SUPPLEMENTARY MATERIAL

Plasma industrial and ruminant *trans* fatty acids and incident type 2 diabetes in the EPIC-Potsdam cohort

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Supplementary Table 1. Dairy food groups

Dairy groups	Foods included
Low-fat dairy	low-fat milk and milk beverages (≤1.5% fat), low-fat yogurt (≤1.5% fat), kefir (≤1.5% fat), curd cheese (≤5% fat), and low-fat cheese (reduced fat or lean: cream cheese, gouda, emmental, tilsiter, camembert, brie, gorgonzola).
Full-fat dairy	full-fat milk and milk beverages (>1.5% fat), full-fat yogurt (>1.5% fat), curd cheese (>5% fat), heavy cream, and full-fat cheese (full-fat cream cheese, gouda, emmental, tilsiter, camembert, brie, gorgonzola, processed cheese).

Supplementary Table 2. Trans fatty acids profile

Class	Lipid name	Common name	Systematic Name
Ruminant Trans Fatty Acids	16:1n-7*t*	*trans*-palmitoleic acid	9E-hexadecenoic acid
	18:1n-7*t*	*trans*-vaccenic acid	11E-octadecenoic acid
	*c*9*t*11-CLA	Rumenic acid	9Z,11E-octadecadienoic acid
	*t*10*c*12-CLA	10E,12Z-octadecadienoic acid	10E,12Z-octadecadienoic acid
Industrial Trans Fatty Acids	18:1n-9*t*	9-elaidic acid	9E-octadecenoic acid
	18:1n-6*t*	*trans*-12-elaidic acid	12E-octadecenoic acid
	18:2n-6,9*t*	Linoelaidic acid	9E,12E-octadecadienoic acid

Supplementary Table 3. Comparison between full cohort with blood samples and random subcohort before exclusions

Characteristics	Full cohort	Subcohort before exclusions	Subcohort after exclusions
N	26437*	1248	1159
Sex (% women)	59.8	60.1	60.8
Age (median, IQR)	50.7 (42.6-58.4)	50.7 (42.4-58.0)	49.4 (42.1-57.7)
BMI (median, IQR)	25.7 (23.2-28.6)	25.6 (23.2-28.2)	25.4 (23.0-28.0)
Education (%)			
Currently in training/no certificate	3.5	3.8	3.5
Skilled worker	35.0	34.6	35.1
Professional school	24.8	24.0	23.8
High education/university	36.7	37.5	37.5
Smoking status (%)			
Never smoker	46.8	47.5	48.5
Ex-smoker	32.5	31.6	31.4
Smoker <20 units/day	14.8	15.3	14.8
Smoker >=20 units/day	5.8	5.6	5.3

*There were missing values for BMI (n=198), education (n=17), smoking (n=16)

Supplementary Table 4. Baseline characteristics according to quintiles of total dairy trans fatty acids (sum of 16:1n-7*t*, 18:1n-7*t*, *c*9*t*11-CLA and *t*10*c*12-CLA), EPIC-Potsdam subcohort (n=1159)

	Fifths of plasma phospholipid total dairy trans-fatty acid concentration					
	First	Second	Third	Fourth	Fifth	Total
Ruminant trans fatty acids (%) *, †	**0.37 (0.34-0.39)**	**0.44 (0.43-0.46)**	**0.50 (0.49-0.52)**	**0.58 (0.56-0.60)**	**0.71 (0.66-0.78)**	**0.50 (0.43-0.60)**
16:1n-7*t* (%) †	0.03 (0.02-0.03)	0.04 (0.03-0.04)	0.04 (0.03-0.04)	0.04 (0.04-0.05)	0.05 (0.04-0.06)	0.04 (0.03-0.05)
18:1n-7*t* (%) †	0.13 (0.11-0.15)	0.17 (0.15-0.18)	0.19 (0.17-0.21)	0.23 (0.20-0.26)	0.28 (0.25-0.32)	0.19 (0.15-0.24)
*c*9*t*11-CLA (%) †	0.20 (0.17-0.22)	0.23 (0.22-0.25)	0.27 (0.24-0.29)	0.30 (0.27-0.33)	0.37 (0.32-0.43)	0.26 (0.22-0.31)
*t*10*c*12-CLA (%) †	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.01)
Industrial TFA						
18:1n-6*t* (%) †	0.08 (0.06-0.09)	0.09 (0.08-0.11)	0.10 (0.08-0.11)	0.11 (0.09-0.13)	0.11 (0.09-0.16)	0.09 (0.08-0.12)
18:1n-9*t* (%) †	0.14 (0.12-0.16)	0.16 (0.14-0.19)	0.16 (0.14-0.19)	0.18 (0.14-0.23)	0.19 (0.14-0.27)	0.16 (0.13-0.20)
18:2n-6,9*t* (%) †	0.02 (0.02-0.02)	0.02 (0.02-0.03)	0.02 (0.02-0.03)	0.02 (0.02-0.03)	0.02 (0.02-0.03)	0.02 (0.02-0.03)
Age (years) †	48.8 (42.2-56.2)	49.5 (41.7-57.4)	52.7 (42.3-58.5)	46.7 (41.4-57.5)	50.9 (43.0-58.5)	49.4 (42.1-57.7)
Sex (% women)	42.7	56.0	64.5	69.4	71.6	60.8
Body mass index (kg/m²) †	26.4 (24.0-29.5)	26.0 (23.3-29.3)	25.2 (23.3-28.1)	24.5 (22.2-27.4)	24.5 (22.4-26.8)	25.4 (23.0-28.0)
Waist (cm) †	89.3 (82.0-97.0)	87.0 (76.0-96.0)	83.0 (75.2-93.0)	80.8 (73.0-90.3)	80.5 (72.0-90.0)	85.0 (75.0-93.8)
Leisure time physical activity (h/week) †	0 (0-2)	0 (0-1)	0 (0-1)	0 (0-1.5)	0 (0-1.8)	0 (0-1.5)
Alcohol from alcoholic drinks (g/day) †	15.6 (3.4-29.3)	8.6 (3.1-19.3)	7.9 (2.9-16.3)	6.8 (2.4-18.8)	6.1 (2.0-14.9)	8.4 (2.9-20.1)
Type of work. (%)						
(Heavy) manual work	6.9	7.3	4.3	6.5	4.7	6.0
Sedentary occupation	52.6	59.9	61.0	63.4	60.3	59.4
Standing occupation	40.5	32.8	34.6	30.2	34.9	34.6
Hypertension (%)	35.3	33.2	36.4	28.9	33.2	33.4
Education (%)						
High education/university	34.5	36.6	41.6	37.9	37.1	37.5
Currently in training/no certificate	5.2	1.3	4.3	3.0	3.9	3.5
Professional school	19.1	25.4	19.0	28.0	27.6	23.8
Skilled worker	41.4	36.6	35.1	31.0	31.5	35.1

