Dietary Intake of Linoleic Acid, Its Concentrations, and the Risk of Type 2 Diabetes: A Systematic Review and Dose-Response Meta-analysis of Prospective Cohort Studies

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BACKGROUND

Earlier evidence on the association between dietary polyunsaturated fatty acids and risk of diabetes has been conflicting.

PURPOSE

To quantitatively summarize previous studies on the association between dietary LA intake, its biomarkers, and the risk of type 2 diabetes mellitus (T2DM) in the general population.

DATA SOURCES

Our data sources included PubMed/MEDLINE, Scopus, and ISI Web of Science until 24 October 2020; reference lists of all related articles; and key journals.

STUDY SELECTION

We included prospective cohort studies that examined the associations of linoleic acid (LA) with the risk of T2DM in adults.

DATA SYNTHESIS

The inverse variance method was applied to calculate summary relative risk (RR) of LA intake and its biomarkers, and dose-response associations were modeled using restricted cubic splines. Twenty-three publications, covering a total of 31 prospective cohorts, were included; these studies included 297,685 participants (22,639 incident diabetes cases) with dietary intake assessment and 84,171 participants (18,458 incident diabetes cases) with biomarker measurements. High intake of LA was associated with a 6% lower risk of T2DM (summary relative risk [RR] 0.94, 95% CI 0.90, 0.99; $I^2 = 48.5\%$). In the dose-response analysis, each 5% increment in energy from LA intake was associated with a 10% lower risk of T2DM. There was also evidence of a linear association between LA intake and diabetes, with the lowest risk at highest intakes. The summary RR for diabetes per SD increment in LA concentrations in adipose tissue/blood compartments was 0.85 (95% CI 0.80, 0.90; $I^2 = 66.2\%$). The certainty of the evidence was assessed as moderate.

LIMITATIONS

A limitation of our work was the observational design of studies included in the analyses.

CONCLUSIONS

We found that a high intake of dietary LA and elevated concentrations of LA in the body were both significantly associated with a lower risk of T2DM. These findings support dietary recommendations to consume dietary LA.

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META-ANALYSIS

Polyunsaturated fatty acids (PUFAs) are lipid molecules containing two or more double bonds along their carbon chain and are categorized into n-9, n-6, and n-3 fats (1). Linoleic acid (LA) (18:2n-6), an essential fatty acid that constituted 85–90% of dietary n-6 PUFAs in the U.S. and derived mostly from plant oils and nuts (2), is recommended for health in most dietary guidelines. For example, the Dietary Guidelines for Americans and the American Heart Association each recommended consumption of LA as 5–10% of total calories (3). In randomized controlled feeding trials, LA consumption reduced total, LDL, and VLDL cholesterol as well as serum triglycerides, and raised HDL cholesterol (4). Randomized controlled feeding trials also demonstrate that PUFA intake (predominantly LA) improves insulin resistance and glycemia (5). A pooled analysis and a meta-analysis of prospective cohort studies indicated an inverse association between dietary LA consumption and its biomarkers and risk of coronary heart disease and stroke (6). Greater intakes of LA, assessed by dietary methods or biomarkers, were also associated with a lower risk of all-cause and cancer mortality (7).

However, some scientists have raised concerns that LA could have adverse health effects. LA is a precursor of arachidonic acid (AA) (20:4n-6), which can, in turn, result in elevated proinflammatory eicosanoids (8), but also other specialized molecules that actively resolve inflammation (9). In addition, feeding studies have found little or no impact of dietary LA on plasma and adipose tissue AA concentrations, which appear to be tightly metabolically regulated (10).

Findings on the association of dietary LA intake and incidence of type 2 diabetes mellitus (T2DM) remain uncertain. Despite some reports showing an inverse association between dietary LA and risk of T2DM (11), others found no association (12,13). Some inconsistencies have also been observed in studies of biomarkers of LA (14–18). A pooled meta-analysis of de novo cohort-level and individual-level analyses across 20 prospective cohorts demonstrated that higher levels of LA biomarkers were associated with 35% lower risk of T2DM (19). However, not all global cohorts participated in that de novo analysis (15–18,20–24). In addition, the dietary intake of LA was not considered. It must be noted that LA biomarkers are not strongly correlated with dietary intakes of this fatty acid, and they might be affected by other factors such as an individual's metabolism. Therefore, the current study was done to quantitatively summarize findings from published prospective cohort studies on the association between dietary intakes and biomarkers of LA and risk of T2DM in the general population.

METHODS

The present meta-analysis was reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (25). The protocol of this study was retrospectively registered at PROSPERO (reg. no. CRD42020221029, https://www.crd.york.ac.uk/PROSPERO).

