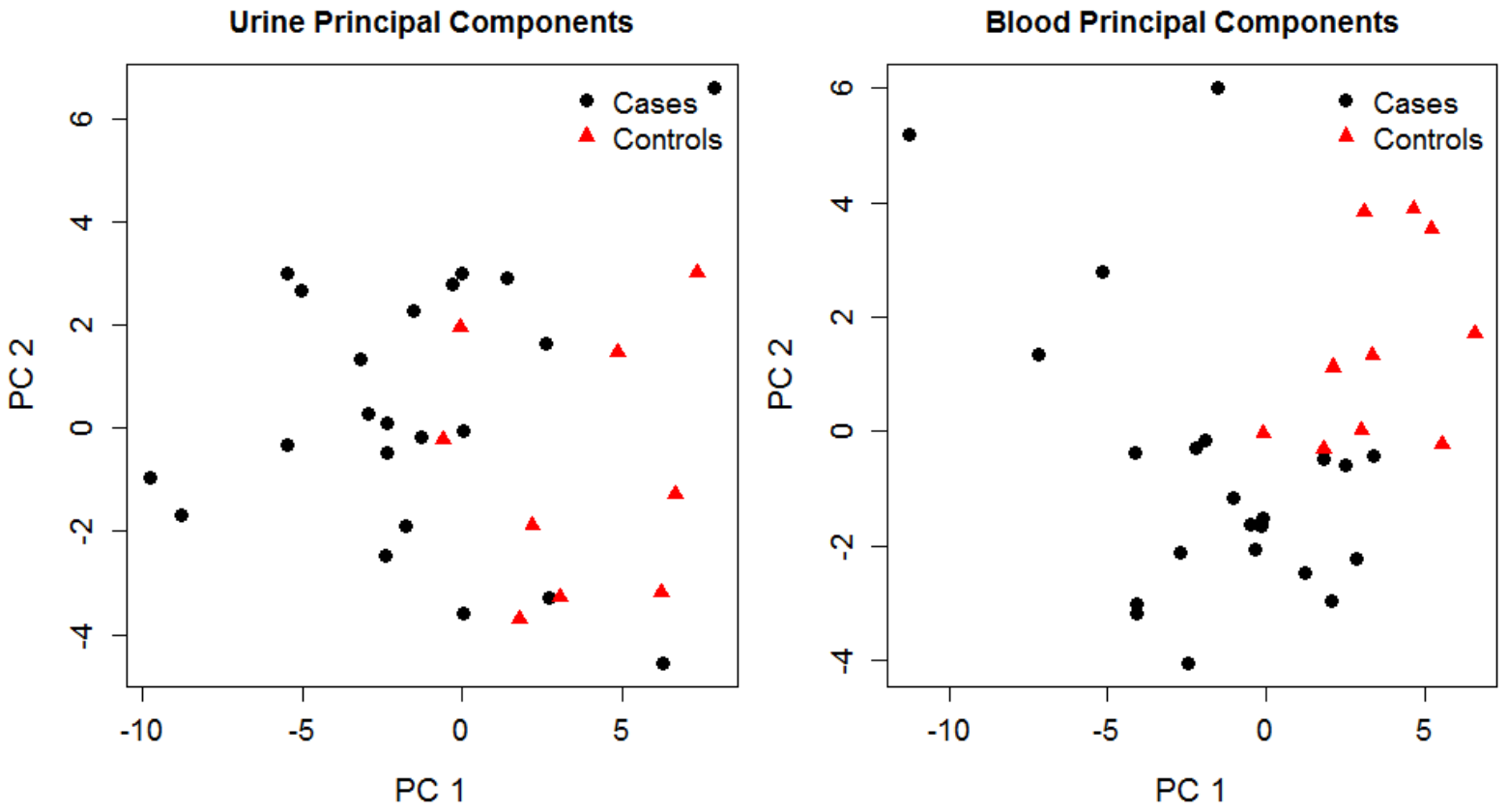
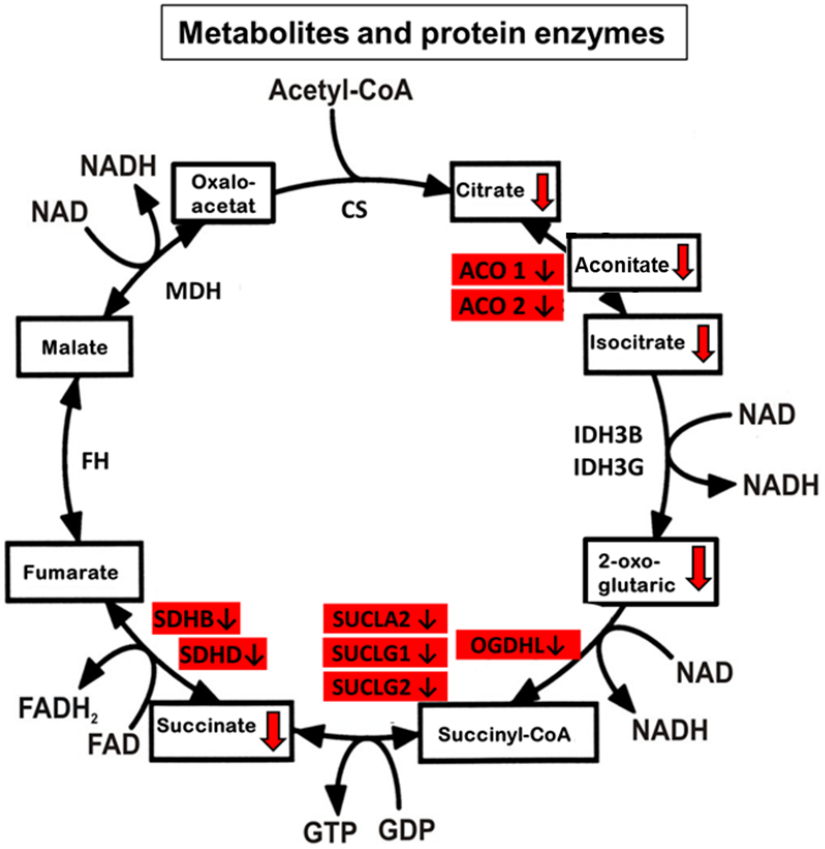
Supplemental data

