Associations Between Linoleic Acid Intake and Incident Type 2 Diabetes Among U.S. Men and Women

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EPIDEMIOLOGY/HEALTH SERVICES RESEARCH

OBJECTIVE

To investigate the association between intakes of n-6 polyunsaturated fatty acids (PUFAs) and type 2 diabetes risk in three prospective cohort studies of U.S. men and women.

RESEARCH DESIGN AND METHODS

We followed 83,648 women from the Nurses' Health Study (NHS) (1980–2012), 88,610 women from NHSII (1991–2013), and 41,771 men from the Health Professionals Follow-Up Study (HPFS) (1986–2012). Dietary data were collected every 2–4 years by using validated food-frequency questionnaires. Self-reported incident diabetes, identified biennially, was confirmed by using a validated supplementary questionnaire.

RESULTS

During 4.93 million person-years of follow-up, 18,442 type 2 diabetes cases were documented. Dietary n-6 PUFAs accounted for 4.4–6.8% of total energy, on average, and consisted primarily of linoleic acid (LA) (≥98%). In multivariate-adjusted models, hazard ratios (95% CIs) of type 2 diabetes risk comparing extreme n-6 PUFA quintiles (highest vs. lowest) were 0.91 (0.85, 0.96) (P_{trend} = 0.002) for total n-6 PUFAs and 0.92 (0.87, 0.98) (P_{trend} = 0.01) for LA. In an isocaloric substitution model, diabetes risk was 14% (95% CI 5%, 21%) (P = 0.002) lower when LA isocalorically replaced saturated fats (5% of energy), 17% (95% CI 9%, 24%) (P < 0.001) lower for *trans* fats (2% of energy), or 9% (95% CI 17%, 0.1%) (P = 0.047) lower for carbohydrates (5% of energy). Replacing n-3 PUFAs or monounsaturated fats with LA was not significantly associated with type 2 diabetes risk.

CONCLUSIONS

Our study provides additional evidence that LA intake is inversely associated with risk of type 2 diabetes, especially when replacing saturated fatty acids, *trans* fats, or carbohydrates.

Polyunsaturated fatty acids (PUFAs) in the American diet are mostly n-6 PUFAs, particularly linoleic acid (LA) (1). Given the compelling evidence supporting the benefits of dietary n-6 PUFAs in coronary heart disease, LA is recommended as a healthy energy source for maintaining long-term health (2). However, the effects of n-6 PUFAs on type 2 diabetes risk remain unclear (3).

To date, large clinical trials that examine the effects of n-6 PUFA intake on type 2 diabetes risk are lacking, and findings from prospective cohort studies are mixed

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(4–12). Most early investigations assessed diet only once and did not capture potential changes in food composition over time. In addition, the association between dietary n-6 PUFAs and type 2 diabetes risk has not been evaluated explicitly with respect to other macronutrients in an isocaloric context (i.e., by a substitution model) (13). Recent studies focusing on fatty acid biomarkers showed that proportions of LA in blood or adipose tissue were independently associated with lower risk for type 2 diabetes (14,15). Given the modest correlations between n-6 PUFA biomarkers and intake (16), however, the extent to which these associations can be ascribed to the intake of specific fatty acids is debatable.

In this study, we investigated the association between intake of n-6 PUFAs, including LA and arachidonic acid (AA), and type 2 diabetes risk in three large prospective cohort studies of American men and women. Specifically, we estimated type 2 diabetes risk when LA replaces other macronutrients, especially saturated fatty acids (SFAs), *trans* fats, and carbohydrates, in an isocaloric substitution model.

RESEARCH DESIGN AND METHODS

Study Populations

The Nurses' Health Study (NHS), NHSII, and Health Professionals Follow-Up Study (HPFS) are ongoing prospective cohort studies. The NHS includes 121,700 female registered nurses who were aged 30–55 years when enrolled in 1976 (17); the NHSII includes 116,671 female registered nurses who were aged 25–44 years when enrolled in 1989; and the HPFS consists of 51,529 male health professionals who were aged 40–75 years when enrolled in 1986. Participants in all studies have been followed through questionnaires, mailed biennially, in order to collect and update information on lifestyles, health-related behaviors, and medical histories. The institutional review boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health approved the study protocol. The completion of self-administered questionnaires was considered to imply informed consent.

Among participants who completed baseline food frequency questionnaires (FFQs) (NHS in 1980, n = 92,468; NHSII in 1991, n = 97,605; and HPFS in 1986, n = 51,530), we excluded individuals if they *1*) reported a diagnosis of diabetes, cardiovascular disease, or cancer at baseline (5,313 participants in NHS, 5,130 in NHSII, and 6,926 in HPFS); *2*) had daily energy intake outside of a normal range ($<$500 or $>$3,500 kcal/day for NHS and $<$800 or $>$4,200 kcal/day for the HPFS) or missing dietary fat data (402 participants in NHS, 2,164 in NHSII, and 1,282 in HPFS); *3*) had a missing date for diagnosis of type 2 diabetes (2,206 participants in NHS, 1,025 in NHSII, and 402 in HPFS); or *4*) completed only the baseline questionnaire or were missing age at baseline (900 participants in NHS, 676 in NHSII, and 1,149 in HPFS). This left 83,647 participants from NHS, 88,610 from NHSII, and 41,771 from HPFS for the current analysis.

