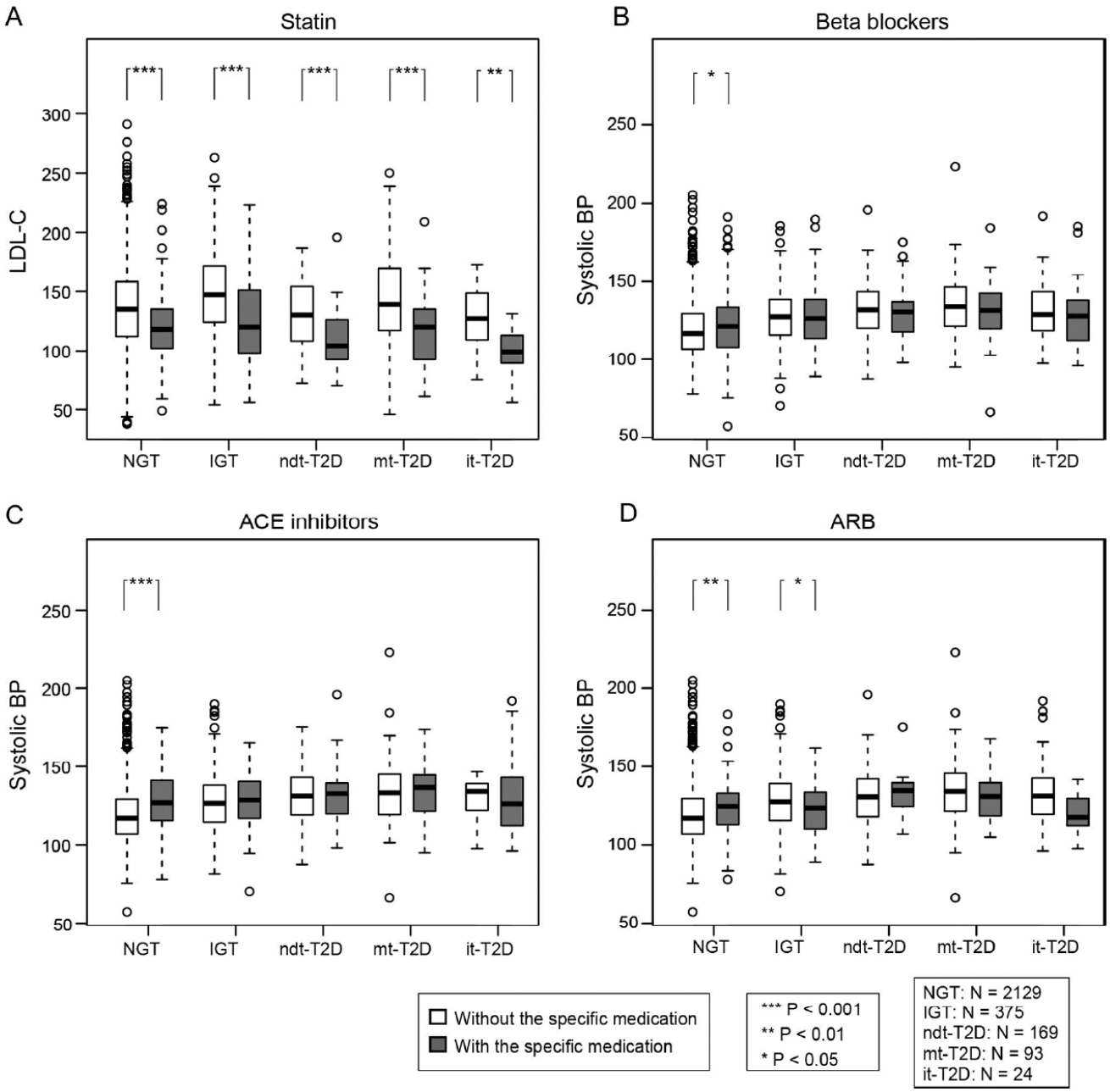
SUPPLEMENTARY DATA

Effects of metformin on metabolite profiles and LDL cholesterol in type 2 diabetes patients

Supplementary Figure 1. Influence of non-antihyperglycemic agents on type 2 diabetes risk factors

