h plasma glucose ≥ 11.1mmol/L), or on HbA1c measurements (≥6·5 %) at the follow-up visit, or on the National Drug Reimbursement registry data (drug treatment started for diabetes during the follow-up).
NHS	154	20·2/22·8	Incident cases of T2D are identified by self-reports on the mail questionnaires and confirmed by supplementary information collected about the diagnosis using the following criteria from the National Diabetes Data Group (NDDG) up until 1998: (1) manifestation of classic symptoms such as excessive thirst, polyuria, weight loss and hunger, in conjunction with elevated fasting glucose ≥7.8mmol/L or non-fasting glucose levels ≥11.1mmol/L (2) asymptomatic but elevated plasma glucose in two separate occasions or abnormal glucose tolerance test results and (3) receiving any hypoglycemic treatment for diabetes. After 1998 a fasting glucose concentration ≥7.0mmol/L was adopted per the new diagnostic criteria of the American Diabetes Association (ADA). Medical records were obtained for a subset of the subjects diagnosed with diabetes to validate the information obtained by the supplemental questionnaire. This supplemental questionnaire has been validated as a confirmation tool for diabetes diagnosis with high reliability (>98% of cases confirmed for those who provided records).
PIVUS	69	10/10·9	Diabetes incidence during follow-up was identified by medical records, repeated blood sampling and self-reports at follow-ups after 5 and 10 years. Type 2 diabetes was defined using one of four criteria: a) fasting blood glucose ≥6.1mmol/L (corresponds to fasting plasma glucose ≥ 7.0mmol/L), b) self-reported diabetes, c) diabetes diagnosis reported in medical records, d) use of insulin or oral hypoglycemic agents.
3C	36	8·0/13·0	Participants were followed up at 2, 4,7,10,12 years post baseline. Type 2 diabetes was self -reported or defined by use of insulin or oral hypoglycemic medication.
ULSAM-50	246	21·4/42·3	Incident diabetes during follow-up was identified using the Swedish Hospital Discharge and Cause of Death registers, and supplemented with ULSAM clinical assessments that occurred at throughout follow-up. All participants were followed regarding incidence of diabetes until December 31, 2011. Using registry data, diabetes was identified according to International Classification of Disease 9th (ICD-9) and 10th revision (ICD-10), codes 250 and E10-E14, respectively. Diabetes prevalence at baseline and subsequent ULSAM clinical assessments were determined as fasting blood glucose ≥6.1mmol/L (corresponds to fasting plasma glucose ≥ 7.0mmol/L) or fasting plasma glucose ≥7.0mmol/L, or the use of glucose-lowering medication.
ULSAM-70	99	14·1/21·5	Incident diabetes during follow-up was identified using the Swedish Hospital Discharge and Cause of Death registers, and supplemented with ULSAM clinical assessments that occurred at throughout follow-up. All participants were followed regarding incidence of diabetes until December 31, 2011. Using registry data, diabetes was identified according to International Classification of Disease 9th (ICD-9) and 10th revision (ICD-10), codes 250 and E10-E14, respectively. Diabetes prevalence at baseline and subsequent ULSAM clinical assessments were determined as fasting blood glucose ≥6.1mmol/L (corresponds to fasting plasma glucose ≥ 7.0mmol/L) or fasting plasma glucose ≥7.0mmol/L, or the use of glucose-lowering medication.
WHIMS	502	11·0/14·1	Incident diabetes was defined as a positive answer to the question (asked annually) regarding "newly prescribed treatment for diabetes with pills or insulin shots." The date of diabetes onset was assigned as the midpoint between the dates between the survey when diabetes was self-reported and the previous survey.

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eTable 3. Continuous covariates at baseline in participants with measured fatty acid (FA) biomarker data in 20 participating cohort studies[a,b]

Study	Age (years)		Body mass index (kg/m^2)		Omega-3 polyunsaturated fatty acids[c]	
	n	Mean ± SD (min - max)	n	Mean ± SD (min - max)	n	Biomarker: Mean ± SD (min - max)
AGES-Reykjavik	753	75·5 ± 5·2 (67·0 – 93·0)	753	27·0 ± 4·0 (15·8 – 40·6)	753	Plasma phospholipid: 10·2 ± 3·0 (4·1 – 21·1)
AOC	2888	69·0 ± 5·6 (58·5 – 81·0)	2886	27·4 ± 3·6 (17·2 – 52·5)	2888	Cholesterol esters: 1·3 ± 0·8 (0 – 12·7)
ARIC	3494	53·7 ± 5·6 (44·0 – 65·0)	3494	26·8 ± 4·4 (15·9 – 51·1)	3494	Plasma phospholipid: 4·3 ± 1·1 (1·5 – 15·6)
CCCC	616	59·2 ± 9·9 (35·0 – 88·0)	616	23·3 ± 3·0 (14·6 – 36·5)	666	Total plasma: 2·2 ± 0·8 (0·6 – 10·6)
CHS	3179	72·4 ± 5·2 (65·0 – 95·0)	3179	26·3 ± 4·5 (13·5 – 53·3)	3179	Plasma phospholipid: 4·5 ± 1·3 (1·5 – 16·6)
EPIC-Norfolk	383	63·9 ± 8·1 (41·0 – 77·0)	383	28·1 ± 4·4 (16·3 – 45·5)	383	Erythrocyte phospholipid: 9·0 ± 1·6 (2·3 – 14·9)
EPIC-Norfolk					383	Plasma phospholipid: 7·2 ± 2·0 (2·6 – 13·9)
EPIC-Potsdam	1724	49·4 ± 8·9 (20·0 – 66·0)	1724	26·0 ± 4·2 (16·4 – 54·8)	1724	Erythrocyte phospholipid: 7·9 ± 1·6 (1·0 – 14·0)
FHS	1913	64·4 ± 8·3 (40·0 – 88·0)	1909	27·8 ± 5·0 (16·5 – 56·3)	1913	Erythrocyte phospholipid: 8·4 ± 1·9 (4·2 – 18·7)
HPFS	1545	64·7 ± 8·6 (46·6 – 80·8)	1494	25·8 ± 3·3 (16·0 – 43·3)	1545	Erythrocyte phospholipid: 6·0 ± 1·7 (1·7 – 14·8)
HPFS	1497	64·6 ± 8·6 (46·6 – 80·8)	1448	25·8 ± 3·3 (16·0 – 43·3)	1497	Total plasma: 2·7 ± 1·2 (0·5 – 10·9)
IRAS	719	55·1 ± 8·5 (40·0 – 70·0)	719	28·4 ± 5·6 (14·2 – 55·4)	719	Total plasma: 2·4 ± 0·8 (0·8 – 7·7)
KIHD	3145	55·5 ± 7·1 (42·0 – 72·6)	3134	27·1 ± 3·9 (17·1 – 50·0)	3145	Serum: 4·8 ± 1·6 (1·7 – 15·6)
MCCS	4046	55·3 ± 8·6 (36·0 – 72·6)	4043	27·0 ± 4·5 (15·4 – 51·7)	4046	Plasma phospholipid: 6·3 ± 1·4 (2·4 – 18·6)
MESA	2230	60·9 ±10·1 (44·0 – 84·0)	2230	27·5 ± 5·4 (15·9 – 54·5)	2230	Plasma phospholipid: 6·1 ± 2·4 (2·2 – 23·5)
METSIM	1301	55·0 ± 5·6 (45·0 – 68·0)	1301	26·4 ± 3·5 (17·4 – 48·1)	1302	Cholesterol esters: 3·5 ±1·4 (0·9 – 11·7)
METSIM					1302	Erythrocyte phospholipid: 10·3 ±1·8 (5·0 – 18·2)
METSIM					1302	Plasma phospholipid: 9·4 ± 2·5 (3·7 – 21·0)
NHS	1500	60·4 ± 6·4 (42·6 – 69·6)	1453	25·2 ± 4·4 (15·5 – 57·1)	1464	Erythrocyte phospholipid: 4·9 ± 1·3 (1·3 – 11·2)
NHS	1595	60·4 ± 6·4 (42·6 – 69·6)	1586	25·3 ± 4·4 (15·5 – 57·1)	1548	Total plasma: 2·2 ± 0·8 (0 – 9·2)
PIVUS	863	70·2 ± 0·2 (70·0 – 70·7)	863	26·8 ± 4·1 (16·6 – 49·7)	822	Cholesterol esters: 3·3 ± 1·3 (0·8 – 9·1)
PIVUS					861	Plasma phospholipid: 8·5 ± 2·2 (3·2 – 18·2)
3C	574	76·6 ± 4·9 (65·0 – 90·4)	574	25·6 ± 4·1 (15·0 – 42·7)	574	Erythrocyte phospholipid: 6·2 ± 1·4 (0·8 – 10·9)
3C	1220	74·4 ± 4·8 (65·6 – 89·4)	1220	26·3 ± 4·2 (14·2 – 43·3)	1220	Total plasma: 3·9 ± 1·3 (1·1 – 9·7)
ULSAM-50	1891	49·7 ± 0·6 (48·6 – 51·1)	1891	25·0 ± 3·2 (15·2 – 42·1)	1891	Cholesterol esters: 2·1 ± 0·8 (0·4 – 7·4)
ULSAM-70	738	71·0 ± 0·6 (69·4 – 73·6)	734	26·2 ± 3·2 (18·1 – 40·2)	738	Adipose tissue: 0·7 ± 0·3 (0·1 – 1·9)
WHIMS	5799	70·1 ± 3·8 (63·0 – 81·0)	5799	28·1 ± 5·5 (13·8 – 66·3)	5799	Erythrocyte phospholipid: 7·8 ± 1·8 (2·3 – 22·3)