Data Sources and Searches

We comprehensively searched four medical databases, including PubMed/MEDLINE, Scopus, ISI Web of Science, and Google Scholar, to identify prospective cohort studies from inception up to 24 October 2020. The combination of MESH and non-MESH terms was used for the study exposure (i.e., "linoleic acid" or "essential fatty acid" or "omega 6 fatty acids") AND outcome (i.e., "diabetes mellitus" or "type 2 diabetes") AND study design (i.e., "cohort studies" or "prospective studies"); a full list of search terms separately in each database are provided in Supplementary Table 1. No limitations were imposed in terms of language. The literature search was complemented by reviewing reference lists of all related articles and reviews and hand searching key journals to make sure no eligible articles were missed. In addition, PubMed's e-mail alert service was activated to ensure awareness of new articles that may have been published on this topic after our initial search.

Study Selection

SMM did the screening of the titles and abstracts of the identified articles, and pertinent articles were independently reviewed in full text by two investigators (S.M.M. and Y.J.). In case of discrepancies in the inclusion or exclusion of some studies, the consensus was reached in consultation with A.E. Studies were eligible for inclusion in the analysis if they met the following criteria: *1*) prospective observational studies including prospective cohort studies or nested case-control/case-cohort studies, *2*) studies that were conducted among the general population age ≥18 years, *3*) studies that reported multivariable-adjusted risk estimates (odds ratios, relative risks, hazard ratios, or risk ratios [RRs]) of the association between LA (dietary intake and/or biomarkers concentrations) as the exposure and T2DM as the outcome of interest. For the dose-response analyses, studies that provided adequate information on the number of cases and participants/person-years or noncases and adjusted relative risks across three or more quantitative categories of dietary LA or reported a continuous estimation from the association were included. For studies published from the same data set, the study with the most complete data or a higher number of cases was included.

We excluded studies that were conducted on children, adolescents, pregnant women, and populations with a preexisting disease; interventional, cross-sectional, and retrospective case-control studies; and letters, editorials, meta-analyses, commentaries, and ecological studies.

Data Extraction and Quality Assessment

Two investigators (S.M.M. and Y.J.) independently extracted the following data: study characteristics, first author's name, cohort name, publication year, follow-up duration, study location; participant characteristics, age, sex, range or mean age at study baseline, sample size, number of cases; exposure assessments, dietary assessment method, exposure dose, tissue types, laboratory assays method; risk estimates; and the corresponding precision in multivariable-adjusted analysis. The risk estimates with the largest number of adjusted variables were considered. However, in studies that controlled for intermediate variables, such as blood glucose, insulin, HOMA of insulin resistance, and HbA_{1c}, in their maximally adjusted model, the alternative model without these intermediate variables was considered in the meta-analyses. If the alternative model was adjusted for age only, a multivariate model including intermediate variables was included. Additionally, GetData Graph Digitizer software was used to extract numerical estimates from graphs. Assessment of studies' quality and

quality of evidence was done using the nine-star Newcastle-Ottawa Scale (NOS) (26) and the newly developed NutriGrade scoring system (with a maximum score of 10) (27), respectively. Any disagreements were discussed and resolved by a chief investigator (A.E.), and consensus was reached in all cases.

Data Synthesis and Analysis

The relative risks and 95% CIs were considered as the effect size in the present meta-analysis. The hazard ratios and odds ratios presented in original studies were considered as equal to relative risks (28). Between-studies heterogeneity was assessed using the Cochrane Q test and I^2 statistics (29). The pooled risk estimates were calculated using an inverse variance–weighted model. For dietary LA, three sets of analyses were performed. First, we performed a pairwise meta-analysis as the main analysis. For this purpose, we combined study-specific effect sizes for the highest compared with the lowest category of dietary LA by using a fixed-effects model. Second, we performed a fixed-effects dose-response meta-analysis to estimate the relative risk for T2DM per additional 5% energy using the method introduced by Greenland and colleagues (30,31). To do this, distribution of cases and person-years and the reported effect estimates across categories of dietary LA was needed. The median of dietary LA in each category was considered. When the studies reported dietary LA as a range of intake, we estimated each category's midpoint by calculating the average of the lower and upper bounds. For studies that reported dietary LA intake as grams per day (12), we converted grams per day to the percent of total energy from LA. In order to calculate LA as a percent of total energy intake, we multiplied the grams of LA by 9.1 kcal, the amount of calories provided by 1 g fat, and then we divided it by the mean energy intake of the corresponding category. Finally, a potential nonlinear association was examined by modeling dietary LA intakes using a one-stage linear mixed-effects meta-analysis (32). With this method the study-specific slope lines are estimated and combined to obtain an overall average slope in a single stage, and this method also allows inclusion of studies with only two categories of exposures in the dose-response analysis.

