SUPPLEMENTARY DATA

Supplementary Table 1. Baseline Characteristics of 4207 participants by quartile of dietary TFAs

	Quartile of Dietary TFA		Quartile of Dietary Trans-18:1		Quartile of Dietary Trans-18:2	
	I	IV	I	IV	I	IV
Mean±SD, g/day	2.17±0.50	5.39±0.83	1.19±0.27	2.96±0.44	0.33±0.06	0.76±0.11
Range, g/day	0.49-2.82	4.45-9.44	0.26-1.54	2.46-5.17	0.04-0.41	0.62-1.38
Age, years	73.1±5.7	73.4±5.9	73.1±5.7	73.3±5.9	73.4±5.7	72.9±5.8
Gender, %male	29.9	55.6*	29.4	56.3	29.7	55.1*
Race, %White	86.1	88	85	88	83.8	90.9
Education, %>High School	53.4	36.2*	53.3	36.6*	52.2	34.7*
Cardiovascular Disease†,%	21.4	20.3	20.8	20.3	22.4	19.7
Hypertension ‡, %	43.2	41.4	44.1	42	43.0	41.3
Current Smoker, %	8.7	13.9*	8.8	14.5*	9.1	15.3
Former Smoker, %	40.9	43.7	41.5	44.3	39.0	42.4
Alcohol, drinks/week	2.3±5.6	2.4±6.3	2.4±5.7	2.7±6.8	2.3±5.5	2.6±6.4
Physical Activity, kcal/week	1948±2025	1710±2067*	1948±2015	1697±2040*	1886±2026	1737±2023*
Body-mass-index, kg/m2	25.9±4.5	26.4±4.5	25.9±4.5	26.4±4.5	26.0±4.5	26.4±4.6
Waist Circumference, cm	91.3±12.9	94.3±12.4*	91.1±12.9	94.5±12.3*	91.4±13.0	94.3±12.5*
Dietary factors						
Coffee, servings/week	2.6±3.0	3.1±3.2*	2.6±3.0	3.1±3.2*	2.6±3.2	3.1±3.1*
Decaffeinated Coffee, servings/week	2.9±3.0	2.3±2.9*	2.8±3.0	2.4±2.9*	2.8±3.0	2.3±2.8*
Red meat, servings/week	2.0±2.2	3.7±2.6*	1.9±2.2	3.8±2.7*	2.3±2.5	3.5±2.5*
Fiber, g/day	36.0±14.1	23.7±10.2*	35.9±14.2	23.6±10.2*	36.4±14.1	24.3±10.4*
Glycemic Load	12375±5014	11318±3804*	12203±5140	11281±3786*	12064±5399	11719±3248*
SFA, %energy	7.5±3.1	11.0±3.6*	7.5±3.2	11.0±3.6*	7.3±3.3	11.2±3.1*
PUFA, %energy	6.5±2.9	6.9±2.9*	6.5±3.1	6.8±2.8	5.8±2.9	7.6±2.8*
Total energy	2036±825	2049±639	2036±832	2040±635	2066±843	2068±623

Abbreviations: N, number; SFA, saturated fat; PUFA, polyunsaturated fat

Baseline characteristics among adults in the lowest and highest quartile categories for each trans-fatty acid levels are presented. Values for continuous variable represent mean ± standard deviation; values for categorical variables represent percentage.

* P <0.01 for trend across quartile categories

†Cardiovascular Disease includes congestive heart failure, coronary heart disease, myocardial infarction, stroke, atrial fibrillation

‡ Hypertension defined as a systolic and diastolic blood pressures ≥140/90 mmHg or use of antihypertensive treatment

SUPPLEMENTARY DATA

Supplementary Table 2. Stratified analyses for prospective association of circulating and dietary trans fatty acids (TFA) with incident diabetes among participants stratified by preceding insulin resistance and beta-cell function.*

