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

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Summary

Background **The metabolic effects of omega-6 polyunsaturated fatty acids (PUFAs) remain contentious, and little evidence is available regarding their potential role in primary prevention of type 2 diabetes. We aimed to assess the associations of linoleic acid and arachidonic acid biomarkers with incident type 2 diabetes.**

Methods **We did a pooled analysis of new, harmonised, individual-level analyses for the biomarkers linoleic acid and its metabolite arachidonic acid and incident type 2 diabetes. We analysed data from 20 prospective cohort studies from ten countries (Iceland, the Netherlands, the USA, Taiwan, the UK, Germany, Finland, Australia, Sweden, and France), with biomarkers sampled between 1970 and 2010. Participants included in the analyses were aged 18 years or older and had data available for linoleic acid and arachidonic acid biomarkers at baseline. We excluded participants with type 2 diabetes at baseline. The main outcome was the association between omega-6 PUFA biomarkers and incident type 2 diabetes. We assessed the relative risk of type 2 diabetes prospectively for each cohort and lipid compartment separately using a prespecified analytic plan for exposures, covariates, effect modifiers, and analysis, and the findings were then pooled using inverse-variance weighted meta-analysis.**

Findings **Participants were 39 740 adults, aged (range of cohort means) 49–76 years with a BMI (range of cohort means) of 23·3–28·4 kg/m², who did not have type 2 diabetes at baseline. During a follow-up of 366 073 person-years, we identified 4347 cases of incident type 2 diabetes. In multivariable-adjusted pooled analyses, higher proportions of linoleic acid biomarkers as percentages of total fatty acid were associated with a lower risk of type 2 diabetes overall (risk ratio [RR] per interquintile range 0·65, 95% CI 0·60–0·72, p<0·0001; I^2=53·9%, $p_{heterogeneity}$=0·002). The associations between linoleic acid biomarkers and type 2 diabetes were generally similar in different lipid compartments, including phospholipids, plasma, cholesterol esters, and adipose tissue. Levels of arachidonic acid biomarker were not significantly associated with type 2 diabetes risk overall (RR per interquintile range 0·96, 95% CI 0·88–1·05; p=0·38; I^2=63·0%, $p_{heterogeneity}$<0·0001). The associations between linoleic acid and arachidonic acid biomarkers and the risk of type 2 diabetes were not significantly modified by any prespecified potential sources of heterogeneity (ie, age, BMI, sex, race, aspirin use, omega-3 PUFA levels, or variants of the *FADS* gene; all $p_{heterogeneity}$≥0·13).**

Interpretation **Findings suggest that linoleic acid has long-term benefits for the prevention of type 2 diabetes and that arachidonic acid is not harmful.**

Funding **Funders are shown in the appendix.**

Introduction

The influence of omega-6 polyunsaturated fatty acids (PUFAs), in particular linoleic acid—the predominant omega-6 PUFA—on health remains disputed.[1,2] Most major guidelines, including those from the American Heart Association and Dietary Guidelines for Americans,[3,4] recommend that 5–10% of energy is obtained from linoleic acid, which is primarily derived from vegetable oils. However, some researchers have hypothesised that linoleic acid might be harmful because it competes with omega-3 PUFA or because its metabolite arachidonic acid might have harmful effects.[5,6] In response to such concerns, French national guidelines[7] have recommended limiting linoleic acid consumption to no more than 4% of energy.

Although many studies[4,8] have investigated the cardiovascular effects of omega-6 PUFAs, less is known about their influence on other major outcomes, such as

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Research in context

Evidence before this study

We searched MEDLINE from inception to Feb 10, 2016, using the search terms "omega-6", "linoleic acid", "arachidonic acid", "diabetes mellitus", "cohort studies", "prospective studies", and "nested case control studies", for articles published in English, manually searched reference lists of previous original publications and systematic reviews, and contacted experts to identify prospective observational studies that assessed the association between linoleic acid (the main dietary omega-6 polyunsaturated fat) and its downstream metabolite, arachidonic acid, and the risk of incident type 2 diabetes. We identified few previous studies that had investigated the association between linoleic acid and arachidonic acid biomarkers and type 2 diabetes; most relied on estimated levels of consumption from self-reported questionnaires, for which evidence has been considered weak. Although biomarkers of linoleic acid and arachidonic acid offer objective assessment of exposure that is free of recall bias, only a handful of prospective studies have evaluated associations between linoleic acid or arachidonic acid biomarkers and type 2 diabetes, with potential limitations of publication bias, and inadequate power to evaluate interactions by population characteristics.

Added value of this study

We did a new, harmonised analysis of individual-level data from 20 prospective cohort studies to assess the association between levels of linoleic acid and arachidonic acid biomarkers and the risk of incident type 2 diabetes. Data from 366 703 person-years of follow-up of more than 39 000 adults without type 2 diabetes at baseline showed a linear inverse association between levels of the biomarker linoleic acid and the incidence of type 2 diabetes, with similar findings across different lipid compartments. Conversely, overall levels of the biomarker arachidonic acid were not significantly associated with type 2 diabetes. To the best of our knowledge, this is the largest and most detailed assessment of objective biomarkers of omega-6 polyunsaturated fatty acids and the incidence of type 2 diabetes. The breadth and scope of the cohorts allowed further assessment of heterogeneity. Despite the diversity of the 20 contributing cohorts, evidence did not indicate that the associations differed by age, BMI, sex, race, omega-3 polyunsaturated fatty acid levels, aspirin use, or variation in the genes encoding fatty acid desaturase.