For the analysis of biomarkers of LA, we estimated the relative risk for each SD increment in biomarkers of LA (7) and then pooled study-specific relative risks by using a random-effects model. When the studies reported direct relative risk per 1 SD increment in biomarkers of LA, the relative risk was included in the meta-analysis as reported. For studies that reported relative risks across categories of biomarkers, we used the method of Danesh et al. (33) to convert the relative risks for the highest versus lowest category to relative risks for 1 SD increment in biomarkers. For studies that reported effect estimates for LA biomarkers across different tissues, the overall pooled estimates were obtained based on the biomarker that best reflects long-term consumption, in the following order: adipose tissue, erythrocyte phospholipids, plasma phospholipids, total plasma or serum, and cholesterol esters (34). In addition, we separately calculated the pooled estimates for each lipid compartment. We did not perform a dose-response analysis for biomarkers of LA due to a lack of sufficient data in primary studies. To examine the potential impact of each individual study on the overall estimates, sensitivity analyses were performed using the leave-one-out method. To explore potential sources of heterogeneity, we performed subgroup and meta-regression analyses by sex, study location, duration of follow-up, number of cases, dietary assessment tools, and adjustment for key covariates. Assessment of publication bias was done with the Egger test as well as through visual inspection of funnel plots. All statistical analyses were conducted with Stata software, version 15.0 (StataCorp, College Station, TX), and P values <0.05 were considered significant.

RESULTS

A total of 10,221 articles were retrieved in the primary search of databases. Of them, 1,447 duplicates were removed, and 8,774 unique records remained. Details of the literature search can be found in Supplementary Fig. 1. After screening articles based on titles and abstracts and an additional review of the reference lists, 88 records were considered for a detailed full-text screen. Of these studies, 65 articles were excluded: studies that did not examine LA as the exposure ($n = 41$), those with a nonrelevant outcome ($n = 2$), studies with interventional and cross-sectional designs or nonoriginal articles including letters, reviews, and editorials ($n = 8$), and those with insufficient data ($n = 1$) and lack of reporting of the relevant risk estimate ($n = 1$). In addition, 10 duplicate studies and 2 studies that were conducted in patient populations ($n = 2$) were excluded. Finally, 23 articles covering 31 cohorts met the selection criteria and were included in the meta-analysis. Of 23 articles, 3 reported information for both dietary and biomarkers of LA (14,35,36). One article included data from three large cohort studies (11). Three articles were published based on data from the METabolic Syndrome In Men (METSIM) study and reported information for LA levels in different tissues (21,37,38). One study was a de novo individual cohort-specific participant data analysis of 20 prospective cohort studies, of the 11 studies had no separate publication; therefore, we got the required information for these 11 cohorts from the study of Wu et al. (19).

Finally, nine cohorts reported associations for dietary LA (11–14,35,36,39) and 27 cohorts for biomarkers (14–24,35–38, 40–43). Detailed information about the excluded articles and the reasons are summarized in Supplementary Table 2.

LA Intake and Risk of T2DM

Nine prospective cohorts (11–14,35,36,39) reported on the association between dietary LA intake and risk of T2DM, including 297,685 participants and 22,639 incident cases. Median follow-up duration varied from 4 to 32 years, and participants' age ranged between 25 and 80 years. Three studies were conducted in women only (11,12) and one was conducted in men (11), and five studies included both sexes (13,14,35,36,39). Three studies were from the U.S. (11), four studies from Europe (12,35,36,39), one from Asia (13), and one from Australia (14). Assessment of dietary LA intake in all studies was done using a validated food-frequency questionnaire (FFQ), at baseline only for five studies and with use of repeated dietary assessments for four studies (Supplementary Table 3). The mean (median) NOS scores of these studies were 8.0 (8.0) (Supplementary Table 5).

In comparison of the highest versus the lowest category of dietary LA, the

pooled multivariable-adjusted relative risk from the fixed-effects model indicated a marginally inverse association between dietary LA intake and T2DM (relative risk 0.94, 95% CI 0.90, 0.99; $P = 0.02$), with moderate evidence of between-study heterogeneity ($I^2 = 48.5\%$, $P_{heterogeneity} = 0.05$) (Fig. 1). In the sensitivity analysis, we found that this association was influenced by the results of the Health Professionals Follow-up Study (HPFS) (11). When this study was excluded from the analysis, the pooled effect size was not statistically significant (relative risk 0.97, 95% CI 0.92, 1.02) (Supplementary Fig. 2). Six prospective studies reported sufficient data for inclusion in the dose-response meta-analysis (11,12,36,39). The linear trend estimation suggested that an additional 5% of energy from LA was associated with a 10% lower risk of T2DM (relative risk 0.90, 95% CI 0.84, 0.98) (Supplementary Fig. 3). The dose-response analysis showed an inverse linear association between dietary LA intake and risk of T2DM ($P_{nonlinearity} = 0.73$, $P_{linearity} = 0.01$), with the lowest risk at highest intakes (Fig. 2 and Supplementary Table 6). The association disappeared with exclusion of the HPFS from the dose-response analysis ($P_{nonlinearity} = 0.22$, $P_{linearity} = 0.37$) (Supplementary Fig. 4). The overall quality of the evidence was rated moderate on the basis of the NutriGrade score (NutriGrade score = 6.8) (Supplementary Table 7).