		Quartile of plasma phospholipid and dietary TFAs				
		I	II	III	IV	P for trend
Participants with preceding higher insulin resistance						
Circulating TFAs (cohort at risk: n=1093)						
Trans16:1n9	Cases (Person-Years)	38 (2951)	34 (2960)	27 (2862)	23 (2670)	
	Hazard Ratio (95% CI)†	1.00 (ref)	1.15 (0.69, 1.91)	1.31 (0.73, 2.35)	1.61 (0.82, 3.15)	0.16
Trans18:1	Cases (Person-Years)	30 (2875)	41 (2840)	29 (2900)	22 (2829)	
	Hazard Ratio (95% CI)†	1.00 (ref)	1.73 (1.03, 2.90)	1.53 (0.84, 2.80)	1.87 (0.89, 3.92)	0.16
Cis/Trans18:2	Cases (Person-Years)	32 (2949)	36 (2917)	33 (2872)	21 (2706)	
	Hazard Ratio (95% CI)†	1.00 (ref)	1.34 (0.82, 2.20)	1.20 (0.72, 1.99)	0.89 (0.49, 1.61)	0.67
Trans/Cis18:2	Cases (Person-Years)	33 (2916)	33 (2876)	32 (2915)	24 (2737)	
	Hazard Ratio (95% CI)†	1.00 (ref)	1.27 (0.77, 2.11)	1.02 (0.61, 1.70)	0.82 (0.47, 1.42)	0.33
Trans/Trans18:2	Cases (Person-Years)	29 (3062)	38 (3082)	30 (2739)	25 (2561)	
	Hazard Ratio (95% CI)†	1.00 (ref)	1.68 (1.01, 2.79)	1.80 (1.04, 3.12)	1.48 (0.82, 2.67)	0.19
Dietary TFAs (cohort at risk: n=1505)						
Total TFA	Cases (Person-Years)	37 (4566)	42 (4354)	46 (4398)	55 (4130)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	0.97 (0.59, 1.60)	1.04 (0.63, 1.74)	1.26 (0.73, 2.17)	0.31
Trans18:1	Cases (Person-Years)	37 (4642)	42 (4309)	47 (4373)	54 (4125)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	0.87 (0.52, 1.43)	1.02 (0.62, 1.68)	1.21 (0.70, 2.09)	0.34
Trans18:2	Cases (Person-Years)	41 (4665)	37 (4364)	39 (4368)	63 (4051)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	1.00 (0.60, 1.66)	0.93 (0.55, 1.60)	1.69 (0.98, 2.91)	0.05
Participants with preceding lower β-cell function						
Circulating TFAs (cohort at risk: n=1115)						
Trans16:1n9	Cases (Person-Years)	21 (3020)	14 (3327)	20 (2749)	24 (2758)	
	Hazard Ratio (95% CI)†	1.00 (ref)	0.60 (0.29, 1.23)	1.15 (0.58, 2.31)	1.33 (0.61, 2.88)	0.20
Trans18:1	Cases (Person-Years)	22 (2973)	16 (3117)	18 (2973)	23 (2791)	
	Hazard Ratio (95% CI)†	1.00 (ref)	0.60 (0.30, 1.20)	0.75 (0.26, 1.56)	1.88 (0.37, 2.08)	0.97
Cis/Trans18:2	Cases (Person-Years)	30 (3189)	12 (2998)	20 (3010)	17 (2657)	
	Hazard Ratio (95% CI)†	1.00 (ref)	0.45 (0.23, 0.90)	0.80 (0.44, 1.45)	0.67 (0.35, 1.35)	0.42
Trans/Cis18:2	Cases (Person-Years)	26 (2945)	20 (3295)	12 (2860)	21 (2754)	
	Hazard Ratio (95% CI)†	1.00 (ref)	0.70 (0.38, 1.27)	0.48 (0.24, 0.98)	0.88 (0.47, 1.65)	0.77
Trans/Trans18:2	Cases (Person-Years)	20 (3266)	18 (3237)	22 (2742)	19 (2609)	
	Hazard Ratio (95% CI)†	1.00 (ref)	0.87 (0.45, 1.70)	1.29 (0.68, 2.47)	0.95 (0.46, 1.97)	0.90
Dietary TFAs (cohort at risk: n=1530)						
Total TFA	Cases (Person-Years)	26 (4863)	21 (4811)	24 (4644)	23 (4468)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	0.64 (0.35, 1.16)	0.78 (0.42, 1.47)	0.76 (0.37, 1.56)	0.64
Trans18:1	Cases (Person-Years)	26 (4919)	20 (4856)	24 (4533)	24 (4478)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	0.68 (0.37, 1.22)	0.74 (0.39, 1.39)	0.74 (0.36, 1.53)	0.52
Trans18:2	Cases (Person-Years)	30 (4962)	16 (4785)	26 (4631)	22 (4409)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	0.84 (0.47, 0.52)	0.98 (0.53, 1.81)	0.77 (0.36, 1.63)	0.57
Participants with both preceding higher insulin resistance and lower β-cell function						
Circulating TFAs (cohort at risk: n=336)						
Trans16:1n9	Cases (Person-Years)	24 (804)	22 (872)	9 (860)	16 (743)	