[a]Cohort medians were presented for prospective studies; medians for sub-cohort members only were determined for case-cohort studies (MCCS and EPIC-Potsdam).
[b]Participants were excluded from analysis for missing continuous covariates (0% missing, n=12 cohorts; ≤0.5% missing, n=6 cohorts; <3.3% missing, n=2 cohorts).
[c]n-3 polyunsaturated fatty acids represent the sum of eicosapentaenoic acid (EPA) + docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) in the cohorts except for the AOC (EPA only), METSIM (for the cholesterol esters fraction, EPA+DHA), and ULSAM-50 (EPA+DHA). Fatty acid values are % total fatty acids.
AGES-Reykjavik: Age, Gene/Environment Susceptibility Study (Reykjavik); AOC: Alpha Omega Cohort; ARIC: Atherosclerosis Risk in Communities Study; CCCC, Chin-Shan Community Cardiovascular study; CHS: Cardiovascular Health Study; EPIC-Norfolk: European Prospective Investigation into Cancer (Norfolk); EPIC-Potsdam: European Prospective Investigation into Cancer (Potsdam); FHS: Framingham Heart Study; HPFS: Health Professionals Follow-up

Study; IRAS: Insulin Resistance Atherosclerosis Study; KIHD: Kuopio Ischaemic Heart Disease Risk Factor Study; MCCS: Melbourne Collaborative Cohort Study; MESA: Multi-Ethnic Study of Atherosclerosis; METSIM: Metabolic Syndrome in Men Study; NHS: Nurses' Health Study; PIVUS: Prospective Investigation of the Vasculature in Uppsala Seniors; 3C Study: Three City Study; ULSAM 50 & 70: Uppsala Longitudinal Study of Adult Men. WHIMS: Women's health initiative memory study.

eTable 4. Frequencies of categorical covariates (%) at baseline in participants with measured fatty acid (FA) biomarker data in 20 participating cohort studies[a,b]

Study	Sex (male)	Race	Smoking	Alcohol	Physical activity assessment*	Education	Drug-treated hypertension	Drug-treated high cholesterol	Prevalent CHD
AGES-Reykjavik	40·5	100 (Caucasian)	12·0 (Current) 43·5 (Former) 44·5 (Never)	32·4 (None) 62·7 (<1 drink/d) 3·1 (1-2 drinks/d) 1·9 (>2 drinks/d)	Frequency of moderate or vigorous physical activities in the last 12 months were assessed by self-reported questionnaire and classified as never, rarely, occasionally, or moderate to high	21·8 (< high school) 50·2 (high school) 28·0 (> high school)	58·2	23·4	23·9
AOC	79·1	99·0 (Caucasian) 1·0 (Asian/Black)	16·5 (Current) 66·2 (Former) 17·3 (Never)	18·6 (None) 38·5 (<1 drink/d) 18·9 (1-2 drinks/d) 23·9 (>2 drinks/d)	Assessed with a validated Physical Activity Scale for the Elderly (PASE) questionnaire, Participants were categorized based on METs.	20·6 (< high school) 66·8 (high school) 12·6 (> high school)	12·5	15·4	100
ARIC	48·0	100 (Caucasian)	23·0 (Current) 40·2 (Former) 36·8 (Never)	46·0 (None) 30·3 (<1 drink/d) 15·9 (1-2 drinks/d) 7·8 (>2 drinks/d)	Questionnaire measured sport-type physical activities during leisure time. Participants were classified based on sports index score.	6·1 (< high school) 34·3 (high school) 59·7 (> high school)	22·9	0	3·8
CCCC	59·9	100 (Asian)	43·2 (Current) 56·8 (Not current)	67·1 (None) 33·0 (regular drinker)	Questionnaire assessed if participants exercised (defined as exercise lasting >20 min, exercise that led to sweating, or laborious job) or not in the past week.	94·5 (< 9 years school) 5·5 (≥ 9 years school)	30·0	n/a	6·5
CHS	38·6	88·9 (Caucasian) 11·1 (Black)	9·2 (Current) 43·9 (Former) 46·9 (Never)	52·3 (None) 34·2 (<1 drink/d) 6·8 (1-2 drinks/d) 6·7 (>2 drinks/d)	Leisure-time activity assessed by the Minnesota Leisure-time Activities questionnaire, exercise intensity and usual walking habits assessed by self-report. Participants categorized according to energy expanded on physical activity.	24·6 (< high school) 28·3 (high school) 47·1 (> high school)	16·0	6·9	20·5

EPIC-Norfolk	53·3	100 (Caucasian)	11·5 (Current) 50·4 (Former) 38·1 (Never)	25·9 (None) 43·9 (<1 drink/d) 15·1 (1-2 drinks/d) 15·1 (>2 drinks/d)	Validated Cambridge Physical Activity questionnaire.	51·2 (< high school) 10·4 (high school) 38·4 (> high school)	27·2	1·8	5·7
EPIC-Potsdam	37·7	100 (Caucasian)	20·8 (Current) 32·1 (Former) 47·1 (Never)	3·1 (None) 61·5 (<1 drink/d) 21·1 (1-2 drinks/d) 14·3 (>2 drinks/d)	Leisure time (sports activities and biking) activities assessed by a computer-guided interview, participants categorized according to hours spent on leisure time physical activity per week.	3·5 (no or currently undertaking training) 34·0 (vocational training) 62·5 (technical school or university degree)	15·8	4·4	7·2
FHS	42·8	100 (Caucasian)	9·1 (Current) 90·9 (Former or never)	23·0 (None) 50·2 (<1 drink/d) 21·3 (1-2 drinks/d) 5·5 (>2 drinks/d)	Typical activities and rest hours per day were assessed by a physical activity questionnaire and participants were categorized based on METs.	2·5 (< high school) 23·9 (high school) 73·5 (> high school)	40·6	37·1	8·1
HPFS	100	93·9 (Caucasian) 6·1 (Non-Caucasian)	8·8 (Current) 48·3 (Former) 42·9 (Never)	24·1 (None) 44·3 (<1 drink/d) 17·3 (1-2 drinks/d) 14·3 (>2 drinks/d)	Questionnaires ascertained amount of time spent per week on different types of physical activities and walking pace. Participants were categorized based on METs	100 (>high school)	24·0	7·0	2·3
IRAS	44·2	42·3 (Caucasian) 33·2 (Hispanic) 24·5 (Black)	14·6 (Current) 39·2 (Former) 46·2 (Never)	11·4 (None) 37·1 (1-2 drinks/d) 51·5 (>2 drinks/d)	Assessed using a questionnaire and a structured interview, and participants were categorized based on METs.	11·3 (< high school) 25·5 (high school) 63·3 (> high school)	24·5	7·9	2·2
KIHD	74·4	100 (Caucasian)	25·8 (Current) 29·2 (Former) 44·9 (Never)	17·7 (None) 57·7 (<1 drink/d) 12·5 (1-2 drinks/d) 11·9 (>2 drinks/d)	Assessed using a 12-month leisure time physical activity questionnaire administered in an interview, and participants were categorized based on METs.	52·5 (< high school) 39·4 (high school) 8·1 (> high school)	26·2	1·7	24·2
MCCS	44·8	100 (Caucasian)	11·1 (Current) 31·6 (Former) 57·2 (Never)	31·0 (None) 36·0 (<1 drink/d) 16·5 (1-2 drinks/d) 16·6 (>2 drinks/d)	Physical activity score calculated based on frequency of walking, vigorous exercise, and	55·2 (< high school) 21·3 (high school) 23·6 (> high school)	23·0	n/a	5·3