Implications of all the available evidence

The prevalence of type 2 diabetes is escalating rapidly around the world, so identification of dietary and other modifiable risk factors for the prevention of the disease is of clinical, scientific, and public health importance. Several dietary guidelines recommend increased linoleic acid consumption to improve blood cholesterol levels and reduce cardiovascular risk. Our analysis provides novel findings that, when combined with in-vitro experimental and shorter-term interventions for metabolic risk factors, suggest that linoleic acid has an additional role for prevention of type 2 diabetes in healthy populations. Additionally, our findings do not corroborate concerns about potential harmful effects of arachidonic acid. Consistent with these findings, a recent systematic review found that levels of the biomarker arachidonic acid were associated with lower incidence of coronary heart disease.

type 2 diabetes. A meta-analysis[9] of randomised controlled feeding trials indicated that total PUFA consumption (predominantly linoleic acid) improves both glycaemia and insulin resistance. However, whether such short-term benefits translate to primary prevention of type 2 diabetes remains unclear. Most longitudinal studies[10] of linoleic acid and incident type 2 diabetes have relied on self-reported dietary estimates of intake that might be affected by errors or bias in recall. Linoleic acid cannot be synthesised by human beings, and thus biomarker measurements of linoleic acid can provide objective assessments that are free of memory errors, recall bias, or inaccuracies of food databases.[11] Biomarker measurements are also crucial for studying the effects of arachidonic acid, for which levels are tightly regulated and less correlated with dietary intake.[12] However, only a handful of prospective studies[10] have evaluated associations between linoleic acid or arachidonic acid biomarkers and type 2 diabetes, resulting in potential limitations of publication bias and inadequate power to assess interactions by demographic, medical, or genetic characteristics. Thus, the potential effects of omega-6 PUFAs, including linoleic acid and its metabolite arachidonic acid, on type 2 diabetes remain unresolved and are of considerable clinical, scientific, and public health importance. To address these questions, we did a pooled analysis of new, harmonised, individual-level data within the Fatty Acids and Outcomes Research Consortium.[13] Our primary aim was to assess the associations of linoleic acid and arachidonic acid biomarkers with incident type 2 diabetes, with additional aims to assess factors that might modify these associations. We hypothesised that the level of linoleic acid biomarkers, but not arachidonic acid biomarkers, would be inversely associated with type 2 diabetes risk.

Methods

Study population

In this pooled analysis, we identified prospective cohort studies that had assessed circulating or tissue biomarkers of linoleic acid and arachidonic acid, and incidence of type 2 diabetes. Studies were identified by

contacting experts, manual searches of reference lists of previous original publications and systematic reviews, and online searches of MEDLINE from inception to Feb 10, 2016, using the search terms "omega-6", "linoleic acid", "arachidonic acid", "diabetes mellitus", "cohort studies", "prospective studies", and "nested case control studies".

Participants included in the analysis were aged 18 years or older, with available data for linoleic acid and arachidonic acid biomarkers at baseline. Participants with type 2 diabetes at baseline were excluded. Each cohort receieved institutional review board approval from their respective institutions and written consent was obtained from all participants.

Uniform analysis protocol

A standardised analysis protocol was developed and provided to researchers for each participating cohort. To reduce heterogeneity, the analysis plan included harmonised specifications for population inclusion, exposures, covariates, effect modifiers, outcomes, and analysis, and specifications for methods for pooling results. Individual scientists analysed individual-level data from each cohort and provided the results using prespecified standardised electronic forms, which were sent to JHYW for pooling.

Procedures

Fatty acid levels were assessed in each study in various lipid compartments and expressed as the proportion of total fatty acids (appendix).

Incident type 2 diabetes was defined by whichever of the following criteria were met first: a fasting glucose concentration of 126 mg/dL (7·0 mmol/L) or higher, a glucose concentration of 200 mg/dL (11·1 mmol/L) or higher as measured by a 2 h post-oral glucose tolerance test, new use of insulin or oral hypoglycaemic medication, fasting or non-fasting HBA_{1c} concentrations of 6·5% or more, or by self-reported physician diagnosis in some cohorts (appendix).

On the basis of biological interest and well established associations with type 2 diabetes risk, prespecified covariates were age, sex, race, site of patient recruitment if applicable, BMI, education, smoking, physical activity, alcohol intake, prevalent coronary heart disease, treatment for hypertension, treatment for hypercholesterolaemia, and biomarker omega-3 PUFA concentrations (appendix). Participants with missing categorical covariates were included via missing indicator categories.

To minimise concerns about multiple testing, we prespecified all potential sources of heterogeneity on the basis of demographic, anthropometric, or biological importance. Cohort-specific analyses were stratified by age, sex, race, BMI, long-chain omega-3 PUFA biomarker concentrations, aspirin use (which might promote formation of arachidonic acid-derived resolvers of inflammation), and common genetic variations in the fatty acid desaturase (FADS) genes (ie, *FADS1* [single nucleotide polymorphism rs174547]), which most strongly associates with omega-6 PUFA levels (appendix).[14]

Cohort analyses

For prospective cohorts with time-to-event data, Cox proportional hazards were used to obtain the hazard ratio (HR) and SE. For studies with a case-cohort design, weighted Cox models were used.[15] Participants were followed up from time of fatty acid measurement to time of diagnosis of type 2 diabetes, death, or censoring at the end of follow-up. For a prospective case-cohort[16] and prospective case-control study[17] without time-to-event data, logistic regression (weighted for case-cohort studies) was used to obtain the odds ratio (OR) and SE for incident type 2 diabetes. All analyses used robust SEs.

To reduce likelihood of reverse causation as a result of prevalent subclinical disease, sensitivity analyses were done in each cohort, excluding cases diagnosed in the first 2 years of follow-up. To minimise exposure misclassification due to changes in fatty acid levels over time, we also did a sensitivity analysis for each cohort, censoring participants after the initial 6 years of follow-up.