Supplemental Figure 1. Principal components analyses for urine excretion of metabolites per day (A) and blood concentrations of metabolites (B) show segregation of CKD vs. healthy controls. The figures show the plot of principal components 1 vs. 2 (x vs. y axis). Black circles and red triangles denote CKD and healthy controls, respectively.



Supplemental Figure 2. The urinary excretion of citric acid (TCA) cycle metabolites and the renal expression of genes that regulate these metabolites were significantly reduced among participants with versus without non-diabetic CKD. Urine excretion of citric acid cycle metabolites in the proximal part of the pathway were reduced in samples from patients with non-diabetic CKD, as was the mRNA expression of the enzymes catalyzing the proximal steps of the citric acid cycle in the glomerular compartment in biopsies of patients with non-diabetic CKD (tubulointerstitial compartment shown in main text).



Protein enzymes

Gene Name	Fold Change	Q value
ACO1	**0.8**	**<0.001**
ACO2	**0.9**	**0.036**
IDH1	1.1	0.417
IDH2	1.1	0.417
IDH3A	1.1	0.202
IDH3B	1.1	0.491
IDH3G	1.0	0.241
OGDH	1.0	0.519
OGDHL	**0.7**	**<0.001**
SUCLA2	**0.9**	**0.036**
SUCLG1	**0.9**	**<0.001**
SUCLG2	**0.8**	**<0.001**
SDHA		
SDHB	**0.9**	**0.036**
SDHC		
SDHD	**0.9**	**<0.001**
FH	1.0	0.500
MDH1	1.0	1.000
MDH2	1.0	0.473

Supplemental Table 1. Influence of glomerular and tubular kidney function on metabolite levels.