Smoking status (%)						
Never smoker	35.8	43.1	54.5	51.3	57.8	48.5
Ex-smoker	37.9	36.2	29.0	28.0	25.9	31.4
Smoker < 20 units/day	18.1	15.5	12.6	15.9	12.1	14.8
Smoker ≥ 20 units/day	8.2	5.2	3.9	4.7	4.3	5.3
HbA_{1c} (%) †	5.4 (5.2-5.8)	5.4 (5.1-5.8)	5.4 (5.2-5.8)	5.3 (5.1-5.6)	5.4 (5.1-5.6)	5.4 (5.1-5.7)
Triglycerides (mg/dL) †	126 (82-184)	108 (74-170)	102 (77-148)	94 (70-136)	99 (72-143)	106 (74-161)
Non-HDL cholesterol (mg/dL) †	154 (124-178)	149 (123-180)	146 (122-172)	145 (118-166)	141 (115-173)	147 (119-173)
HDL cholesterol (mg/dL) †	52 (45-63)	55 (46-63)	55 (47-64)	57 (48-67)	57 (47-66)	55 (46-65)
Adiponectin (µg/ml) †	6.7 (4.8-9.1)	7.3 (5.6-9.8)	8.3 (5.5-11.6)	8.5 (6.2-11.9)	8.4 (6.0-11.6)	7.8 (5.5-10.8)
Fetuin (µg/ml) †	257 (224-299)	268 (222-302)	265 (224-312)	271 (230-316)	270 (233-312)	265 (227-308)
hsCRP (mg/dL) †	0.07 (0.02-0.27)	0.10 (0.04-0.25)	0.06 (0.02-0.18)	0.07 (0.02-0.15)	0.07 (0.02-0.16)	0.07 (0.02-0.20)
GGT (units/L) †	25 (14-45)	19 (12-35)	16 (10-28)	14 (10-27)	15 (10-27)	17 (12-32)

* Ruminant trans fatty acids are the sum of the relative concentrations of the fatty acids 16:1n-7*t*, 18:1n-7*t*, *c*9*t*11-CLA, *t*10*c*12-CLA
† Values are presented as median (interquartile range)
HbA_{1c}, hemoglobin A_{1c}; hsCRP, high-sensitivity C-reactive protein; GGT, γ-glutamyl transferase

Supplementary Table 5. Pearson correlation coefficients between specific plasma phospholipid trans fatty acids adjusted for age and sex, EPIC-Potsdam subcohort (n=1159)

		Ruminant TFA				Industrial TFA			Cis FA or SFA												
		16:1n-7t	18:1n-7t	c9t11-CLA	t10c12-CLA	18:1n-6t	18:1n-9t	18:2n-6,9t	15:0	16:0	16:1n-7c	17:0	18:0	18:1n-9c	18:1n-7c	18:2n-6c	20:3n-6	20:4n-6	20:5n-3	22:5n-3	22:6n-3
	16:1n-7t	1																			
	18:1n-7t	0.70	1																		
	c9t11-CLA	0.34	0.47	1																	
	t10c12-CLA	0.49	0.39	0.12	1																
	18:1n-6t	0.61	0.66	0.16	0.54	1															
	18:1n-9t	0.39	0.50	0.05	0.34	0.75	1														
	18:2n-6,9t	0.46	0.29	-0.04	0.53	0.41	0.32	1													
	15:0	0.46	0.49	0.50	0.16	0.17	-0.12	0.06	1												
	16:0	0.00	-0.23	0.28	-0.11	-0.22	-0.20	-0.07	0.09	1											
	16:1n-7c	-0.27	-0.32	0.36	-0.18	-0.16	-0.03	-0.21	-0.17	0.40	1										
	17:0	0.52	0.62	0.28	0.44	0.40	0.22	0.41	0.51	-0.22	-0.42	1									
	18:0	-0.05	0.11	-0.22	0.12	0.10	0.10	0.07	-0.13	-0.79	-0.29	0.18	1								
	18:1n-9c	-0.20	-0.18	0.41	-0.10	-0.13	0.03	-0.20	-0.09	0.23	0.70	-0.27	-0.21	1							
	18:1n-7c	-0.06	-0.16	0.02	-0.03	-0.07	0.06	0.10	-0.26	0.14	0.26	-0.07	-0.28	0.27	1						
	18:2n-6c	0.23	0.33	-0.24	0.10	0.18	0.15	0.17	0.11	-0.32	-0.47	0.18	0.04	-0.37	-0.21	1					
	20:3n-6	-0.16	-0.21	0.12	-0.10	-0.10	-0.11	-0.23	0.03	0.16	0.26	-0.17	0.02	0.07	-0.14	-0.31	1				
	20:4n-6	-0.14	-0.22	-0.24	-0.09	-0.13	-0.09	-0.06	-0.17	-0.01	-0.08	-0.08	0.00	-0.22	0.15	-0.51	0.03	1			
	20:5n-3	-0.05	0.03	0.16	0.00	0.04	-0.05	-0.04	0.06	0.02	0.02	0.07	0.06	-0.06	-0.09	-0.44	-0.12	0.05	1		
	22:5n-3	-0.04	0.06	0.17	0.08	0.06	0.00	-0.03	0.14	-0.28	0.04	0.19	0.31	0.12	-0.01	-0.37	-0.04	0.11	0.36	1	
	22:6n-3	0.10	0.02	0.04	0.07	0.10	-0.09	0.08	0.08	0.02	-0.14	0.15	0.04	-0.27	0.04	-0.39	-0.15	0.07	0.56	0.29	1

Supplementary Table 6. Factor loadings of the seven trans fatty acids on the retained principal component