Ascertainment of Diet and n-6 PUFA Intake

In 1980, 1984, and 1986, and every 4 years thereafter, NHS participants completed a validated FFQ to assess their habitual diet over the preceding year. A similar FFQ has been sent to NHSII and HPFS participants every 4 years since 1991 and 1986, respectively (18). The questionnaires inquire about how often, on average, participants had consumed specific foods and the types of fats, oils, and margarines they used during cooking and at the table in the previous year. Nutrient intake was calculated on the basis of U.S. Department of Agriculture and Harvard University food composition databases, which have been updated over time to include new food items and reflect changes in food composition. Multiple validation studies have demonstrated the validity of FFQ assessments of dietary fats (19–21). In the most recent validation study using NHS participants, deattenuated Spearman correlation coefficients of energy-adjusted nutrient intake assessments by FFQs versus multiple 7-day diet records were 0.55 ($P < 0.001$) for LA and 0.52 ($P < 0.001$) for AA.

We calculated cumulative averages of intake of nutrients or foods based on valid assessments from baseline to the end of follow-up in order to better represent long-term diet. To reduce the possibility of reverse causation bias, we stopped updating dietary information if a participant reported a diagnosis of cardiovascular disease or cancer. Nutrient intakes were adjusted for total energy by using the residual method.

Ascertainment of Incident Type 2 Diabetes

Participants who reported a diagnosis of diabetes were mailed a supplementary questionnaire regarding diagnosis date, symptoms, diagnostic tests, and hypoglycemic therapy. The diagnosis of type 2 diabetes was considered confirmed if at least one of the following was reported on the supplementary questionnaire, according to the National Diabetes Data Group criteria (22): one or more classic symptoms (excessive thirst, polyuria or frequent urination, weight loss, hunger) plus fasting plasma glucose $\geq$7.8 mmol/L or random plasma glucose $\geq$11.1 mmol/L; two or more elevated plasma glucose concentrations on different occasions (fasting glucose $\geq$7.8 mmol/L, random plasma glucose $\geq$11.1 mmol/L, plasma glucose $\geq$11.1 mmol/L after $\geq$2 h as shown by an oral glucose tolerance test, or all three) in the absence of symptoms; or treatment with hypoglycemic medication. The diagnostic criteria changed in June 1998, and fasting plasma glucose of 7.0 mmol/L (instead of 7.8 mmol/L) was considered to be the threshold for the diagnosis of diabetes according to the American Diabetes Association criteria. In validation studies, 61 of 62 randomly selected cases of type 2 diabetes (98%) in NHS—a number that was confirmed by using the supplementary questionnaire—were reconfirmed after an endocrinologist blinded to the disease status of patients reviewed medical records (23); in the HPFS, 57 of 59 cases (97%) were reconfirmed (24).

Statistical Analysis

Macronutrients were analyzed as percentages of energy by dividing the energy from a specific macronutrient by total energy intake. Spearman correlation coefficients among dietary fatty acids were calculated by using data assessed at baseline, the midpoint of follow-up, and the last FFQ before the end of follow-up. Person-years of follow-up for each participant were calculated from the return date of the baseline questionnaire to the date when the participant received a diagnosis of type 2 diabetes, the date of their death, or the end of follow-up, whichever occurred first. Hazard ratios (HRs) and 95% CIs of incident type 2 diabetes were estimated by using a time-dependent Cox proportional hazards regression model in each cohort, with follow-up

Table 1—Age-standardized participant characteristics at baseline according to quintiles of total n-6 PUFA intake