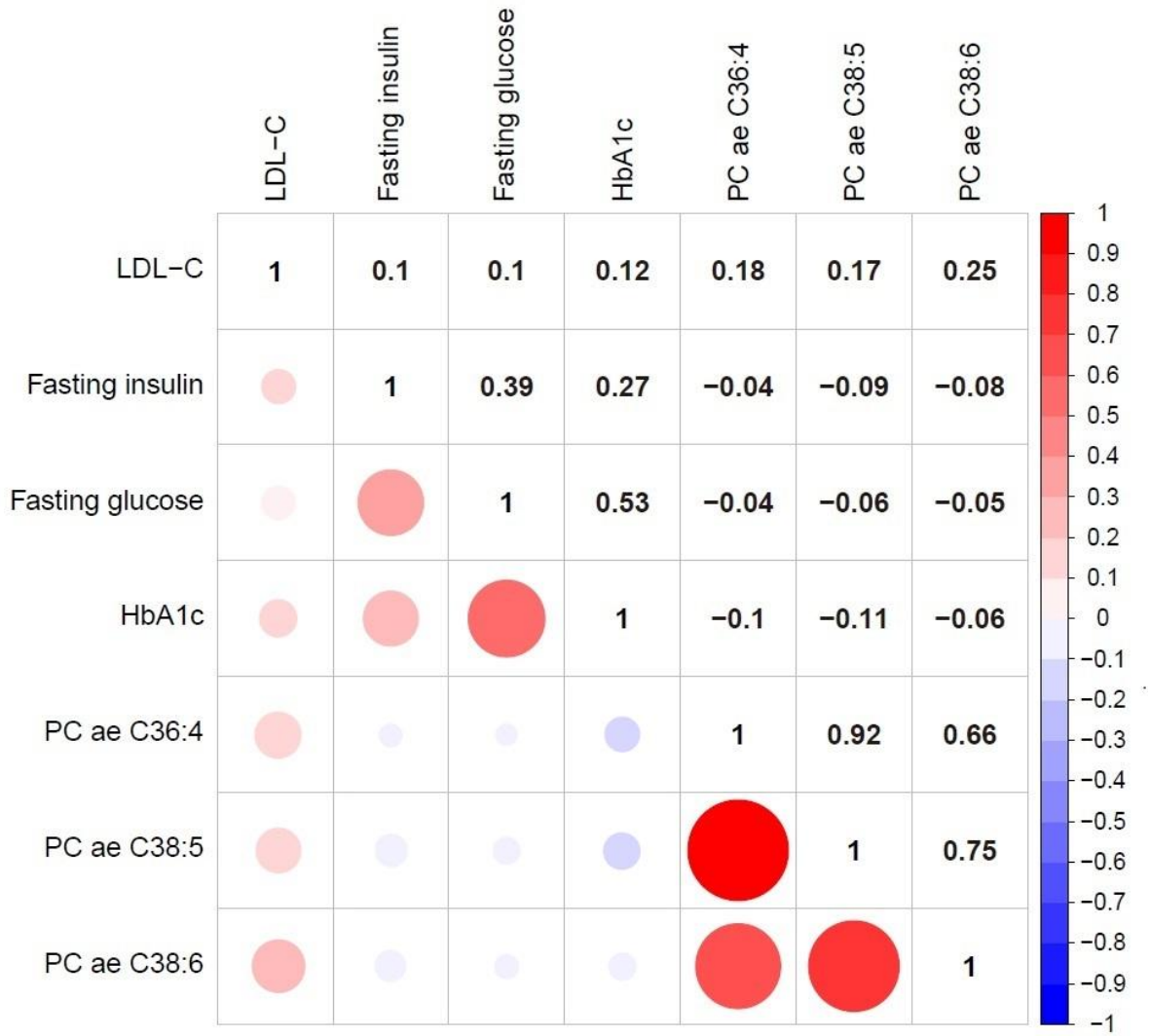
Statin usage was associated with lower levels of low density lipoprotein cholesterol (LDL-C) in all five groups: NGT, IGT, ndt-T2D, mt-T2D and it-T2D (plot A); treatment with beta blockers (plot B), angiotensin-converting-enzyme (ACE) inhibitors (plot C), or angiotensin receptor blockers (ARB) (plot D) are associated with different levels of systolic blood pressure (BP). NGT, normal glucose tolerance; IGT, impaired glucose tolerance; ndt-T2D, non-drug treated type 2 diabetes; mt-T2D, metformin-treated type 2 diabetes, it-T2D, insulin-treated type 2 diabetes.



SUPPLEMENTARY DATA

Supplementary Figure 2. Correlation of the three metformin-associated metabolites with risk factors of type 2 diabetes

The spearman correlation coefficients between the three metformin-associated metabolites and conventional risk factors of type 2 diabetes including LDL-C, fasting insulin, fasting glucose, and HbA1c, in the cross-sectional KORA F4 study are shown. Both the size of the cycle and intensity of color indicate the degree of correlation between the metabolites and the risk factors. The numeric values of spearman correlation coefficients are shown in the upper triangle.



SUPPLEMENTARY DATA

Supplementary Table 1. Characteristics of the KORA S4 → F4 prospective study samples

Percentages of individuals or means (SD) are shown for each variable and each group. Abbreviations: w/o, without; w/, with; ndt-T2D, non-anti-diabetic drug treated type 2 diabetes; mt-T2D, metformin-treated type 2 diabetes; BMI, body mass index; h, hour; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers. Significant *P* values are indicated bold (*P* value<0.05).