TFA	Principal Component 1 (PC1)*
16:1n-7*t*	0.83
18:1n-7*t*	0.83
*c*9*t*11-CLA	0.38
*t*10*c*12-CLA	0.69
18:1n-6*t*	0.88
18:1n-9*t*	0.73
18:2n-6,9*t*	0.60

*53% explained total variance

Supplementary Table 7. Prospective associations of TFA and T2D risk, with and without adjusting for alcohol intake and according to quartiles of alcohol intake

Fatty acid	Hazard Ratio per 1-SD (95%CI)* by Quartiles of Alcohol Intake				p-interaction ‡
	First: 1.0 (0.5-1.9) † (n=501)	Second: 5.2 (4.0-6.7) † (n=472)	Third: 12.8 (10.0-16.1) † (n=468)	Fourth: 33.9 (24.5-46.4) † (n=486)	
18:1n-7*t*	0.44 (0.27-0.72)	0.72 (0.47-1.10)	0.62 (0.39-0.98)	0.67 (0.45-0.99)	0.05
***c*9*t*11-CLA**	1.70 (1.25-2.30)	1.47 (1.09-1.99)	1.20 (0.88-1.62)	1.42 (1.08-1.87)	0.74
***t*10*c*12-CLA**	0.76 (0.55-1.04)	0.75 (0.57-0.98)	1.12 (0.80-1.57)	0.78 (0.59-1.02)	0.35

* Hazard Ratios (HR) and 95% confidence intervals (95% CI) derived from a multivariable Cox regression model adjusted for age (stratum variable), sex, waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or >=5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, skilled worker, technical school, or university degree), red meat intake (energy-adjusted), coffee intake (energy-adjusted), fiber intake (energy-adjusted), fasting status, total energy intake and all the other trans fatty acids (16:1n-7*t*, 18:1n-6*t*, 18:1n-7*t*, 18:1n-9*t*, 18:2n-6,9*t*, *c*9*t*11-CLA, *t*10*c*12-CLA)

† Values are median (interquartile range) of grams of alcohol intake per day

‡ Derived from tests for statistical interactions of trans fatty acids concentrations with quartiles of alcohol intake by including cross-product terms in the fully-adjusted models

Supplementary Table 8. Prospective associations of plasma trans fatty acids with risk of type 2 diabetes in men (total n=904, cases n=462) and women (total n=1023, cases n=334) from EPIC-Potsdam

Fatty acid	Men	Women	p- interaction *
16:1n-7*t*			
Median concentration (IQR) (%) †	0.04 (0.03-0.04)	0.04 (0.03-0.05)	
Hazard Ratio per 1 SD (95% CI) ‡	1.05 (0.80-1.38)	1.32 (1.00-1.72)	0.73
18:1n-7*t*			
Median concentration (IQR) (%) †	0.17 (0.14-0.22)	0.20 (0.16-0.25)	
Hazard Ratio per 1 SD (95% CI) ‡	0.74 (0.56-0.99)	0.55 (0.40-0.75)	0.82
***c*9*t*11-CLA**			
Median concentration (IQR) (%) †	0.25 (0.21-0.29)	0.27 (0.23-0.32)	
Hazard Ratio per 1 SD (95% CI) ‡	1.39 (1.13-1.72)	1.45 (1.18-1.79)	0.80
***t*10*c*12-CLA**			
Median concentration (IQR) (%) †	0.01 (0.01-0.01)	0.01 (0.01-0.01)	
Hazard Ratio per 1 SD (95% CI) ‡	1.03 (0.85-1.25)	0.59 (0.46-0.74)	0.06
18:1n-6*t*			
Median concentration (IQR) (%) †	0.09 (0.07-0.11)	0.10 (0.08-0.12)	
Hazard Ratio per 1 SD (95% CI) ‡	0.91 (0.65-1.25)	1.23 (0.87-1.74)	0.59
18:1n-9*t*			
Median concentration (IQR) (%) †	0.16 (0.13-0.19)	0.16 (0.13-0.21)	
Hazard Ratio per 1 SD (95% CI) ‡	1.08 (0.82-1.43)	1.15 (0.87-1.52)	0.15
18:2n-6,9*t*			
Median concentration (IQR) (%) †	0.02 (0.02-0.03)	0.02 (0.02-0.03)	
Hazard Ratio per 1 SD (95% CI) ‡	0.82 (0.67-1.00)	1.13 (0.89-1.43)	0.13

* Derived from tests for statistical interactions of trans fatty acids concentrations with sex by including cross-product terms in the continuous fully-adjusted models

† Plasma TFA concentrations based on the subcohort sample (men n=454, women n=705)

‡ The standard deviation (SD) was derived from full cohort. Hazard ratio (HR) and 95% confidence interval (CI) per-1SD increase of each trans fatty acid, after accounting for age (stratum variable), sex, waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or >=5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, skilled worker, technical school, or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/d), red meat intake (energy-adjusted), coffee intake (energy-adjusted), fiber intake (energy-adjusted), fasting status, total energy intake and all other TFA subtypes (16:1n-7*t*, 18:1n-6*t*, 18:1n-7*t*, 18:1n-9*t*, 18:2n-6,9*t*, *c*9*t*11-CLA, *t*10*c*12-CLA)

Supplementary Table 9. Prospective associations of plasma trans fatty acids with risk of type 2 diabetes according to BMI (above and below the median) in EPIC-Potsdam

Fatty acid	Hazard Ratio per 1-SD (95%CI) * BMI ≤27 (n = 955)	BMI > 27 (n = 972)	p- interaction BMI †
Ruminant TFA			
16:1n-7*t*	1.35 (0.98-1.86)	1.00 (0.79-1.27)	0.42
18:1n-7*t*	0.57 (0.41-0.79)	0.76 (0.58-1.01)	0.47
***c*9*t*11-CLA**	1.55 (1.21-2.00)	1.31 (1.07-1.59)	0.38
***t*10*c*12-CLA**	0.76 (0.59-0.98)	0.81 (0.68-0.98)	0.90
Industrial TFA			
18:1n-6*t*	1.34 (0.90-2.01)	1.04 (0.76-1.43)	0.72
18:1n-9*t*	0.80 (0.53-1.18)	1.06 (0.84-1.35)	0.56
18:2n-6,9*t*	1.05 (0.76-1.45)	0.99 (0.83-1.19)	0.96