In the subgroup analysis, the association appears significantly different by several subgroups, suggesting an inverse association in studies conducted in the U.S., those that used repeated dietary assessments during follow-up, studies with a long follow-up duration (≥10 years), publications with a high number of case subjects (>1,000 people), and those with adjustment in the analysis for total energy intake, physical activity, smoking status, dietary fiber and *trans* fats, and family history of diabetes (Supplementary Table 8). In addition, meta-regression analysis suggested that sex might explain between-study heterogeneity ($P < 0.05$). However, after examination of the geographical location, follow-up duration, number of cases, dietary assessment method, and adjustments for some covariates, no significant heterogeneity source was detected (Supplementary Table 8).

Biomarkers of LA and Risk of T2DM

Twenty-seven prospective cohorts (17 publications) (14–24,35–38,40–43), with 84,171 participants and 18,458 incident cases, reported the association between biomarkers of LA and risk of T2DM. Median follow-up duration varied from 4 to 21.4 years, and participants' mean age ranged between 33.5 and 76.6 years. Five studies enrolled only men (19,37,42), 3 studies recruited only women (19,24,41), and 19 remaining studies included both sexes. Fourteen studies were from Europe (15,18–20,22,24,35–37,42), 8 from the U.S. (19,40,41,43), and 4 from Asia (16,17, 19,23), and 1 other study was from Australia (14). Gas chromatography, gas-liquid chromatography, and nuclear MRS were used to measure LA concentrations in adipose tissue (1 cohort) or in different blood compartments, including erythrocyte phospholipids (10 cohorts), plasma phospholipids (7 cohorts), total plasma/serum (8 cohorts), and cholesteryl esters (1 cohort) (Supplementary Table 4). The mean (median) NOS scores were 7.8 (8.0), where 15 cohorts had a score of ≥8 (Supplementary Table 5).

With combining effect sizes from all tissue types, the pooled multivariable-adjusted relative risk from the random-effects meta-analysis for each 1 SD increment in LA levels was 0.83 (95% CI 0.81, 0.85), with moderate heterogeneity ($I^2 = 70.6\%$, $P_{heterogeneity} < 0.001$) (Fig. 3).