Data pooling and meta-analysis

We used HRs and ORs to approximate relative risks (RRs) and pooled the data to generate summary results using inverse-variance weighted meta-analysis. We also used random effects models in sensitivity analyses.[18] Because fatty acids were measured in different lipid compartments (phospholipids, plasma, cholesterol esters, and adipose tissue) using differing methods, linoleic acid and arachidonic acid were evaluated continuously per study-specific interquintile range (the distance between the midpoint of the first and fifth quintiles) to facilitate pooling. We pooled results separately for each lipid compartment and across all studies. For studies with multiple measures, we prioritised the overall pooled analysis on the basis of the biomarkers that would best reflect long-term intake, as specified in the following ordered list: adipose tissue, erythrocyte phospholipids, plasma phospholipids, total plasma or serum, and cholesterol esters.[12]

We assessed potential non-linear relationships by pooling the HR or OR for each study-specific quintile, established as an indicator variable against the lowest quintile as the reference; and in each compartment by multivariate inverse-variance weighted meta-regression, modelling the fatty acid quintile results using restricted cubic splines.[19,20] Because findings across compartments could not be pooled using restricted cubic splines, these analyses were considered exploratory. Heterogeneity was assessed using the I^2 statistic. Statistical significance of differences between prespecified subgroups was assessed using inverse-variance weighted meta-regression. We used STATA (version 13.1) with a two-sided α level of 0·05 for all meta-analyses.

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See **Online** for appendix

	Country	Baseline year(s) of blood sampling	Study design	Number of participants (n)	Number of men (%)	Age (years)	BMI (kg/m²)	Biomarker compartment assessed	Incident type 2 diabetes cases (n)	Maximum follow-up (years)
AGES-Reykjavik	Iceland	2002–06	PC	753	309 (41%)	76 (5·2)	27 (4)	Plasma phospholipids	28	7·8
AOC	Netherlands	2002–06	PC	2888	2282 (79%)	69 (5·6)	27 (4)	Cholesterol esters	154	4·8
ARIC	USA	1987–89	PC	3494	1642 (47%)	54 (5·6)	27 (4)	Plasma phospholipids	304	9·0
CCCC	Taiwan	1992–93	PC	616	370 (60%)	59 (9·9)	23 (3)	Total plasma	128	8·1
CHS	USA	1992–93	PC	3179	1240 (39%)	72 (5·2)	26 (5)	Plasma phospholipids	284	18·0
EPIC-Norfolk	UK	1993–97	PCC	383	203 (53%)	64 (8·1)	28 (4)	Erythrocyte phospholipids, plasma phospholipids	199	12·1
EPIC-Potsdam	Germany	1994–98	PNC	2165	823 (38%)	49 (8·9)	26 (4)	Erythrocyte phospholipids	488	10·1
FHS	USA	2005–08	PC	1913	823 (43%)	64 (8·3)	28 (5)	Erythrocyte phospholipids	98	9·0
HPFS	USA	1994	PC	1545	1545 (100%)	65 (8·6)	26 (3)	Erythrocyte phospholipids, Total plasma	113	17·6
IRAS	USA	1992–94	PC	719	316 (44%)	55 (8·5)	28 (6)	Total plasma	146	5·0
KIHD	Finland	1991–92 (men) 2003 (women)	PC	3145	2327 (74%)	56 (7·1)	27 (4)	Serum	595	26·8
MCCS	Australia	1990–94	PNC	4046	1821 (45%)	55 (8·6)	27 (5)	Plasma phospholipids	336	9·9
MESA	USA	2000–02	PC	2230	1026 (46%)	61 (10·1)	28 (5)	Plasma phospholipids	297	11·2
METSIM	Finland	2006–10	PC	1301	1301 (100%)	55 (5·6)	26 (3)	Cholesterol esters, erythrocyte phospholipids, plasma phospholipids	71	7·9
NHS	USA	1990	PC	1595	0	60 (6·4)	25 (4)	Erythrocyte phospholipids, total plasma	154	22·8
PIVUS	Sweden	2001–04	PC	861	422 (49%)	70 (0·2)	27 (4)	Cholesterol esters, plasma phospholipids	69	10·9
3C	France	1999–00	PC	1220	464 (38%)	74 (4·8)	26 (4)	Erythrocyte phospholipids, total plasma	36	13·0
ULSAM-50	Sweden	1970–73	PC	1891	1891 (100%)	50 (0·6)	25 (3)	Cholesterol esters	246	42·3
ULSAM-70	Sweden	1991–95	PC	738	738 (100%)	71 (0·6)	26 (3)	Adipose tissue	99	21·5
WHIMS	USA	1996	PC	5799	0	70 (3·8)	28 (5)	Erythrocyte phospholipids	502	14·1

Data are n, n (%), or mean (SD), unless otherwise specified. Characteristics were correct at the time of fatty acid biomarker measurement. References for all studies are shown in the appendix. AGES-Reykjavik=Age, Gene/Environment Susceptibility Study (Reykjavik). AOC=Alpha Omega Cohort. ARIC=Atherosclerosis Risk in Communities. CCCC=Chin-Shan Community Cardiovascular Cohort 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=Three City Study. ULSAM-50=Uppsala Longitudinal Study of Adult Men-50. ULSAM-70=Uppsala Longitudinal Study of Adult Men-70. WHIMS=Women's Health Initiative Memory Study. PC=prospective cohort. PCC=prospective nested case-control. PNC=prospective nested case-cohort.

***Table 1*: Baseline cohort characteristics from 20 studies with linoleic acid and arachidonic acid biomarker measures and follow-up data for incident type 2 diabetes**

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, writing of the report, or the decision to submit for publication. The corresponding author had full access to all the data. All authors had final responsibility for the decision to submit for publication.

Results

20 (77%) of the 26 studies identified agreed to participate by February, 2016. Overall, we included 39 740 adults from 20 cohorts in ten countries (USA, Iceland, the Netherlands, Germany, Finland, the UK, Sweden, France, Australia, and Taiwan) in the analyses. Participants with missing continuous covariates were excluded (maximum exclusion for an individual covariate was 3·3%). Our analyses included 17 prospective cohort studies, two prospective case-cohort studies, and one nested case-control cohort study. Table 1 shows the baseline characteristics of the studies and the participants. The ranges of the mean cohort ages (49–76 years) and BMI (23·3–28·4 kg/m²) were wide. Within cohorts, even wider age ranges and BMI ranges were represented (appendix). Most participants were of European descent, although several cohorts included greater than 10% of individuals of African (Insulin Resistance Atherosclerosis Study [IRAS; 24·5%], Multi-Ethnic Study of Atherosclerosis [MESA; 23·9%], Cardiovascular Health Study [11·1%]), Asian (Chin-Shan Community Cardiovascular Cohort Study [100%], MESA [25·6%]), or Hispanic (IRAS [33·2%], MESA [22·2%]) descent (appendix).

