"Erythrocyte n-6 polyunsaturated fatty acids, gut microbiota and incident type 2 diabetes: a prospective cohort study"

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SUPPLEMENTAL MATERIALS

Study Population

Guangzhou Nutrition and Health Study (GNHS), is a community-based prospective cohort study conducted in urban areas of southern China. There were two waves of participant recruitment

using the same criteria as described previously(1): between 2008 and 2010 (n=3169), and between 2012 and 2013 (n=879).

At baseline, socio-demographic characteristics, lifestyles and dietary factors, and medical history of the participants were collected by face-to-face interviews using a structured questionnaire. Habitual dietary intakes over the past 12 months were collected by a validated food frequency questionnaire (FFQ) which included 79 items (2). Dietary macronutrients and fatty acids were adjusted for total energy intake using the residual method(3). Physical activity was assessed as total metabolic equivalent for task (MET) hours per day using a physical activity questionnaire including 19 items (4). Anthropometric parameters including height, weight, waist circumference and hip circumference were measured by trained nurses at the site after questionnaire interview. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$, and waist-to-hip ratio (WHR) was calculated as waist circumference (cm)/hip circumference (cm).

Fasting blood samples at each visit were used to measure the standard clinical chemistry of participants. Erythrocytes were aliquoted within 2 hours of blood sampling and stored at -80°C. Fatty acid moieties of erythrocyte membranes were trans-methylated and measured as proportions (%) of total fatty acids by using gas chromatography (7890 GC, DB-23 capillary column 60m×0.25mm internal diameter×0.15μm film, Agilent, California, USA). Commercially available standards (Nu-Chek Prep, Minnesota, USA) were used to identify individual fatty acids (n=37) and quantify a relative peak strength of each. The intra-assay coefficients of variation for LA, GLA and AA were 6.4%, 12.8%, and 8.04%, respectively. Glycated hemoglobin (HbA1c) was measured by high performance liquid chromatography using Bole D-10 Hemoglobin A1c Program on Bole D-10 Hemoglobin Testing System. Fasting glucose, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol (TC) and triglycerides (TG) were measured by colorimetric methods using a Roche cobas 8000 c702 automated analyzer (Roche Diagnostics, Shanghai, China). Fasting insulin was determined by electrochemiluminescence immunoassay using a Roche Cobas 8000/e602 immunoanalyzer (Roche Diagnostics, Shanghai, China). Insulin resistance was evaluated by the homeostasis model assessment of insulin resistance (HOMA-IR) calculated as fasting insulin (μIU/mL) × fasting glucose (mmol/ mL)/22.5. β-cell function was evaluated by the homeostasis model assessment model (HOMA-β) calculated as 20 × fasting insulin (μIU/mL)/ (fasting glucose (mmol/ mL) − 3.5). Non-HDL was calculated as TC (mmol/L) – HDL-C(mmol/L). The intra-assay coefficients of variation were 0.75% for HbA1c, 2.5% for glucose, 4.3% for HDL, 3.1% for LDL, 3.1% for TC, 5.8% for TG, and 5.8% for insulin respectively.

Fecal sample collection and 16S rRNA gene sequencing

Fecal samples were collected on the examination day during follow-up visits and then were kept at 4 ℃ within four hours of donation before keeping at -80 ℃. Microbial DNA was extracted from each sample using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instruction. DNA concentrations were determined using the Qubit quantification system (Thermo Scientific, Delaware, US). All extracted DNA was stored at -20 °C for further sequencing.

The V3-V4 hypervariable region of the 16S rRNA gene was amplified from genomic DNA using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). PCR reaction was performed in microtiter plates with a 50 µL mixture consisting of 1X KAPA HiFi Hot start Ready Mix, 0.1µM primer 341 F, 0.1 µM primer 805 R, and 12.5 ng template DNA. Reactions were run in a T100 PCR thermocycle (BIO-RAD) according to the following cycling program: 3 min of denaturation at 94 °C, followed by 18 cycles of 30 s at 94 °C (denaturing), 30 s at 55 °C (annealing), and 30 s at 72 °C (elongation), with a final extension at 72 °C for 5 min. Subsequently, the amplified products were checked by 2% agarose gel electrophoresis and ethidium bromide staining. Amplicons were quantified using the Qubit quantification system (Thermo Scientific, Wilmington, DE, US) following the manufacturers' instructions. Sequencing primers and adaptors were added to the amplicon products in the second PCR step as follows 2 µL of the diluted amplicons were mixed with a reaction solution consisting of 1×KAPA HiFi Hotstart ReadyMix, 0.5µM fusion forward and 0.5µM fusion reverse primer, 30 ng Meta-gDNA (total volume 50 µL). The PCR was run according to the cycling program above except with cycling number of 12. The amplification products were purified with Agencourt AMPure XP Beads (Beckman Coulter Genomics, MA, USA) according to the manufacturer's instructions and quantified as described above. Equimolar amounts of the amplification products were pooled together in a single tube. The concentration of the pooled libraries was determined by the Qubit quantification system.

Amplicon sequencing was performed on the Illuimina MiSeq System (Illumina, San Diego, USA). Fastq-files were demultiplexed, merge-paired, quality filtered by Quantitative Insights into Microbial Ecology (QIIME) software (version 1.9.0) (5). To obtain effective reads, marker gene Illumina sequence data, chimeric sequences ('consensus') and low-quality regions of the sequences were detected and filtered. Filtered sequences were clustered into operational taxonomic units (OTUs) with 97% similarity. Taxonomy of the OTUs was assigned using the Greengenes Database (version 13_8)(6). To calculate α-diversity, four indicators were applied: Observed OTUs and Chao index (representing community richness), Shannon's diversity index and Simpson index (representing community diversity).