	Baseline S4: w/o metformin Follow-up F4: w/o metformin (n=869)			**Baseline S4: w/o metformin Follow-up F4: w/ metformin (n=43)**		
	S4	**F4**	***P*-value**	**S4**	**F4**	***P*-value**
Age	63.1 (5.4)	70.2 (5.4)	-	63.9 (4.4)	71.1 (4.6)	-
Male	51	51	-	55	55	-
Weight, kg	77.0 (12.5)	77.6 (13.3)	**8.9E-05**	89.6 (14.2)	87.1 (14.8)	**3.4E-04**
BMI, kg/m^2	28.0 (4.0)	28.3 (4.2)	**6.4E-09**	32.6 (4.2)	31.6 (4.4)	**2.8E-03**
Physical activity, > 1h per week	52.8	47.5	**2.2E-03**	67.4	58.2	0.29
High alcohol intake[†]	20.0	17.2	**0.05**	27.9	20.9	0.37
Smoker	11.9	7.4	**1.8E-08**	18.6	11.6	0.37
Systolic BP, mmHg	133.5 (18.9)	127.9 (19.4)	**1.5E-13**	144.0 (17.7)	130.8 (17.6)	**4.7E-05**
HDL-C, mg/dL	59.3 (16.5)	56.7 (14.1)	**2.6E-12**	53.2 (11.6)	52.7 (8.9)	0.66
LDL-C, mg/dL	155.5 (39.5)	141.8 (36.4)	**1.0E-21**	143.1 (37.2)	125.5 (24.4)	**5.6E-03**
Total cholesterol, mg/dL	245.4 (41.2)	223.7 (40.8)	**1.5E-26**	233.9 (41.7)	203.37(33.91)	**3.5E-04**
Triglycerides, mg/dL	127.4 (71.1)	127.1 (71.4)	0.76	171.8 (165.3)	151.2 (148.3)	0.08
HbA_{1C}, %	5.6 (0.4)	5.7 (0.5)	**0.01**	6.4 (0.9)	6.6 (0.7)	0.35
HbA_{1C}, mmol/mol	38 (4.4)	39 (5.5)	**0.01**	46 (9.8)	49 (7.7)	0.35
Fasting glucose, mg/dL	99.8 (11.1)	99.8 (17.4)	0.58	134.7 (33.1)	130.9 (27.3)	0.35
Statin usage	9.4	22.7	**1.5E-21**	9.3	30.2	**2.7E-02**
Beta blocker usage	18.1	31.5	**1.2E-18**	20.9	37.2	**4.6E-02**
ACE inhibitor usage	9.4	24.4	**9.3E-23**	20.9	51.2	**3.6E-03**
ARB usage	3.7	12.4	**1.6E-15**	2.3	13.9	0.07

[†] ≥40g/day in men; ≥20g/day in women

SUPPLEMENTARY DATA

Supplementary Table 2. Characteristics of the ERF study samples

Percentages of individuals or means (SD) are shown for each variable and each group. NGT, normal glucose tolerance; ndt-T2D, non-anti-diabetic drug treated type 2 diabetes; mt-T2D, metformin-treated type 2 diabetes; it-T2D, insulin-treated type 2 diabetes; BMI, body mass index; h, hour; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ACE, angiotensin-converting-enzyme; ARB, angiotensin receptor blockers.

Clinical parameters	**NGT**	**ndt-T2D**	**mt-T2D**	**it-T2D**
n	2396	29	32	3
Age, years	47.9 (14.2)	55.7 (11.4)	60.7 (11.8)	54.4 (15.5)
Males	43.8	51.0	58.8	20.0
BMI, kg/m^2	26.7 (4.5)	28.6 (4.5)	32.4 (6.1)	28.1 (5.8)
Physical activity, > 1h per week	43.3	77.0	70.6	66.6
High alcohol intake†	7.2	10.3	8.7	33.3
Active Smokers	39.3	40	42.9	100
Systolic BP, mmHg	138.9 (19.7)	154.4 (22.1)	151.5 (19.2)	152.50 (35.91)
HDL-C, mg/dL	49.4 (13.9)	47.1 (13.9)	39.4 (9.3)	40.15 (10.42)
LDL-C, mg/dL	144.8 (28.2)	145.2 (42.1)	108.5 (30.9)	122.39 (38.99)
Triglycerides, mg/dL	115.0 (64.6)	161.1 (107.1)	163.7 (92.0)	151.32 (77.87)
HbA_{1C}, %	NA	NA	NA	NA
HbA_{1C}, mmol/mol	NA	NA	NA	NA
Fasting glucose, mg/dL	79.8 (11.4)	145.0 (21.8)	139.3 (49.0)	170.09 (78.91)
2-h post-glucose load, mg/dL	NA	NA	NA	NA
Statin usage	17.5	34.4	58.8	100
Beta blocker usage	15.7	18.8	29.9	66.6
ACE inhibitor usage	5.6	9.4	19.6	66.6
ARB usage	7.8	5.9	17.6	33.3
Metformin usage	0	0	100	0
Insulin therapy	0	0	0	100