Fatty acid biomarkers were measured in phospholipids (n=14 cohorts), total plasma or serum (n=6), cholesterol esters (n=4), and adipose tissue (n=1); and in six cohorts, measurements were done in more than two lipid compartments. With the exception of the Uppsala Longitudinal Study of Adult Men-50 (1970–73) cohort,

	Studies (n)†	Cases (n)†	Continuous analysis‡			Quintile 5 *vs* quintile 1		
			I^2 (%)	Relative risk fixed effect	Relative risk random effect	I^2 (%)	Relative risk fixed effect	Relative risk random effect
Linoleic acid								
Phospholipids	14	2979	58·4%	0·69 (0·61–0·77)	0·64 (0·53–0·78)	56·3%	0·60 (0·52–0·70)	0·57 (0·45–0·72)
Total plasma or serum	6	1220	40·7%	0·55 (0·47–0·64)	0·55 (0·44–0·69)	1·0%	0·47 (0·38–0·59)	0·47 (0·38–0·59)
Cholesterol esters	4	624	0%	0·58 (0·46–0·73)	0·58 (0·46–0·73)	0%	0·54 (0·41–0·73)	0·54 (0·41–0·73)
Adipose tissue	1	99	..	0·82 (0·49–1·35)	0·82 (0·49–1·35)	..	0·76 (0·38–1·53)	0·76 (0·38–1·53)
Overall	20	4347	53·9%	0·65 (0·60–0·72)	0·64 (0·56–0·74)	46·3%	0·57 (0·51–0·64)	0·57 (0·48–0·67)
Arachidonic acid								
Phospholipids	14	2979	64·2%	0·99 (0·89–1·10)	1·01 (0·84–1·22)	54·6%	0·99 (0·86–1·14)	0·97 (0·78–1·22)
Total plasma or serum	6	1220	63·8%	0·73 (0·62–0·86)	0·74 (0·54–1·03)	66·5%	0·64 (0·52–0·79)	0·65 (0·43–0·99)
Cholesterol esters	4	624	12·3%	1·12 (0·90–1·40)	1·14 (0·90–1·46)	0%	1·22 (0·94–1·59)	1·22 (0·94–1·59)
Adipose tissue	1	99	..	1·56 (0·84–2·89)	1·56 (0·84–2·89)	..	1·67 (0·72–3·91)	1·67 (0·72–3·91)
Overall	20	4347	63·0%	0·96 (0·88–1·05)	1·01 (0·87–1·18)	61·2%	0·93 (0·83–1·04)	0·96 (0·79–1·17)

Data are relative risk (95% CI). *Effect estimates were pooled using inverse-variance weighted or random effects meta-analysis. †Multiple biomarkers were available in some studies, but only one biomarker per study was included for estimation of overall relative risks, therefore the overall number of studies and cases does not equal the sum of studies and cases per biomarker. ‡Fatty acids were modelled as continuous variables and relative risks were estimated per interquintile range (ie, the distance between the midpoints of the first and fifth quintiles).

Table 2: **Pooled relative risks of type 2 diabetes according to levels of linoleic acid and arachidonic acid biomarkers***

baseline blood was sampled between 1987–89 and 2002–06. All studies used gas chromatography to measure fatty acid biomarkers, with interassay coefficients of variation less than or equal to 15% (appendix). The median percentage of linoleic acid in total fatty acid in each cohort ranged from 8·3% in erythrocyte phospholipids to 54·5% in plasma cholesterol esters (appendix). The median percentage of arachidonic acid in total fatty acid ranged from 0·3% in adipose tissue to 17·0% in erythrocyte phospholipids (appendix). Spearman correlations across lipid compartments within the six studies that included more than one measure ranged from 0·38 to 0·84 for linoleic acid and from 0·48 to 0·92 for arachidonic acid (appendix).

During 366 073 person-years, 4347 participants developed type 2 diabetes (appendix). In pooled analyses, linoleic acid levels were inversely associated with incidence of type 2 diabetes, with a lower risk in continuous analyses per interquintile range (fixed-effect RR 0·65, 95% CI 0·60–0·72, p<0·0001) and in categorical analysis (quintile 5 *vs* quintile 1; 0·57, 0·51–0·64, p<0·0001; table 2). Findings were similar across lipid compartments (figure 1; table 2), although not statistically significant in adipose tissue, for which only one study provided data (99 incident cases out of 738 participants). Heterogeneity in the overall pooled analysis was moderate (I^2=53·9% for continuous analyses, 46·3% for categorical analyses; table 2).

Arachidonic acid biomarkers were not associated with incidence of type 2 diabetes overall (RR per interquintile range 0·96, 95% CI 0·88–1·05, p=0·38; table 1, figure 2). Arachidonic acid biomarker concentrations in separate lipid compartments were not associated with type 2 diabetes, with the exception of total plasma, whereby an inverse association was identified (RR per interquintile range 0·73, 95% CI 0·62–0·86, p=0·0003; I^2=63·8%; table 1, figure 2).

Categorical analysis across quintiles showed that participants in each of the higher quintiles (2–5) of linoleic acid biomarker had significantly lower risk than participants within the lowest quintile (figure 3). Additionally, the dose–response association between linoleic acid biomarker and type 2 diabetes appeared monotonic (appendix).