SUPPLEMENTARY DATA

	Hazard Ratio (95% CI)†	1.00 (ref)	1.10 (0.58, 2.07)	0.52 (0.22, 1.24)	1.71 (0.73, 4.01)	0.53
Trans18:1	Cases (Person-Years)	19 (808)	19 (886)	17 (806)	16 (781)	
	Hazard Ratio (95% CI)†	1.00 (ref)	1.29 (0.63, 2.64)	2.33 (1.05, 5.18)	3.67 (1.44, 9.33)	0.004
Cis/Trans18:2	Cases (Person-Years)	14 (917)	21 (830)	17 (809)	19 (724)	
	Hazard Ratio (95% CI)†	1.00 (ref)	1.86 (0.90, 3.86)	1.97 (0.92, 4.33)	2.48 (1.15, 5.37)	0.02
Trans/Cis18:2	Cases (Person-Years)	18 (927)	20 (795)	18 (806)	15 (752)	
	Hazard Ratio (95% CI)†	1.00 (ref)	1.48 (0.77, 2.87)	1.55 (0.77, 3.16)	1.55 (0.74, 3.23)	0.27
Trans/Trans18:2	Cases (Person-Years)	23 (957)	16 (700)	17 (875)	15 (749)	
	Hazard Ratio (95% CI)†	1.00 (ref)	0.94 (0.47, 1.86)	0.96 (0.49, 1.90)	1.11 (0.53, 2.32)	0.81
Dietary TFAs (cohort at risk: n=577)						
Total TFA	Cases (Person-Years)	23 (1751)	35 (1631)	26 (1529)	33 (1478)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	2.75 (1.46, 5.18)	2.36 (1.19, 4.65)	3.14 (1.49, 6.62)	0.01
Trans18:1	Cases (Person-Years)	26 (1763)	34 (1597)	23 (1566)	34 (1463)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	1.94 (1.06, 3.57)	1.62 (0.84, 3.11)	2.63 (1.29, 5.33)	0.02
Trans18:2	Cases (Person-Years)	25 (1788)	32 (1628)	30 (1495)	30 (1478)	
	Hazard Ratio (95% CI)‡	1.00 (ref)	1.66 (0.92, 2.98)	1.78 (0.94, 3.40)	2.04 (1.00, 4.18)	0.10

*Participants were stratified by preceding insulin resistance and beta-cell function based on the median levels of homeostatic model assessment for insulin resistance and β-cell function assessed in the 1992-93.

†Hazard ratio (95% confidence interval) adjusted for age, sex, race, education, enrollment site, smoking status, alcohol consumption, physical activity, BMI, waist circumference, prevalence of cardiovascular disease, hypertension, and plasma phospholipid 16:0 and 18:0 fatty acids

‡Hazard ratio (95% confidence interval) adjusted for age, sex, race, education, enrollment site, smoking status, alcohol consumption, physical activity, BMI, waist circumference, cardiovascular disease, hypertension, dietary score, and total energy

Contrast tests revealed significant differences between the associations of dietary TFAs, but not phospholipid TFAs, with incident DM among participants with varying preceding insulin resistance and β-cell function: the associations of dietary total TFA (p-contrast=0.008), *t*-18:1 (p-contrast=0.005), and *t*-18:2 (p-contrast=0.01) with DM were significantly higher among participants having both preceding higher insulin resistance and lower β-cell function than among participants having only preceding lower β-cell function.