	NHS, 1980			NHSII, 1991			HPFS, 1986		
	Q1 (*n* = 16,729)	Q3 (*n* = 16,730)	Q5 (*n* = 16,729)	Q1 (*n* = 17,722)	Q3 (*n* = 17,724)	Q5 (*n* = 17,722)	Q1 (*n* = 8,354)	Q3 (*n* = 8,355)	Q5 (*n* = 8,354)
n-6 PUFAs, % energy	2.55 ± 0.42	4.17 ± 0.20	6.70 ± 1.25	3.31 ± 0.42	4.76 ± 0.18	6.91 ± 1.13	3.42 ± 0.49	5.14 ± 0.20	7.65 ± 1.34
Age, years	47.6 ± 7.1	45.8 ± 7.2	45.0 ± 7.1	35.7 ± 4.7	36.1 ± 4.6	36.6 ± 4.6	54.6 ± 9.7	52.7 ± 9.5	52.6 ± 9.2
Caucasian race	97	97	98	94	96	97	94	95	96
Alcohol intake, g/day	9.4 ± 14.1	5.9 ± 9.5	4.7 ± 7.9	3.7 ± 7.9	3.1 ± 5.7	2.7 ± 5.0	15.3 ± 20.1	11.1 ± 14.2	8.4 ± 11.7
Current smoking	32	27	28	13	12	12	11	10	9
Physical activity, MET h/week	15.9 ± 23.6	14.0 ± 18.4	12.6 ± 18.5	25.0 ± 32.0	20.5 ± 26.3	18.2 ± 24.0	23.7 ± 32.0	21.1 ± 29.3	19.5 ± 26.6
BMI, kg/m^2	24.1 ± 4.2	24.4 ± 4.3	24.3 ± 4.6	24.0 ± 4.9	24.5 ± 5.2	24.9 ± 5.6	24.8 ± 4.8	25.0 ± 4.9	24.9 ± 4.8
Family history of diabetes	24	25	25	16	16	16	18	19	18
Multivitamin use	36	34	32	48	44	40	43	42	41
Any use of hormone after menopause	15	15	15	3	3	3	–	–	–
Current use of oral contraceptives	–	–	–	11	10	11	–	–	–
Hypertension	17	15	14	6	6	6	20	19	18
Hypercholesterolemia	5	5	5	14	15	14	10	10	11
Total energy, kcal	1,535 ± 499	1,590 ± 489	1,559 ± 514	1,758 ± 540	1,802 ± 555	1,779 ± 549	1,955 ± 619	2,011 ± 614	2,032 ± 643
Total fats, % energy	34.3 ± 8.9	38.7 ± 7.0	43.2 ± 6.7	25.4 ± 5.2	30.4 ± 4.4	35.2 ± 4.8	25.3 ± 6.3	30.6 ± 4.7	35.6 ± 5.1
PUFAs, % energy	3.06 ± 0.46	4.72 ± 0.23	7.31 ± 1.27	3.80 ± 0.47	5.34 ± 0.23	7.68 ± 1.26	4.34 ± 1.01	5.77 ± 0.74	7.94 ± 1.44
n-3 PUFAs, % energy	0.52 ± 0.11	0.56 ± 0.09	0.61 ± 0.13	0.48 ± 0.15	0.58 ± 0.13	0.77 ± 0.22	0.52 ± 0.19	0.63 ± 0.16	0.79 ± 0.25
LA, % energy	2.46 ± 0.42	4.08 ± 0.20	6.61 ± 1.25	3.24 ± 0.42	4.68 ± 0.18	6.83 ± 1.13	3.35 ± 0.49	5.05 ± 0.21	7.57 ± 1.34
AA, mg/day	136 ± 60	154 ± 64	153 ± 70	148 ± 780	163 ± 78	162 ± 81	156 ± 74	177 ± 76	178 ± 83
Trans fats, % energy	1.62 ± 0.47	2.22 ± 0.54	2.87 ± 0.85	1.31 ± 0.50	1.67 ± 0.57	1.89 ± 0.65	1.02 ± 0.45	1.31 ± 0.48	1.42 ± 0.54
SFAs, % energy	15.4 ± 4.3	15.7 ± 3.4	15.5 ± 3.2	10.3 ± 2.7	11.3 ± 2.3	11.9 ± 2.2	10.2 ± 3.4	11.2 ± 2.6	11.5 ± 2.4
Cis-MUFAs, % energy	14.2 ± 4.3	16.1 ± 3.5	17.5 ± 3.3	10.0 ± 2.3	12.1 ± 2.0	13.7 ± 2.2	10.1 ± 2.7	12.3 ± 2.1	14.3 ± 2.5
Protein, % energy	19.9 ± 4.5	19.2 ± 3.7	18.0 ± 3.5	19.5 ± 4.0	19.5 ± 3.3	18.7 ± 3.3	18.5 ± 3.7	18.6 ± 3.3	18.1 ± 3.2
Carbohydrates, % energy	39.8 ± 10.8	38.8 ± 8.7	38.3 ± 8.5	54.1 ± 8.3	49.6 ± 6.7	46.2 ± 6.6	50.3 ± 10.2	46.6 ± 7.5	43.9 ± 7.5
Fruits and vegetables, servings/day	4.63 ± 2.44	4.03 ± 1.90	3.39 ± 1.69	4.71 ± 3.16	4.32 ± 2.55	4.06 ± 2.41	5.99 ± 3.29	5.34 ± 2.63	4.97 ± 2.44
Cereal fiber, g/day	2.11 ± 1.51	2.50 ± 1.47	2.74 ± 1.53	5.53 ± 3.50	5.66 ± 2.95	5.62 ± 2.94	5.97 ± 4.16	5.80 ± 3.65	5.68 ± 3.82

Data are mean (SD) or percentage and are standardized to the age distribution of the study population.