					less vigorous exercise assessed by a questionnaire.				
MESA	46·1	28·4 (Caucasian) 22·2 (Hispanic) 23·9 (Black) 25·6 (Asian)	13·2 (Current) 31·8 (Former) 55·1 (Never)	37·2 (None) 40·1 (<1 drink/d) 15·1 (1-2 drinks/d) 7·6 (>2 drinks/d)	Total METs quantified with the Typical Week Physical Acitivity Survey. Participants were categorized based on METs.	18·7 (< high school) 18·2 (high school) 63·1 (> high school)	30·9	13·2	0
METSIM	100	99·5 (Caucasian) 0·5 (Non-Caucasian)	18·1 (Current) 38·0 (Former) 43·9 (Never)	20·0 (None) 42·0 (<1 drink/d) 22·5 (1-2 drinks/d) 15·4 (>2 drinks/d)	Leisure time physical activity was assessed by self-report. Participants categorized according to time spent on leisure time physical activity per week.	n/a	12·5	13·7	0·2
NHS	0	99·2 (Caucasian) 0·8 (Non-Caucasian)	18·5 (Current) 39·6 (Former) 41·9 (Never)	40·0 (None) 47·2 (<1 drink/d) 7·7 (1-2 drinks/d) 5·1 (>2 drinks/d)	Questionnaires ascertained amount of time spent per week on different types of physical activities and walking pace. Participants were categorized based on METs	100 (> high school)	27·9	3·9	1·1
PIVUS	49·0	100 (Caucasian)	11·0 (Current) 39·0 (Former) 50·0 (Never)	34·0 (None) 53·0 (<1 drink/d) 11·0 (1-2 drinks/d) 3·0 (>2 drinks/d)	Time in spent in moderate and vigorous activity each week was assessed in a questionnaire, and participants were categorized based on METs.	55·0 (< high school) 18·0 (high school) 26·0 (> high school)	27·0	14·0	7·0
3C – erythrocyte phospholipid	35·4	100 (Caucasian)	5·6 (Current) 30·8 (Former) 63·6 (Never)	20·7 (None) 23·3 (<1 drink/d) 28·9 (1-2 drinks/d) 24·2(>2 drinks/d)	Assessed by questionnaire, participants were classified as those who exercised regularly vs. not.	49·1 (< high school) 21·8 (high school) 29·1 (> high school)	75·4	56·8	9·4
3C – total plasma	38·1	100 (Caucasian)	4·9 (Current) 29·9 (Former) 65·2 (Never)	20·4 (None) 21·6 (<1 drink/d) 31·4 (1-2 drinks/d) 24·3(>2 drinks/d)		49·8 (< high school) 20·0 (high school) 30·0 (> high school)	77·9	56·2	9·3
ULSAM-50	100	100 (Caucasian)	51·0 (Current) 23·0 (Former) 25·0 (Never)	n/a	Assessed by questionnaire, participants were classified according to the duration and vigorousness of their	63·0 (≤ 7yrs school) 26·0 (8-13 yrs school) 10·0 (> 13 yrs school)	3·9	15·0	0·3

					leisure time physical activity				
ULSAM-70	100	100 (Caucasian)	20·0 (Current) 49·0 (Former) 30·0 (Never)	32·0 (None) 53·0 (<1 drink/d) 14·0 (1-2 drinks/d) 1·0 (>2 drinks/d)	Assessed by questionnaire, participants were classified according to the duration and vigorousness of their leisure time physical activity	56·0 (≤ 7yrs school) 31·0 (8-13 yrs school) 13·0 (> 13 yrs school)	32·0	8·9	7·3
WHIMS	0	88·4 (Caucasian) 2·1 (Hispanic) 6·0 (Black) 1·7 (Asian) 1·7 (Other)	7·2 (Current) 38·5 (Former) 54·4 (Never)	43·8 (None) 43·4 (<1 drink/d) 8·2 (1-2 drinks/d) 4·6 (>2 drinks/d)	Self-reported information on sedentary behavior and usual physical activity obtained via questionnaire, and participants were categorized based on METs.	7·0 (< high school) 22·3 (high school) 70·7 (> high school)	28·4	16·9	16·2

[a]Cohort frequencies were used for prospective studies; frequencies for sub-cohort members only were determined for case-cohort studies (MCCS and EPIC-Potsdam).

[b]For statistical analyses, covariates were pre-specified and standardized across studies with harmonized definitions and categorization: sex (male, female), race (study-specific categories), field or clinical center if applicable (study-specific categories), education (less than high school graduate, high school graduate, some college or vocational school, college graduate; or study-specific categories if these not available), smoking (current, never, former [unless former not assessed]), physical activity (quartiles of metabolic equivalents (METs) per week; or if METs were not available, four categories of physical or leisure activity as measured in each study), alcohol intake (none, 1-6 drinks/week, 1-2 drink/day, >2 drink/day[with 1 drink defined as containing 14 grams of alcohol]; or study-specific categories if these not available,), prevalent coronary heart disease (yes or no, with study-specific definition), treated hypertension (yes or no; based on hypertension drug use; if unavailable, then diagnosed/history of hypertension per each study), and treated hypercholesterolemia (yes or no; based on lipid-lowering drug use; if unavailable, then diagnosed/history of hypercholesterolemia per each study).

AGES-Reykjavik, Age, Gene/Environment Susceptibility Study (Reykjavik); AOC: Alpha Omega Cohort; ARIC: Atherosclerosis Risk in Communities Study; CCCC, Chin-Shan Community Cardiovascular study; CHS: Cardiovascular Health Study; EPIC-Norfolk: European Prospective Investigation into Cancer (Norfolk); EPIC-Potsdam: European Prospective Investigation into Cancer (Potsdam); FHS: Framingham Heart Study; HPFS: Health Professionals Follow-up Study; IRAS: Insulin Resistance Atherosclerosis Study; KIHD: Kuopio Ischaemic Heart Disease Risk Factor Study; MCCS: Melbourne Collaborative Cohort Study; MESA: Multi-Ethnic Study of Atherosclerosis; METSIM: Metabolic Syndrome in Men Study; NHS: Nurses' Health Study; PIVUS: Prospective Investigation of the Vasculature in Uppsala Seniors; 3C Study: Three City Study; ULSAM 50 & 70: Uppsala Longitudinal Study of Adult Men; WHIMS: Women's health initiative memory study.

eTable 5. Genotype information for cohorts contributing to the single nucleotide polymorphism and fatty acid interaction analysis

Study	SNP[a]	Coded allele	Coded allele frequency	Minor allele	Minor allele frequency	Imputation quality
ARIC	rs174547	T	0·66	C	0·34	Genotyped
CHS	rs174547	T	0·68	C	0·32	1·0
EPIC-Norfolk	rs174547	T	0·66	C	0·34	1·0
EPIC-Potsdam	rs174546	C	0·67	T	0·33	Genotyped
FHS	rs174547	T	0·66	C	0·34	Genotyped
MESA	rs174547	T	0·67	C	0·33	Genotyped
METSIM	rs174550	T	0·57	C	0·43	Genotyped
PIVUS	rs174546	C	0·64	T	0·36	Genotyped
3C	rs174546	C	0·71	T	0·29	Genotyped
ULSAM50	rs174547	T	0·65	C	0·35	Genotyped
ULSAM70	rs174547	T	0·65	C	0·35	Genotyped
WHIMS	rs174547	T	0·66	C	0·34	1·0