SUPPLEMENTARY DATA

Supplementary Table 3. Sensitivity analyses for circulating and dietary *trans* fatty acids

		Quartile of *trans* fatty acids			
	I	II	III	IV	P for trend
Circulating trans fatty acids†					
Defining DM without post-challenge glucose test*					
Trans16:1n9	1.00 (ref)	1.29 (0.96, 1.73)	1.23 (0.88, 1.72)	1.78 (1.22, 2.60)	0.006
Trans18:1	1.00 (ref)	1.41 (1.05, 1.90)	1.25 (0.88, 1.78)	1.89 (1.25, 2.87)	0.009
Cis/Trans18:2	1.00 (ref)	1.07 (0.79, 1.43)	1.20 (0.89, 1.61)	1.11 (0.80, 1.54)	0.43
Trans/Cis18:2	1.00 (ref)	1.09 (0.81, 1.45)	0.86 (0.64, 1.17)	1.07 (0.79, 1.45)	0.92
Trans/Trans18:2	1.00 (ref)	1.06 (0.80, 1.41)	1.03 (0.76, 1.39)	1.02 (0.74, 1.41)	0.92
Censoring the cohort at year 9					
Trans16:1n9	1.00 (ref)	1.32 (0.89, 1.94)	1.25 (0.80, 1.9)	1.50 (0.90, 2.52)	0.16
Trans18:1	1.00 (ref)	1.41 (0.94, 2.11)	1.57 (1.00, 2.49)	2.03 (1.16, 3.55)	0.02
Cis/Trans18:2	1.00 (ref)	1.15 (0.77, 1.70)	1.37 (0.92, 2.03)	1.16 (0.74, 1.81)	0.39
Trans/Cis18:2	1.00 (ref)	1.19 (0.81, 1.75)	0.82 (0.54, 1.23)	1.16 (0.77, 1.73)	0.78
Trans/Trans18:2	1.00 (ref)	1.12 (0.77, 1.61)	0.94 (0.63, 1.43)	0.87 (0.56, 1.36)	0.47
Excluding cases occurred in the first 2 years					
Trans16:1n9	1.00 (ref)	1.17 (0.83, 1.65)	1.11 (0.75, 1.64)	1.63 (1.05, 2.52)	0.04
Trans18:1	1.00 (ref)	1.40 (0.99, 1.99)	1.25 (0.83, 1.89)	2.05 (1.27, 3.31)	0.009
Cis/Trans18:2	1.00 (ref)	1.41 (0.81, 1.60)	1.23 (0.87, 1.74)	1.10 (0.74, 1.61)	0.57
Trans/Cis18:2	1.00 (ref)	1.29 (0.92, 1.80)	0.93 (0.65, 1.32)	1.16 (0.81, 1.66)	0.77
Trans/Trans18:2	1.00 (ref)	1.11 (0.80, 1.54)	1.09 (0.77, 1.56)	1.01 (0.69, 1.47)	0.97
Dietary trans fatty acids‡					
Defining DM without post-challenge glucose test*					
Total TFA	1.00 (ref)	1.28 (0.95, 1.74)	1.40 (1.02, 1.92)	1.64 (1.17, 2.30)	0.003
Trans18:1	1.00 (ref)	1.26 (0.93, 1.70)	1.36 (0.99, 1.86)	1.53 (1.09, 2.16)	0.02
Trans18:2	1.00 (ref)	1.35 (1.00, 1.82)	1.33 (0.97, 1.84)	1.72 (1.23, 2.31)	0.005
Censoring the cohort at year 9					
Total TFA	1.00 (ref)	1.16 (0.83, 1.63)	0.92 (0.63, 1.33)	1.10 (0.74, 1.64)	0.75
Trans18:1	1.00 (ref)	1.08 (0.77, 1.51)	0.81 (0.55, 1.18)	1.14 (0.77, 1.69)	0.61
Trans18:2	1.00 (ref)	0.90 (0.64, 1.27)	0.99 (0.69, 1.42)	1.06 (0.72, 1.57)	0.71
Excluding cases occurred in the first 2 years					
Total TFA	1.00 (ref)	1.08 (0.79, 1.48)	1.10 (0.79, 1.53)	1.26 (0.88, 1.80)	0.19
Trans18:1	1.00 (ref)	0.93 (0.68, 1.37)	0.98 (0.71, 1.37)	1.16 (0.81, 1.65)	0.32
Trans18:2	1.00 (ref)	1.03 (0.75, 1.41)	1.05 (0.75, 1.44)	1.27 (0.89, 1.81)	0.21
Circulating total trans18:2					
Multivariate§	1.00 (ref)	1.14 (0.83, 1.57)	0.97 (0.69, 1.35)	0.91 (0.65, 1.29)	0.42
Multivariate+FA†	1.00 (ref)	1.21 (0.88, 1.66)	1.09 (0.78, 1.52)	1.07 (0.75, 1.52)	0.91