Restricted cubic spline regression analysis within each lipid compartment found little evidence for non-linearity in the relationship between linoleic acid biomarkers in cholesterol esters or total plasma and incident type 2 diabetes ($p_{non\text{-}linearity}$≥0·4 each; $p_{linearity}$<0·001 each; appendix). A potentially non-linear association was identified in erythrocyte phospholipids ($p_{non\text{-}linearity}$=0·005) and plasma phospholipids ($p_{non\text{-}linearity}$=0·03; appendix); risk for each association declined steeply initially then plateaued (but did not significantly increase) at very high levels. For arachidonic acid, levels of biomarker in total plasma were associated with lower risk ($p_{linearity}$<0·001), with little evidence for non-linear associations within any of the compartments ($p_{non\text{-}linearity}$≥0·47; appendix). Although overall arachidonic acid biomarker levels in phospholipids were not associated with type 2 diabetes (table 2, figure 2), exploratory restricted cubic spline analyses, which assessed erythrocyte phospholipids ($p_{linearity}$=0·001) and plasma phospholipids ($p_{linearity}$=0·03) separately, suggested divergent linear associations with type 2 diabetes (appendix).

The associations of linoleic acid and arachidonic acid biomarkers with incident type 2 diabetes did not significantly vary according to any prespecified potential sources of heterogeneity ($p_{heterogeneity}$≥0·13 each; appendix). In the 12 cohorts with available genetic data, genetic variants of the *FADS* genes had no significant interaction

on the association between either linoleic acid or arachidonic acid biomarker levels and incident type 2 diabetes ($p_{interaction} \geq 0 \cdot 47$; appendix).

Compared with the main analyses, similar results were observed for linoleic acid and arachidonic acid biomarkers after exclusion of type 2 diabetes cases identified in the first 2 years of follow-up, and censoring of follow-up at 6 years after baseline (appendix).

Discussion

In this consortium of 20 prospective studies across ten countries, biomarker levels of linoleic acid were inversely associated with incident type 2 diabetes, whereas levels of arachidonic acid biomarkers were not associated with type 2 diabetes. The magnitude of the association between linoleic acid biomarkers and type 2 diabetes was substantial, with high linoleic acid levels associated with a 43% lower relative risk of type 2 diabetes across quintiles in the categorical analysis. To the best of our knowledge, this is the largest and most detailed biomarker assessment of omega-6 PUFA and type 2 diabetes, including across multiple lipid compartments. Despite the breadth and scope of the cohorts, associations did not seem to differ by age, BMI, sex, race, omega-3 PUFA levels, aspirin use, or variation in the genes encoding FADS.

Incorporation of linoleic acid into phospholipids alters membrane fluidity and might modulate insulin receptor activity.[21] In a meta-analysis[9] of 102 randomised controlled feeding trials, dietary PUFAs (predominantly linoleic acid) improved glycaemia, insulin resistance, and insulin secretion capacity, compared with carbohydrate, saturated fat, and for some endpoints even monounsaturated fat. In other randomised controlled trials,[22] linoleic acid-rich vegetable oil reduced markers of inflammation, visceral fat deposition, and hepatic steatosis. Because dietary linoleic acid intake correlates with levels of circulating and tissue linoleic acid,[12] our biomarker-based findings extend and expand these previous results by providing evidence that linoleic acid might have long-term benefits for preventing the onset of type 2 diabetes, supporting clinical recommendations to increase dietary intake of linoleic acid-rich vegetable oils. Our novel findings also support the need for future studies to establish the potential influence and clinical effects of other influences (eg, pharmacological) on these fatty acid biomarkers, identify the downstream biological mediating pathways of these fatty acid biomarkers on risk of type 2 diabetes, and investigate potential novel influences