[a]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.
ARIC: Atherosclerosis Risk in Communities Study; CHS: Cardiovascular Health Study; EPIC-Norfolk: European Prospective Investigation into Cancer (Norfolk); EPIC-Potsdam: European Prospective Investigation into Cancer (Potsdam); FHS: Framingham Heart Study; MESA: Multi-Ethnic Study of Atherosclerosis; METSIM: Metabolic Syndrome in Men Study; PIVUS: Prospective Investigation of the Vasculature in Uppsala Seniors; 3C Study: Three City Study; ULSAM 50 & 70: Uppsala Longitudinal Study of Adult Men. WHIMS: Women's health initiative memory study.

eTable 6. Relative risk of incident type 2 diabetes by omega-6 fatty acid biomarkers, according to pre-specified potential sources of heterogeneity[a,b]

	n-6 PUFA biomarkers	
Sources of heterogeneity	Linoleic acid RR (95% CI)	Arachidonic acid RR (95% CI)
Age (y)		
<60	0·59 (0·51-0·68)	0·90 (0·79-1·04)
≥60	0·69 (0·60-0·79)	1·05 (0·93-1·19)
$P_{heterogeneity}$	0·67	0·37
Sex		
Female	0·72 (0·63-0·83)	0·96 (0·83-1·10)
Male	0·60 (0·53-0·68)	0·99 (0·87-1·12)
$P_{heterogeneity}$	0·36	0·43
Race		
African American	0·73 (0·46-1·15)	1·12 (0·72-1·76)
Asian	0·89 (0·60-1·33)	1·55 (0·99-2·41)
Caucasian	0·61 (0·55-0·68)	0·95 (0·86-1·05)
Hispanic	0·77 (0·47-1·26)	0·98 (0·58-1·63)
$P_{heterogeneity}$	≥ 0·16	≥ 0·20
BMI (kg/m^2)		
<25	0·73 (0·59-0·90)	0·99 (0·80-1·21)
≥25	0·61 (0·55-0·68)	0·96 (0·86-1·06)
$P_{heterogeneity}$	0·27	0·93
n-3 PUFA		
<Median	0·63 (0·55-0·72)	0·91 (0·80-1·03)
≥Median	0·63 (0·55-0·73)	1·09 (0·95-1·25)
$P_{heterogeneity}$	0·91	0·19
Regular Aspirin use[c]		
No	0·74 (0·65-0·85)	0·92 (0·80-1·05)
Yes	0·57 (0·45-0·74)	0·81 (0·64-1·04)
$P_{heterogeneity}$	0·13	0·35

[a]Pooled relative risks (RR) of type 2 diabetes mellitus (T2D) are shown per interquintile range of linoleic acid and arachidonic acid in the specified subgroups. Interquintile range is the difference between the midpoints of the first and fifth quintiles. Association was assessed in multivariable models for each subgroup in each cohort, and results pooled using inverse-variance weighted meta-analysis. If multiple biomarkers were available within a study, one was chosen for the overall analysis with order of preference based on the biomarker that may best reflect long-term dietary intake, i.e. adipose tissue >

phospholipid > total plasma > cholesterol ester. Similarly, erythrocyte phospholipid was preferred over plasma phospholipid if both were available from a cohort.
[b]$P_{heterogeneity}$ for each potential source of heterogeneity was assessed by meta-regression including all subgroup risk estimates for the given effect modifier. For race, p-heterogeneity from meta-regression was obtained for each indicator category relative to Caucasians as the reference category.
[c]Data on aspirin use was available in 11 cohorts.

eTable 7. Interaction of omega-6 fatty acid biomarkers with polymorphism in the FADS genes and risk of incident type 2 diabetes

	Linoleic acid		Arachidonic acid	
Number of T allele	RR (95% CI)[a]	P-interaction[b]	RR (95% CI)[a]	P-interaction[b]
0	0·68 (0·52-0·89)	0·91	0·97 (0·77-1·23)	0·47
1	0·68 (0·67-0·69)		0·98 (0·96-1·00)	
2	0·72 (0·71-0·74)		1·02 (0·99-1·04)	

[a]Pooled RR ± 95% CI representing the RR change per each additional T allele of SNPs rs174547. Interaction terms for each SNP were constructed by creating a cross-product term of the omega-6 fatty acid exposure of interest (continuous) by the SNP (ordinal; 0, 1, or 2 T alleles) and added to the fully adjusted model: $S(x)=\exp(\beta \text{ n6 PUFA } + \beta \text{ SNP} + \beta \text{ n6 PUFA} \times \text{SNP} + \text{covariates})$.
[b]Interaction terms were pooled using inverse-variance weighted meta-analysis.

eTable 8. Sensitivity analysis for the association of omega-6 fatty acid biomarkers and incident type 2 diabetes

	n-6 PUFA biomarkers	
Analyses	Linoleic acid RR (95% CI)	Arachidonic acid RR (95% CI)
Main analysis	0·65 (0·60-0·72)	0·96 (0·88-1·05)
Censored at 6yr of follow-up[a]	0·71 (0·62-0·81)	0·99 (0·86-1·14)
Excluded cases ≤ 2 yr of baseline[b]	0·76 (0·70-0·83)	0·95 (0·86-1·06)

[a]Participants were censored in main (continuous) models at the first 6 years of follow-up to minimize exposure misclassification due to within-person variation over time.
[b]Cases identified in first 2 years after biomarker sampling were excluded in main (continuous) models to minimize the effect of reverse causation due to a pre-existing health condition

Appendix

Contributing Cohorts

20 cohort studies contributed participant data for the current investigation, including: the Age, Gene/Environment Susceptibility Study (Reykjavik) (AGES-Reykjavik); the Alpha Omega Cohort (AOC); the Atherosclerosis Risk in Communities Study (ARIC); the Chin-Shan Community Cardiovascular (CCCC) study; the Cardiovascular Health Study (CHS); the European Prospective Investigation into Cancer-Norfolk (EPIC-Norfolk) and Potsdam (EPIC-Potsdam) studies: the Framingham Heart Study (FHS); the Health Professionals Follow-up Study (HPFS); the Insulin Resistance Atherosclerosis Study (IRAS); the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study; the Melbourne Collaborative Cohort Study (MCCS); the Multi-Ethnic Study of Atherosclerosis (MESA); the Metabolic Syndrome in Men Study (METSIM); Nurses' Health Study (NHS); the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study; the Three City Study (3C); the Uppsala Longitudinal Study of Adult Men (ULSAM 50 and 70); and the Women's Health Initiative Memory Study (WHIMS).

Study samples, fatty acid measurement, and genotyping methods in the contributing cohorts

Age, Gene/Environment Susceptibility Study (Reykjavik)

The AGES-Reykjavik Study is a random sample of 5,764 men and women who were drawn from an established single center population based cohort; the Reykjavik Study, begun in 1967 to study heart disease. 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.[1] 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.[1]

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.[2] Phospholipids were separated from other lipids by using one-dimensional thin-layer chromatography.[3] Fatty acid methyl esters were prepared by direct transesterification,[4] and separated by using gas chromatography (Agilent Technologies 7890 Gas Chromatograph flame ionization detector detector; Supelco fusedsilica 100-m capillary column SP-2560; initially at 1608C for 16 min, ramped up at 3.08C/min to 2408C, 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 were < 2.5%.[1]

Alpha Omega Cohort

The Alpha Omega Cohort (www.alphaomegatrial.com) is a cohort of 4837 non-hospitalized patients who experienced a myocardial infarction up to 10 years before enrolment. The study includes a trial phase (Alpha Omega Trial, 3-year intervention with low doses of n-3 fatty acids, until 2009). It is now used as prospective cohort study for risk prediction in post-MI patients. [5,6] The patients were recruited in collaboration with cardiologists from 32 Dutch hospitals. At baseline (2002-2006), data were collected on diet, lifestyle, cardiovascular risk factors, medical history, and medication use. Subjects were physically examined by trained research nurses, which included anthropometry, blood pressure, heart rate, and blood sampling. Examinations were repeated after 20 months (midterm examination in n=800) and 40 months (final examination). Patients have been continuously followed for cause-specific mortality, also after the trial ended.