† ≥ 20g/day for women; ≥ 40g/day for men

SUPPLEMENTARY DATA

Supplementary Table 3. Characteristics of the NTR study samples

Percentages of individuals or means (SD) are shown for each variable and each group. Non-T2D, non-type 2 diabetes; ndt-T2D, non-anti-diabetic drug treated type 2 diabetes; mt-T2D, metformin-treated type 2 diabetes; it-T2D, insulin-treated type 2 diabetes; BMI, body mass index; h, hour; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ACE, angiotensin-converting-enzyme; ARB, angiotensin receptor blockers.

Clinical Parameters	**non-T2D**	**ndt-T2D**	**mt-T2D**	**it-T2D**
n	1256	73	29	9
Age, years	50.8 (14.1)	56.6 (11.0)	62.6 (6.5)	49.3 (18.9)
Male	66	75	59	67
BMI, kg/m^2	25.7 (3.6)	28.6 (4.2)	30.1 (5.4)	25.1 (3.8)
Physical activity, > 1h per week	NA	NA	NA	NA
High alcohol intake	NA	NA	NA	NA
Smoker	22	19	17	0
Systolic BP, mmHg	NA	NA	NA	NA
HDL-C, mg/dL	52.1 (14.5)	44.5 (14.0)	44.6 (9.7)	56.3 (16.5)
LDL-C, mg/dL	123.1 (35.7)	120.5 (39.3)	100.1 (32.9)	97.7 (22.8)
Triglycerides, mg/dL	131.9 (74.5)	190.1 (102.0)	156.1 (97.9)	77.7 (29.2)
HbA_{1C}, %	5.2 (1.1)	6.0 (1.3)	6.0 (0.8)	6.7 (1.04)
HbA_{1C}, mmol/mol	33 (12.0)	42 (14.2)	42 (8.7)	50 (11.4)
Fasting glucose, mg/dL	98.4 (10.0)	151.8 (35.6)	144.6 (34.1)	146.6 (41.6)
2-h post-glucose load, mg/dL	NA	NA	NA	NA
Statin usage	10	27	55	33
Beta blocker usage	9	18	28	22
ACE inhibitor usage	4	15	38	44
ARB usage	4	10	14	11
Metformin usage	0	0	100	0
Insulin therapy	0	0	0	100

SUPPLEMENTARY DATA

Supplementary Table 4. Variances of the six metabolites in other pair-wise comparisons in the discovery KORA F4 study

Effect estimates were calculated using multivariable linear regression analysis with the fully adjusted model. Significant *P* values are indicated bold (*P* value<3.8E-04).

Metabolite	mt-T2D vs. NGT		mt-T2D vs. IGT		mt-T2D vs. it-T2D	
	Effect estimate (95% CI)	***P*-value**	**Effect estimate (95% CI)**	***P*-value**	**Effect estimate (95% CI)**	**P-value**
PC ae C36:4	-0.64(-0.93,-0.35)	**1.65E-05**	-0.55(-0.85,-0.24)	4.99E-04	-0.73(-1.21,-0.25)	3.53E-03
PC ae C38:5	-0.59(-0.88,-0.30)	**7.69E-05**	-0.49(-0.80,-0.18)	2.08E-03	-0.65(-1.14,-0.16)	1.14E-02
PC ae C38:6	-0.77(-1.05,-0.49)	**1.09E-07**	-0.66(-0.95,-0.36)	**1.60E-05**	-0.32(-0.77,0.14)	1.76E-01
PC aa C36:0	-0.74(-1.03,-0.45)	**4.47E-07**	-0.69(-1.02,-0.37)	**3.84E-05**	0.25(-0.24,0.74)	3.29E-01
PC aa C38:0	-0.65(-0.94,-0.36)	**8.75E-06**	-0.64(-0.96,-0.32)	**1.07E-04**	-0.21(-0.70,0.28)	4.05E-01
Ornithine	-0.70(-0.98,-0.43)	**5.71E-07**	-0.41(-0.74,-0.09)	1.38E-02	-0.59(-1.12,-0.07)	2.85E-02