Table 2—Spearman correlations among specific dietary fats at baseline, midpoint, and end of follow-up

	n-6 PUFAs	LA	AA	n-6 PUFAs	LA	AA	n-6 PUFAs	LA	AA
	NHS, 1980			NHSII, 1991			HPFS, 1986		
% Energy, mean ± SD	4.37 ± 1.54	4.28 ± 1.54	0.09 ± 0.03	4.92 ± 1.34	4.84 ± 1.34	0.08 ± 0.04	5.31 ± 1.58	5.23 ± 1.58	0.08 ± 0.03
n-3 PUFAs	0.28*	0.28*	0.31*	0.58*	0.57*	0.26*	0.47*	0.46*	0.32*
SFAs	0.003	<0.001	0.18*	0.23*	0.24*	−0.02*	0.18*	0.18*	0.06*
Trans fats	0.60*	0.60*	−0.06*	0.34*	0.35*	−0.09*	0.29*	0.29*	−0.09*
MUFAs	0.29*	0.29*	0.31*	0.53*	0.53*	0.06*	0.52*	0.52*	0.15*
	NHS, 1994			NHSII, 1999			HPFS, 1998		
% Energy, mean ± SD	4.77 ± 1.42	4.70 ± 1.41	0.07 ± 0.03	5.55 ± 1.62	5.49 ± 1.62	0.06 ± 0.03	5.46 ± 1.53	5.39 ± 1.52	0.07 ± 0.03
n-3 PUFAs	0.47*	0.47*	0.22*	0.67*	0.67*	0.21*	0.44*	0.44*	0.25*
SFAs	0.27*	0.27*	0.17*	0.34*	0.33*	0.22*	0.31*	0.31*	0.14*
Trans fats	0.34*	0.34*	0.02*	0.30*	0.29*	0.13*	0.31*	0.32*	−0.004
MUFAs	0.52*	0.51*	0.20*	0.53*	0.53*	0.25*	0.55*	0.55*	0.16*
	NHS, 2010			NHSII, 2011			HPFS, 2010		
% Energy, mean ± SD	6.44 ± 2.04	6.36 ± 2.03	0.08 ± 0.04	6.76 ± 1.85	6.67 ± 1.85	0.09 ± 0.05	6.28 ± 1.95	6.19 ± 1.95	0.09 ± 0.05
n-3 PUFAs	0.65*	0.64*	0.34*	0.55*	0.54*	0.31*	0.60*	0.59*	0.42*
SFAs	0.12*	0.12*	−0.05*	0.11*	0.11*	−0.03*	0.19*	0.19*	0.001
Trans fats	−0.16*	−0.15*	−0.18*	0.05*	0.05*	−0.08*	−0.21*	−0.20*	−0.22*
MUFAs	0.46*	0.46*	0.14*	0.56*	0.56*	0.16*	0.51*	0.51*	0.12*

*$P < 0.001$.

duration as the time scale. Regression models were stratified jointly by age in months and calendar year in order to better control for their confounding and possible interactions; they were further adjusted for ethnicity, family history of diabetes, menopausal status and hormone use after menopause (NHS and NHSII participants only), oral contraceptive use (NHSII participants only), multivitamin use, smoking status, alcohol intake, physical activity, baseline hypertension, baseline hypercholesterolemia, updated BMI, total energy intake, and intake of fruits and vegetables. We further adjusted for percentages of energy from total fats, *trans* fats, *cis*-monounsaturated fatty acids (*cis*-MUFAs), and other PUFAs to estimate the main associations of n-6 PUFAs with type 2 diabetes compared with SFAs. We tested for linear trend by modeling the median value of n-6 PUFAs in each category as a continuous variable. We used the likelihood ratio test to examine proportional hazards assumption by fitting a model that included interaction terms between n-6 PUFAs and duration of follow-up; the assumption was unlikely to be violated ($P > 0.05$ for all tests).

We estimated type 2 diabetes risk when LA isocalorically replaces energy from SFAs, *trans* fats, *cis*-MUFAs, or carbohydrates. Dietary covariates included total calories, total fats (for the fat-fat substitution models only), AA, and all other macronutrients except the one being replaced. In such models, regression coefficients for LA can be interpreted as the estimated effect of isocalorically substituting LA for the specific nutrient while holding constant the intake of total energy and other macronutrients. We analyzed the three cohorts separately, and then we pooled the results in a fixed-effect model when the *P* value for heterogeneity was >0.05.

We performed several sensitivity analyses to examine the robustness of findings from the substitution analyses: *1*) adjusting for hypertension and hypercholesterolemia diagnosed during follow-up, *2*) using the averages of nutrient intake from the two most recent FFQ assessments for each 4-year follow-up period, or *3*) using baseline dietary data only. Statistical analyses were performed by using SAS 9.4 (SAS Institute, Cary, NC). All *P* values were two-sided; statistical significance was defined as $P < 0.05$.

RESULTS

Baseline characteristics of study participants are presented in Table 1. Those with higher total n-6 PUFA intake were more likely to be Caucasian but less likely to smoke, to drink alcohol, to engage in physical activities, or to use multivitamins. NHS and HPFS participants who consumed more n-6 PUFAs were younger and less likely to have hypertension, whereas NHSII participants who consumed more n-6 PUFAs were older and heavier. In terms of dietary factors, participants with higher n-6 PUFA intake also consumed more MUFAs and *trans* fats, and fewer carbohydrates, proteins, fruits, and vegetables.