SUPPLEMENTARY FIGURES

Figure S1. Flow chart of participants included in the present study

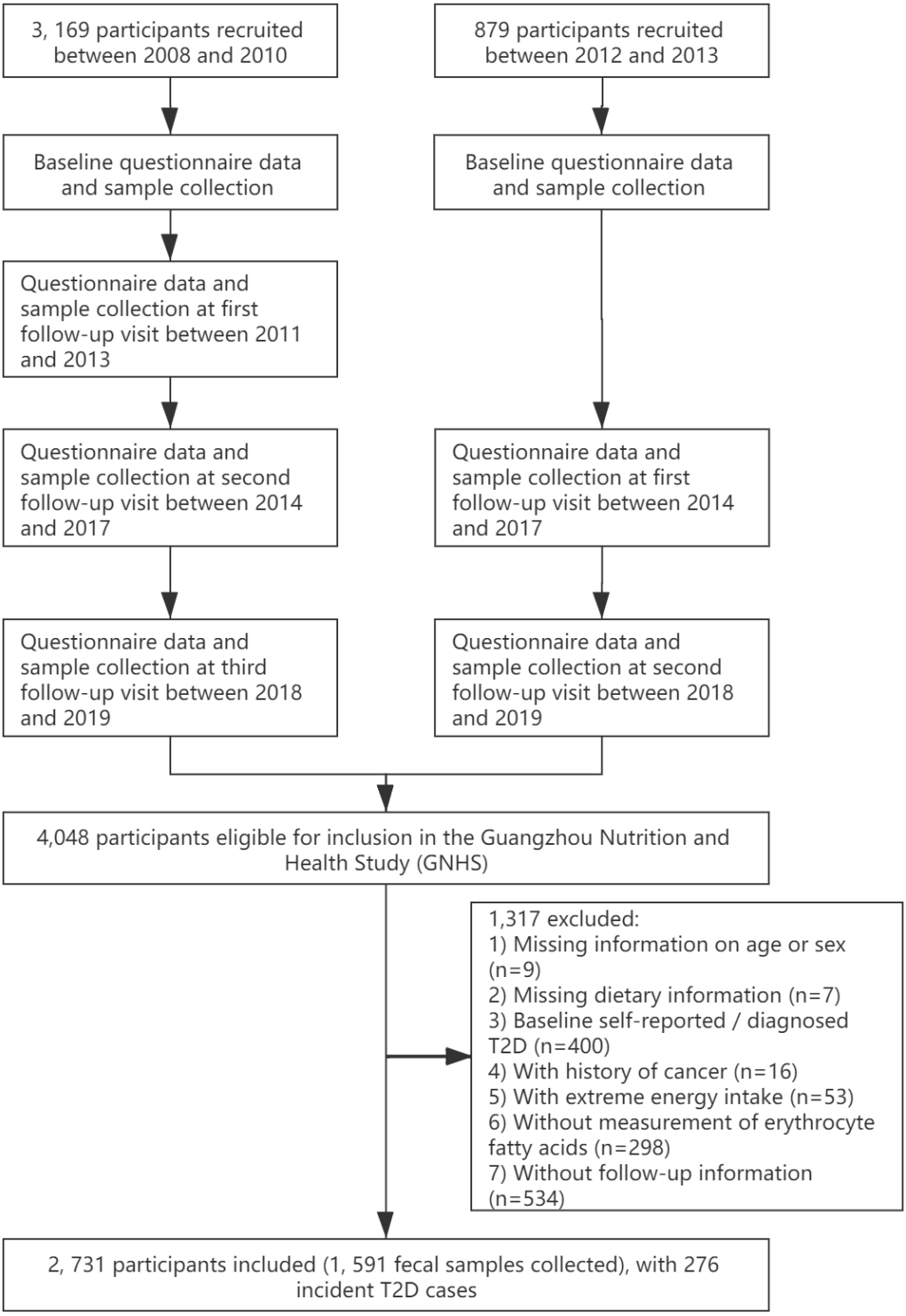
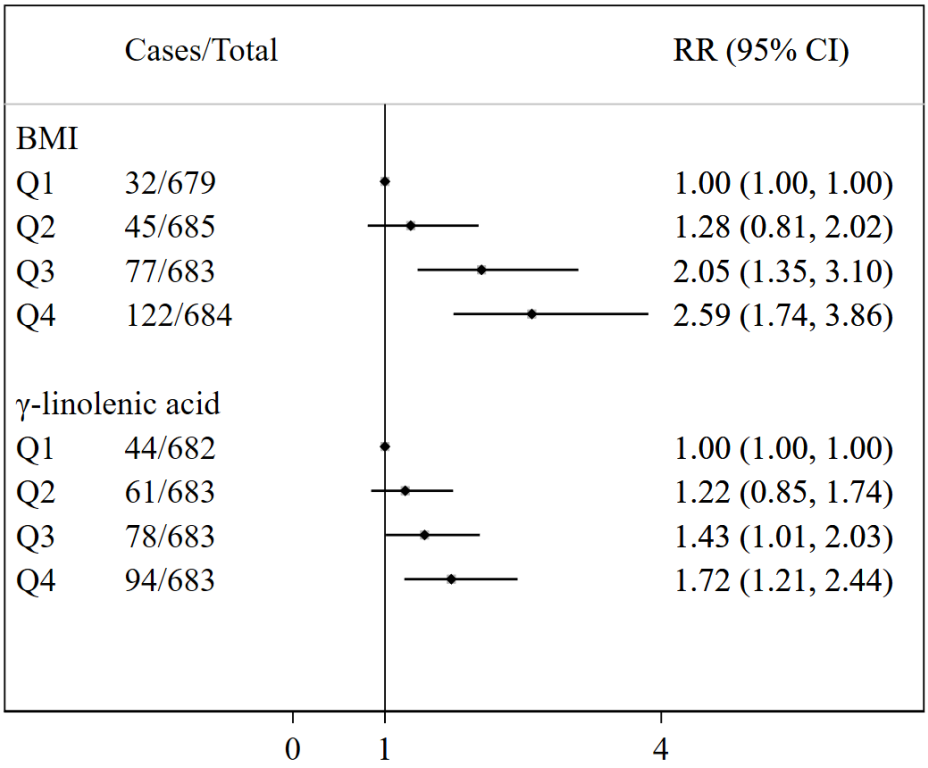


Figure S2. Relative risks of type 2 diabetes according to the interquartile range of γ-linolenic acid and BMI*



*Multivariable-adjusted RRs (95% CIs) were calculated for Q2-Q4 of the erythrocyte n-6 fatty acids and BMI using Q1 as the reference group. Covariates included age, sex, BMI, waist-hip ratio, education, household income, smoking and alcohol drinking status, physical activity, total energy intake, family history of diabetes, baseline erythrocyte total n-3 PUFAs and fasting glucose. Abbreviations: BMI, body mass index; CI, confidence interval; PUFAs, polyunsaturated fatty acids; RR, risk ratio.

SUPPLEMENTAL TABLES

Table S1. Baseline population characteristics by quartiles of erythrocyte linoleic acid and arachidonic acid (N=2,731) *

	Linoleic acid (C18:2n6)				Arachidonic acid (C20:4n6)			
	Q1 (N=682)	**Q2 (N=683)**	**Q3 (N=683)**	**Q4 (N=683)**	**Q1 (N=682)**	**Q2 (N=683)**	**Q3 (N=683)**	**Q4 (N=683)**
Age (year)	58.2 (5.4)	58.3 (5.7)	58.0 (5.8)	57.9 (5.8)	57.7 (5.0)	58.9 (6.0)	58.5 (6.1)	57.2 (5.3)
Sex, % of women	179 (26%)	192 (28%)	207 (30%)	248 (36%)	197 (29%)	233 (34%)	225 (33%)	171 (25%)
BMI (kg/m^2)	23.3 (3.2)	23.5 (2.8)	23.1 (3.0)	23.1 (3.1)	23.4 (3.1)	23.6 (3.1)	23.3 (3.0)	22.7 (3.0)
WHR	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
Education level, n (%)								
Middle school or lower	216 (32%)	193 (28%)	172 (25%)	184 (27%)	204 (30%)	213 (31%)	193 (28%)	155 (23%)
High school or professional college	312 (46%)	330 (48%)	329 (48%)	314 (46%)	309 (45%)	288 (42%)	331 (48%)	357 (52%)
University and upper	154 (23%)	160 (23%)	182 (27%)	185 (27%)	169 (25%)	182 (27%)	159 (23%)	171 (25%)
Household income (Chinese Yuan/month/person)								
≤500	15 (2%)	10 (1%)	14 (2%)	16 (2%)	14 (2%)	17 (2%)	11 (2%)	13 (2%)
500-1500	198 (29%)	162 (24%)	175 (26%)	169 (25%)	200 (29%)	160 (23%)	153 (22%)	191 (28%)
1500-3000	367 (54%)	405 (59%)	392 (57%)	392 (57%)	336 (49%)	412 (60%)	438 (64%)	370 (54%)
>3000	102 (15%)	106 (16%)	102 (15%)	106 (16%)	132 (19%)	94 (14%)	81 (12%)	109 (16%)
Family history of diabetes, %	76 (11%)	73 (11%)	67 (10%)	71 (10%)	80 (12%)	79 (12%)	65 (10%)	63 (9%)
Current smoking, %	83 (12%)	95 (14%)	101 (15%)	133 (19%)	97 (14%)	119 (17%)	108 (16%)	88 (13%)
Current alcohol drinking, %	38 (6%)	36 (5%)	54 (8%)	46 (7%)	36 (5%)	53 (8%)	50 (7%)	35 (5%)
Physical activity (MET•hours/d)	41.3 (14.7)	41.7 (14.7)	41.0 (15.0)	41.8 (15.2)	42.9 (15.9)	41.4 (15.0)	40.1 (13.8)	41.4 (14.8)
Total energy intake (kcal/d)	1740 (470)	1769 (473)	1744 (488)	1819 (509)	1805 (482)	1746 (513)	1737 (484)	1784 (462)
Dairy intake (g/d)	16.0 (13.9)	16.2 (16.5)	16.8 (13.6)	17.3 (13.2)	17.3 (15.4)	16.2 (15.0)	15.7 (13.6)	17.1 (13.4)
Red and processed meat intake (g/d)	83.3 (53.4)	80.4 (47.5)	85.1 (54.8)	86.8 (57.3)	83.7 (51.8)	80.6 (54.4)	86.2 (53.6)	85.0 (53.7)