	it-T2D vs. NGT		it-T2D vs. IGT		it-T2D vs. ndt-T2D	
	Effect estimate (95% CI)	***P*-value**	**Effect estimate (95% CI)**	***P*-value**	**Effect estimate (95% CI)**	***P*-value**
PC ae C36:4	0.04(-0.47,0.55)	8.78E-01	0.15(-0.43,0.73)	6.11E-01	-0.08(-0.57,0.41)	7.57E-01
PC ae C38:5	0.01(-0.49,0.51)	9.76E-01	0.12(-0.46,0.70)	6.91E-01	-0.09(-0.57,0.38)	7.03E-01
PC ae C38:6	-0.44(-0.93,0.05)	8.12E-02	-0.32(-0.88,0.23)	2.57E-01	-0.24(-0.69,0.22)	3.11E-01
PC aa C36:0	-0.61(-1.10,-0.11)	1.60E-02	-0.44(-1.05,0.18)	1.63E-01	-0.54(-1.03,-0.05)	3.26E-02
PC aa C38:0	-0.19(-0.68,0.31)	4.55E-01	-0.17(-0.78,0.43)	5.77E-01	-0.34(-0.82,0.14)	1.69E-01
Ornithine	-0.29(-0.76,0.19)	2.34E-01	0.20(-0.42,0.82)	5.27E-01	0.05(-0.44,0.54)	8.39E-01

	ndt-T2D vs. NGT		ndt-T2D vs. IGT		NGT vs. IGT	
	Effect estimate (95% CI)	***P*-value**	**Effect estimate (95% CI)**	***P*-value**	**Effect estimate (95% CI)**	***P*-value**
PC ae C36:4	0.04(-0.16,0.24)	6.80E-01	-0.02(-0.23,0.18)	8.24E-01	-0.09(-0.21,0.02)	1.20E-01
PC ae C38:5	0.03(-0.17,0.22)	7.81E-01	-0.04(-0.25,0.16)	6.78E-01	-0.11(-0.22,0.00)	6.04E-02
PC ae C38:6	0.16(-0.03,0.35)	1.01E-01	0.05(-0.14,0.25)	6.04E-01	-0.14(-0.25,-0.03)	1.56E-02
PC aa C36:0	0.08(-0.11,0.28)	4.13E-01	-0.02(-0.23,0.20)	8.91E-01	-0.17(-0.28,-0.05)	3.99E-03
PC aa C38:0	-0.08(-0.27,0.12)	4.27E-01	-0.10(-0.31,0.11)	3.63E-01	-0.09(-0.20,0.03)	1.29E-01
Ornithine	0.24(0.05,0.43)	1.16E-02	-0.02(-0.24,0.19)	8.23E-01	**-0.21(-0.32,-0.10)**	**1.82E-04**

SUPPLEMENTARY DATA

Supplementary Table 5. Sensitivity analysis for the comparison between mt-T2D and ndt-T2D in the discovery KORA F4

Effect estimates and *P* values were calculated with multivariable linear regression analysis adjusted for the full set of covariates and additionally 1) the years since the T2D diagnosis; 2) waist; 3) LDL-C; and 4) LDL-C with insulin. All newly diagnosed T2D patients in the follow up were assigned with 0 years for the duration of diabetes. CI denotes confidence interval. Significant *P* values are indicated bold (*P* value<3.8E-04).

Metabolite	Full model + duration of diabetes		Full model + waist		Full model + LDL-C		Full model + LDL-C + insulin	
	Effect estimate (95% CI)	*P*-value	Effect estimate (95% CI)	*P*-value	Effect estimate (95% CI)	*P*-value	Effect estimate (95% CI)	*P*-value
PC ae C36:4	-0.69(-1.06,-0.31)	**6.08E-05**	-0.66(-0.91,-0.41)	**6.54E-07**	-0.60(-0.85,-0.35)	**6.09E-06**	-0.61(-0.87,-0.36)	**4.21E-06**
PC ae C38:5	-0.56(-0.97,-0.15)	**9.02E-05**	-0.62(-0.88,-0.36)	**4.79E-06**	-0.54(-0.80,-0.29)	**5.10E-05**	-0.55(-0.81,-0.29)	**5.23E-05**
PC ae C38:6	-0.64(-1.04,-0.25)	**1.82E-04**	-0.58(-0.82,-0.33)	**5.69E-06**	-0.47(-0.71,-0.23)	**1.42E-04**	-0.48(-0.72,-0.24)	**1.04E-04**
PC aa C36:0	-0.69(-1.12,-0.26)	2.33E-03	-0.56(-0.83,-0.29)	**5.57E-05**	-0.49(-0.76,-0.22)	**4.64E-04**	-0.50(-0.77,-0.23)	**3.51E-04**
PC aa C38:0	-0.74(-1.15,-0.32)	7.2E-04	-0.68(-0.94,-0.42)	**6.59E-07**	-0.57(-0.82,-0.32)	**1.72E-05**	-0.57(-0.83,-0.32)	**1.67E-05**
Ornithine	-0.26(-0.68,0.16)	0.23	-0.58(-0.85,-0.32)	**3.10E-05**	-0.57(-0.84,-0.29)	**6.81E-05**	-0.58(-0.86,-0.30)	**6.11E-05**