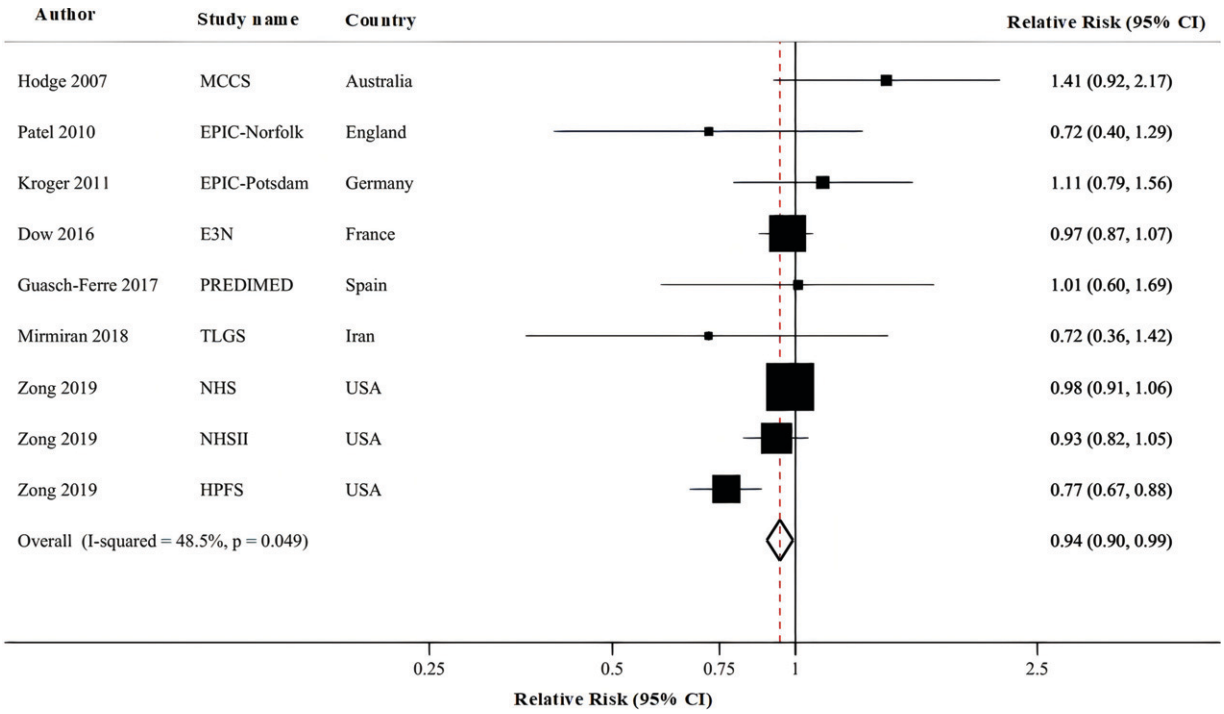

Figure 1—Summary relative risk and 95% CIs of T2DM for the highest compared with the lowest category of LA intake. EPIC, European Prospective Investigation into Cancer and Nutrition; E3N, Etude Epidémiologique auprés des femmes de la Mutuelle Générale de l'Education Nationale; MCCS, Melbourne Collaborative Cohort Study; TLGS, Tehran Lipid and Glucose Study.

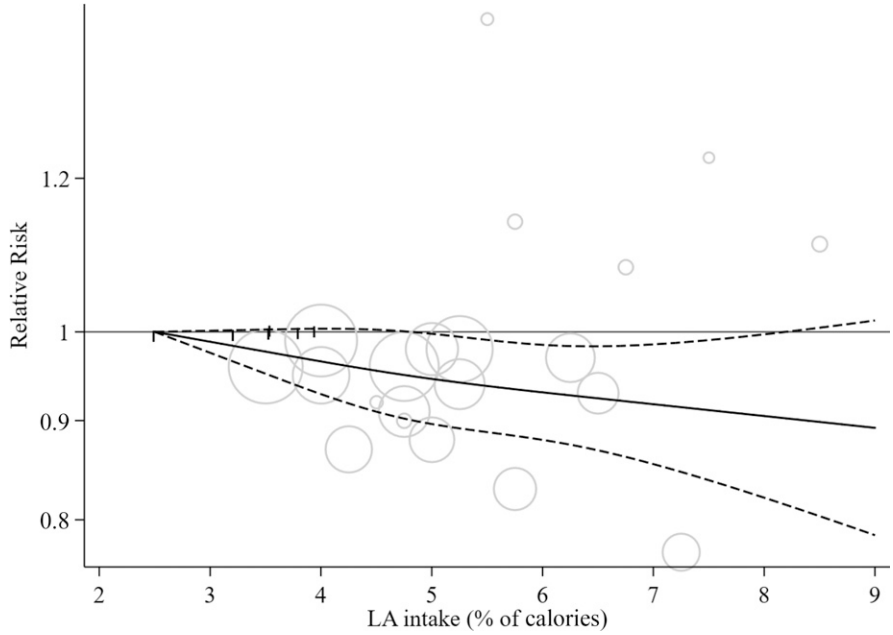

Figure 2—Nonlinear dose-response association between LA intake and the risk of T2DM ($P_{nonlinearity} = 0.73$, $P_{linearity} = 0.01$). Solid line represents nonlinear dose response, and dotted lines represent 95% CI. Circles represent relative risk point estimates for LA intake categories from each study, with circle size proportional to inverse of SE. Small vertical black lines indicate baseline LA intake categories in each study.

Findings from the sensitivity analysis showed that a significant inverse association was robust, such that exclusion of each study at a time did not change the pooled effect size (Supplementary Fig. 5). The pooled estimates were similar across tissue types apart from adipose tissue, for which only one study with a nonsignificant association was included (Supplementary Fig. 6 and Supplementary Table 2). The association varied by sex and BMI adjustment; however, an inverse association between LA biomarkers and T2DM was observed (Supplementary Table 9). Based on meta-regression analysis, adjustment for smoking status ($P = 0.01$) might be a potential source of heterogeneity. The heterogeneity was not explained by tissue type, sex, geographical location, follow-up duration, number of cases, or adjustments for other confounding factors (Supplementary Table 9). The quality of evidence was rated moderate (NutriGrade score = 6.4) (Supplementary Table 7).

Small Study Effects and Publication Bias

Visual inspection of the funnel plots demonstrated no evidence of asymmetry in the plots for the association between dietary and biomarkers of LA and T2DM (Supplementary Fig. 7). These observations were affirmed by no considerable evidence of publication bias in Egger tests for dietary intakes ($P = 0.93$) or biomarkers ($P = 0.68$).