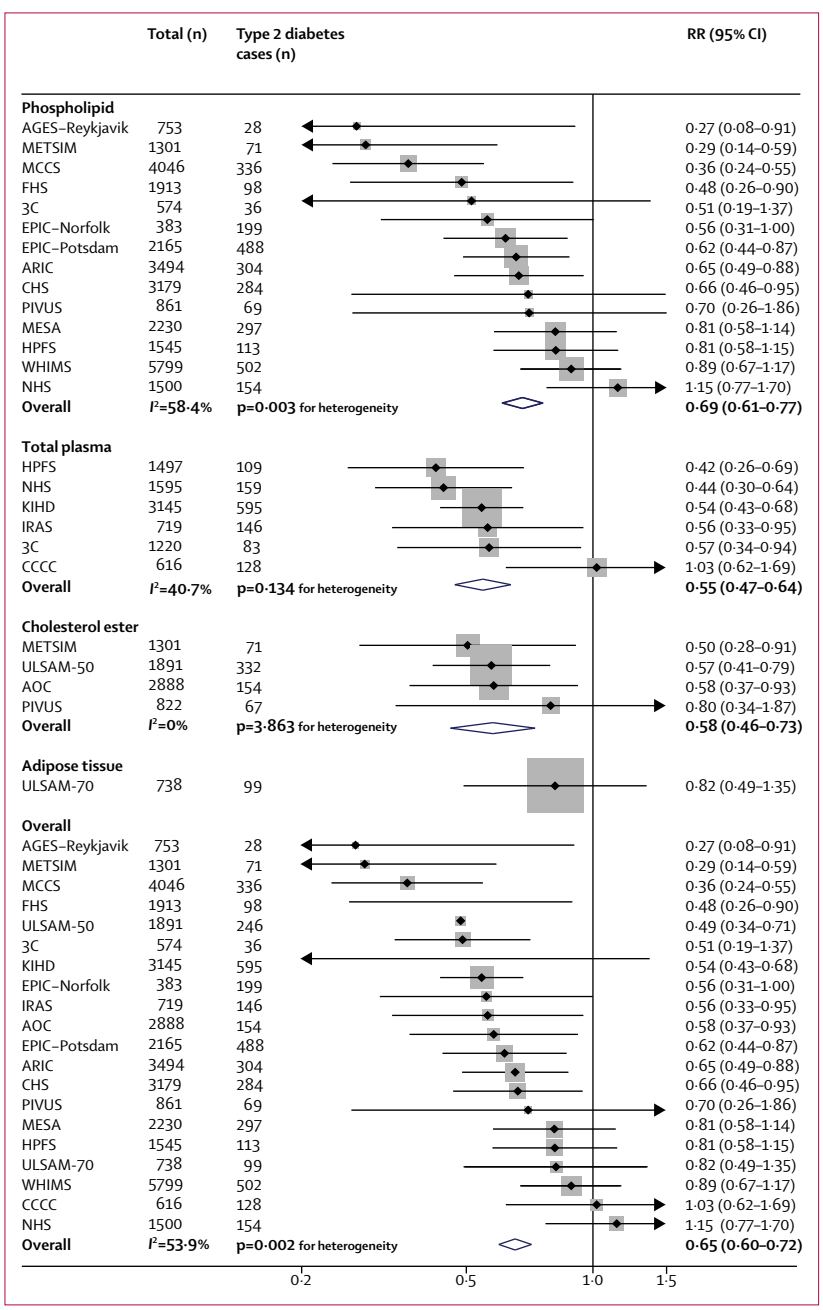

***Figure 1*: Pooled relative risks of type 2 diabetes according to interquintile range* of linoleic acid biomarker, per lipid compartment**

The association between linoleic acid and type 2 diabetes was assessed in multivariable models for each cohort, and the results were pooled using inverse-variance weighted fixed effects meta-analysis. If multiple biomarkers were available within a study, one was chosen for the overall analysis on the basis of its ability to reflect long-term dietary intake (in the following order of preference): adipose tissue, phospholipids, total plasma, and cholesterol esters. Similarly, data for erythrocyte phospholipids were preferred over plasma phospholipids if both were available from a cohort. References for all studies are shown in the appendix. RR=relative risk. AGES-Reykjavik=Age, Gene/Environment Susceptibility Study (Reykjavik). METSIM=Metabolic Syndrome in Men Study. MCCS=Melbourne Collaborative Cohort Study. FHS=Framingham Heart Study. 3C=Three City Study. EPIC-Norfolk=European Prospective Investigation into Cancer (Norfolk). EPIC-Potsdam=European Prospective Investigation into Cancer (Potsdam). ARIC=Atherosclerosis Risk in Communities. CHS=Cardiovascular Health Study. PIVUS=Prospective Investigation of the Vasculature in Uppsala Seniors. MESA=Multi-Ethnic Study of Atherosclerosis. HPFS=Health Professionals Follow-up Study. WHIMS=Women's Health Initiative Memory Study. NHS=Nurses' Health Study. KIHD=Kuopio Ischaemic Heart Disease Risk Factor Study. IRAS=Insulin Resistance Atherosclerosis Study. CCCC=Chin-Shan Community Cardiovascular Cohort Study. ULSAM-50=Uppsala Longitudinal Study of Adult Men-50. AOC=Alpha Omega Cohort. ULSAM-70=Uppsala Longitudinal Study of Adult Men-70. *Difference between the midpoints of the first and fifth quintiles.

(eg, pharmacological and lifestyle) on these downstream biological mediating pathways. Mendelian randomisation studies[23] should also assess the association between the common genetic variants that influence fatty acid concentrations and type 2 diabetes.

Despite the established benefits of PUFAs for blood cholesterol levels and glucose-insulin homoeostasis,[9] some scientists maintain that omega-6 PUFA is harmful for health.[24] A main theorised harm relates to the conversion of linoleic acid to arachidonic acid, which has been considered as pro-inflammatory and potentially harmful for glucose metabolism, weight regulation, and eating behaviour.[6] However, multiple studies indicate that variations in both dietary linoleic acid and arachidonic acid have little effect on circulating arachidonic acid levels, indicating close endogenous regulation of the metabolite.[25] Additionally, arachidonic acid has important metabolites that actively resolve inflammation,[26] and systematic reviews of trials have not identified pro-inflammatory effects of linoleic acid consumption.[27] Indeed, a systematic review[8] found that higher biomarker levels of arachidonic acid were associated with lower incidence of coronary heart disease. We found no evidence to suggest that arachidonic acid contributes to the development of type 2 diabetes. Together with the findings of previous experimental and interventional studies on metabolic risk factors, our findings do not suggest that high levels of dietary omega-6 PUFA are harmful. Additionally, although omega-3 and omega-6 PUFA has been hypothesised to compete, we did not identify any evidence of a physiologically relevant interaction in this large, well powered consortium analysis.

A 2016 nested case-cohort analysis from the European Prospective Investigation into Cancer (EPIC) cohort,[28] published during the preparation of our manuscript, found an inverse association between plasma phospholipid linoleic acid and type 2 diabetes (HR per SD increase 0·80, 95% CI 0·77–0·83), and no significant association between arachidonic acid and type 2 diabetes (HR 1·02, 0·98–1·06). Our findings are consistent with this report, and include a worldwide perspective, using data from multiple lipid compartments and detailed assessment of potential effect modification, including by variation in the genes encoding FADS. Our study also