Vegetable intake (g/d)	381.2 (257.5)	378.1 (163.7)	373.3 (254.0)	396.9 (207.9)	400.2 (318.6)	368.4 (168.3)	378.2 (199.2)	382.6 (176.7)
Fruit intake (g/d)	153.9 (120.3)	150.9 (109.8)	137.1 (92.5)	149.5 (115.6)	153.9 (119.4)	141.3 (103.8)	147.9 (112.3)	148.2 (104.3)
Fish intake (g/d)	52.2 (41.0)	58.1 (47.4)	47.4(31.5)	48.8 (38.4)	52.6 (65.8)	51.4 (40.0)	52.9 (60.0)	49.3 (48.5)
Dietary fiber intake (g/d)	11.4 (4.4)	11.2 (3.1)	11.1 (4.5)	11.5 (3.4)	11.7 (5.6)	11.2 (3.1)	11.1 (3.2)	11.2 (3.0)
Erythrocyte n-3 PUFAs (%)	6.3 (2.3)	7.3 (1.7)	7.2 (1.5)	6.8 (1.4)	5.4 (2.1)	7.1 (1.3)	7.5 (1.4)	7.7 (1.4)
Fasting blood glucose (mmol/L)	4.6 (0.7)	4.8 (0.7)	4.7 (0.6)	4.7 (0.7)	4.6 (0.7)	4.8 (0.7)	4.7 (0.6)	4.6 (0.6)
Serum TG (mmol/L)	1.5 (0.9)	1.6 (1.0)	1.5 (1.0)	1.6 (1.4)	1.7 (1.2)	1.8 (1.4)	1.5 (0.8)	1.2 (0.7)
Serum HDL (mmol/L)	1.4 (0.3)	1.4 (0.3)	1.4 (0.3)	1.4 (0.4)	1.3 (0.3)	1.4 (0.3)	1.4 (0.3)	1.5 (0.3)
Serum LDL (mmol/L)	3.6 (0.9)	3.7 (0.9)	3.6 (0.9)	3.5 (0.9)	3.7 (1.0)	3.6 (0.9)	3.5 (0.9)	3.5 (0.8)

*Data are presented as mean (SD) for continuous variables, and n (%) for categorical variables. Abbreviations: BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PUFAs, polyunsaturated fatty acids; Q, quartile; TG, total triglycerides; WHR, waist-to-hip ratio.

Table S2. Baseline characteristics for participants with and without follow-up information (N=3,265) *

	Participants with follow-up information (N=2,731)	Participants lost to follow-up (N=534)	*p*
Age (year)	58.1 (5.7)	58.8 (7.2)	0.015
Sex, % of women	826 (30%)	174 (33%)	0.28
BMI (kg/m2)	23.2 (3.0)	23.3 (3.1)	0.51
WHR	0.9 (0.1)	0.9 (0.1)	0.055
Education level			<0.001
Middle school or lower	765 (28%)	215 (40%)	
High school or professional college	1,285 (47%)	215 (40%)	
University and upper	681 (25%)	104 (19%)	
Household income (Chinese Yuan/month/person)			0.26
≤500	55 (2%)	14 (3%)	
500-1500	704 (26%)	145 (27%)	
1500-3000	1,556 (57%)	310 (58%)	
>3000	416 (15%)	65 (12%)	
Family history of diabetes	287 (11%)	56 (10%)	0.99
Current smoking	412 (15%)	109 (20%)	0.002
Current alcohol drinking	174 (6%)	42 (8%)	0.20
Physical activity (MET•hours/d)	41.5 (14.9)	39.6 (14.3)	0.008
Total energy intake (kcal/d)	1768 (486)	1717 (530)	0.029
Dairy intake (g/d)	16.6 (14.4)	15.2 (14.5)	0.051
Red and processed meat intake (g/d)	83.9 (53.4)	81.7 (53.3)	0.4
Vegetable intake (g/d)	382.4 (224.2)	351.4 (167.2)	0.002
Fruit intake (g/d)	147.8 (110.2)	142.5 (116.6)	0.32
Fish intake (g/d)	55.3 (73.4)	50.0 (55.7)	0.12
Dietary fiber intake (g/d)	11.3 (3.9)	11.2 (3.6)	0.65
Erythrocyte n-3 PUFAs (%)	6.9 (1.8)	6.8 (1.8)	0.34
Fasting blood glucose (mmol/L)	4.7 (0.7)	4.9 (0.7)	<0.001
Serum TG (mmol/L)	1.5 (1.1)	1.6 (1.1)	0.38
Serum HDL (mmol/L)	1.4 (0.3)	1.4 (0.4)	0.53
Serum LDL (mmol/L)	3.6 (0.9)	3.6 (0.9)	0.53

*Data are presented as mean (SD) for continuous variables, and n (%) for categorical variables. *p*-value for the difference between the two groups was calculated by chi-square test for categorical variables and by ANOVA for continuous variables. Abbreviations: BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PUFAs, polyunsaturated fatty acids; TG, total triglycerides; WHR, waist-to-hip ratio.

Table S3. Baseline characteristics for participants with and without 16S profiling (N=2,731) *

	Participants with 16S profiling (N=1,591)	Participants without 16S profiling (N=1,140)	*p*
Age (year)	57.8 (5.3)	58.3 (5.9)	0.024
Sex, % of women	316 (28%)	510 (32%)	0.015
BMI (kg/m2)	23.3 (3.2)	23.2 (3.0)	0.21
WHR	0.9 (0.1)	0.9 (0.1)	<0.001
Education level			0.17
Middle school or lower	336 (29%)	429 (27%)	
High school or professional college	538 (47%)	747 (47%)	
University and upper	266 (23%)	415 (26%)	
Household income (Chinese Yuan/month/person)			<0.001
≤500	32 (3%)	23 (1%)	
500-1500	339 (30%)	365 (23%)	
1500-3000	565 (50%)	991 (62%)	
>3000	204 (18%)	212 (13%)	
Family history of diabetes	122 (11%)	165 (10%)	0.78
Current smoking	176 (15%)	236 (15%)	0.66
Current alcohol drinking	63 (6%)	111 (7%)	0.13
Physical activity (MET•hours/d)	42.3 (15.7)	40.9 (14.3)	0.012
Total energy intake (kcal/d)	1801 (495)	1745 (478)	0.003
Dairy intake (g/d)	16.9 (14.5)	16.3 (14.2)	0.29
Red and processed meat intake (g/d)	84.6 (54.9)	83.4 (52.3)	0.57
Vegetable intake (g/d)	394.6 (242.6)	373.7 (209.8)	0.017
Fruit intake (g/d)	152.0 (112.8)	144.8 (108.2)	0.094
Fish intake (g/d)	51.5 (53.8)	60.5 (94.0)	0.002
Dietary fiber intake (g/d)	11.1 (3.7)	11.6 (4.1)	0.004
Erythrocyte n-3 PUFAs (%)	6.7 (1.9)	7.0 (1.8)	<0.001
Fasting blood glucose (mmol/L)	4.6 (0.7)	4.7 (0.7)	<0.001
Serum TG (mmol/L)	1.5 (0.9)	1.6 (1.2)	0.028
Serum HDL (mmol/L)	1.4 (0.3)	1.4 (0.3)	0.10
Serum LDL (mmol/L)	3.6 (0.9)	3.6 (0.9)	0.34

*Data are presented as mean (SD) for continuous variables, and n (%) for categorical variables. *p*-value for the difference between the two groups was calculated by chi-square test for categorical variables and by ANOVA for continuous variables. Abbreviations: BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PUFAs, polyunsaturated fatty acids; TG, total triglycerides; WHR, waist-to-hip ratio.

Table S4. Correlation of dietary n-6 fatty acids and erythrocyte n-6 fatty acids*

	Erythrocyte Total n-6 PUFAs	Erythrocyte Linoleic acid (C18:2n6)	Erythrocyte γ-linolenic acid (γC18:3n6)	Erythrocyte Arachidonic acid (C20:4n6)	Dietary Total n-6 PUFAs	Dietary Linoleic acid (C18:2n6)	Dietary Arachidonic acid (C20:4n6)
Erythrocyte total n-6 PUFAs	1						
Erythrocyte linoleic acid (C18:2n6)	0.70†	1					
Erythrocyte γ-linolenic acid (γC18:3n6)	-0.32†	-0.26†	1				
Erythrocyte arachidonic acid (C20:4n6)	0.84†	0.27†	-0.32†	1			
Dietary total n-6 PUFAs	0.08‡	0.14†	0.01	0.02	1		
Dietary linoleic acid (C18:2n6)	0.08‡	0.11†	0.02	0.02	0.99†	1	
Dietary arachidonic acid (C20:4n6)	0.03	-0.01	-0.05	-0.04	0.46†	0.02	1

* Spearman's correlation coefficients were calculated between dietary n-6 fatty acids and erythrocyte n-6 fatty acids (n=2,731).