DISCUSSION

In the present systematic review and meta-analysis of prospective cohort studies, we found an inverse association between dietary intake and biomarkers of LA and the risk of T2DM. The magnitude of associations, based on the dose-response analysis, revealed that each 5% increment in energy from LA intake was associated with a 10% lower risk of T2DM, while a 15% reduction in diabetes risk was observed per SD increase in biomarker levels of LA. There was also evidence of a nonlinear association between LA intake and diabetes, with the lowest risk at highest intakes. The association with biomarker levels of LA was robust, as it persisted in sensitivity analyses; however, this was not the case with dietary LA intakes because with exclusion of one study at a time, the association with dietary LA became nonsignificant. This is the first meta-analysis to investigate both dietary intake and biomarkers of LA in relation to the incidence of T2DM.

Previous meta-analyses have focused on the association between n-6 PUFA and CVD end points (7,44,45). However, less attention has been given to the risk of diabetes. Our findings align with the current U.S. recommendations for consumption of 5–10% of energy from LA. However, some other guidelines recommended lower intakes of LA (no more than 4% of energy intake) (3,46). In addition, previous research showed that replacing 5% energy intake from carbohydrate or saturated fatty acids (SFAs) with PUFA significantly lowered fasting glucose, HbA_{1c}, HOMA of insulin resistance, and insulin secretion capacity (5). Similar to our results, the authors found beneficial effects for LA and total PUFA rather than only n-3 PUFA, which is in line with other studies that did not show significant effects of supplemental, dietary intakes or blood levels of n-3 PUFA on fasting glucose or diabetes risk (47). Interestingly, based on the aforementioned findings, it seems that potential beneficial effects of PUFA in a diet may largely be due to n-6 PUFA intake, especially LA. On the other side, although some have raised concern over the conversion of LA to AA (48), the evidence suggests that this conversion is tightly regulated and not dependent on the level of dietary LA intake (49). In a pooled analysis of 20 prospective cohort studies, AA biomarker levels