SUPPLEMENTARY DATA

Supplementary Table 6. Longitudinal analysis of the effect of metformin on metabolite profiles
Linear mixed effect model (with metabolite as dependent and the group as independent variable) adjusted for both the crude and the full set of covariates in the longitudinal study of 912 participants with no anti-diabetic medical treatment at baseline KORA S4. Of these participants, 43 started metformin treatment after the baseline KORA S4 study. A sensitivity analysis was conducted in a subset of 55 participants who were ndt-T2D patients at KORA S4, 36 of them took metformin during the follow-up. Significant *P* values are indicated bold (*P* value<0.05).

Metabolites	Crude model Effect estimate (95% CI)	*P*-value	Full model Effect estimate (95% CI)	*P*-value
With 912 participants				
PC ae C36:4	-0.65 (-0.92,-0.36)	**2.38E-06**	-0.67 (-0.94,-0.41)	**1.01E-06**
PC ae C38:5	-0.65 (-0.91,-0.37)	**1.53E-06**	-0.60 (-0.87,-0.35)	**5.18E-06**
PC ae C38:6	-0.60 (-0.85,-0.35)	**2.32E-06**	-0.54 (-0.78,-0.30)	**1.30E-05**
Sensitivity analysis with subset of 55 participants				
PC ae C36:4	-0.69 (-1.13, -0.25)	**3.7E-03**	-0.70 (-1.15, -0.24)	**4.35E-03**
PC ae C38:5	-0.43 (-0.86, 0.01)	0.06	-0.45 (-0.89, -0.01)	**0.05**
PC ae C38:6	-0.36 (-0.80, 0.06)	0.10	-0.45 (-0.86, -0.03)	**0.04**

SUPPLEMENTARY DATA

Supplementary Table 7. Associations between metformin treatment and change in lipid profile in the prospective KORA S4→F4 study

The associations were calculated using linear mixed effect models of 912 participants with no anti-diabetic medical treatment at baseline KORA S4. Of these participants, 43 started metformin treatment after the baseline KORA S4 study. Statin users (n = 247) in either S4 or F4 were excluded from the analysis to rule out any potential influence of statin use. In total, 757 participants with longitudinal KORA S4→F4 data were used for the sensitivity analysis, 28 of them took metformin only during the follow-up. The full models were modified for investigation of the HDL-C and triglycerides. The linear mixed effect models (with the lipid levels as outcome and the grouping variable as predictor) were adjusted for age and sex (crude model). The full model was additionally adjusted for BMI, physical activity, alcohol intake, smoking, systolic BP, HbA_{1c}, fasting glucose, and usage of statin, beta blockers, ACE inhibitors, and ARB. Associations for LDL-C and total cholesterol were additionally adjusted for HDL-C and triglycerides, whereas the associations for HDL-C and triglycerides were additionally adjusted for LDL-C and total cholesterol. Significant *P* values are indicated bold (*P* value<0.05).

	Crude model		**Full model**	
	Effect estimate (95% CI)	***P*-value**	**Effect estimate (95% CI)**	***P*-value**
With 912 participants				
LDL-C	-13.14 (-22.88, -3.40)	**8.34E-03**	-11.83 (-21.51, -2.15)	**0.02**
HDL-C	0.05 (-3.03, 3.12)	0.98	0.38 (-2.60, 3.36)	0.80
Total cholesterol	-19.16 (-29.77, -8.55)	**4.23E-04**	-16.18 (-26.03, -6.34)	**0.02**
Triglycerides	-7.44 (-23.45, 8.57)	0.36	-2.01 (-17.54, 13.52)	0.80
Sensitivity analysis with subset of 757 participants				
LDL-C	-9.77(-19.49, -0.05)	**0.04**	-8.68(-19.92, -0.85)	**0.04**
HDL-C	-0.78(-4.62, 3.05)	0.69	0.40(-3.35, 4.15)	0.83
Total cholesterol	-14.03(-24.47, -3.58)	**0.01**	-11.63(-21.23, -2.04)	**0.02**
Triglycerides	-1.59(-20.35, 17.17)	0.87	-5.18(-23.35, 12.99)	0.58

SUPPLEMENTARY DATA

Supplementary Table 8. Mediation analysis of the associations between metformin, LDL-C and the three metabolites

The associations were calculated using multivariable linear regression models with crude and full adjustments for 912 participants with no anti-diabetic medical treatment at baseline KORA S4. Of these participants, 43 started metformin treatment after the baseline KORA S4 study. Significant *P* values are indicated bold (*P* value<0.05).