* Hazard ratio (HR) and 95% confidence interval (CI) per-1SD increase of each trans fatty acid in the EPIC-Potsdam subcohort (total n= 1927, cases n=796), after accounting for age (stratum variable), sex, waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or >=5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, skilled worker, technical school, or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/d), red meat intake (energy-adjusted), coffee intake (energy-adjusted), fiber intake (energy-adjusted), fasting status, total energy intake and all other TFA subtypes (16:1n-7*t*, 18:1n-6*t*, 18:1n-7*t*, 18:1n-9*t*, 18:2n-6,9*t*, *c*9*t*11-CLA, *t*10*c*12-CLA)

† Derived from tests for statistical interactions of trans fatty acids concentrations with BMI≤27 and BMI>27 by including cross-product terms in the continuous fully-adjusted models

Supplementary Table 10. Prospective associations of plasma trans fatty acids with risk of type 2 diabetes according to polymorphisms in the *PPARG* gene, in EPIC-Potsdam

	Hazard Ratio per 1-SD (95%CI) *		
Fatty acid	***PPARG* Pro12Pro (Wild type) (n = 1362)**	***PPARG* Pro12Ala (Ala carriers) (n = 485)**	**p-interaction †**
***c*9*t*11-CLA**	1.37 (1.13-1.65)	1.48 (1.12-1.96)	0.92
***t*10*c*12-CLA**	0.78 (0.66-0.92)	0.82 (0.59-1.14)	0.50

* Hazard ratio (HR) and 95% confidence interval (CI) per-1SD increase of each trans fatty acid in the EPIC-Potsdam subcohort (total n= 1927, cases n=796), after accounting for age (stratum variable), sex, waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or >=5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, skilled worker, technical school, or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/d), red meat intake (energy-adjusted), coffee intake (energy-adjusted), fiber intake (energy-adjusted), fasting status, total energy intake and other TFA subtypes (16:1n-7*t*, 18:1n-6*t*, 18:1n-7*t*, 18:1n-9*t*, 18:2n-6,9*t*, *c*9*t*11-CLA, *t*10*c*12-CLA)

† Derived from tests for statistical interactions of conjugated linoleic acid concentrations with *PPARG* polymorphisms (wild type vs ala carriers) by including cross-product dichotomous terms in the continuous fully-adjusted models

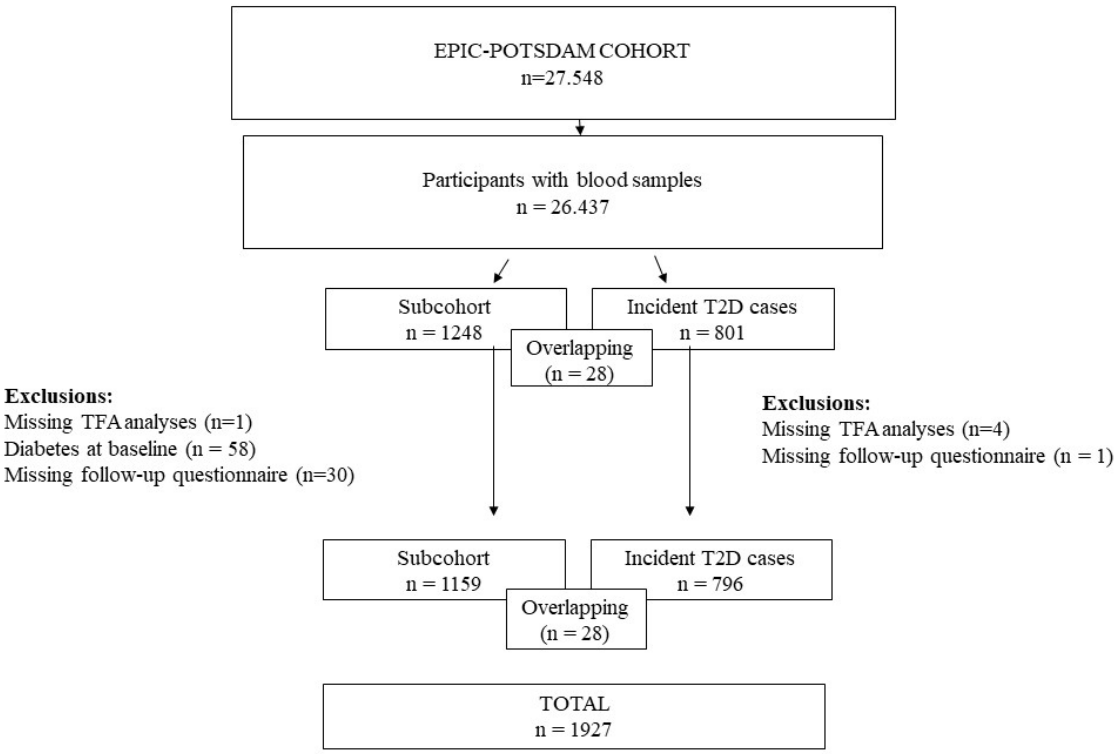
Supplementary Table 11. Mediation analysis of associations of trans fatty acids with type 2 diabetes by metabolic markers and liver fat in EPIC-Potsdam (total n= 1927, cases n=796)

	18:1n-7t			*c*9*t*11-CLA			*t*10*c*12-CLA		
	Hazard ratio per 1 SD (95%-CI)		% change (95%-CI) *	Hazard ratio per 1 SD (95%-CI)		% change (95%-CI) *	Hazard ratio per 1 SD (95%-CI)		% change (95%-CI) *
Reference Model †	**0.61**	**(0.47-0.79)**	-	**1.53**	**(1.29; 1.87)**	-	**0.78**	**(0.60-0.98)**	-
+ Triglycerides	0.60	(0.45-0.79)	5 (-16; 24)	1.40	(1.15-1.73)	-20 (-47; -5)	0.85	(0.63; 1.10)	-35 (-189; -6)
+ Non-HDL cholesterol	0.61	(0.47-0.81)	-0.8 (-9; 4)	-	-	-	0.79	(0.6; 1.01)	-7 (-77; 19)
+ HDL-cholesterol	-	-	-	-	-	-	0.78	(0.58; 1.00)	1 (-45; 30)
+ HbA_{1c}	0.54	(0.37-0.75)	27 (-24; 116)	1.42	(1.12-1.88)	-16 (-65; 28)	-		-
+ GGT	0.57	(0.43-0.75)	16 (-13; 49)	-	-	-	0.81	(0.61; 1.04)	-12 (-107; 21)
+ Adiponectin	0.59	(0.45-0.79)	6 (-9; 24)	-	-	-	-		-
+ hsCRP	0.53	(0.39-0.74)	29 (1; 68)	-	-	-	0.78	(0.59; 0.98)	0 (-38; 53)
+ Fetuin	-	-	-	-	-	-	-		-
+ FLI ‡	0.60	(0.45; 0.78)	6 (-23; 39)	1.37	(1.12-1.71)	-26 (-58; -6)	0.86	(0.64; 1.10)	-38 (-205; -6)