Baseline blood samples of 10 mL of non-fasting venous blood were drawn at the patients' home or at the hospital. For cholesteryl ester fatty acid analysis, blood was collected in EDTA containing vacutainers, packed in a sealed envelope and sent over postal mail to a central laboratory. At the laboratory, the EDTA samples were centrifuged for 10 minutes at 1200 g and plasma was stored at -80°C. 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. Within-run and between-run coefficients of variation (CV) of the control pool were calculated as measure of repeatability for palmitic acid (16:0), alpha-linolenic acid (18:3 n-3), EPA (20:5 n-3), DHA (22:6 n-3), linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6). The CVs ranged from 0.35 to 1.44 (within-run) and 1.88 to 6.71 (between-run).

The Atherosclerosis Risk in Communities Study

ARIC is a multi-center prospective investigation of atherosclerotic disease in a predominantly bi-racial population[7]. White and African American men and women aged 45-64 years at baseline were recruited from 4 communities: Forsyth County, North Carolina; Jackson, Mississippi; suburban areas of Minneapolis, Minnesota; and Washington County, Maryland. A total of 15,792 individuals participated in the baseline examination in 1987-1989, but only baseline fasting blood from the Minnesota field center were analyzed for participants with all data plus plasma fatty acids (n=3494).
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 [8]. Lipids were extracted with chloroform/methanol and separated by thin layer chromatography. Fatty acid methyl esters were prepared from the phospholipid fraction and separated by gas chromatography using an HP-5890 gas chromatograph (Hewlett- Packard, Palo Alto, CA) with a 100-m capillary Varian CP7420 column. We identified 29 fatty acids. The concentration of each fatty acid was expressed as to percentage of total fatty acids. ARIC Study samples were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, California. Autosomal SNPs were used for imputation after exclusion of SNPs with HWE deviation $p<1 \times 10^{-5}$, call rate <95%, or MAF<1%.

Chin-Shan Community Cardiovascular Study

The Chin-Shan Community Cardiovascular Cohort study begun in 1990, following 1703 men and 1899 women aged 35 years old and above, through to 1999-2000.[9] The population was homogeneous in Chinese ethnicity, and were recruited from northern Taiwan for the study of cardiovascular diseases. The cohort was assembled from permanent resident registry data of the Ministry of Interior, and the participants were recruited by house-to-house visits (response rate, 83%). The study was approved by the IRB in the National Taiwan University Hospital. Participants received baseline health examination at community health centers. Persons with a baseline history of cardiovascular disease and missing metabolic syndrome status were excluded. A total of 3602 subjects were included in the CCCC. In the survey, all of the study participants were individually interviewed from a structured questionnaire administered by trained examiners, 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. Participants with prevalent diabetes was ascertained in 1992-1993 (considered the baseline for the current analysis) and excluded.

For fatty acid measurement, a 10-mL tube of EDTA anti coagulated blood was collected in 1992-1993, 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. Inter-assay CVs were 6.5% and 4.3% for LA and AA, respectively.

The Cardiovascular Health Study

The CHS is a population-based longitudinal study of risk factors for cardiovascular disease and stroke in adults 65 years of age or older, recruited at four field centers (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Pittsburgh, PA) [10]. Overall, 5201 predominantly Caucasian individuals were recruited in 1989-1990 from random samples of Medicare eligibility lists, followed by an additional 687 African-Americans recruited in 1992-1993 (total n=5,888). The CHS genome-wide association study (GWAS), which had the primary aim of studying incident cardiovascular events, focused on 3980 CHS participants who were free of clinical cardiovascular disease at study baseline, consented to genetic testing, and had DNA available for genotyping. A total of 1,908 persons were excluded from the GWAS study sample due to the presence at study baseline of coronary heart disease, congestive heart failure, peripheral vascular disease, valvular heart disease, stroke, or transient ischemic attack. Fatty acids were measured on samples collected in the 3rd year of follow-up.

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

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

European Prospective Investigation into Cancer-Norfolk

The EPIC-Norfolk study is a population-based cohort study with 25.639 participants, aged 60-79 ye at baseline (1993-1997), who resided in and around Norwich, England. There were 892 incident diabetes cases that occurred until 31 December 2005. A set of randomly selected noncases (n = 1025) was compiled from the entire EPIC-Norfolk cohort at baseline. From this set of cases (n = 892) and noncases (n = 1025), a subsample of 397 diabetes cases and noncases were selected for whom measured plasma phospholipid fatty acid concentrations were available. After excluding 14 prevalent cases with diabetes, 383 participants became available for this analysis.[11] Nonfasting venous blood samples were collected from each participant in citrated tubes at the baseline health check. The samples were stored in a dark container overnight at 4–7 °C and then centrifuged at 2100 g for 15 min at 4 °C, transported, and subsequently stored in liquid nitrogen at 196 °C. The average time interval from blood draw to storage in freezers was 2 d. Plasma samples were stored for an average of 10 y, and erythrocyte samples were stored for 12 y before fatty acid analysis.

Fatty acid measurement for erythrocyte phospholipids

Erythrocyte-membrane phospholipid fatty acid analysis was conducted on stored baseline samples at the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) Laboratory at the National Institute for Public Health and the Environment in Bilthoven, Netherlands. Relative concentrations as % of total phospholipid fatty acids were assayed after trans-methylation with a GC-3900 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with a flame ionization detector. Quality control was assured by between-run samples, which were analyzed with every 100 samples. The CV values for 18:2n–6 and 20:4n–6 were 8.5% and 0.7%, respectively.

Fatty acid measurement for plasma phospholipids

Plasma fatty acid analysis was performed in the Nutrition and Hormones Laboratory at the International Agency for Research on Cancer (IARC) in Lyon, France. Relative concentrations as % of total phospholipid fatty acids were assayed after trans-methylation with a HP-5980 gas chromatograph (Agilent, Palo Alto, CA) equipped with a flame ionization detector. The details are reported elsewhere.[12] Quality control was conducted by using a daily in-house standard plasma sample (n = 137). The CV values for 18:2n–6 and 20:4n–6 were 0.9% and 4.0%, respectively.

Genotyping

SNP genotyping was according to Affymetrix BioBank Axiom chips, imputed using ShapeIT v2.r790, IMPUTE 2.3.1 and associate 1000G phase 3 reference panel. Criteria for quality control included sample call rate <97%, SNP call rate (95% minimum, male-specific on Y), cluster pattern (using Affymetrix SNPolisher), plate effect on minor allele frequency, and HWE p < 1e-8.

European Prospective Investigation into Cancer-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. A case-cohort study within the prospective EPIC-Potsdam study was designed. 2,500 individuals were randomly selected from all participants of the EPIC-Potsdam study population who provided blood samples (*n*=26,444) for a subcohort. The subcohort and the incident cases of T2D form the case-cohort study population for the current investigation.[13]

30mL 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.

Genotyping

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.

The Framingham Heart Study Offspring Study

The Framingham Heart Study (FHS) Offspring sample is a population based longitudinal study of families living in Framingham, Massachusetts.[14] The offspring study was initiated in 1971 and consisted of a sample of 5,124 individuals, offspring of the original cohort and their spouses. Blood samples for fatty acid measurement and covariate data were collected during wave 8 of the study (2005-2008), and participants were followed till 2015.

The fatty acid composition of erythrocyte 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.[15] 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%.

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.

Health Professional Follow-up Study

The Health Professionals Follow-up Study (HPFS) started in 1986, with 51,529 male health professionals, who were 40 – 75 years of age at recruitment in 1986. Blood samples were collected from HPFS participants in 1994. For this study we utilized previously measured fatty acid concentrations in stored blood used for nested case-control studies of incident CVD.[16] Subjects were free of CVD, cancer and diabetes at the time of blood sampling.
Blood samples were sent to the lab with an ice pack via overnight courier and the majority of the samples arrived within 24 hours. Upon arrival, samples were centrifuged and divided into aliquots for plasma, white blood cell, and red blood cells, and stored in liquid nitrogen freezers at ≤-130°C. Fatty acid concentrations were measured in stored total plasma and erythrocyte samples using gas-liquid chromatography. Concentrations of individual circulating fatty acids were expressed as a percentage of total fatty acids either in plasma or erythrocyte membranes The average intra-assay CV were 10% for erythrocyte linoleic acid, 10% for erythrocyte arachidonic acid, 7% for plasma linoleic acid, and 10% for plasma arachidonic acid.