	Crude model		**Full model**	
	Effect estimate (95% CI)	***P*-value**	**Effect estimate (95% CI)**	***P*-value**
A) Association between metformin treatment and LDL-C adjusted for different metabolites (Direct effect of metformin on LDL-C)				
Adjusted for PC ae C36:4	-10.1 (-19.74,-0.47)	**0.04**	-5.71 (-14.39,2.96)	0.2
Adjusted for PC ae C38:5	-9.98 (-19.62,-0.33)	**0.04**	-5.65 (-14.34,3.04)	0.2
Adjusted for PC ae C38:6	-7.74 (-17.21,1.73)	0.11	-3.41 (-11.95,5.12)	0.43
Adjusted for summed concentration*	-9.16 (-18.76,0.43)	0.06	-5.04 (-13.69,3.62)	0.25
B) Association between metformin treatment and metabolites				
PC ae C36:4	-0.62 (-0.88,-0.36)	**2.51E-06**	-0.53 (-0.78,-0.28)	**3.72E-05**
PC ae C38:5	-0.63 (-0.89,-0.37)	**1.97E-06**	-0.49 (-0.74,-0.25)	**9.80E-05**
PC ae C38:6	-0.60 (-0.85,-0.35)	**2.76E-06**	-0.45 (-0.69,-0.22)	**1.71E-04**
Summed concentration*	-1.85 (-2.56,-1.14)	**3.12E-07**	-1.47 (-2.15,-0.79)	**2.16E-05**
C) Association between metabolites and LDL-C adjusted for metformin treatment				
PC ae C36:4	4.81 (3.19,6.44)	**7.22E-09**	4.76 (2.94,6.57)	**2.95E-07**
PC ae C38:5	4.59 (2.97,6.21)	**3.22E-08**	4.77 (2.94,6.60)	**3.64E-07**
PC ae C38:6	8.59 (6.97,10.21)	**9.57E-25**	8.81 (6.95,10.66)	**2.69E-20**
Summed concentration*	2.31 (1.72,2.90)	**1.49E-14**	2.33 (1.67,3.00)	**6.87E-12**
D) Association between metformin treatment and total cholesterol adjusted for different metabolites (Direct effect of metformin on total cholesterol)				
Adjusted for PC ae C36:4	-16.45 (-26.93,-5.96)	**2.13E-03**	-8.54 (-17.67,0.59)	0.07
Adjusted for PC ae C38:5	-16.63 (-27.10,-6.16)	**1.87E-03**	-8.57 (-17.70,0.56)	0.07
Adjusted for PC ae C38:6	-15.45 (-25.76,-5.13)	**3.38E-03**	-6.66 (-15.55,2.23)	0.14
Adjusted for summed concentration*	-15.71 (-26.11,-5.31)	**3.09E-03**	-7.10 (-16.12,1.91)	0.12
E) Association between metabolites and total cholesterol adjusted for metformin treatment				
PC ae C36:4	8.02 (6.32,9.73)	**4.45E-20**	5.81 (3.86,7.76)	**5.87E-09**
PC ae C38:5	7.94 (6.24,9.63)	**7.76E-20**	5.60 (3.64,7.57)	**2.59E-08**
PC ae C38:6	11.69 (10.01,13.37)	**5.52E-41**	9.19 (7.19,11.1)	**3.24E-19**
Summed concentration*	3.58 (2.97,4.19)	**3.61E-30**	2.64 (1.92,3.35)	**5.25E-13**

*The summed concentration refers to the overall concentration of the three metabolites (PC ae C36:4, PC ae C38:5, PC ae C38:6)

SUPPLEMENTARY DATA

Supplementary Table 9. Mediation effects of the three metabolites for the association between metformin treatment and LDL-C reduction after removing statin users

Statin users (n = 247) in either S4 or F4 were excluded from the analysis to rule out any potential influence of statin use. In total, 757 participants with longitudinal KORA S4→F4 data were used for the sensitivity mediation analysis. 28 of these participants took metformin only during the follow-up. The mediation effects for three metformin-associated metabolites were assessed individually and as a combined total concentration using both the crude model and the full model. Significant *P* values are indicated bold (*P* value<0.05).

	Crude model		**Full model**	
	Effect estimate (95% CI)	***P*-value**	**Effect estimate (95% CI)**	***P*-value**
A) Mediation of the effect of metformin on LDL-C reduction				
PC ae C36:4	-3.34 (-4.97, -1.71)	**2.59E-04**	-1.11 (-1.74, -0.48)	**0.04**
PC ae C38:5	-3.28 (-4.85, -1.71)	**2.45E-04**	-0.84 (-1.29, -0.39)	0.07
PC ae C38:6	-5.42 (-8.81, -2.03)	**3.80E-05**	-2.77 (-4.73, -0.81)	**0.006**
Summed concentration	-4.18 (-6.28, -2.08)	**5.46E-05**	-1.73 (-2.75, -0.71)	**0.016**
B) Mediation of the effect of metformin on total cholesterol reduction				
PC ae C36:4	-2.85 (-5.48, -0.21)	**0.01**	-1.10 (-1.87, -0.33)	0.08
PC ae C38:5	-2.63 (-4.90, -0.36)	**0.01**	-0.71 (-1.16, -0.26)	0.14
PC ae C38:6	-4.15 (-8.51, -1.76)	**0.01**	-1.46 (-2.89, -0.03)	0.09
Summed concentration	-3.74 (-7.26, -0.22)	**0.01**	-1.26 (-2.20, -0.32)	0.07