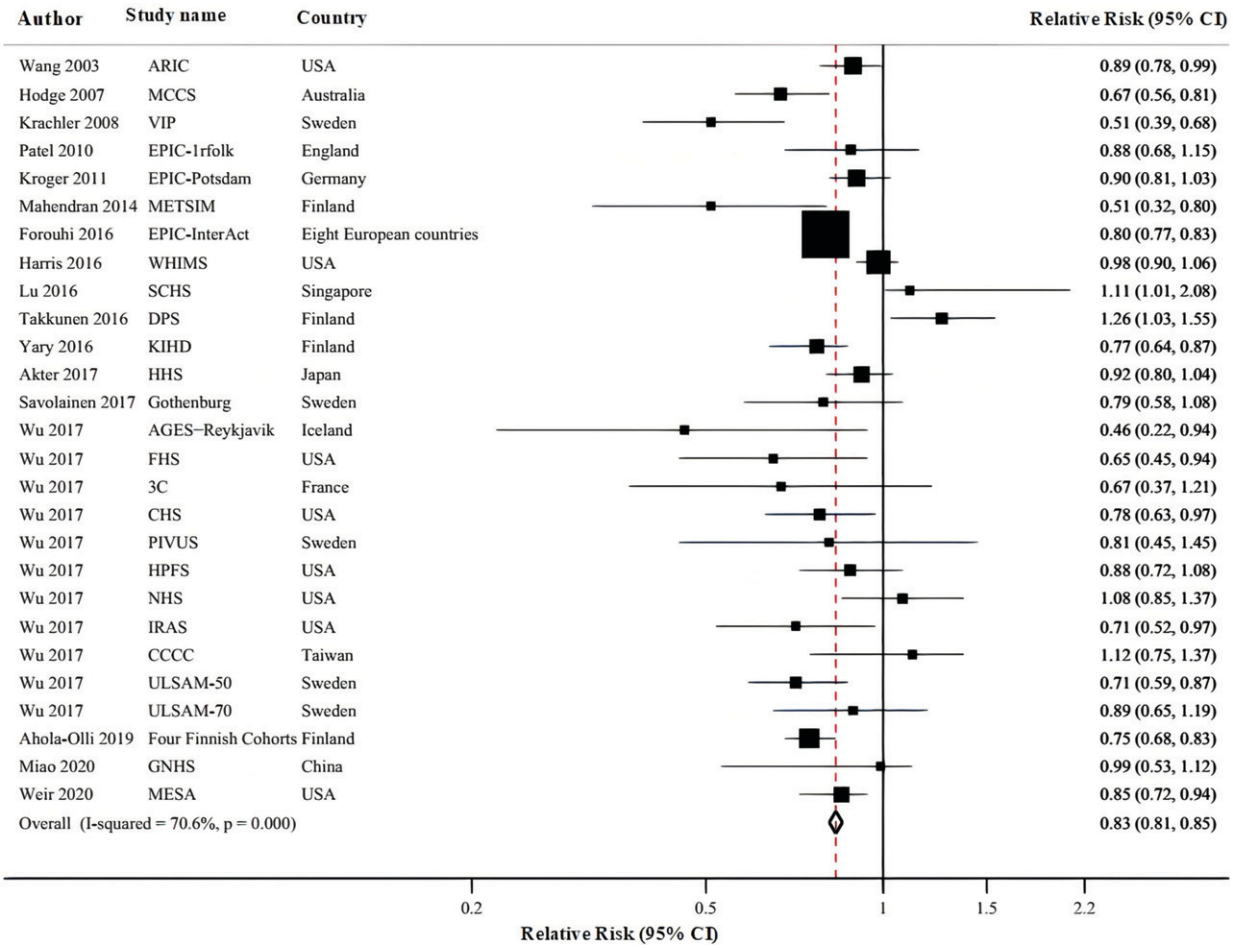

Figure 3—Summary relative risk and 95% CIs of T2DM for each SD increment in LA biomarkers concentrations. AGES, Age, Gene/Environment Susceptibility; ARIC, Atherosclerosis Risk in Communities; CCCC, Chin-Shan Community Cardiovascular Cohort Study; CHS, Cardiovascular Health Study; DPS, Diabetes Prevention Study; EPIC, European Prospective Investigation into Cancer and Nutrition; FHS, Framingham Heart Study; GNHS, Guangzhou Nutrition and Health Study; HHS, Hitachi Health Study; IRAS, Insulin Resistance Atherosclerosis Study; KIHD, Kuopio Ischemic Heart Disease Risk Factor; MCCS, Melbourne Collaborative Cohort Study; MESA, Multi-Ethnic Study of Atherosclerosis; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; SCHS, Singapore Chinese Health Study; ULSAM, Uppsala Longitudinal Study of Adult Men; VIP, Västerbotten Intervention Programme; WHIMS, Women's Health Initiative Memory Study.

were not associated with the risk of T2DM (19). In addition, according to a systematic review and meta-analysis, higher biomarker levels of AA were associated with a lower incidence of coronary heart disease (50). Moreover, dietary LA and AA had no significant effects on inflammatory markers, platelet function, or immune activation (51). Therefore, together with the findings as mentioned above from different studies, our findings provide further supportive evidence that high levels of LA do not have harmful effects.

While metabolism influences circulating and adipose tissue levels of LA, these biomarkers remain valuable markers of diet, as they increased in a dose-response manner to dietary LA (34). Our findings of a 15% lower risk of T2DM for each 1 SD increment in LA levels are in line with a previous pooled de novo analysis of 20 prospective cohort studies, in which each interquartile range increment in LA biomarkers was associated with 35% reduction in incident T2DM (19). While no well-established mechanisms are available explaining the observed inverse associations between dietary and biomarker levels of LA and risk of diabetes, some possible explanations are as follows: incorporation of unsaturated fats like LA in the cell membrane might improve cell fluidity and functions such as GLUT translocation, cell signaling, ion permeability, and insulin receptor binding and affinity, which could lead to higher insulin sensitivity (52). LA can also play a role in the regulation of genes like *SREBP1* (sterol regulatory element–binding transcription factor 1), which balances fatty acid synthesis and oxidation, as a suggested mechanism for LA to reduce hepatic fat contents (53). In addition, a diet with high levels of LA was effective in improving abdominal fat distribution and insulin sensitivity (54). Another possible mechanism could be that higher LA intake leads to lower intake of other macronutrients. It was shown that substitution of PUFAs for SFAs or carbohydrates was associated with a lower risk for T2DM (55). This highlights the importance of the quality of dietary fats for the prevention of diabetes. With regard to the LA intake, pooling data from three large prospective studies, including Nurses' Health Study (NHS), NHSII, and HPFS, indicated that substitution of 5% of total calories from SFAs, *trans* fats, and carbohydrates with