†P-value for correlation < 0.001.

‡P-value for correlation between 0.001 and 0.05

Table S5. Dietary sources of n-6 polyunsaturated fatty acids*

Food		Linoleic acid (C18:2n6)	γ-linolenic acid (γC18:3n6)	Arachidonic acid (C20:4n6)
Oil	Sunflower oil	60.36	-	-
	Corn oil	53.47	-	-
	Soybean oil	49.37	-	-
	Peanut oil	36.19	-	
	Rapeseed Oil	15.57	-	-
Fish or sea food	Grass carp	0.61	-	0.022
	Carp	0.41	-	0.015
	Prawn	0.054	-	-
	River crab	0.12		-
	Clam	0.36		-
Legumes	Soybean	7.88	-	-
	Tofu	1.76	-	-
Meat	Pork, loin	0.74	-	0.014
	Pork, liver	0.48	-	0.19
	Mutton, lion	0.12	-	0.021
	Chicken, wing	2.28	-	0.022
Nuts	Watermelon seeds, hulled, dried	28.68	-	-
	Walnut, hulled. dried	35.97	-	-
	Hazelnuts, hulled. dried	24.00	-	-
	Peanut, dried	15.87	-	-

* Contents of n-6 polyunsaturated fatty acids in selected plant and animal-based foods were expressed as g/100g, according to the Chinese Food consumption Table, 2002(7).

Table S6. The association between erythrocyte n-6 fatty acids and type 2 diabetes after imputing missing values of type 2 diabetes (N=3,265) *

Erythrocyte n-6 fatty acids		Multivariable-adjusted RRs (95% CIs)				
		Q1(N=397)	Q2(N=398)	Q3(N=398)	Q4(N=398)	*p* for trend
Linoleic acid (C18:2n6)	Simple imputation†	1.00 (Ref)	0.94 (0.69, 1.29)	0.92 (0.67, 1.27)	0.96 (0.70-1.31)	0.78
	Multiple imputation‡	1.00 (Ref)	0.93 (0.70, 1.24)	0.96 (0.70, 1.32)	0.95(0.70, 1.27)	0.78
γ-linolenic acid (γC18:3n6)	Simple imputation†	1.00 (Ref)	1.25 (0.87, 1.81)	1.49 (1.04, 2.13)	1.68 (1.16, 2.42)	0.003
	Multiple imputation‡	1.00 (Ref)	1.15 (0.80, 1.66)	1.28 (0.93, 1.76)	1.47 (1.05, 2.07)	0.014
Arachidonic acid (C20:4n6)	Simple imputation†	1.00 (Ref)	0.94 (0.67, 1.31)	0.97 (0.68, 1.37)	1.03 (0.73, 1.47)	0.80
	Multiple imputation‡	1.00 (Ref)	0.95 (0.71, 1.27)	1.02 (0.74, 1.42)	1.05 (0.74, 1.48)	0.68
Total n-6 PUFAs	Simple imputation†	1.00 (Ref)	1.08 (0.77, 1.50)	0.94 (0.67, 1.33)	1.10 (0.78, 1.54)	0.83
	Multiple imputation‡	1.00 (Ref)	1.06 (0.78, 1.44)	1.04 (0.74, 1.46)	1.07 (0.78, 1.46)	0.74

*Multivariable-adjusted RRs (95% CIs) were calculated for Q2-Q4 of the erythrocyte n-6 fatty acids using Q1 as the reference group. Covariates included age, sex, BMI, waist-hip ratio, education, household income, smoking and alcohol drinking status, physical activity, total energy intake, family history of diabetes, baseline erythrocyte total n-3 PUFAs and fasting glucose. *p* value for trend was calculated based on per quartile increase in the corresponding PUFA.
Abbreviations: BMI, body mass index; CIs, confidence intervals; PUFAs, polyunsaturated fatty acids; Q, quartile; RRs, risk ratios.
†Simple imputation: assuming the participants with missing outcome data did not develop T2D.
‡Multiple imputation: data were imputed using multivariate imputation with a logistic imputation model for the outcome for 10 rounds.

Table S7. The association between erythrocyte n-6 fatty acids and type 2 diabetes excluding cases ascertained only by fasting glucose (N=2,679) *

Erythrocyte n-6 fatty acids	Multivariable-adjusted RRs (95% CIs)				
	Q1	Q2	Q3	Q4	*p* for trend
Linoleic acid (C18:2n6)	1.00 (Ref)	0.92 (0.65, 1.30)	0.92 (0.65, 1.29)	1.01 (0.71-1.41)	0.95
γ-linolenic acid (γC18:3n6)	1.00 (Ref)	1.37 (0.93, 2.03)	1.47 (0.99, 2.19)	1.86 (1.25, 2.75)	0.002
Arachidonic acid (C20:4n6)	1.00 (Ref)	0.81 (0.55, 1.16)	0.96 (0.66, 1.39)	1.00 (0.69, 1.47)	0.70
Total n-6 PUFAs	1.00 (Ref)	1.01 (0.70, 1.45)	1.04 (0.72, 1.51)	1.12 (0.77, 1.63)	0.53

*Multivariable-adjusted RRs (95% CIs) were calculated for Q2-Q4 of the erythrocyte n-6 fatty acids using Q1 as the reference group. Covariates included age, sex, BMI, waist-hip ratio, education, household income, smoking and alcohol drinking status, physical activity, total energy intake, family history of diabetes, baseline erythrocyte total n-3 PUFAs and fasting glucose. *p* value for trend was calculated based on per quartile increase in the corresponding PUFA.
Abbreviations: BMI, body mass index; CIs, confidence intervals; PUFAs, polyunsaturated fatty acids; Q, quartile; RRs, risk ratios.

Table S8. Numbers of type 2 diabetes cases diagnosed with fasting glucose, HbA1c, or self-reported diabetes medication

Diagnostic criteria	Fasting glucose	HbA1c	Self-reported diabetes medication	HbA1c or self-reported diabetes medication
N of cases diagnosed by the factor listed above	115/276*	143/276	106/276	224/276

*276 is the total number of type 2 diabetes cases ascertained in the present study using fasting glucose, HbA1c or self-reported diabetes medication.

Table S9. Association of erythrocyte n-6 fatty acid biomarkers with incident type 2 diabetes adjusting for additional potential covariates *