The Insulin Resistance Atherosclerosis Study

The Insulin Resistance Atherosclerosis Study (IRAS) is a multi-ethnic, multi-center observational cohort study.[17] Participants were recruited at 4 clinical centers between October 1992 and April 1994. Ethnicity was determined by self-report. Two of the clinical centers (Los Angeles, CA, and Oakland, CA) were assigned to recruit African American and non-Hispanic white participants. In these centers, individuals were sampled from the members of a nonprofit health maintenance organization. In the other 2 clinical centers (San Luis Valley, CA, and San Antonio, TX), Hispanic and non-Hispanic white participants were recruited from ongoing population-based epidemiologic studies. All participants provided informed consent as approved by their respective field center's institutional review board. For the purpose of fatty acids, a subcohort of individuals without baseline diabetes was chosen (n = 749). Participants with missing fatty acids (n = 30) were excluded from the present investigation.

Fatty acids were measured in fasting plasma samples, utilizing a targeted, quantitative gas chromatography approach. Briefly, the lipids from plasma were extracted in the presence of authentic internal standards using chloroform:methanol (2:1 v/v), the Folch method[2]. The total lipid extract was trans-esterified in 1% sulfuric acid in methanol in a sealed vial under a nitrogen atmosphere at 100°C for 45 min. The resulting samples were neutralized with 6% potassium carbonate and the fatty acid methyl esters (FAME) re-extracted with hexane and prepared for gas chromatography. Fatty acid methyl esters were separated and quantified by capillary gas chromatography (Agilent Technologies model 6890) equipped with a 30 m DB-88MS capillary column (Agilent Technologies) and a flame-ionization detector. Quantitative results were obtained by comparing each fatty acid to its internal standard control. All fatty acid concentrations analyzed here passed internal quality assurance and quality control processes. Each fatty acid was expressed as a percentage of the total fatty acid. Inter-assay CVs were 7% for linoleic acid and 5% for arachidonic acid.

The Kuopio Ischaemic Heart Disease Risk Factor Study

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.[18] 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. The baseline examinations were followed by the 4-year examination round (1991-1993) in which 1038 men from the second cohort (88% of the eligible) participated. At the 11-year examination round (1998-2001), all men from the second cohort were invited and 854 men (95% of the eligible) participated. These examinations were also the baseline for 920 postmenopausal women (78.4% of the 1173 eligible women) from the same area, aged 53-73 years. During the 20-year examination round, all eligible men from the first and second baseline cohorts and all women were invited to the study site. A total of 1241 men (80% of the eligible) and 634 women (81.0% of the eligible) participated.

Venous blood samples were collected between 8AM and 10AM 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 fatty acids were expressed as percentages of the total fatty acids. The coefficient of variation (CV) for was 9.6% for linoleic acid and 9.2% for arachidonic acid at study baseline.

The Melbourne Collaborative Cohort Study

The MCCS is a prospective cohort study of 41,514 residents (17,045 men) of Melbourne, Australia aged between 27 and 75 years at baseline (99.3% were ages 40– 69 years). Italian and Greek migrants were deliberately recruited to extend the range of lifestyle exposures. Recruitment occurred between 1990 and 1994. Participants were recruited via the electoral rolls (registration to vote is compulsory for adults in Australia), advertisements, and community announcements in local media (e.g., television, radio, and newspapers). Comprehensive lists of Italian and Greek surnames also were used to target southern European migrants in the phone book and electoral rolls. Biomarker fatty acids were measured in plasma collected at recruitment in a sub-group of around 6900 participants based on a random cohort of around 4000 people and cases of cancer, CVD and diabetes.[19]

Samples for participants in the subcohort were selected in random order and aliquoted in batches. To each batch were added 10% plasma matrix quality control samples. The % plasma phospholipid fatty acid assay has been described in detail elsewhere Hodge et al.[20] Briefly, samples were retrieved from liquid nitrogen storage and aliquotted on ice under red light conditions before being refrozen and transported to the laboratory. Total lipids were extracted from plasma and separated by thin-layer chromatography into PPLs, triacylglycerol and cholesterol esters on silica gel plates (Silica gel 60H Merck Darmstadt Germany). Plasma phospholipid fatty acid methyl esters 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. The between batch coefficients of variation were between 1% and 12%.

The Multi-Ethnic Study of Atherosclerosis

The Multi-Ethnic Study of Atherosclerosis[21] is a National Heart, Lung and Blood Institute-sponsored, population-based investigation of subclinical cardiovascular disease and its progression. A total of 6,814 individuals, aged 45 to 84 years, were recruited from six US communities (Baltimore City and County, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN) between July 2000 and August 2002. Participants were excluded if they had physician-diagnosed cardiovascular disease prior to enrollment, including angina, myocardial infarction, heart failure, stroke or TIA, resuscitated cardiac arrest or a cardiovascular intervention (e.g., CABG, angioplasty, valve replacement, or pacemaker/defibrillator placement). Pre-specified recruitment plans identified four racial/ethnic groups (White European-American, African-American, Hispanic-American, and Chinese-American) for enrollment, with targeted oversampling of minority groups to enhance statistical power. For the present analysis, fatty acid measurement was available for a subset of 2230 participants.

Phospholipid fatty acids were extracted and measured at the University of Minnestoa (Minneapolis, MN). Plasma was diluted in saline and lipids were extracted from with a mixture of chloroform:methanol, and cholesterol, triglycerides and phospholipid subclasses were separated on a silica thin-layer chromatography plate in a solvent mixture of petroleum ether, diethyl ether, and glacial acetic acid. The band of phospholipids was harvested for the formation of methyl esters. Fatty acid methyl esters (FAMEs) were prepared with 14% boron trifluoride in methanol, incubated at 80ºC for 90 minutes, and extracted with petroleum ether. The final product was dissolved in heptane and injected onto a capillary Varian CP7420 100-m column with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a HP6890A autosampler. The GC is configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation software. Separation of individual fatty acids was obtained over an 80-minute run. Individual fatty acid values are expressed as percentage of total fatty acids. Inter-assay CVs were less than 10%.

Genotyping was done using a high-density SNP marker platform (Affymetrix 6.0). Samples with call rates below 95%, monomorphic SNPs, SNPs with observed heterozygosity > 53%, and SNPs with missing rate > 5% at genotyped markers were excluded.

The Metabolic Syndrome in Men Study

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.[22]

Fatty acid measurement for phospholipids and cholesterol esters:

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. Interassay (between runs) CVs: LA: 3.21 %; AA 2.55%

Fatty acid measurement for erythrocyte phospholipids:

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: LA: 3.21 %; AA 2.55%

Genotyping

In METSIM, genotyping was done using the HumanOmniExpress BeadChip-12v1 (Illumina). Call rate was >99%. SNP rs 174550 was directly genotyped (no imputation) and was in HWE (P>0.05).

Nurses' Health Study

The Nurses Health Study (NHS) was established in 1976 by recruiting 121,700 female nurses aged 30 to 55 who responded to a questionnaire with information related to their health, lifestyle practices and occurrence of chronic diseases. Blood samples were collected from NHS participants in 1989-1990. For this study we utilized previously measured fatty acid concentrations in stored blood used for nested case-control studies of incident CVD. Subjects were free of CVD, cancer and diabetes at the time of blood sampling.[16]
Blood samples were sent to the lab with an ice pack via overnight courier and the majority of the samples arrived within 24 hours. Upon arrival, samples were centrifuged and divided into aliquots for plasma, white blood cell, and red blood cells, and stored in liquid nitrogen freezers at ≤-130°C. Fatty acid concentrations were measured in stored total plasma and erythrocyte samples using gas-liquid chromatography. Concentrations of individual circulating fatty acids were expressed as a percentage of total fatty acids either in plasma or erythrocyte membranes. CVs were 10% for linoleic acid and arachidonic acid for erythrocyte membrane, 7% for linoleic in plasma, and 10% for arachidonic acid in plasma.