calories from LA was associated with a 14%, 17%, and 9% lower risk of T2DM, respectively (11). However, there were not enough studies to assess different substitutions in our meta-analysis. Since this meta-analysis was performed on observational studies, the causality of the observed associations is unclear; therefore, more experimental, molecular, and clinical studies can help to elucidate a more comprehensive assessment. Nevertheless, some important points should be noted regarding the metabolism of LA in interpreting the results of this study. As we know, LA converts to the long-chain PUFAs such as γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA), and AA through the function of two key enzymes, Δ-5 and Δ-6 desaturases. Both n-3 and n-6 PUFAs usually compete for these enzymes, and it is well established that n-3 PUFAs have a greater affinity for such enzymes (56). Several inconsistent results have been found regarding LA by-products, GLA and DGLA, and disease risk. Some studies showed high activity of Δ-6 desaturase and increased ratio of GLA to LA were associated with increased risk of T2DM, while the greater activity of Δ-5 desaturase and increased ratio of AA to DGLA were linked to a reduced risk (19,57). In contrast, an inverse association has been found between circulating level of DGLA and the odds of diabetic nephropathy (58). Some studies showed modifying effects of variants in the *FADS1*/*FADS2*/*FADS3* gene cluster in response to dietary LA for some cardiovascular risk factors (6,59). In contrast, a study did not confirm the modulating effect of the *FADS1* rs174547 variant related to LA and AA levels and risk of T2DM (58). Therefore, because of these discrepancies in the metabolism of LA and the importance of genetic variants, caution should be taken in recommending PUFAs to people generally.

We found some important points in the subgroup analyses that need to be considered in future studies. Our analyses indicated that concentrations of LA biomarkers in all compartments, except for adipose tissue, were significantly associated with a lower risk of T2DM, showing a class effect of LA rather than the primacy of any single compartment. Only one study evaluated LA biomarkers in the adipose tissue, and there is a need for further studies in this area. We also found that LA dietary intake and biomarkers were more protective against T2DM incidence in men than women. This might be explained by the LA's conversion rate to other n-6 PUFAs and AA, which is lower in men than in women (60). In addition, a more robust inverse association was seen for studies that used repeated FFQs, which demonstrates the fact that repeated measurements of diet may reduce random errors and can quickly reflect long-term dietary intakes (19).

Strength and Limitations

Our study has several strengths. We included a large number of prospective cohort studies from different continents, including the U.S., Europe, and Asia, which increases the generalizability of the findings. The analyses included a large number of participants and cases, which increased statistical power to detect significant associations and facilitated the investigation of several effect modifiers. We also conducted linear and nonlinear dose-response analyses to clarify the strength and shape of the observed associations. Our results regarding the associations between LA biomarkers levels and risk of incident T2DM did not change in the sensitivity analyses, suggesting the robustness of findings. Other advantages included examination of the associations between both dietary and biomarkers of LA and risk of incident T2DM. Finally, the use of NutriGrade score, that considers several aspects of the meta-analysis quality, is another strength. However, some limitations must be noted. Although we considered the levels of LA biomarkers in all compartments, only one study detected LA in the adipose tissue. The fatty acid composition of adipose tissue is the gold standard for representing dietary fatty acids, and further studies are therefore needed of adipose tissue LA and T2DM risk. While we considered both dietary and biomarker levels of LA, the metabolism of LA biomarkers was not considered in the current study. The biomarker levels of LA were only assessed at baseline of the included studies, and their changes over time might lead to misclassification, which would tend to attenuate the observed associations. In addition, dose-response analysis in light of the usually semiquantitative nature of dietary intakes might have led to under- or overestimating relations, and we had to use different approximations that might affect the results. Therefore, it is possible that measurement errors have impacted the shape of the dose-response curves. While some covariates were adjusted for in primary studies, effect of residual confounding due to other unmeasured or imprecisely measured covariates cannot be excluded. Most studies had applied FFQs, with their own limitations, to assess dietary intakes. Different methods were used to ascertain T2DM, leading to some outcome misclassification and underestimation of the actual associations. Finally, the results of dietary LA intake were not robust in influence analyses and were dependent on the HPFS study; thus, further studies are needed before firm conclusions can be drawn regarding the association between dietary LA intake and T2DM.

Conclusion

The present meta-analysis of 31 prospective cohort studies showed that high intake of dietary LA and elevated concentrations of LA in the body were both significantly associated with a lower risk of T2DM. In addition, the association between LA and reduced diabetes risk was significant at an intake of 5.5–7.0% of energy from LA. Although further studies are needed before these findings can be considered conclusive, these findings suggest that dietary recommendations to increase LA intake may reduce the risk of diabetes.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.
Author Contributions. S.M.M. and A.E. contributed to the study conception and literature search, screened the articles, contributed to data extraction and data analysis, and wrote the manuscript. Y.J. and E.K. contributed to the literature search, data extraction, and manuscript drafting. D.A. and B.L. contributed to manuscript drafting. D.M. and W.C.W. contributed to the study conception and data analysis. All authors acknowledge full responsibility for the analyses and interpretation of the report and have read and approved the final manuscript. A.E. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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