Erythrocyte n-6 fatty acids		Multivariable-adjusted RRs (95% CIs)				
		Q1	Q2	Q3	Q4	*p* for trend
Linoleic acid	Model 3	1.00 (Ref)	0.84 (0.57, 1.25)	1.03 (0.71, 1.49)	0.76 (0.51, 1.15)	0.36
(C18:2n6)	Model 3a	1.00 (Ref)	0.94 (0.70, 1.26)	0.93 (0.68, 1.26)	0.91 (0.67-1.23)	0.54
	Model 3b	1.00 (Ref)	1.91 (0.68, 1.23)	0.93 (0.69, 1.26)	0.91 (0.68, 1.24)	0.61
	Model 3c	1.00 (Ref)	0.92 (0.70, 1.24)	0.94 (0.70, 1.26)	0.97 (0.71, 1.31)	0.86
γ-linolenic acid	Model 3	1.00 (Ref)	1.10 (0.70, 1.74)	1.26 (0.80, 1.98)	1.83 (1.18, 2.85)	0.0038
(γC18:3n6)	Model 3a	1.00 (Ref)	1.21 (0.84, 1.73)	1.48 (1.04, 2.11)	1.69 (1.19, 2.41)	0.0012
	Model 3b	1.00 (Ref)	1.22 (0.85, 1.74)	1.42 (1.00, 2.02)	1.70 (1.20, 2.42)	0.0014
	Model 3c	1.00 (Ref)	1.22 (0.86, 1.74)	1.41 (0.99, 1.99)	1.69 (1.19, 2.40)	0.0016
Arachidonic	Model 3	1.00 (Ref)	1.08 (0.73, 1.61)	1.02 (0.66, 1.58)	1.25 (0.81, 1.93)	0.41
acid (C20:4n6)	Model 3a	1.00 (Ref)	0.88 (0.64, 1.21)	0.92 (0.66, 1.30)	1.00 (0.71, 1.40)	0.91
	Model 3b	1.00 (Ref)	0.89 (0.65, 1.22)	0.98 (0.70, 1.38)	1.02 (0.72, 1.45)	0.72
	Model 3c	1.00 (Ref)	0.90 (0.66, 1.23)	0.99 (0.71, 1.39)	1.03 (0.74, 1.45)	0.68
Total n-6	Model 3	1.00 (Ref)	0.89 (0.59, 1.34)	1.02 (0.68, 1.53)	1.07 (0.71, 1.61)	0.58
PUFAs	Model 3a	1.00 (Ref)	1.02 (0.74, 1.39)	0.96 (0.69, 1.34)	1.03 (0.74, 1.44)	0.96
	Model 3b	1.00 (Ref)	1.02 (0.75, 1.40)	0.99 (0.71, 1.38)	1.08 (0.77, 1.50)	0.73
	Model 3c	1.00 (Ref)	1.04 (0.76, 1.41)	1.00 (0.72, 1.39)	1.13 (0.81, 1.57)	0.54

*Multivariable-adjusted RRs (95% CIs) were calculated for Q2-Q4 of the erythrocyte n-6 fatty acids using Q1 as the reference group. Model 3 was adjusted for age, sex, BMI, waist-hip ratio, physical activity, education, alcohol drinking, smoking, household income, family history of diabetes, total energy intake, fasting blood glucose and erythrocyte total n-3 PUFAs. Model 3a (n=2,707) included covariates in model 3 + additional dietary variables (dietary intake of dairy products, red and processed meat, fish, vegetable, and fruit, in quartiles). Model 3b (n=2,731) included covariates in model 3 + baseline total triglycerides and low-density lipoprotein cholesterol. Model 3c (n=2,731) included covariates in model 3 + prevalent coronary heart disease, treatment for hypertension and hyperlipidemia. *p* value for trend was calculated based on per quartile increase in the corresponding PUFA. Abbreviations: BMI, body mass index; CIs, confidence intervals; PUFAs, polyunsaturated fatty acids; Q, quartile; RRs, risk ratios.

Table S10. Association of erythrocyte n-6 fatty acid biomarkers with microbiota α-diversity (N=1,591) *

Erythrocyte n-6 fatty acids		Observed OTUs		Chao index		Shannon' diversity index		Simpson index	
		beta (95% CIs)	*p*	beta (95% Cis)	*p*	beta (95% Cis)	*p*	beta (95% Cis)	*p*
Linoleic acid	Q1	reference		reference		reference		reference	
(C18:2n6)	Q2	-0.14 (-0.27, -0.02)	0.06	-0.13 (-0.26,0)	0.08	-0.11 (-0.24,0.02)	0.12	-0.06 (-0.19,0.08)	0.30
	Q3	-0.02 (-0.15,0.1)	0.45	-0.02 (-0.15,0.11)	0.48	0.02 (-0.11,0.15)	0.48	0.04 (-0.09,0.18)	0.38
	Q4	-0.06 (-0.18,0.07)	0.28	-0.05 (-0.18,0.08)	0.32	-0.03 (-0.16,0.1)	0.42	-0.05 (-0.18,0.08)	0.34
	per quartile	-0.01 (-0.04,0.03)	0.48	0 (-0.04,0.03)	0.49	0 (-0.03,0.04)	0.49	-0.01 (-0.04,0.03)	0.48
γ-linolenic acid	Q1	reference		reference		reference		reference	
(γC18:3n6)	Q2	-0.16 (-0.28, -0.03)	0.052	-0.14 (-0.27, -0.01)	0.067	-0.13 (-0.26,0)	0.083	-0.06 (-0.19,0.07)	0.28
	Q3	-0.14 (-0.26, -0.01)	0.070	-0.13 (-0.26,0)	0.077	-0.14 (-0.27, -0.01)	0.074	-0.13 (-0.26,0.01)	0.089
	Q4	-0.26 (-0.39, -0.13)	0.021	-0.26 (-0.39, -0.12)	0.023	-0.23 (-0.37, -0.1)	0.028	-0.15 (-0.29, -0.01)	0.067
	per quartile	-0.08 (-0.11, -0.04)	0.025	-0.08 (-0.11, -0.04)	0.026	-0.07 (-0.11, -0.03)	0.031	-0.05 (-0.09, -0.02)	0.057
Arachidonic	Q1	reference		reference		reference		reference	
acid (C20:4n6)	Q2	0.03 (-0.11,0.16)	0.46	0.02 (-0.12,0.16)	0.47	0.03 (-0.11,0.17)	0.44	0.06 (-0.09,0.2)	0.33
	Q3	-0.01 (-0.14,0.13)	0.50	-0.01 (-0.15,0.13)	0.50	-0.04 (-0.18,0.11)	0.42	-0.07 (-0.21,0.08)	0.29
	Q4	0.08 (-0.06,0.22)	0.20	0.1 (-0.04,0.24)	0.15	0.06 (-0.08,0.21)	0.29	0.06 (-0.09,0.21)	0.33
	per quartile	0.02 (-0.01,0.06)	0.25	0.03 (-0.01,0.07)	0.18	0.01 (-0.02,0.05)	0.40	0 (-0.03,0.04)	0.49
Total n-6	Q1	reference		reference		reference		reference	
PUFAs	Q2	-0.04 (-0.18,0.09)	0.38	-0.05 (-0.18,0.09)	0.38	-0.06 (-0.2,0.08)	0.31	-0.05 (-0.19,0.09)	0.36
	Q3	-0.05 (-0.18,0.09)	0.36	-0.03 (-0.16,0.11)	0.46	-0.03 (-0.17,0.1)	0.43	0.02 (-0.12,0.16)	0.48
	Q4	0.05 (-0.08,0.19)	0.33	0.06 (-0.07,0.2)	0.27	0.02 (-0.12,0.15)	0.48	-0.03 (-0.17,0.11)	0.44
	per quartile	0.02 (-0.02,0.05)	0.31	0.02 (-0.01,0.06)	0.22	0.01 (-0.03,0.05)	0.44	0 (-0.04,0.04)	0.50

* Beta values (95% CIs) were calculated for Q2-Q4 of the erythrocyte n-6 fatty acids using Q1 as the reference group. α-diversity metrics were standardized to have mean 0 and SD 1 and then were modeled as responses in linear mixed models with technical confounders including sequencing depth and Bristol scale as fixed effects, and sequencing batch as random effect. Linear regression was conducted with the residuals of α-diversity metrics as dependent variables and baseline quartiles of individual n-6 fatty acid biomarkers as independent variables. Covariates included age, sex, BMI and waist-hip ratio, education, household income, smoking and alcohol drinking status, physical activity, total energy intake, baseline erythrocyte total n-3 PUFAs. Abbreviations: BMI, body mass index; CIs, confidence intervals; PUFAs, polyunsaturated fatty acids; Q, quartile.