* The change (%) reflects the change of the estimate after additional adjustment for each biomarkers and fatty liver index, relative to the estimate from the reference model. Its stability as well as the corresponding hazard ratio were estimated as median and dispersion from a bootstrapping procedure (500 bootstrap replicates)

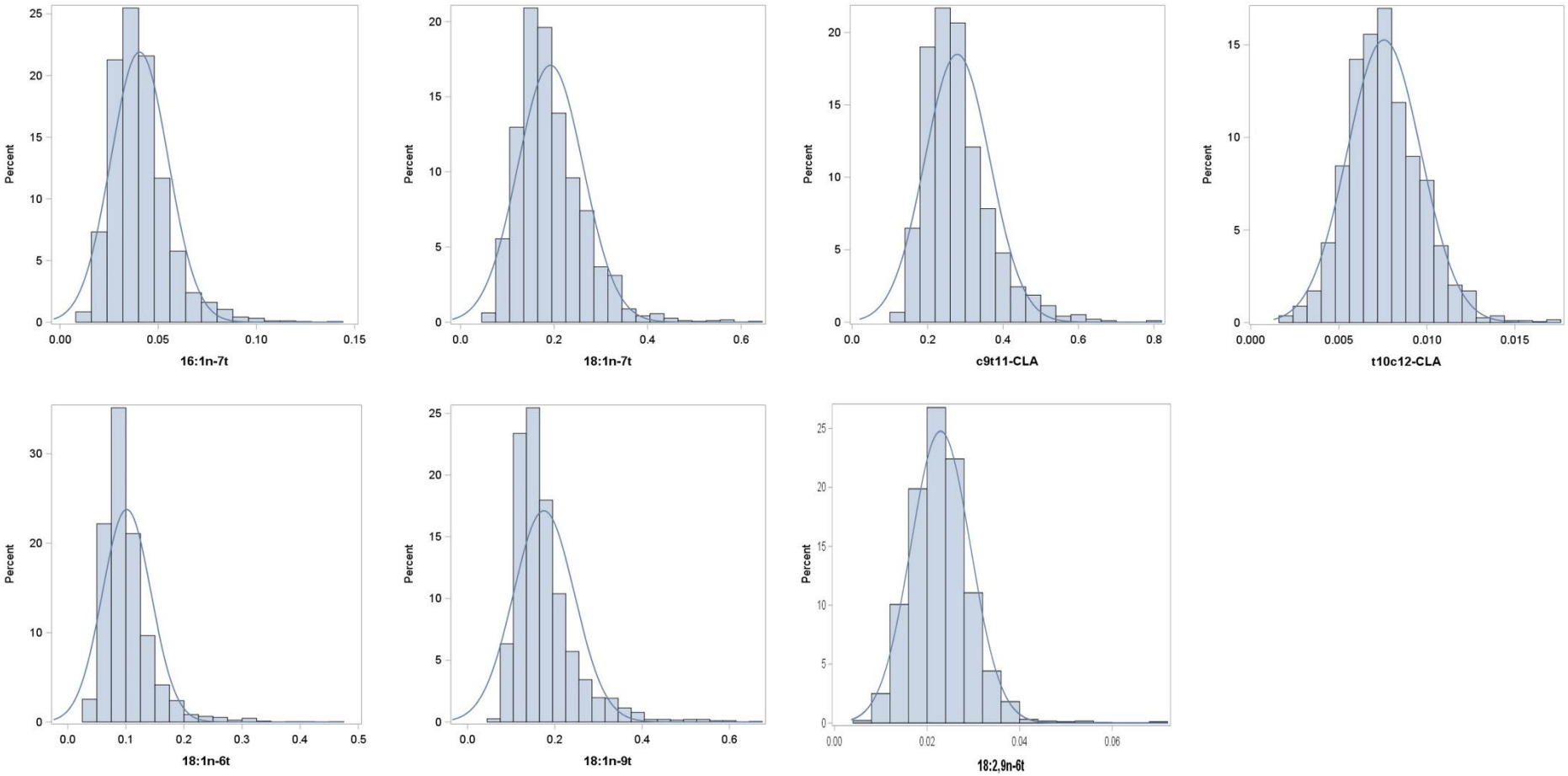
† Hazard ratio (HR) and 95% confidence interval (CI) per-1SD increase of each trans fatty acid, estimated as median and dispersion from a bootstrapping procedure (500 bootstrap replicates). Models adjusted for age (stratum variable), sex, waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or >=5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, skilled worker, technical school, or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/d), red meat intake (energy-adjusted), coffee intake (energy-adjusted), fiber intake (energy-adjusted), fasting status, total energy intake, all other TFA subtypes (16:1n-7*t*, 18:1n-6*t*, 18:1n-7*t*, 18:1n-9*t*, 18:2n-6,9*t*, *c*9*t*11-CLA, *t*10*c*12-CLA)

‡ Fatty liver index (FLI) was calculated with the following formula: $FLI = (e^{0.953*\log e(\text{triglycerides}) + 0.139*BMI + 0.718*\log e(ggt) + 0.053*\text{waist circumference} - 15.745}) / (1 + e^{0.953*\log e(\text{triglycerides}) + 0.139*BMI + 0.718*\log e(ggt) + 0.053*\text{waist circumference} - 15.745}) * 100$