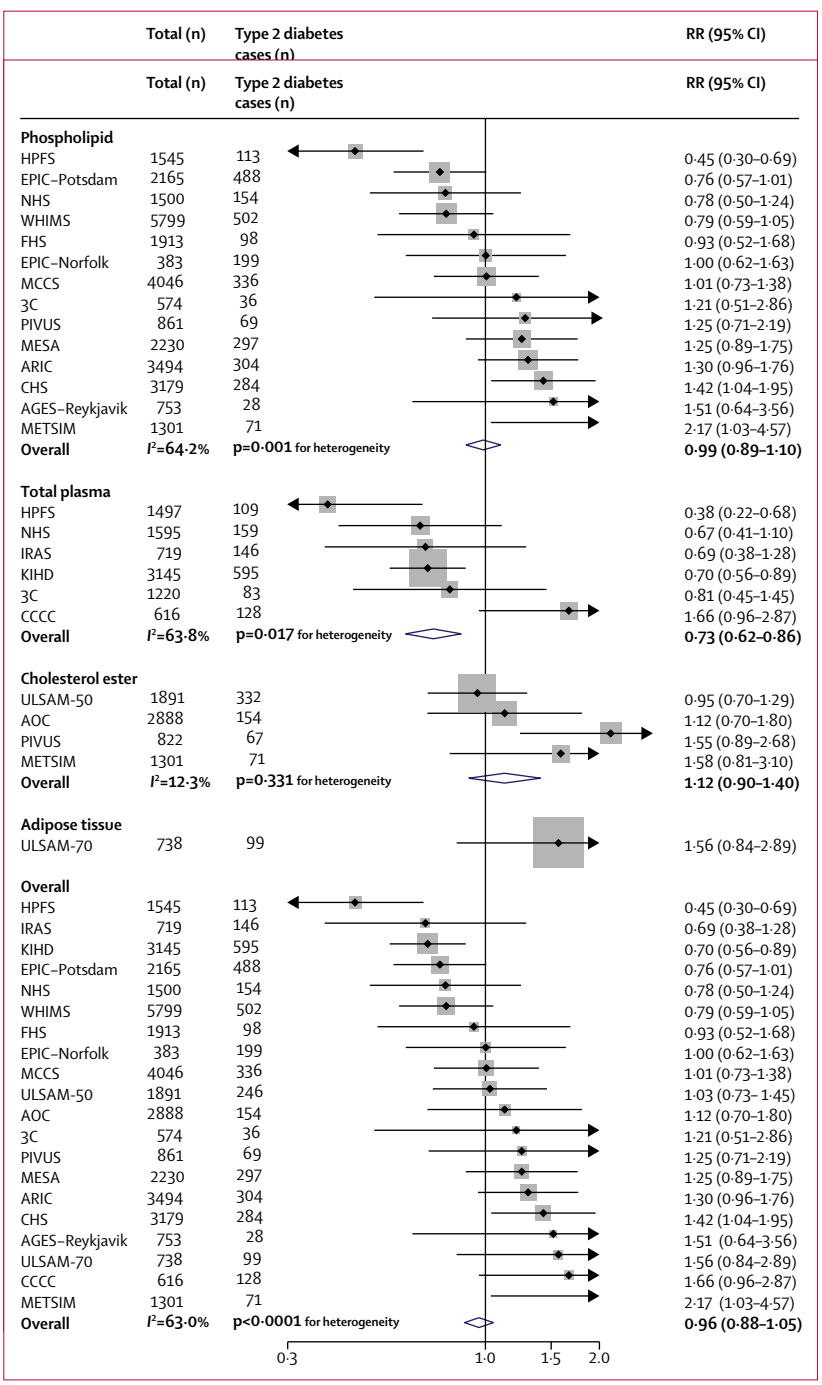

***Figure 2*: Pooled relative risks of type 2 diabetes according to interquintile range* of arachidonic acid biomarker, per lipid compartment**

Association between arachidonic acid and type 2 diabetes was assessed in multivariable models for each cohort, and the results were pooled using inverse-variance weighted fixed effects meta-analysis. If multiple biomarkers were available within a study, one was chosen for the overall analysis on the basis of its ability to reflect long-term dietary intake (in the following order of preference): adipose tissue, phospholipids, total plasma, and cholesterol esters. Similarly, data for erythrocyte phospholipids was preferred over plasma phospholipids if both were available from a cohort. References for all studies are shown in the appendix. RR=relative risk. HPFS=Health Professionals Follow-up Study. EPIC-Potsdam=European Prospective Investigation into Cancer (Potsdam). NHS=Nurses' Health Study. WHIMS=Women's Health Initiative Memory Study. FHS=Framingham Heart Study. EPIC-Norfolk=European Prospective Investigation into Cancer (Norfolk). MCCS=Melbourne Collaborative Cohort Study. 3C=Three City Study. PIVUS=Prospective Investigation of the Vasculature in Uppsala Seniors. MESA=Multi-Ethnic Study of Atherosclerosis. ARIC=Atherosclerosis Risk in Communities. CHS=Cardiovascular Health Study. AGES-Reykjavik=Age, Gene/Environment Susceptibility Study (Reykjavik). METSIM=Metabolic Syndrome in Men Study. IRAS=Insulin Resistance Atherosclerosis Study. KIHD=Kuopio Ischaemic Heart Disease Risk Factor Study. CCCC=Chin-Shan Community Cardiovascular Cohort Study. ULSAM-50=Uppsala Longitudinal Study of Adult Men-50. AOC=Alpha Omega Cohort. ULSAM-70=Uppsala Longitudinal Study of Adult Men-70. *Difference between the midpoints of the first and fifth quintiles.

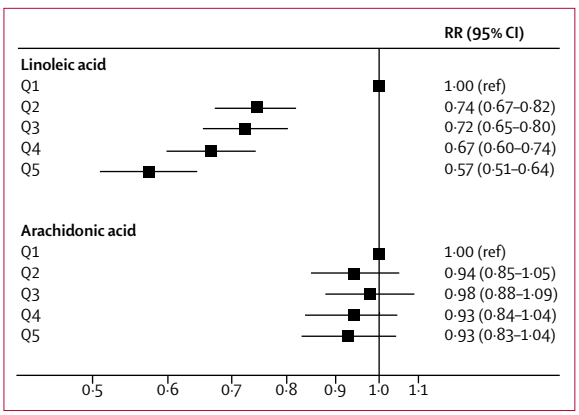

***Figure 3*: Pooled relative risks of type 2 diabetes per quintile of linoleic acid and arachidonic acid biomarker**

Association of linoleic acid and arachidonic acid levels with type 2 diabetes was assessed in multivariable models for each cohort, and results were pooled using inverse-variance weighted meta-analysis. The lowest quintile was used as the reference group. For studies in which multiple biomarkers were available, one was chosen for the overall analysis on the basis of its ability to reflect long-term dietary intake (in the following order of preference): adipose tissue, phospholipids, total plasma, and cholesterol esters. Similarly, data for erythrocyte phospholipids was preferable to plasma phospholipids if data on both biomarkers were available. The Age, Gene/Environment Susceptibility Study (Reykjavik) was excluded from these analyses due to the small number of patients who developed incident type 2 diabetes, so the effect estimates were pooled from the other 19 cohorts. RR=relative risk. Q=quintile.

appreciably reduces the possibility of chance findings or publication bias, compared with individual cohort reports, because we included most of the available cohorts with measured fatty acid biomarkers and assessment of incident type 2 diabetes. The inclusion of EPIC-InterAct in our pooled analysis would be unlikely to affect the conclusions of our study.