As shown in Table 2, the primary dietary n-6 PUFA was LA (≥98%); AA intake contributed only 2% of total n-6 PUFAs and 0.07–0.09% of total energy. LA intake ranged from 4.3% of total energy (NHS in 1980) to 6.7% during the follow-up (NHSII in 2011). The Spearman correlation coefficients between LA and *trans* fats were between 0.29 (HPFS in 1986) and 0.60 (NHS in 1980) in early follow-up cycles and were substantially attenuated in later cycles (*r* between 0.05 [NHSII in 2011] and −0.20 [HPFS in 2010]). Top

Table 3—Associations between total n-6 PUFAs and LA and type 2 diabetes risk in NHS, NHSII, and HPFS*

	Quintiles of fatty acid intake (% energy)					
	Q1	Q2	Q3	Q4	Q5	*P* for trend
n-6 PUFAs						
NHS						
Median (range)	2.62 (0.66, 3.09)	3.47 (3.09, 3.82)	4.16 (3.82, 4.53)	4.95 (4.53, 5.49)	6.32 (5.49, 20.8)	
Cases/person-years	1,800/447,983	1,780/448,767	1,922/448,995	1,882/448,948	1,991/448,694	
Model 1	1	1.01 (0.94, 1.07)	1.10 (1.03, 1.17)	1.08 (1.01, 1.15)	1.16 (1.08, 1.23)	<0.001
Model 2	1	0.96 (0.90, 1.03)	1.02 (0.95, 1.09)	0.97 (0.90, 1.03)	1.01 (0.95, 1.08)	0.71
Model 3	1	0.95 (0.89, 1.02)	1.00 (0.93, 1.07)	0.94 (0.87, 1.01)	0.97 (0.90, 1.06)	0.53
NHSII						
Median (range)	3.41 (0.87, 3.84)	4.17 (3.84, 4.46)	4.76 (4.46, 5.08)	5.43 (5.08, 5.88)	6.60 (5.88, 24.77)	
Cases/person-years	953/355,884	996/357,336	1,061/358,269	1,189/358,491	1,261/358,163	
Model 1	1	1.04 (0.95, 1.13)	1.09 (0.99, 1.19)	1.19 (1.09, 1.30)	1.22 (1.12, 1.33)	<0.001
Model 2	1	0.96 (0.88, 1.05)	0.95 (0.86, 1.03)	0.99 (0.91, 1.08)	0.98 (0.90, 1.07)	>0.99
Model 3	1	0.93 (0.85, 1.02)	0.91 (0.82, 1.00)	0.94 (0.85, 1.04)	0.91 (0.80, 1.02)	0.21
HPFS						
Median (range)	3.53 (0.94, 4.04)	4.43 (4.04, 4.79)	5.13 (4.79, 5.50)	5.91 (5.50, 6.43)	7.24 (6.43, 21.1)	
Cases/person-years	691/179,560	688/181,090	756/181,399	743/181,415	729/180,777	
Model 1	1	1.00 (0.90, 1.11)	1.12 (1.01, 1.24)	1.09 (0.98, 1.21)	1.07 (0.96, 1.19)	0.11
Model 2	1	0.94 (0.84, 1.04)	1.03 (0.92, 1.14)	0.98 (0.88, 1.09)	0.97 (0.87, 1.08)	0.83
Model 3	1	0.86 (0.77, 0.96)	0.90 (0.80, 1.01)	0.82 (0.73, 0.92)	0.74 (0.65, 0.85)	<0.001
Pooled†						
Model 2	1	0.96 (0.91, 1.00)	1.00 (0.95, 1.05)	0.98 (0.93, 1.02)	0.99 (0.95, 1.04)	0.87
Model 3	1	0.93 (0.88, 0.97)	0.95 (0.91, 1.00)	0.92 (0.87, 0.97)	0.91 (0.85, 0.96)	0.001
LA						
NHS						
Median (range)	2.54 (0.65, 3.01)	3.39 (3.01, 3.73)	4.07 (3.73, 4.44)	4.86 (4.44, 5.40)	6.23 (5.40, 20.8)	
Cases/person-years	1,800/447,964	1,792/448,793	1,907/448,907	1,898/449,043	1,978/448,681	
Model 1	1	1.01 (0.95, 1.08)	1.09 (1.02, 1.16)	1.09 (1.02, 1.16)	1.15 (1.08, 1.22)	<0.001
Model 2	1	0.97 (0.91, 1.04)	1.02 (0.95, 1.08)	0.98 (0.92, 1.05)	1.01 (0.95, 1.08)	0.65
Model 3	1	0.96 (0.89, 1.02)	0.99 (0.93, 1.07)	0.96 (0.89, 1.03)	0.98 (0.91, 1.06)	0.70
NHSII						
Median (range)	3.33 (0.86, 3.76)	4.08 (3.76, 4.38)	4.68 (4.38, 4.99)	5.35 (4.99, 5.80)	6.51 (5.80, 24.7)	
Cases/person-years	957/355,840	1,012/357,413	1,057/358,191	1,175/358,537	1,259/358,161	
Model 1	1	1.05 (0.96, 1.14)	1.08 (0.99, 1.18)	1.17 (1.08, 1.28)	1.22 (1.12, 1.33)	<0.001
Model 2	1	0.97 (0.89, 1.07)	0.94 (0.86, 1.03)	0.99 (0.91, 1.08)	0.98 (0.90, 1.07)	0.93
Model 3	1	0.95 (0.87, 1.04)	0.91 (0.82, 1.00)	0.94 (0.85, 1.04)	0.93 (0.82, 1.05)	0.33
HPFS						
Median (range)	3.45 (0.77, 3.96)	4.35 (3.96, 4.71)	5.05 (4.71, 5.42)	5.83 (5.42, 6.35)	7.16 (6.35, 21.0)	
Cases/person-years	698/179,551	694/181,094	741/181,452	745/181,349	729/180,794	
Model 1	1	1.00 (0.90, 1.11)	1.08 (0.97, 1.20)	1.08 (0.97, 1.20)	1.06 (0.95, 1.17)	0.15
Model 2	1	0.94 (0.84, 1.05)	1.00 (0.90, 1.11)	0.98 (0.88, 1.09)	0.97 (0.87, 1.08)	0.80
Model 3	1	0.87 (0.78, 0.97)	0.88 (0.79, 0.99)	0.83 (0.74, 0.94)	0.77 (0.67, 0.88)	<0.001
Pooled						
Model 2	1	0.97 (0.92, 1.01)	0.99 (0.94, 1.04)	0.98 (0.94, 1.03)	0.99 (0.95, 1.04)	0.87
Model 3	1	0.94 (0.89, 0.98)	0.95 (0.90, 1.00)	0.93 (0.88, 0.98)	0.92 (0.87, 0.98)	0.005