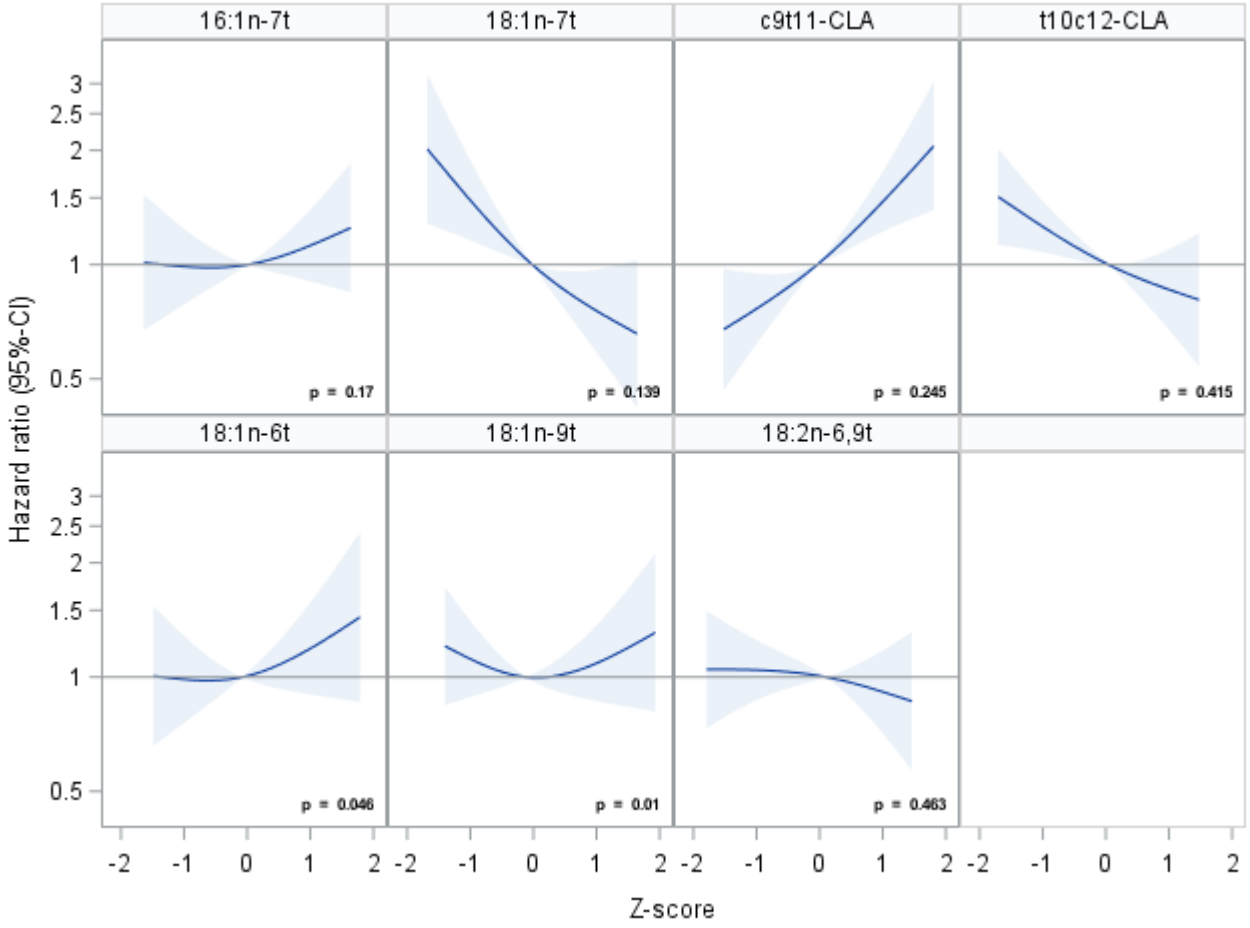


Supplementary Figure 1. Flow chart describing study sample

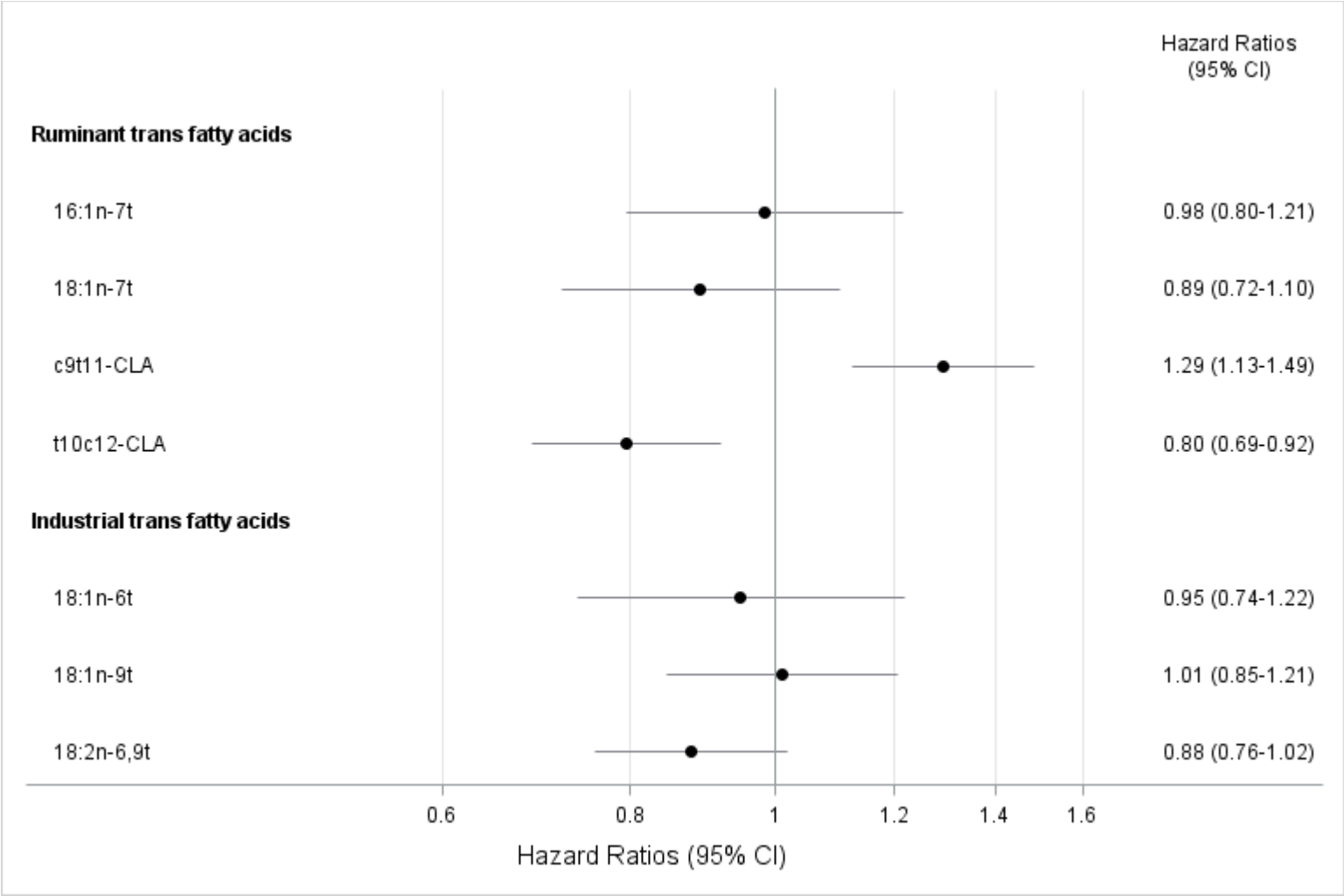
T2D: type 2 diabetes



Supplementary Figure 2. Trans fatty acids histograms

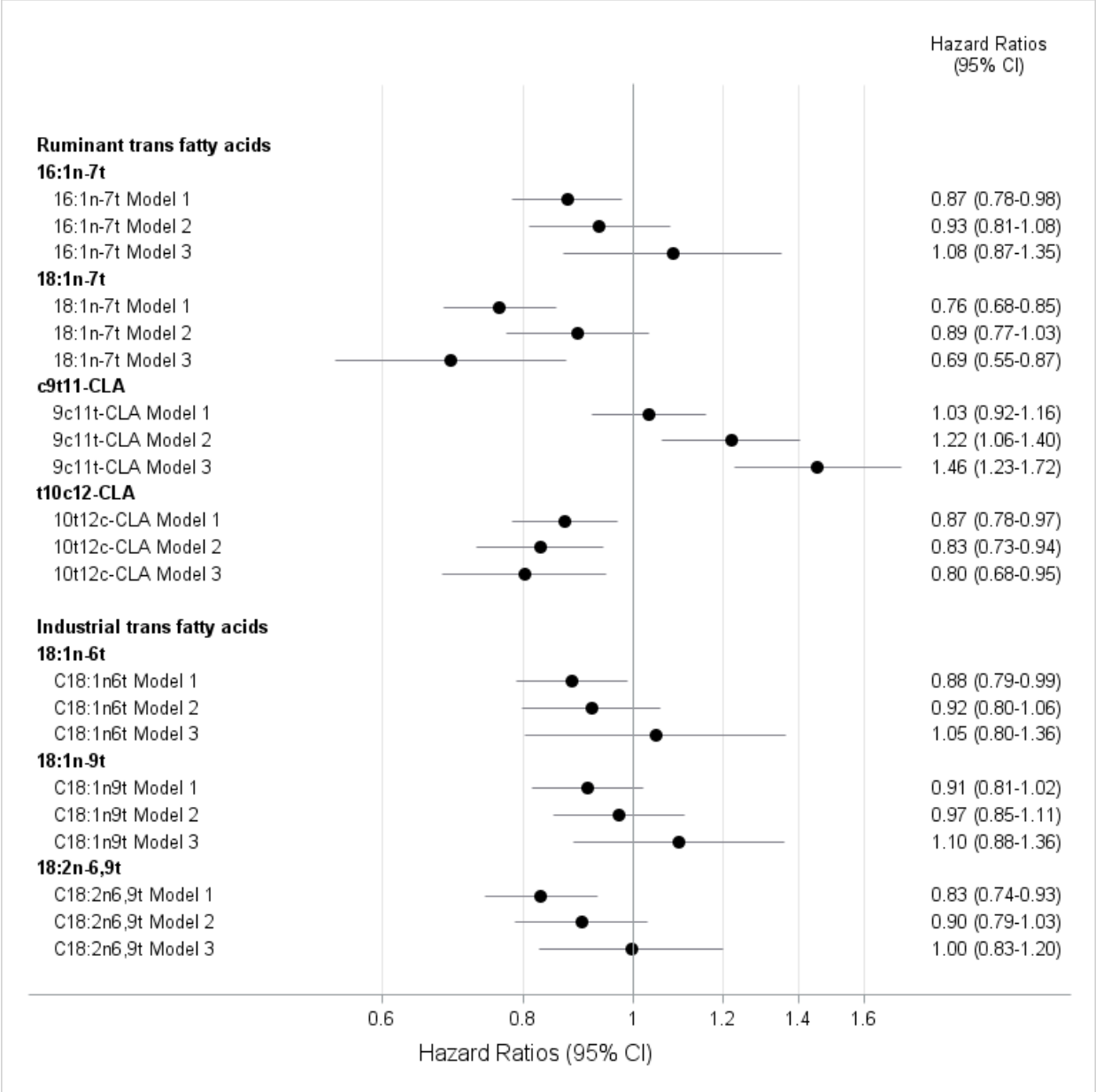


Supplementary Figure 3. Prospective associations between trans fatty acids and incidence of T2D modelled using a restricted cubic spline function with 3 knots placed at the 10th, 50th and 90th percentiles in the EPIC Potsdam (total n= 1927, cases n=796). Shaded areas are 95% confidence intervals. Numbers in the bottom right of each panel are p-values based on the likelihood ratio test



Supplementary Figure 4. Prospective associations of plasma trans fatty acids with risk of type 2 diabetes in EPIC-Potsdam (total n= 1927, cases n=796), with a model further adjusted for PC score. Hazard ratio (HR) and 95% confidence interval (CI) per-1SD increase of each trans fatty acid

Model adjusted for age (stratum variable), sex, waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or >=5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, skilled worker, technical school, or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/d), red meat intake (energy-adjusted), coffee intake (energy-adjusted), fiber intake (energy-adjusted), fasting status, total energy intake and PC score