Table S11. Association between erythrocyte n-6 fatty acid biomarkers and microbiota α-diversity, additionally adjusted for dietary fiber intake (N=1,581) *

Erythrocyte n-6 fatty acids		Observed OTUs		Chao index		Shannon' diversity index		Simpson index	
		beta (95% CIs)	*p*	beta (95% Cis)	*p*	beta (95% Cis)	*p*	beta (95% Cis)	*p*
Linoleic acid	Q1	reference		reference		reference		reference	
(C18:2n6)	Q2	-0.14(-0.27, -0.02)	0.06	-0.14(-0.27, -0.01)	0.07	-0.11(-0.24,0.02)	0.11	-0.05(-0.19,0.08)	0.32
	Q3	-0.03(-0.15,0.1)	0.44	-0.02(-0.15,0.1)	0.45	0.01(-0.12,0.14)	0.49	0.04(-0.09,0.17)	0.39
	Q4	-0.07(-0.19,0.06)	0.23	-0.06(-0.19,0.06)	0.26	-0.04(-0.17,0.08)	0.36	-0.06(-0.19,0.07)	0.30
	per quartile	-0.01(-0.04,0.02)	0.45	-0.01(-0.04,0.03)	0.46	0(-0.04,0.03)	0.50	-0.01(-0.04,0.03)	0.46
γ-linolenic acid	Q1	reference		reference		reference		reference	
(γC18:3n6)	Q2	-0.15(-0.28, -0.03)	0.05	-0.14(-0.27, -0.01)	0.07	-0.13(-0.25,0)	0.09	-0.06(-0.19,0.08)	0.30
	Q3	-0.14(-0.27, -0.01)	0.07	-0.14(-0.27, -0.01)	0.07	-0.14(-0.27, -0.01)	0.07	-0.13(-0.27,0)	0.08
	Q4	-0.26(-0.39, -0.13)	0.02	-0.25(-0.39, -0.12)	0.02	-0.23(-0.37, -0.09)	0.03	-0.15(-0.29, -0.01)	0.07
	per quartile	-0.08(-0.11, -0.04)	0.03	-0.08(-0.11, -0.04)	0.03	-0.07(-0.11, -0.03)	0.03	-0.05(-0.09, -0.02)	0.06
Arachidonic	Q1	reference		reference		reference		reference	
acid (C20:4n6)	Q2	0.03(-0.11,0.16)	0.44	0.02(-0.12,0.16)	0.47	0.03(-0.11,0.17)	0.43	0.06(-0.08,0.2)	0.30
	Q3	-0.01(-0.15,0.13)	0.49	-0.02(-0.16,0.13)	0.48	-0.04(-0.19,0.1)	0.39	-0.07(-0.22,0.08)	0.27
	Q4	0.08(-0.06,0.22)	0.23	0.09(-0.05,0.24)	0.17	0.06(-0.09,0.2)	0.34	0.05(-0.1,0.2)	0.36
	per quartile	0.02(-0.02,0.06)	0.29	0.03(-0.01,0.06)	0.21	0.01(-0.03,0.05)	0.45	0(-0.04,0.04)	0.50
Total n-6	Q1	reference		reference		reference		reference	
PUFAs	Q2	-0.05(-0.18,0.09)	0.37	-0.05(-0.19,0.09)	0.35	-0.06(-0.2,0.08)	0.29	-0.05(-0.19,0.1)	0.37
	Q3	-0.05(-0.18,0.09)	0.37	-0.03(-0.16,0.11)	0.46	-0.03(-0.17,0.1)	0.43	0.02(-0.12,0.16)	0.48
	Q4	0.04(-0.09,0.18)	0.38	0.05(-0.08,0.19)	0.33	0(-0.13,0.14)	0.50	-0.04(-0.18,0.1)	0.42
	per quartile	0.01(-0.02,0.05)	0.35	0.02(-0.02,0.06)	0.26	0.01(-0.03,0.04)	0.47	0(-0.04, 0.03)	0.49

* Beta values (95% CIs) were calculated for Q2-Q4 of the erythrocyte n-6 fatty acids using Q1 as the reference group. α-diversity metrics were standardized to have mean 0 and SD 1 and then were modeled as responses in mixed models with technical confounders including sequencing depth and Bristol scale as fixed effects, and sequencing batch as random effect. Linear regression was conducted with the residuals of α-diversity metrics as dependent variables and baseline quartiles of individual n-6 fatty acid biomarkers as independent variables. Covariates included age, sex, BMI and waist-hip ratio, education, household income, smoking and alcohol drinking status, physical activity, total energy intake, baseline erythrocyte total n-3 PUFAs, and dietary fiber intake. Abbreviations: BMI, body mass index; CIs, confidence intervals; PUFAs, polyunsaturated fatty acids; Q, quartile.

Table S12. Association of erythrocyte n-6 fatty acid biomarkers with incident type 2 diabetes among participants with 16S profiling (N=1,591) *

Erythrocyte n-6 fatty acids		Multivariable-adjusted RRs (95% CIs)				
		Q1(N=397)	Q2(N=398)	Q3(N=398)	Q4(N=398)	*p* for trend
Linoleic acid	Median, %	8.11	9.43	10.29	11.41	
(C18:2n6)	No. of cases	45	39	45	35	
	Model 1	1.00 (Ref)	0.84 (0.56, 1.25)	1.05 (0.71, 1.54)	0.82 (0.54-1.24)	0.60
	Model 2	1.00 (Ref)	0.86 (0.58, 1.28)	1.09 (0.74, 1.60)	0.84 (0.56, 1.27)	0.68
	Model 3	1.00 (Ref)	0.84 (0.57, 1.25)	1.03 (0.71, 1.49)	0.76 (0.51, 1.15)	0.36
γ-linolenic acid	Median, %	0.02	0.03	0.04	0.07	
(γC18:3n6)	No. of cases	27	34	42	61	
	Model 1	1.00 (Ref)	1.21 (0.75, 1.95)	1.50 (0.95, 2.36)	2.18 (1.42, 3.35)	<0.001
	Model 2	1.00 (Ref)	1.22 (0.76, 1.96)	1.44 (0.91, 2.28)	2.09 (1.35, 3.24)	<0.001
	Model 3	1.00 (Ref)	1.10 (0.70, 1.74)	1.26 (0.80, 1.98)	1.83 (1.18, 2.85)	0.0038
Arachidonic	Median, %	7.73	10.9	12.02	13.41	
acid (C20:4n6)	No. of cases	47	45	37	35	
	Model 1	1.00 (Ref)	0.82 (0.55, 1.21)	0.71 (0.48, 1.07)	0.80 (0.53, 1.20)	0.21
	Model 2	1.00 (Ref)	0.87 (0.58, 1.30)	0.76 (0.50, 1.14)	0.83 (0.55, 1.25)	0.27
	Model 3	1.00 (Ref)	1.08 (0.73, 1.61)	1.02 (0.66, 1.58)	1.25 (0.81, 1.93)	0.41
Total n-6	Median, %	16.76	20.77	22.32	23.97	
PUFAs	No. of cases	45	42	39	38	
	Model 1	1.00 (Ref)	0.83 (0.56, 1.24)	0.84 (0.56, 1.26)	0.92 (0.61, 1.37)	0.68
	Model 2	1.00 (Ref)	0.89 (0.59, 1.33)	0.91 (0.61, 1.37)	0.92 (0.61, 1.38)	0.72
	Model 3	1.00 (Ref)	0.89 (0.59, 1.34)	1.02 (0.68, 1.53)	1.07 (0.71, 1.61)	0.58

* Multivariable-adjusted RRs (95% CIs) were calculated for Q2-Q4 of the erythrocyte n-6 fatty acids using Q1 as the reference group. Covariates included in model 1 were age, sex, BMI and waist-hip ratio; model 2, model 1 + education, household income, smoking and alcohol drinking status, physical activity, total energy intake, family history of diabetes; and model 3, as model 2 + baseline erythrocyte total n-3 PUFAs and fasting glucose. *p* value for trend was calculated based on per quartile increase in the corresponding PUFA. Abbreviations: BMI, body mass index; CIs, confidence intervals; PUFAs, polyunsaturated fatty acids; Q, quartile; RRs, risk ratios.

Table S13. Summary statistics of the mediation analysis for α-diversity indicators*

	n	ACME	*p*	ADE	*p*	%
Observed OTUs	1563	0.0011	0.006	0.14	0.01	7.9
Chao index	1563	0.00097	0.011	0.012	0.0093	7.1
Shannon's diversity index	1563	0.00098	0.005	0.012	0.01	7.1

* Models were built to test whether the association between γ-linolenic acid and type 2 diabetes was mediated by gut microbial α-diversity indices. The direct and indirect effects are calculated using the quasi-Bayesian Monte Carlo method with 2,000 simulations (R {*mediation*}). Covariates included age, sex, BMI and waist-hip ratio, education, household income, smoking and alcohol drinking status, physical activity, total energy intake, family history of diabetes, baseline erythrocyte total n-3 polyunsaturated fatty acids and fasting glucose. Abbreviations: BMI, body mass index.