*HRs and 95% CIs were calculated through the use of a Cox proportional hazards model. Model 1 was adjusted for age. Model 2 was adjusted for the model 1 variable as well as ethnicity (Caucasian, African American, Asian, or other ethnicity), smoking status (never, former, current [1–14, 15–24, or ≥25 cigarettes/day], or missing), alcohol intake (0.0, 0.1–4.9, 5.0–14.9, or >15.0 g/day in women and 0.0, 0.1–4.9, 5.0–29.9, or >30.0 g/day in men, or missing), family history of diabetes (yes/no), menopausal status and postmenopausal hormone use (premenopause, postmenopause [never, former, or current hormone use], or missing; for women only), physical activity (<3.0, 3.0–8.9, 9.0–17.9, 18.0–26.9, or ≥27.0 MET h/week or missing), multivitamin use (yes/no), baseline hypertension, baseline hypercholesterolemia, updated BMI (<23.0, 23.0–24.9, 25.0–29.9, 30.0–34.9, or >35.0 kg/m^2 or missing), total energy intake, and intake of fruits and vegetables. Model 3 was adjusted for the model 1 and 2 variables as well as total fats, *trans* fats, MUFAs, and other PUFAs. †Study estimates from three cohorts were pooled by using a fixed effects model.

food sources of LA included items containing plant oils, margarines (before the year 2000), and nuts, whereas AA mainly came from animal products such as poultry, fish, red meat, and eggs (Supplementary Table 1).

Total n-6 PUFAs were associated with a higher risk for type 2 diabetes in the age-adjusted model, but the associations were greatly attenuated after controlling for established type 2 diabetes risk factors, including BMI (Table 3, model 2). After further adjusting for total fats, MUFAs, *trans* fats, and n-3 PUFAs (Table 3, model 3), we observed an inverse association between n-6 PUFA intake and type 2 diabetes risk: HRs (95% CIs) for low to high quintiles were 1 (reference), 0.93 (0.88, 0.97), 0.95 (0.91, 1.00), 0.92 (0.87, 0.97), and 0.91 (0.85, 0.96) (P_{trend} = 0.001). The association between LA intake and type 2 diabetes risk in model 3 was similar after controlling for other fats: HRs (95% CIs) were 1 (reference), 0.94 (0.89, 0.98), 0.95 (0.89, 1.00), 0.93 (0.88, 0.98), and 0.92 (0.87, 0.98)