Little is known about how differences in fatty acid function between lipid compartments relate to health. Our analyses provided novel assessment of dose–response associations between omega-6 PUFA and type 2 diabetes in different lipid compartments. For linoleic acid biomarkers, all compartments (with the exception of adipose tissue, which was only assessed in one study) showed significant linear inverse associations with type 2 diabetes, suggesting a class effect of linoleic acid rather than primacy of any single compartment. In exploratory analyses, the protective association between linoleic acid and type 2 diabetes seemed to be linear in cholesterol esters and total plasma, but non-linear in phospholipids, where benefit appeared to plateau at very high levels. The biological and clinical relevance of this discrepancy warrants further investigation. Studies are also needed to define the dose–response relationship between a broad range of markers of linoleic acid intake and biomarker concentrations in different lipid compartments. For arachidonic acid biomarkers, there was little evidence for non-linearity for any of the lipid compartments. The opposing associations of erythrocyte phospholipids and plasma phospholipids with arachidonic acid identified by semiparametric analyses require further investigation; these results could be due to chance because arachidonic acid concentrations in these two compartments are highly correlated and are known to readily interexchange.[29] Consistent with this suggestion, in the EPIC cohort,[28] levels of plasma phospholipid arachidonic acid were not associated with type 2 diabetes. Our new findings of a protective association between arachidonic acid in total plasma and incident type 2 diabetes, based on findings in six cohorts, should be explored further.

Our investigation has important strengths. We included prospective cohorts, which minimised the likelihood of selection bias. Our use of biomarkers avoided recall bias associated with self-reported intake and allowed objective assessment of linoleic acid and arachidonic acid levels. Collaboration between 20 cohorts enabled simultaneous investigation of multiple lipid compartments, which could be cost prohibitive in a single study. Harmonised, predefined analysis protocols standardised exposures, outcomes, covariates, and statistical modelling, reducing post-hoc-driven reporting and heterogeneity across studies. The prespecified analytic plan and inclusion of 20 (77%) of the 26 identified global cohorts greatly reduced publication bias. The large numbers of participants and events increased statistical power to explore effect modification. Results were consistent in sensitivity analyses, increasing confidence in the robustness of findings and underlying model assumptions. Inclusion of multiple cohorts and nations with diverse demographic, lifestyle, and dietary characteristics enhanced generalisability.

Our study also has limitations. Few data were available on adipose tissue, reducing power and precision to assess its relevance for type 2 diabetes. Although multiple races and ethnicities were included, most participants were of European descent and statistical power was low with respect to differences in other ethnic groups, although central risk estimates for linoleic acid biomarkers were protective in each group. Fatty acid biomarker levels were assessed at baseline, and changes over time would attenuate findings toward the null hypothesis, causing underestimation of magnitudes of true associations. Linoleic acid biomarkers reflect dietary intake and other factors such as metabolism, so differences in type 2 diabetes risk should not be interpreted as entirely attributable to dietary linoleic acid. We did not assess other fatty acid biomarkers, which should be the subject of future investigations—particularly saturated fatty acids such as palmitic acid, which has shown pro-diabetogenic effects in experimental studies.[30–32] In addition, the reliability of type 2 diabetes ascertainment was likely to have differed across the cohorts, which might have caused some outcome misclassification and underestimation of true associations. Our findings are relevant for the incidence of type 2 diabetes, but not type 1 diabetes. Residual confounding by unmeasured or imprecisely

measured covariates, including by other fatty acid biomarkers, cannot be fully excluded. However, the magnitude of the observed association between linoleic acid biomarkers and the incidence of type 2 diabetes, consistency across biomarker compartments, inclusion of varied populations with diverse underlying characteristics, and supportive biological plausibility from interventional trials of risk factors suggest that our findings are not solely due to statistical chance and uncontrolled confounding.

In conclusion, this international collaboration of 20 prospective cohorts showed that biomarker levels of linoleic acid, the major dietary omega-6 PUFA, were inversely associated with the risk of incident type 2 diabetes, whereas levels of arachidonic acid were not significantly associated with risk of the disease.

Contributors

All authors conceived the study concept and design. JW, MM, FI, NT, AVAK, JdG, XZ, W-SY, MCdOO, JK, WQ, JKV, JKB, T-AC, ML, AVS, and KR did the data analysis. All authors interpreted the data. JW, MM, RM, FI, RL, and DM wrote the first draft of the Article. All authors reviewed and edited the report.

Declaration of interests

JHYW received research grants from Unilever for this study. LCDG received ad-hoc consulting fees from the Life Sciences Research Organization. CH received fees for a conference from Novartis. JMG received funding from Unilever for epidemiological studies of dietary and circulating fatty acids and cardiometabolic disease. RM received research grants from Unilever for this study. DM received ad-hoc honoraria and consulting fees from the Life Sciences Research Organization, AstraZeneca, Boston Heart Diagnostics, Global Organization for EPA and DHA Omega-3, DSM, Nutrition Impact, the Haas Avocado Board, and Pollock Communications; and chapter royalties from UpToDate. All other authors declare no competing interests.

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