Table S14. Association of erythrocyte n-6 fatty acid biomarkers with microbiota β-diversity (N=1,591) *

Erythrocyte n-6 fatty acids	Genus scaled relative abundances				OTU scaled relative abundances			
	Df	F	R^2	*p*	Df	F	R^2	*p*
Linoleic acid (C18:2n6)	3	1.17962	0.00214	0.14	3	1.05695	0.002	0.31
γ-linolenic acid (γC18:3n6)	3	1.63964	0.00298	0.002	3	1.46483	0.003	0.001
Arachidonic acid (C20:4n6)	3	1.66591	0.00303	0.002	3	1.42632	0.003	0.006
Total n-6 PUFAs	3	1.36979	0.00249	0.03	3	1.21031	0.002	0.062

* The dissimilarities in gut composition between quartiles of n-6 PUFA biomarkers (β-diversity) were assessed with PERMANOVA (R function adonis {vegan}, 999 permutations) based on the Bray-Curtis distance calculated at the genus and OTU level. Scaled (that is, divided by the standard deviation) relative abundances were used. The potential confounders included in the PERMANOVA were sequencing depth, sequencing batch, Bristol scale, age, sex, BMI, waist-hip ratio, education, household income, smoking status, alcohol drinking status, physical activity, total energy intake, and baseline erythrocyte total n-3 PUFAs. Abbreviations: BMI, body mass index, PUFAs, polyunsaturated fatty acids.

Table S15. Results from Pairwise PERMANOVA analysis of γ-linolenic acid (γC18:3n6) verses microbiota β diversity (N=1,591) *

γ-linolenic acid (γC18:3n6)	Genus scaled relative abundances				OTU scaled relative abundances			
	F	R^2	*p*	*p* adjusted	F	R^2	*p*	*p* adjusted
Q1 vs Q2	1.35734	0.00171	0.118	0.708	1.1686	0.00147	0.18	1
Q1 vs Q3	2.46699	0.00310	0.002	0.012	1.89583	0.00239	0.001	0.006
Q1 vs Q4	2.55441	0.00321	0.001	0.006	2.15991	0.00272	0.002	0.012
Q2 vs Q3	0.75098	0.00095	0.795	1	1.05105	0.00132	0.36	1
Q2 vs Q4	1.39985	0.00176	0.087	0.522	1.45270	0.00183	0.035	0.21
Q3 vs Q4	0.97721	0.00123	0.49	1	1.07135	0.00135	0.34	1

* The dissimilarities in gut composition between different quartiles of γ-linolenic acid (β-diversity) were assessed with Pairwise PERMANOVA (R function pairwise.adonis {vegan}, 999 permutations) based on the Bray-Curtis distance calculated at the genus and OTU level. Scaled (that is, divided by the standard deviation) relative abundances were used. *p* values were adjusted for multiple testing using Bonferroni method. Abbreviations: Q, quartile.

Table S16. Cross-sectional association of microbiota α-diversity with type 2 diabetes (N=1,563) *

α-diversity	ORs (95%CIs)	*p*
Observed OTUs	0.72 (0.61, 0.84)	<0.001
Chao index	0.75 (0.64, 0.87)	0.0024
Shannon's diversity index	0.73 (0.64, 0.84)	<0.001
Simpson index	0.84 (0.75, 0.95)	0.02

*Results from logistic regression analysis of microbiota α-diversity metrics versus type 2 diabetes, adjustment for sequencing batch, sequencing depth, Bristol scale, age, sex, BMI, waist-hip ratio, education, household income, smoking status, alcohol drinking status, prevalent hypertension and dyslipidemia. Abbreviations: BMI, body mass index; CIs, confidence intervals; ORs, odds ratios.

Table S17. Cross-sectional association of microbiota β-diversity with type 2 diabetes (N=1,563) *

	Genus scaled relative abundances				OTU scaled relative abundances			
	Df	**F**	**R^2**	***p***	**Df**	**F**	**R^2**	***p***
Type 2 diabetes	1	4.02387	0.00248	0.001	1	2.46126	0.00152	0.001

*The dissimilarities in gut composition across type 2 diabetes (β-diversity) were assessed with PERMANOVA (R function adonis {vegan}, 999 permutations) based on the Bray-Curtis distance calculated at the genus and OTU level. Scaled (that is, divided by the standard deviation) relative abundances were used. The potential confounders included in the PERMANOVA were sequencing depth, sequencing batch, Bristol scale, age, sex, BMI, waist-hip ratio, education, household income, smoking status, alcohol drinking status, prevalent hypertension and dyslipidemia.

Table S18. Association of taxonomic biomarkers of γ-linolenic acid (γC18:3n6) with type 2 diabetes-related traits *

	Total triglycerides (mmol/L)		Low-density lipoprotein cholesterol (mmol/L)		High-density lipoprotein cholesterol (mmol/L)		Total cholesterol (mmol/L)		Non-HDL (mmol/L)	
	rho	Bonferroni-adjusted *p*	rho	Bonferroni-adjusted *p*	rho	Bonferroni-adjusted *p*	rho	Bonferroni-adjusted *p*	rho	Bonferroni-adjusted *p*
Bacteroides	0.0618	1	0.0163	1	0.0033	1	0.0176	1	0.0216	1
[Eubacterium]	-0.1298	0.0001	0.0032	1	0.0088	1	0.0183	1	0.0273	1
Turicibacter	-0.0748	1	0.0355	1	0.0301	1	0.0329	1	0.014	1
Streptophyta Other	-0.0098	1	-0.0285	1	0.0245	1	0.0184	1	0.0188	1
Paraprevotella	-0.0527	1	0.0259	1	0.0396	1	0.0611	1	0.0413	1
S24-7 Other	-0.1311	0.0001	-0.0122	1	0.0925	0.11	0.0145	1	0.0404	1
Butyrivibrio	-0.0838	0.39	0.0355	1	0.02	1	0.024	1	0.0178	1
Coriobacteriaceae Other	-0.1653	<0.0001	0.0298	1	0.0645	1	0.0086	1	0.0204	1
Blautia	-0.1130	0.0033	0.0239	1	0.0717	1	0.0161	1	0.0122	1
Christensenellaceae Other	-0.1142	0.0027	0.022	1	0.0627	1	0.0061	1	0.0335	1
Rikenellaceae Other	-0.0730	1	0.0257	1	0.007	1	0.0257	1	0.0245	1
Oscillospira	-0.1362	<0.0001	0.0355	1	0.0882	0.21	0.0085	1	0.0287	1
Odoribacter	-0.1038	0.017	0.0278	1	0.016	1	0.0086	1	0.0081	1
Clostridiales Other	-0.1023	0.022	0.0039	1	0.0159	1	0.0246	1	0.0358	1
Prevotella	-0.0817	0.52	0.0339	1	0.0572	1	0.0324	1	0.0241	1
Rothia	-0.0792	0.73	0.0348	1	0.0797	0.69	0.0559	1	0.0474	1
Coprococcus	-0.0693	1	-0.0026	1	0.0081	1	0.0095	1	0.007	1
Faecalibacterium	-0.1104	0.0054	0.0074	1	0.0502	1	0.0333	1	0.0477	1
Sutterella	-0.0706	1	0.0266	1	0.0227	1	-0.012	1	0.0208	1