* DM defined by new use of insulin or hypoglycemic medication, fasting glucose≥126 mg/dL or nonfasting glucose≥200 mg/dL

†Adjusted for age, sex, race, education, enrollment site, body mass index, waist circumference, smoking status, alcohol consumption, physical activity, prevalence of cardiovascular disease, hypertension at baseline, plasma phospholipid 16:0 and 18:0 fatty acids.

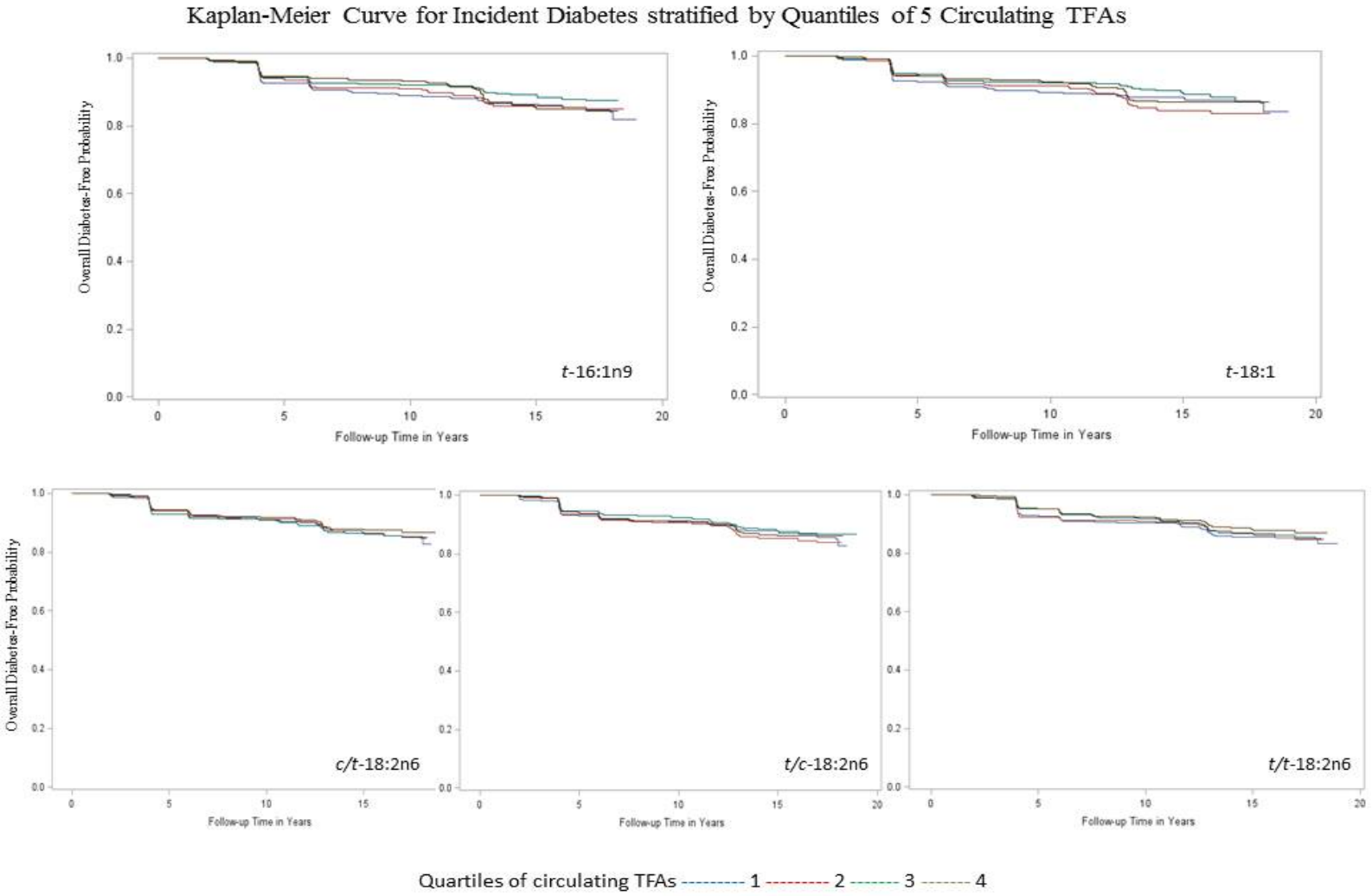
‡Adjusted for age, sex, race, education, enrollment site, smoking status, alcohol consumption, physical activity, BMI, waist circumference, prevalence of cardiovascular disease, hypertension, and total energy and a dietary score consisted of consumption of whole grain, fish, fruit and vegetables, nuts, red/processed meat, sugar-sweetened beverages, and fried potatoes