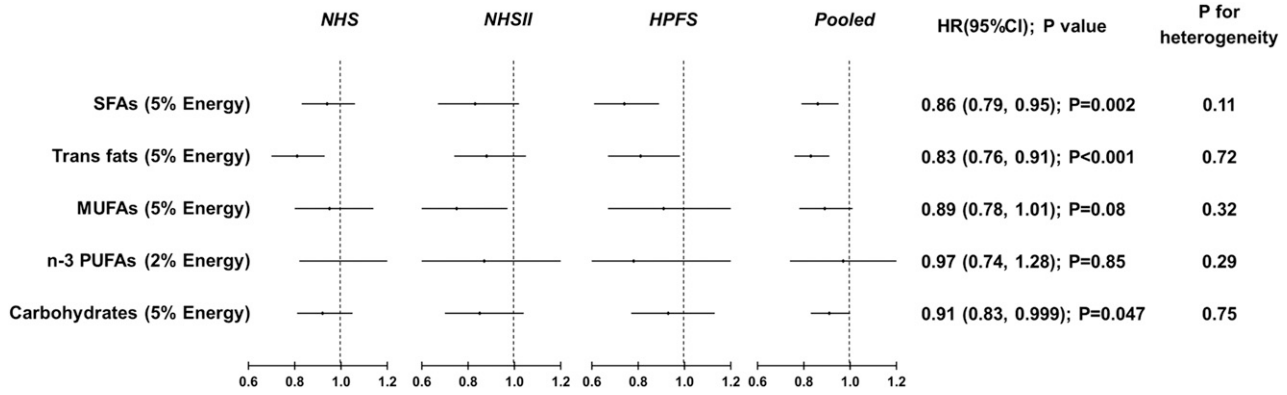

Figure 1—HRs for type 2 diabetes, with LA substituting for energy from other macronutrients. HRs were calculated in Cox proportional hazards model after adjusting for age, ethnicity (Caucasian, African American, Asian, and other ethnicity), smoking status (never, former, current [1–14, 15–24, or ≥25 cigarettes/day], or missing), alcohol intake (0.0, 0.1–4.9, 5.0–14.9, and >15.0 g/day in women; 0.0, 0.1–4.9, 5.0–29.9, and >30.0 g/day in men; or missing), family history of diabetes (yes/no), menopausal status and hormone use after menopause (premenopause, postmenopause hormone use [never, former, or current], or missing; for women only), physical activity (<3.0, 3.0–8.9, 9.0–17.9, 18.0–26.9, and ≥27.0 MET h/week or missing), multivitamin use (yes/no), baseline hypertension, baseline hypercholesterolemia, updated BMI (<23.0, 23.0–24.9, 25.0–29.9, 30–34.9, and >35.0 kg/m^2 or missing), total energy intake, and intake of fruits and vegetables. For fat-fat substitution, we further adjusted for other fats and total fats; for carbohydrate substitution, we further adjusted for energy from protein. Study estimates from the three cohorts were pooled by using a fixed-effects model. Black dots indicate point estimates; the horizontal lines represent the 95% CIs; and the vertical dashed lines represent the reference lines for an HR of 1.

(P_{trend} = 0.005) for low to high quintiles of LA intake.

Intake of AA was positively associated with type 2 diabetes risk in the age-adjusted model (Supplementary Table 2), and the associations were attenuated after adjusting for established type 2 diabetes risk factors and other dietary fats. We further adjusted for major sources of AAs to explore whether these factors could explain the associations. When comparing extreme AA quintiles (low vs. high), HRs (95% CIs) were 1.22 (1.16, 1.29) after controlling for red meat, 1.23 (1.17, 1.30) for processed meat, 1.24 (1.17, 1.31) for poultry, and 1.35 (1.26, 1.44) for fish.

Figure 1 presents estimated type 2 diabetes risk when LA was modeled to specifically replace other macronutrients. Isocalorically replacing energy from SFAs with that from LA (5% of energy) was associated with a 14% (HR 0.86 [95% CI 0.79, 0.95]) lower type 2 diabetes risk, and replacing *trans* fats with LA (2% of energy) was associated with a 17% (HR 0.83 [95% CI 0.76, 0.91]) lower risk. Type 2 diabetes risk was not significantly different when substituting energy from LA for that from MUFAs (HR 0.89 [95% CI 0.78, 1.01]; 5% of total energy) or total n-3 PUFAs (HR 0.97 [95% CI 0.74, 1.28]; 2% of total energy). When LA replaced 5% of energy from carbohydrates, the HR of type 2 diabetes was 0.91 (95% CI 0.83, 0.999) (P = 0.047). We observed no significant between-study heterogeneity in the substitution analyses (all P for heterogeneity >0.10).

In sensitivity analyses, findings from the substitution analyses were largely similar after further adjusting for incident hypertension and hypercholesterolemia (Supplementary Table 3), or when using the two most recent dietary assessments in order to measure long-term diet (Supplementary Table 4). The associations were attenuated, however, when only baseline dietary data, rather than all follow-up dietary data, were used (Supplementary Table 5).

CONCLUSIONS

In three cohort studies of U.S. men and women, we found that higher LA intake was associated with lower type 2 diabetes risk, especially when LA was modeled to isocalorically substitute for SFAs, *trans* fats, or carbohydrates. Accounting for <2% of total n-6 PUFAs, AA was associated with a higher type 2 diabetes risk. These associations were independent of established and potential risk factors of type 2 diabetes and remained in sensitivity analyses.