The Prospective Investigation of the Vasculature in Uppsala Seniors study

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) was initiated in 2001 as a research collaboration between the Department of Medicine and the University Hospital in Uppsala with the primary aim to evaluate the usefulness of different measurements of endothelial function and other techniques to evaluate vascular

function. In June 2004 the last subject was included in the cohort resulting in 1016 subjects aged 70 being randomly selected from the general population in the town of Uppsala. Several secondary aims have also been added to this prospective cohort study and several academic groups have been engaged in the evaluation of this cohort from different aspects. [23]

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 percent of total fatty acids analyzed in each compartment.

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$<1\times10^{-6}$; 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]).

The Three City 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).[24] 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. Four follow-up examinations were performed at 2, 4, 7, and 10 years after baseline. The present study is based on the 12 years of follow-up. Fatty acid composition of red blood cell membrane phospholipids were measured at baseline from fasting blood samples among 670 individuals from the Bordeaux and Montpellier centers.Among them, 78 participants with prevalent diabetes and 18 participants with missing data for diabetes at baseline were excluded leaving574 participants free of diabetes for the analysis sample. This sample is constituted of 342 (59.9%) participants from Bordeaux and 232 (40.4%) participants from Montpelier. In Bordeaux, total plasma fatty acids were also measured at baseline in 1,416 participants. Among them, 145 participants with prevalent diabetes and 51 participants with missing data for diabetes at baseline were excluded leaving 1220 participants free of diabetes for the analysis sample.

Fatty acid measurement for erythrocyte phospholipids

Erythrocyte membrane phospholipid fatty acids were measured at the French Institute for fats and oils (ITERG).Total lipids from red blood cell membranes were extracted by using the method of Peuchant et al.[25] A one dimensional thin-layer chromatography was used to separate total phospholipids of red blood cells from neutral lipids. Total fatty acids of the red blood cell phospholipid fraction were methylated according to the procedure of Morrison and Smith,[26] to obtain fatty acid methyl esters. Individual fatty acid methyl esters were separated using a gas chromatograph (Focus GC, Thermo Scientific, France) equipped with a flam ionization detector and a split injector. A fused silica capillary column (BPX 70, 60m x 0.25mm internal diameter, 0.25mm film; Phenomenex, Germany) was used with H2 as the carrier gas (inlet pressure: 1 bar). The column temperature was programmed to increase from 150 to 200°C at 1.5°C/min for 25 min, and then from 200 to 225°C at 20°C/min and was held at 225°C until the completion of the analysis (20 min). The injection port and detector were maintained at 250 and 280°C, respectively. Data were integrated using the ChromQuest Software (Thermo Scientific). Individual fatty acid methyl esters were identified by comparing their retention times with those of authentic standards eluted in the same conditions (Sigma Chemical Co., Saint Quentin Fallavier, France). The results are expressed as a weight percentage of total fatty acids. The laboratory CV's were The laboratory CV's were 0.5% for linoleic acid and 0.8% for arachidonic acid.

Fatty acid measurement for total plasma

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, Cergy-Pontoise, 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 Chemical Co, Lyon, France), and peak areas were measured with an automatic integrator (DP700; Fisons Instruments, Arcueil, France). The results for each fatty acid were expressed as a percentage of total fatty acids. The laboratory CV's were 0.66% for linoleic acid and 0.02% for arachidonic acid.

The Uppsala Longitudinal Study of Adult Men-50 and 70

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.[23,27] 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.

Fatty acid measurement for cholesterol esters (ULSAM-50)

For analysis of the fatty acid composition of the serum cholesterol esters, serum was extracted with a hexane-isopropanol solution (1+4).[28] Cholesterol esters were separated from the extract by thin layer chromatography before inter-esterification (acidic methanol at 85°C, 2 h), and free cholesterol liberated in the reaction was removed by an

aluminium oxide column to avoid contamination of the gas liquid chromatography column. The percentage composition of methylated fatty acids 14:0 to 22:6 was determined by gas chromatography (a 25 m NB-351 silica capillary column, i.d. 0.32 mm, phase layer 0.20 mm) with use of a flame ionisation detector and with helium as carrier gas. Every 25th sample was a serum control pool. Intraassay CVs were 0.2-5% depending on the fatty acid; and Interassay CVs were 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.

Fatty acid measurement for adipose tissue (ULSAM-70)

Initially the adipose tissue fatty acid composition was analysed in a random subsample of 318 men, whereas the fatty acid composition in serum cholesterol esters was analysed in 611 men. In December 2008, 535 new samples were 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. Subcutaneous adipose tissue was collected as described.[29] Prior to the fatty acid analysis the biopsy was weighed and homogenised. The fatty acid compositions of the serum lipids and subcutaneous adipose tissue were analysed as described in detail by Carlson (1963)[30] and Boberg et al. (1985).[31] An extraction with chloroform in the presence of methanol, butylated hydroxytoluene, and NaH2PO4 was conducted over night, and evaporated under nitrogen. The dry extracts were dissolved in a few drops of chloroform and applicated on thin liquid chromatography plates for separation of the lipids in a solvent system consisting of petroleum ether:diethyl ether:acetic acid (81:18:1, by volume).[31] The lipid fractions were visualised in UV light and scraped off separately. The lipid esters were trans methylated in warm, acidic environment overnight. The methylesters were extracted with petroleum ether and deionised water, and the solvent was evaporated under nitrogen. The fatty acid methyl esters were dissolved in hexane and separated by gas-liquid chromatography (GLC). The Hewlett Packard GLC system used for the analyses was consisted a GC 5890, automatic sampler 7671A, integrator 3392A, and 25 m Quadrex Fused Silica capillary column OV-351, with helium as the carrying gas. The temperature program used during the separation of the fatty acid methyl esters was 130-220°C. The fatty acids were identified by comparison of the retention times of separation was controlled by Nu Check Prep GLC reference standard GLC-68A. Interassay CV was 0.79% (LA), and 3.87% (AA).

Genotyping method

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%.

Women's Health Initiative Memory Study

The Women's Health Initiative Memory Study examined the effects of postmenopausal hormone therapy on cognitive function in women aged 65-80 years.[32] Recruitment began in 1995. Diabetes status was last assessed in August 2009. 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.[33] All fatty acids present at >1% abundance had CVs of ≤6.5%. Genotyping was conducted using the Human Omni Express Exome-8v1_B, with imputation using the 1000 genomes reference panel.

Conflict of interest

Drs. Wu and Micha report research support from Unilever for this work. Dr Del Gobbo reported receiving ad hoc consulting fees from the Life Sciences Research Organization. Dr Geleijnse received funding from Unilever R&D, Vlaardingen, The Netherlands, for epidemiological studies of dietary and circulating fatty acids and cardiometabolic disease. Dr Helmer reported receiving fees for a conference from Novartis. Dr. Mozaffarian reports ad hoc honoraria or consulting from Life Sciences Research Organization, Astra Zeneca, Boston Heart Diagnostics, GOED, DSM, Nutrition Impact, Haas Avocado Board, and Pollock Communications; and chapter royalties from UpToDate. No other disclosures were reported.

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Appendix Table 1 Cohort sources of support

Cohort	Funding and acknowledgement
Age, Gene/Environment Susceptibility Study	Office of Dietary Supplements, NIH contract N01-AG012100, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament).
Alpha Omega Cohort study	The Alpha Omega Trial was supported by the Netherlands Heart Foundation (grant 2000T401), the NIH (NIH/NHLBI and ODS, grant R01HL- 076200), and Unilever R&D, Vlaardingen (margarine production and distribution).
Atherosclerosis Risk in Communities Study	The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.
Chin-Shan Community Cardiovascular study	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).
Cardiovascular Health Study	The Cardiovascular Health Study was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, and N01HC85086 and grant U01HL080295 from the National Heart, Lung, and Blood Institute, with additional contribution from the National Institute of Neurological Disorders and Stroke. Additional support was provided by grant R01AG023629 from the National Institute on Aging. A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.
EPIC-Norfolk	The EPIC-Norfolk study is supported by programme grants from the Medical Research Council UK and Cancer Research UK. NJW, NGF, and FI are supported by 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).
Framingham Heart Study	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.