SUPPLEMENTARY DATA

Supplementary Table 10. Genes with associated common variants linked to the three acyl-alkyl PCs identified by PSEA
Abbreviations, clusters, functions, associated diseases of 17 genes identified by PSEA are reported in the first four columns, respectively. The chromosome number and location (minimum and maximum) are listed in the following columns. The considered number of SNPs considered by using PSEA is shown in the last column. All listed genes showed a P<1.0E-04 in a permutation test in the PSEA.

Gene	Gene cluster	Functions	Associated diseases	Chr.	Position (min. – max.)	No. of SNPs
C7orf42	1	Unclear		7	65913652-66100806	83
SLC26A4	2	Transport (chloride, iodide, sulfate)	Deafness (1)	7	106978397-107183738	146
LOC286002[§]	2	Transcription		7	107045357-107198952	114
DAGLA	3	Insulin secretion, lipid metabolism	Spinocerebellar ataxia (2)	11	61094821-61309777	122
C11orf9	3	Transcription	Retinopathy (3)	11	61166755-61352140	104
FEN1	3	DNA repair, DNA transcription	Cancer (breast (4; 5), ovarian gastrointestinal (6), lung (7))	11	61210766-61360086	96
DKFZP434K028	3	Unclear		11	61238622-61391664	106
FADS2	3	Fatty acid metabolism	Retinopathy (8), Cornary artery disease (9)	11	61244266-61430694	131
C11orf10	3	Unclear		11	61273486-61426522	114
MIR611[§]	3	Transcription		11	61277244-61426522	112
FADS1	3	Fatty acid metabolism	Diabetes Retinopathy (10-13)	11	61287413-61448917	119
MIR1908[§]	3	Transcription		11	61300075-61448917	112
FADS3	3	Fatty acid metabolism	Diabetes (14), Coronary artery disease (15), Hyperlipidemia (16)	11	61358484-61525549	145
BEST1	3	Transport (chloride, calcium)	Retinopathy (17)	11	61365899-61527475	138
RAB3IL1	3	Transport (protein)	Retinopathy (18)	11	61381461-61551489	144
MANSC1	4	Unclear	Cancer (prostate, breast, lymphoma, leukemia) (19; 20)	12	12333540-12502043	159
C20orf94 (*SLX4IP*)	5	Unclear (possibly DNA repair)	Childhood acute lymphoblastic leukemia (21)	20	10254387-10591850	432

[§]Gene has no proteins in *Homo sapiens* in STITCH (22).

SUPPLEMENTARY DATA

Supplementary Table 11. Known metformin target genes

Known metformin target genes were retrieved from DrugBank (23).

Metformin target gene	Full biochemical name
AMPK	AMP-activated protein kinase
SLC22A1 (also known as *OCT1*)	solute carrier family 22, member 1
SLC22A2 (also known as *OCT2*)	solute carrier family 22, member 2
SLC22A3 (also known as *OCT3*)	solute carrier family 22, member 3
SLC47A1 (also known as *MATE1*)	solute carrier family 47, member 1
SLC29A4 (also known as *hENT4* and *PMAT*)	solute carrier family 29, member 4

Supplementary Table 12. Interactions between metformin target genes, pathway related proteins and PSEA identified genes

The first two columns show the names of the pairwise interaction partners in the investigated network. The following columns describe the observed interaction with literature and the underlying species.

Action 1	Action 2	Interaction	Species
Metformin	AMPK	In liver and muscle: Metformin →AMPK (24; 25); In hypothalamus: Metformin -| AMPK (26)	Rat Rat
AMPK	SREBP1c	In liver: AMPK -| SREBP1c (27)	Mouse
Leptin	AMPK	In liver: Leptin →AMPK (28); In hypothalamus: Leptin -| AMPK (29)	Mouse Mouse
SREBP1c	FADS2	In liver: SREBP1c → FADS2 (30)	Mouse
SREBP1c	FADS1	In liver: SREBP1c → FADS1 (30)	Mouse
Leptin	SREBP1c	In liver: Leptin -| SREBP1c (31)	Mouse
Leptin	FADS2	In liver: Leptin -| FADS2 (32)	Rat

SUPPLEMENTARY DATA

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