Existing studies have reported inconsistent results regarding the relation between LA intake and diabetes risk. In a previous analysis of HPFS, dietary LA showed inverse associations with type 2 diabetes risk only among younger and leaner participants (4), whereas several other studies found a null association between baseline LA intake and long-term diabetes risk (5–8). In our study, we found consistent inverse associations between n-6 PUFAs and risk for type 2 diabetes, especially when we modeled the effects of substituting LA for other macronutrients. Of note, the interrelationships between PUFAs and other macronutrients such as *trans* fat changed over time in the cohorts, probably because of changes in the amounts of these macronutrients in some foods during the extended follow-up (25,26). To account for these changes, we derived cumulative means from repeated assessments of diet and used other strategies to minimize reverse causation bias. Overall, the consistent findings in the three cohorts of men and women suggest that important heterogeneity by gender is unlikely.

This analysis suggests that LA, as the dominant PUFA in the diet, could be a healthy source of energy for preventing type 2 diabetes when compared with SFAs, *trans* fats, and carbohydrates. Our data extend earlier findings from the NHS that isocalorically substituting total PUFAs for SFAs, total carbohydrates, and particularly *trans* fats was associated with a lower risk for type 2 diabetes. Likewise, replacing SFAs or carbohydrates with total PUFAs (not including long-chain marine n-3 PUFAs) was associated with a lower risk for type 2 diabetes in the Iowa Women's Health Study. In an intervention study, replacing SFAs or carbohydrates with total PUFAs led to lower hemoglobin

A_{1c}, improved HOMA of insulin resistance, and a better acute insulin response (27). Abundant evidence supports the notions that SFAs and *trans* fats should be replaced by *cis*-unsaturated fats in order to improve blood lipid profile (28) and that replacing SFAs and refined carbohydrates with PUFAs and MUFAs may lead to a lower risk for coronary heart disease (29). This and our earlier study collectively indicate that the quality of dietary fats is not only an important determinant of cardiovascular disease risk but also of type 2 diabetes risk (30).

Potential mechanisms linking LA intake and type 2 diabetes have not been fully elucidated, but several possible explanations have been proposed. For example, incorporating unsaturated fats may improve cell membrane fluidity and functions, such as GLUT translocation, insulin receptor binding and affinity, cell signaling, and ion permeability, that collectively improve insulin sensitivity (31). LA might also affect the balance between fat oxidation and synthesis by regulating related gene expression (such as SREBP1) (32), which could explain the reduced hepatic fat contents of obese participants consuming a diet containing a large amount of LA during a 10-week intervention (33). A high-LA diet may reduce abdominal fat, which is an established risk factor for type 2 diabetes (34). On the other hand, intake of SFAs, *trans* fats, and refined carbohydrates may deteriorate insulin sensitivity and promote inflammation, both of which predispose to the onset of type 2 diabetes (26,35,36).

The positive association between AA intake and type 2 diabetes risk is consistent with previous findings from the E3N (Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale) study but not with those of the Melbourne Collaborative Cohort Study (MCCS). Consumption of AA is very low (mean $<$200 mg/day, $<$0.15% energy) when compared with that of other fatty acids. Thus, we must be cautious when interpreting these results. AA is consumed primarily through animal-based foods, including red meat, processed meat, fish, and poultry, which do not share common associations with type 2 diabetes risk (37). In our analysis, further adjustment for intakes of red meat, processed meat, fish, and poultry did not significantly change the results. AA is known to be a precursor of proinflammatory eicosanoids that might promote the pathogenesis of type 2 diabetes, although anti-inflammatory eicosanoids derived from AA have also been found (38). In studies focusing on circulating fatty acids as biomarkers, AA showed an inverse or a null association with type 2 diabetes risk (14,15). Tissue levels of AA are, however, tightly regulated and do not properly reflect its intake. Two intervention studies found that AA supplementation up to 1.5 g/day did not change platelet AA contents, immune functions, or inflammatory markers among healthy participants (39). Further studies are needed in order to replicate our findings and assess the potential effect of AA intake on diabetes risk.

Strengths of our study include the large sample size, long follow-up duration, and high follow-up rate. Although errors are inevitable when measuring diet, they are more likely to be nondifferential and might bias true associations toward the null, given the prospective study design. A limitation of our study is that participants were exclusively health professionals and primarily Caucasian, limiting the generalizability of the findings to other populations. In addition, we cannot exclude the existence of undiagnosed type 2 diabetes cases, although these would be identified independently of the dietary assessments and thus would be more likely to attenuate true associations of interest. Finally, we cannot exclude the role of residual or unmeasured confounding by other dietary and lifestyle factors in this observational study.

In conclusion, a high intake of LA is associated with a lower risk for type 2 diabetes among U.S. men and women, particularly when LA isocalorically replaces SFAs, *trans* fats, or carbohydrates. Although evidence is needed from intervention studies in order to substantiate the observed associations, our findings suggest that increasing dietary LA at the expense of unhealthy fats and carbohydrates might facilitate the prevention of type 2 diabetes.

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Author Contributions. G.Z. wrote the manuscript. G.Z., A.J.W., P.L.Z., F.B.H., and Q.S. conceived and designed the study, performed statistical analysis, and interpreted data. G.Z., G.L., W.C.W., A.J.W., M.A., P.L.Z., F.B.H., and Q.S. critically revised the manuscript and approved the final version. G.Z. and Q.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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