Health Professionals Follow-up Study	The Health Professionals Follow-up Study was supported by research grants UM1 CA167552, R01 HL35464, AA11181, HL35464, CA55075, HL60712, and P30 DK46200 from the National Institutes of Health.
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Kuopio Ischaemic Heart Disease Risk Factor Study	The Kuopio Ischaemic Heart Disease Risk Factor Study was supported mainly by grants 41471 and 1041086 from the Academy of Finland, Helsinki, Finland.
Melbourne Collaborative Cohort Study	The Melbourne Collaborative Cohort Study recruitment was funded by VicHealth and Cancer Council Victoria and was further supported by grants 209057, 251553, 126403, 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.
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Metabolic Syndrome in Men Study	The METSIM Study was funded by the grants from The European Union, the Academy of Finland, and the Juselius Foundation.
Nurses' Health Study	The Nurses Health Study was supported by research grants UM1 CA186107, R01 CA49449, R01 HL034594, P01CA87969, R01HL034594, and R01HL088521 from the National Institutes of Health.
Prospective Investigation of the Vasculature in Uppsala Seniors	The study was supported by Uppsala University Hospital and the Swedish Research Council for Health, Working Life and Welfare
Three City Study	Conducted under a partnership agreement between the Institut National de la Sante et de la Recherche Medicale, the University Bordeaux 2 Victor Segalen, and Sanofi. The Fondation pour la Recherche Medicale funded the preparation and initiation of the study. The Three-City study was also supported by the Caisse Nationale Maladie des Travailleurs Salaries, Direction Generale de la Sante, MGEN, Institut de la Longevite, Conseils Regionaux d′Aquitaine et Bourgogne, Fondation de France,Ministry of Research–Institut National de la Sante et de la Recherche Medicale Programme Cohortes et collections de donnees 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 Solidarite et l′Autonomie. Dr Samieri was sponsored by a grant from the Fondation Plan Alzheimer.
Uppsala Longitudinal Study of Adult Men	The Uppsala Longitudinal Studies of Adult Men 50 and 70 were funded by the Swedish Research Council for Health, Working Life andWelfare, Uppsala City Council, and Swedish Research Council.
Women's health initiative memory study	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.

References

1. Harris TB, Song X, Reinders I, et al. Plasma phospholipid fatty acids and fish-oil consumption in relation to osteoporotic fracture risk in older adults: the Age, Gene/Environment Susceptibility Study. *The American journal of clinical nutrition* 2015; **101**(5): 947-55.
2. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of biological chemistry* 1957; **226**(1): 497-509.
3. Schlierf G, Wood P. QUANTITATIVE DETERMINATION OF PLASMA FREE FATTY ACIDS AND TRIGLYCERIDES BY THIN-LAYER CHROMATOGRAPHY. *Journal of lipid research* 1965; **6**: 317-9.
4. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *Journal of lipid research* 1986; **27**(1): 114-20.
5. Geleijnse JM, Giltay EJ, Schouten EG, et al. Effect of low doses of n-3 fatty acids on cardiovascular diseases in 4,837 post-myocardial infarction patients: design and baseline characteristics of the Alpha Omega Trial. *American heart journal* 2010; **159**(4): 539-46 e2.
6. Kromhout D, Giltay EJ, Geleijnse JM. n-3 fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med* 2010; **363**(21): 2015-26.
7. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am J Epidemiol* 1989; **129**(4): 687-702.
8. Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin Chem* 2006; **52**(12): 2265-72.
9. Chien KL, Lin HJ, Hsu HC, et al. Comparison of predictive performance of various fatty acids for the risk of cardiovascular disease events and all-cause deaths in a community-based cohort. *Atherosclerosis* 2013; **230**(1): 140-7.
10. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol* 1991; **1**(3): 263-76.
11. Patel PS, Sharp SJ, Jansen E, et al. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. *The American journal of clinical nutrition* 2010; **92**(5): 1214-22.
12. Saadatian-Elahi M, Slimani N, Chajes V, et al. Plasma phospholipid fatty acid profiles and their association with food intakes: results from a cross-sectional study within the European Prospective Investigation into Cancer and Nutrition. *The American journal of clinical nutrition* 2009; **89**(1): 331-46.
13. Kroger J, Zietemann V, Enzenbach C, et al. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *The American journal of clinical nutrition* 2011; **93**(1): 127-42.
14. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol* 1979; **110**(3): 281-90.
15. Harris WS, Pottala JV, Vasan RS, Larson MG, Robins SJ. Changes in erythrocyte membrane trans and marine fatty acids between 1999 and 2006 in older Americans. *The Journal of nutrition* 2012; **142**(7): 1297-303.
16. Malik VS, Chiuve SE, Campos H, et al. Circulating Very-Long-Chain Saturated Fatty Acids and Incident Coronary Heart Disease in US Men and Women. *Circulation* 2015; **132**(4): 260-8.
17. Santaren ID, Watkins SM, Liese AD, et al. Serum pentadecanoic acid (15:0), a short-term marker of dairy food intake, is inversely associated with incident type 2 diabetes and its underlying disorders. *The American journal of clinical nutrition* 2014; **100**(6): 1532-40.

18. Yary T, Voutilainen S, Tuomainen TP, Ruusunen A, Nurmi T, Virtanen JK. Serum n-6 polyunsaturated fatty acids, Delta5- and Delta6-desaturase activities, and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *The American journal of clinical nutrition* 2016; **103**(5): 1337-43.
19. Hodge AM, English DR, O'Dea K, et al. Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. *The American journal of clinical nutrition* 2007; **86**(1): 189-97.
20. Hodge AM, Simpson JA, Gibson RA, et al. Plasma phospholipid fatty acid composition as a biomarker of habitual dietary fat intake in an ethnically diverse cohort. *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2007; **17**(6): 415-26.
21. Bild DE, Bluemke DA, Burke GL, et al. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol* 2002; **156**(9): 871-81.
22. Lankinen MA, Stancakova A, Uusitupa M, et al. Plasma fatty acids as predictors of glycaemia and type 2 diabetes. *Diabetologia* 2015; **58**(11): 2533-44.
23. Hagstrom E, Kilander L, Nylander R, et al. Plasma parathyroid hormone is associated with vascular dementia and cerebral hyperintensities in two community-based cohorts. *The Journal of clinical endocrinology and metabolism* 2014; **99**(11): 4181-9.
24. Samieri C, Maillard P, Crivello F, et al. Plasma long-chain omega-3 fatty acids and atrophy of the medial temporal lobe. *Neurology* 2012; **79**(7): 642-50.
25. Peuchant E, Wolff R, Salles C, Jensen R. One-step extraction of human erythrocyte lipids allowing rapid determination of fatty acid composition. *Analytical biochemistry* 1989; **181**(2): 341-4.
26. Morrison WR, Smith LM. PREPARATION OF FATTY ACID METHYL ESTERS AND DIMETHYLACETALS FROM LIPIDS WITH BORON FLUORIDE--METHANOL. *Journal of lipid research* 1964; **5**: 600-8.
27. Fall T, Salihovic S, Brandmaier S, et al. Non-targeted metabolomics combined with genetic analyses identifies bile acid synthesis and phospholipid metabolism as being associated with incident type 2 diabetes. *Diabetologia* 2016.
28. Hara A, Radin NS. Lipid extraction of tissues with a low-toxicity solvent. *Analytical biochemistry* 1978; **90**(1): 420-6.
29. Beynen AC, Katan MB. Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition. *The American journal of clinical nutrition* 1985; **42**(2): 317-22.
30. Carlson LA. DETERMINATION OF SERUM TRIGLYCERIDES. *Journal of atherosclerosis research* 1963; **3**: 334-6.
31. Boberg J. Separation of labeled plasma and tissue lipids by thin-layer chromatography. A quantitative methodological study. *Clinica chimica acta; international journal of clinical chemistry* 1966; **14**(3): 325-34.
32. Espeland MA, Rapp SR, Shumaker SA, et al. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. *Jama* 2004; **291**(24): 2959-68.
33. Pottala JV, Espeland MA, Polreis J, Robinson J, Harris WS. Correcting the effects of -20 degrees C storage and aliquot size on erythrocyte fatty acid content in the Women's Health Initiative. *Lipids* 2012; **47**(9): 835-46.