Supplementary Figure 5. Sensitivity Analysis, excluding participants with HbA1c>6.5 (Total n=1651, cases n=564)

Model 1: adjusted for age (stratum variable) and sex
Model 2: further adjusted for waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or >=5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, skilled worker, technical school, or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/d), red meat intake (energy-adjusted), coffee intake (energy-adjusted), fiber intake (energy-adjusted), fasting status, total energy intake
Model 3: further adjusted for all other TFA subtypes (16:1n-7*t*, 18:1n-6*t*, 18:1n-7*t*, 18:1n-9*t*, 18:2n-6,9*t*, *c*9*t*11-CLA, *t*10*c*12-CLA

Fatty Acid Measurements

In detail, 50 µL of plasma was transferred into 16 x 100 mm teflon coated screw capped vials. After addition of 1 mL deionized water total lipids were extracted with 3 mL *tert*-butyl methyl ether (MTBE)/methanol solution (2/1, v/v) (MTBE containing 0.01% butyl hydroxytoluene (BHT)). The mixture was vortexed for 15 min. After centrifugation, the upper layer containing the lipid fractions was transferred into another vial to evaporate to dryness under a stream of N_2 at 40°C.
For SPE the dried lipids were redissolved into 500 µL chloroform. The mixture was applied to conditioned SPE columns (1 mL) containing 100 mg of aminopropyl-modified silica. The columns are placed on a vacuum elution apparatus equipped with vents and manometer. Conditioning of columns was performed by washing with 2 x 1 mL *n*-hexane and 1 x 1 mL chloroform/*i*-propanol (2/1, v/v). The vacuum (~10 kPa) was released in time to prevent columns from becoming completely dry. Firstly, neutral lipids and free FA were eluted with 4 x 1 mL chloroform/methanol/acetic acid (100/2/2, v/v). After changing vials the PL were eluted with 2 x 1 mL methanol. Solvents were evaporated under a stream of N_2 at 40°C.
For hydrolysis and methylation of FA the dried PL were redissolved into 230 µL toluene and transferred into GC-vials. 20 µL of trimethyl sulfonium hydroxide solution (TMSH, 0.25 mol/L in methanol, were added to form FA methyl esters (FAME) from FA of PL (except of sphingolipids). Vials were vortexed (30 min, 750 min^{-1}, 40°C). Samples containing the FAMEs were analyzed by GC.
Analysis of FAMEs was performed using an Agilent GC system 7890A equipped with Agilent 7000 GC/MS Triple Quad (Agilent Technologies, Waldbronn, Germany) and a FID. 1 µL of sample was injected using a CIS 4C PTV-type GC-inlet (Gerstel) in splitless mode at 30°C, 30-260°C, ramp 12°C/s, held 2 min, 260-320°C, ramp 12°C/s.
The FAMEs were separated on a GC capillary column (HP-88, 100 m x 0.25 mm I.D., 0.2 µm film thickness, Agilent) using a constant He carrier gas flow of 1.3 mL/min. The eluting gas flow was splitted for FID and MS detection (1:1) using a 2-way splitter.
FID conditions were as follows: heater at 250°C, H_2 flow at 40 mL/min, air flow at 400 mL/min, and makeup flow at 30 mL/min. GC-MS/MS conditions were as follows: EI ion source at 230°C, He quench gas at 2.25 mL/min, and N_2 collision gas at 1.5 mL/min.
The following FAME standards of odd chain fatty acids (OCFA) and trans fatty acids (TFA) were used for calibration: 15:0 and 17:0 (Sigma-Aldrich, Taufkirchen, Germany), 16:1n-7*t*, 18:1n-9*t*, 18:1n-7*t*, 18:1n-6*t*, 18:2n-6*t*, *c*9*t*11-CLA, and *t*10*c*12-CLA (NuChek-Prep, Elysian, USA). The MRM counts were related to corresponding FID counts using calibration standards resulting in individual linear regression curves and conversion factors. The MRM counts of OCFA and TFA of each sample were transformed into FID analogous counts using corresponding conversion factors of each FA. The results were expressed in area percentage of each FA relative to total area of all 9 FA. Simultaneously, a basic FA spectrum of 13 FA can be evaluated by FID detection and expressed in area percentage of each FA relative to total area of all 13 FA. The spectrum consists of the following FA: 15:0, 16:0, 16:1n-7*c*, 17:0, 18:0, 18:1n-9*c*, 18:1n-7*c*, 18:2n-6*c*, 20:3n-6, 20:4n-6, 20:5n-3, 22:5n-3, 22:6n-3.
We did not use lower limits of detection (LOD) because we worked with area sum%. Instead, we used a signal-to-noise ratio better than 3 (S/N=3) for all analytes. This means that all peaks that were at least three times larger than the noise were evaluated. This was true for all fatty acids indicated.

The reproducibility of the analytes was determined by a comparison of intra- and interassay coefficients of variation (CV%, n=10) of the OCFA and TFA detected by MRM (two extractions injected twice) calculated according to Rodbard (1974) were as follows (see table):

Fatty acid	Systematic name	Mean ± SD (area sum%)	Intra-assay CV (%)	Inter-assay CV (%)
15:0	pentadecanoic acid	17.10 ± 3.18	1.57	1.97
16:1n-7*t*	9E-hexadecenoic acid	3.04 ± 0.44	1.67	3.65
17:0	heptadecanoic acid	26.90 ± 2.87	0.69	0.90
18:1n-9*t*	trans-9-octadecenoic acid	16.89 ± 3.99	2.00	2.71
18:1n-7*t*	trans-11-octadecenoic acid	15.94 ± 1.93	1.08	1.11
18:1n-6*t*	trans-12-octadecenoic acid	6.45 ± 1.86	1.21	1.98
18:2n-6,9*t*	9E,12E-octadecadienoic acid	0.55 ± 0.12	4.17	2.50
*c*9*t*11-CLA	9Z,11E-octadecadienoic acid	12.90 ± 5.91	1.59	1.96
*t*10*c*12-CLA	10E,12Z-octadecadienoic acid	0.23 ± 0.06	9.51	4.66

Reference:

Rodbard D (1974) Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Clinical chemistry 20 (10):1255-1270