§Adjusted for age, sex, race, education, enrollment site, body mass index, waist circumference, smoking status, alcohol consumption, physical activity, prevalence of cardiovascular disease, hypertension at baselin

SUPPLEMENTARY DATA

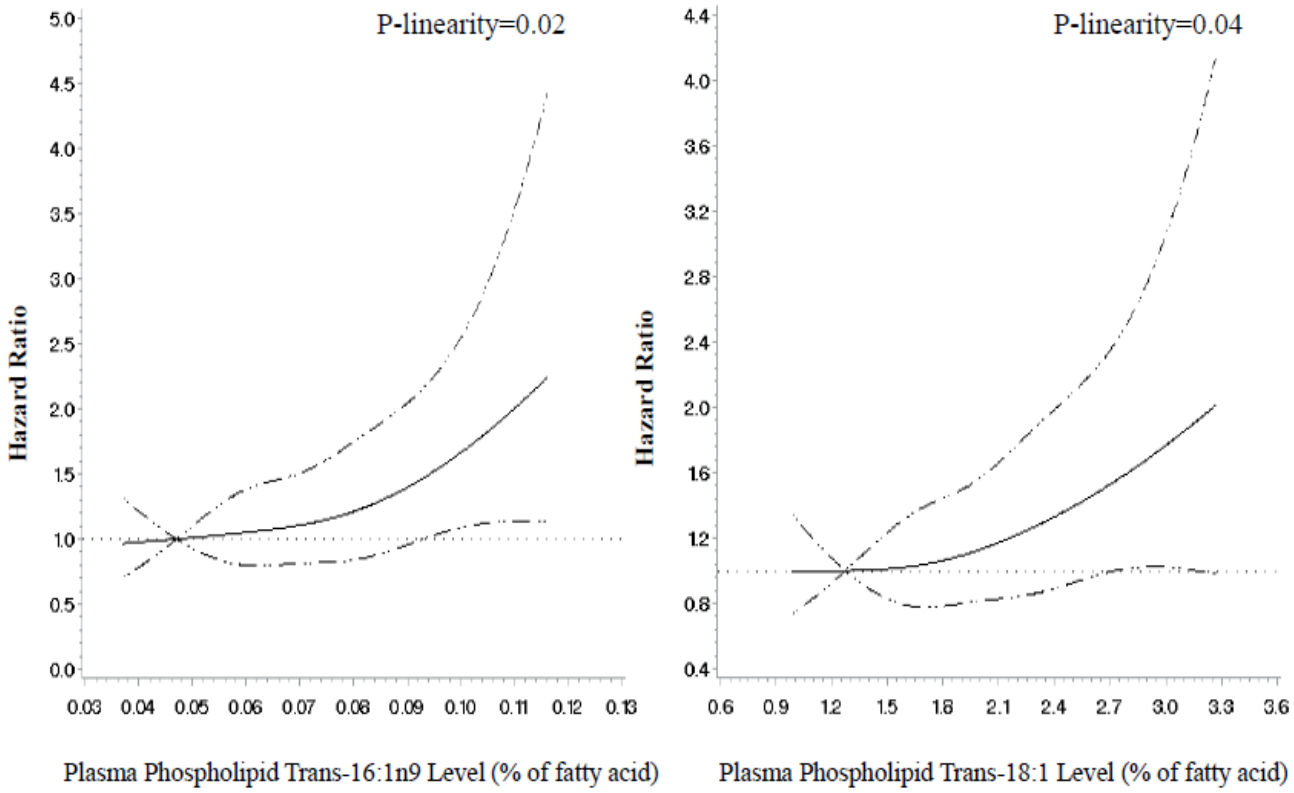
Supplementary Figure 1. Kaplan-Meier curve of crude incident type-2 diabetes mellitus by quartiles of circulating TFAs in the biomarker analysis