	Fasting glucose (mmol/L)		Hb1Ac (%)		Fasting insulin (mIU/L)		HOMA-IR		HOMA-β	
	rho	Bonferroni-adjusted *p*	rho	Bonferroni-adjusted *p*	rho	Bonferroni-adjusted *p*	rho	Bonferroni-adjusted *p*	rho	Bonferroni-adjusted *p*
Bacteroides	-0.0223	1	0.0333	1	-0.0002	1	-0.009	1	0.0034	1
[Eubacterium]	0.0189	1	-0.0112	1	-0.0075	1	-0.015	1	0.0121	1
Turicibacter	-0.0008	1	0.0113	1	-0.0319	1	-0.0496	1	-0.0126	1
Streptophyta Other	-0.0141	1	-0.0022	1	0.0409	1	0.0354	1	0.029	1
Paraprevotella	0.0060	1	-0.03	1	-0.009	1	-0.0032	1	0	1
S24-7 Other	-0.0079	1	-0.0114	1	0.0074	1	0.0004	1	0.0092	1
Butyrivibrio	-0.0100	1	-0.0148	1	-0.0374	1	-0.0457	1	-0.0425	1
Coriobacteriaceae Other	-0.0073	1	-0.0306	1	0.0404	1	0.0306	1	0.0281	1
Blautia	-0.0351	1	-0.0124	1	0.031	1	0.0091	1	0.0374	1
Christensenellaceae Other	0.0004	1	-0.0059	1	0.0558	1	0.0487	1	0.0482	1
Rikenellaceae Other	-0.0416	1	-0.0115	1	0.0575	1	0.0384	1	0.0486	1
Oscillospira	-0.0563	1	-0.0187	1	0.0343	1	0.0023	1	0.0611	1
Odoribacter	-0.0267	1	-0.0131	1	0.023	1	0.0102	1	0.0393	1
Clostridiales Other	-0.0346	1	-0.0418	1	-0.0594	1	-0.0741	1	-0.0428	1
Prevotella	0.0274	1	-0.0102	1	-0.0016	1	-0.013	1	0.0016	1
Rothia	-0.0084	1	0.0103	1	-0.004	1	-0.0035	1	-0.0118	1
Coprococcus	0.0312	1	-0.0087	1	0.0542	1	0.0542	1	0.0333	1
Faecalibacterium	0.0425	1	-0.0014	1	0.0128	1	0.0029	1	0.0118	1
Sutterella	0.0073	1	-0.0311	1	0.0182	1	0.0066	1	0.0242	1

*Spearman correlation with prior adjustment of the type 2 diabetes-related traits for age, sex and BMI by linear regression, with γ-linolenic acid-related microbes. The multiple testing was adjusted by Bonferroni correction.

Table S19. The association between dietary n-6 fatty acids intake and type 2 diabetes (N=2,731) *

Dietary n-6 fatty acids	Multivariable-adjusted RRs (95% CIs)				
	Q1(N=397)	Q2(N=398)	Q3(N=398)	Q4(N=398)	*p* for trend
Linoleic acid (C18:2n6)	1.00 (Ref)	1.44 (1.03, 2.00)	1.20 (0.85, 1.69)	1.51 (1.09-2.09)	0.045
Arachidonic acid (C20:4n6)	1.00 (Ref)	1.04 (0.76, 1.42)	1.03 (0.76, 1.40)	1.08 (0.80, 1.47)	0.63
Total n-6 PUFAs	1.00 (Ref)	1.29 (0.93, 1.78)	1.11 (0.79, 1.56)	1.44 (1.05, 1.98)	0.056

*Multivariable-adjusted RRs (95% CIs) were calculated for Q2-Q4 of the dietary n-6 fatty acids using Q1 as the reference group. Covariates included age, sex, BMI, waist-hip ratio, education, household income, smoking and alcohol drinking status, physical activity, total energy intake, family history of diabetes, fasting glucose and baseline erythrocyte total n-3 PUFAs. *p* value for trend was calculated based on per quartile increase in the corresponding PUFA. Abbreviations: BMI, body mass index; CIs, confidence intervals; PUFAs, polyunsaturated fatty acids; Q, quartile; RRs, risk ratios.

Table S20. Association of dietary n-6 fatty acid intake with microbiota α-diversity (N=1,591) *

Dietary n-6 fatty acids		Observed OTUs		Chao index		Shannon' diversity index		Simpson index	
		beta (95% CIs)	*p*	beta (95% Cis)	*p*	beta (95% Cis)	*p*	beta (95% Cis)	*p*
Linoleic acid	Q1	reference		reference		reference		reference	
(C18:2n6)	Q2	-0.07 (-0.20, 0.05)	0.20	-0.08(-0.21, 0.05)	0.18	-0.10(-0.22,0.03)	0.14	-0.12(-0.25,0.01)	0.10
	Q3	-0.08(-0.20, 0.05)	0.19	-0.08(-0.21 ,0.05)	0.18	-0.09(-0.22,0.04)	0.15	-0.07(-0.2,0.06)	0.24
	Q4	-0.16(-0.23, -0.03)	0.052	-0.18(-0.31, 0.05)	0.043	-0.09(-0.22,0.04)	0.15	-0.04(-0.17,0.1)	0.41
	per quartile	-0.05(-0.08, -0.01)	0.058	-0.05(-0.09, -0.02)	0.048	-0.03(-0.06,0.01)	0.16	-0.01(-0.04,0.03)	0.47
Arachidonic	Q1	reference		reference		reference		reference	
acid (C20:4n6)	Q2	-0.01(-0.14, 0.12)	0.50	0.02(-0.11, 0.15)	0.47	-0.04(-0.17,0.09)	0.40	-0.06(-0.19,0.08)	0.32
	Q3	0.08(-0.05, 0.21)	0.19	0.10(-0.04, 0.23)	0.15	0.07(-0.07,0.20)	0.26	0.05(-0.08,0.19)	0.34
	Q4	0.07(-0.06, 0.21)	0.24	0.08(-0.05, 0.22)	0.19	0.11(-0.03,0.25)	0.13	0.14(-0.004,0.28)	0.09
	per quartile	0.03(-0.005, 0.07)	0.15	0.03(-0.004, 0.07)	0.14	0.04(0.01,0.08)	0.09	0.05(0.02,0.09)	0.06
Total n-6	Q1	reference		reference		reference		reference	
PUFAs	Q2	-0.08(-0.21, 0.04)	0.17	-0.09(-0.21, 0.04)	0.16	-0.10(-0.23,0.03)	0.12	-0.10(-0.23,0.03)	0.13
	Q3	-0.09(-0.21, 0.04)	0.16	-0.10(-0.22, 0.03)	0.14	-0.08(-0.21,0.05)	0.19	-0.04(-0.17,0.1)	0.41
	Q4	-0.19(-0.32, -0.06)	0.037	-0.21(-0.34, -0.08)	0.031	-0.12(-0.25,0.01)	0.09	-0.04(-0.17,0.1)	0.41
	per quartile	-0.06(-0.10, -0.02)	0.041	-0.06(-0.10, -0.03)	0.034	-0.03(-0.07,0.0004)	0.12	-0.005(-0.04,0.03)	0.49

* Beta values (95% CIs) were calculated for Q2-Q4 of the dietary n-6 fatty acids intake using Q1 as the reference group. α-diversity metrics were standardized to have mean 0 and SD 1 and then were modeled as responses in linear mixed models with technical confounders including sequencing depth and Bristol scale as fixed effects, and sequencing batch as random effect. Linear regression was conducted with the residuals of α-diversity metrics as dependent variables and baseline quartiles of individual n-6 fatty acid biomarkers as independent variables. Covariates included age, sex, BMI and waist-hip ratio, education, household income,

smoking and alcohol drinking status, physical activity, total energy intake, baseline erythrocyte total n-3 PUFAs. Abbreviations: BMI, body mass index; CIs, confidence intervals; PUFAs, polyunsaturated fatty acids; Q, quartile.

Table S21. Association of dietary n-6 fatty acid intake with microbiota β-diversity (N=1,591) *

Dietary n-6 fatty acids	Genus scaled relative abundances			
	Df	**F**	**R^2**	***p***
Linoleic acid (C18:2n6)	3	0.8891	0.00168	0.71
Arachidonic acid (C20:4n6)	3	1.5366	0.00285	0.006
Total n-6 PUFAs	3	0.7570	0.0014	0.937

* The dissimilarities in gut composition between quartiles of dietary n-6 PUFA (β-diversity) were assessed with PERMANOVA (R function adonis {vegan}, 999 permutations) based on the Bray-Curtis distance calculated at the genus and OTU level. Scaled (that is, divided by the standard deviation) relative abundances were used. The potential confounders included in the PERMANOVA were sequencing depth, sequencing batch, Bristol scale, age, sex, BMI, waist-hip ratio, education, household income, smoking status, alcohol drinking status, physical activity, total energy intake, and baseline erythrocyte total n-3 PUFAs. Abbreviations: BMI, body mass index, PUFAs, polyunsaturated fatty acids.

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