Figure presents the unadjusted Kaplan-Meier curve stratified by quartile of each circulating TFAs for 2919 participants in the biomarker analysis. The Y-axis shows the probability of participants without incident diabetes and X-axis indicates follow-up time (max=19 years).



SUPPLEMENTARY DATA

Supplementary Figure 2. Multivariable adjusted relationships of plasma phospholipid trans16:1n9 and trans18:1 with incident diabetes mellitus evaluated using restricted cubic splines



SUPPLEMENTARY DATA

Fatty Acid Measurements

All bloods were drawn from CHS participants in 1992 during clinic visits after a 12-hour fast. Multiple aliquots of plasma, serum, and buffy coats were prepared according to specified protocols and stored at -70 ^{o}C before weekly shipping, on dry ice, to the CHS Central Blood Analysis Laboratory (U. of Vermont) for long-term storage at -80 ^{o}C. Under these conditions, phospholipid fatty acids are stable during long-term storage (at least up to 12 years in plasma).

Plasma phospholipid fatty acids were measured in PHS biomarker laboratory at the Fred Hutchinson Cancer Research Center. Total lipids were extracted from plasma using the method of Folch. Phospholipids were then separated from neutral lipids by one dimensional thin layer chromatography (TLC) using TLC Silica gel 60 plates (EMD Millipore, Billerica, MA) and a developing solution consisting of 45:10:0.5 hexane with 0.0005% BHT/ethyl ether/glacial acetic acid. The total lipid extract was evaporated under nitrogen and reconstituted in 80µl of chloroform. Samples were applied as bands onto individual lanes of the TLC plate using an Automatic TLC Sampler 4 (Camag Scientific, Wilmington, NC). After the plate was developed for half an hour and allowed to dry briefly, the phospholipid fractions, which uniquely remain at the origin, were scraped off with a razor onto weighing paper and transferred to clean tubes for direct transesterification to prepare fatty acid methyl esters (FAMEs) using the method of Lepage.

Samples were injected in split mode (1:30) onto the gas-chromatography system (5890 GC Series 2 with flame ionization detector, Agilent Technologies, Pal Alto, CA). Individual FAMEs were separated on a 100m x 0.25mm internal diameter fused silica capillary column with 0.2µm coating (SP-2560 Supelco, Belefonte, PA). GC parameters were as follows: initial oven temperature 160°C for 16 minutes, ramp of 3.7°C/min to 245°C, and held for 15 minutes; injector temperature 240°C; detector temperature 275°C. The carrier gas was helium at 50 PSI for 15 minutes, ramp of 1 PSI/min to 75 PSI; make-up gas was nitrogen with combined flow of 34ml/min. Total run time was 60 minutes. This gas chromatographic technique allowed the measurement of 45 fatty acids. Results were expressed as weight percentages of total phospholipid fatty acids analyzed.