	Correlation (R^2) with eGFR		Fractional excretion (%) showing tubular influence		
Metabolite	**Urine**	**Blood**	**CKD**	**Ctr.**	**Δ (95% CI) p-value**
Acetoacetic acid	0.01	0.16	0.0	0.0	0.0 (0.0 0.0) 0.93
Adipic acid	0.04	0.40	16.4	12.8	5.2 (-0.2 12.3) 0.06
Azelaic acid	0.23	0.00	10.7	7.2	0.8 (-10.8 11.4) 0.66
Benzoic acid	0.01	0.07	4.4	1.8	2.6 (1.0 4.5) <0.001
Cis-Aconitic acid	0.06	0.00	32.0	11.2	13.6 (-0.4 22.9) 0.07
Citric acid	0.23	0.00	4.5	5.5	-0.3 (-2.5 2.4) 0.82
Dodecanoic acid	0.04	0.02	1.5	0.9	0.6 (0.1 1.1) 0.01
Ethylmalonic acid	0.21	0.00	17.4	21.0	-2.4 (-5.7 2.2) 0.19
Fumaric acid	0.06	0.18	3.0	2.5	1.0 (0.0 4.0) 0.05
Glutaconic acid	0.08	0.15	34.8	25.1	6.4 (-10.6 20.4) 0.59
Glutaric acid	0.10	0.07	11.6	9.6	-0.7 (-5.5 5.0) 0.72
Glyceric acid	0.01	0.12	8.6	8.9	1.3 (-3.2 6.7) 0.59
Glycolic acid	0.30	0.34	10.0	7.3	3.7 (1.0 9.0) 0.004
Hexanoylglycine	0.01	0.01	75.2	38.2	5.0 (-43.7 52.2) 0.82
Hippuric acid	0.02	0.16	325	1057	-757 (-7217 -117) 0.008
Homovanillic acid	0.12	0.04	5.5	2.6	2.2 (-0.2 6.0) 0.06
OH-phenyllactic acid	0.01	0.04	3.0	1.9	0.7 (-0.6 2.2) 0.45
OH-propionic acid	0.09	0.03	6.9	5.4	1.0 (-0.8 2.9) 0.30
Isocitric acid	0.10	0.23	26.1	19.3	3.2 (-2.7 10.0) 0.33
L-2-OH-glutaric acid	0.04	0.25	14.9	20.9	-2.1 (-7.4 4.1) 0.42
L-lactic acid	0.01	0.01	0.2	0.1	0.1 (0.0 0.2) <0.001
L-malic acid	0.00	0.23	1.9	2.9	0.3 (-1.5 3.5) 0.62
L-octanoylcarnitine	0.00	0.00	2.5	1.3	1.3 (0.6 2.0) <0.001
Leucinic acid	0.22	0.10	0.8	0.6	0.3 (0.0 1.3) 0.09
Methylglutaric acid	0.00	0.00	151	81	58 (5 184) 0.04
Methylmalonic acid	0.30	0.04	6.8	20.1	-3.1 (-17.8 10.7) 0.59
Methylsuccinic acid	0.01	0.06	4.8	0.5	2.6 (1.1 5.2) <0.001
Mevalonic acid	0.04	0.01	2.7	1.5	0.8 (-1.0 3.6) 0.20
Myristic acid	0.02	0.05	0.0	0.0	0.0 (0.0 0.0) 0.50
N-acetyl-L-aspartic acid	0.04	0.03	12.6	5.2	0.7 (-10.8 10.3) 0.86
Oleic acid	0.05	0.08	0.0	0.0	0.0 (0.0 0.0) 0.45
Orotic acid	0.02	NA	NA	NA	NA
o-OH-phenylacetic acid	0.38	0.06	0.0	30.7	-30.7 (-51.9 0.0) 0.07
Oxoglutaric acid	0.01	0.10	2.0	2.3	0.3 (-1.3 2.5) 0.69
p-OH-phenylacetic acid	0.01	0.00	71.5	98.6	-24.6 (-85.3 4.7) 0.11
Palmitic acid	0.24	0.11	0.1	0.1	0.0 (-0.1 0.0) 0.11
Phenylpyruvic acid	0.01	0.13	1.8	2.0	-0.2 (-1.2 1.2) 0.70

Pyroglutamic acid	0.06	0.00	7.7	5.7	1.8 (-0.7 5.0) 0.15
Pyruvic acid	0.00	0.00	0.3	0.2	0.2 (0.1 0.4) <0.001
Stearic acid	0.16	0.14	0.1	0.2	-0.1 (-0.2 0.0) 0.07
Suberic acid	0.03	NA	77.3	77.5	2.5 (-54.3 82.3) 0.62
Succinic acid	0.01	0.00	3.5	2.8	0.3 (-1.1 3.1) 0.69
Tiglylglycine	0.04	0.03	106.4	88.0	-24.2 (-67.6 46.1) 0.66
Vanillylmandelic acid	0.01	0.36	75.8	97.4	-21.9 (-45.7 13.8) 0.21
(R)-3-hydroxybutyric acid	0.01	0.06	0.1	0.1	0.0 (-0.1 0.0) 0.56
(S)-3-OH-isobutyric acid	0.05	0.00	2.4	2.1	0.5 (-0.1 1.4) 0.07
2-ethylhydracrylic acid	0.01	0.06	2.9	2.7	0.6 (-0.8 1.7) 0.40
2-OH-3-methylbutyric acid	0.05	0.05	0.3	0.2	0.1 (0.0 0.3) 0.01
2-OH-butyric acid	0.03	0.00	0.1	0.1	0.0 (0.0 0.1) 0.14
2-methyl-3-OH-butyric acid	0.37	0.03	10.0	10.5	-0.5 (-3.2 2.4) 0.69
2-methylacetoacetic acid	0.05	0.28	0.0	0.0	0.0 (0.0 0.0) 0.003
2-methylcitric acid	0.04	0.62	27.8	52.9	-18.9 (-30.7 -6.1) 0.009
2-octenedioic acid	0.01	0.07	8.3	6.9	0.8 (-4.4 8.9) 0.73
3-OH-adipic acid	0.01	0.26	13.4	11.0	2.9 (-1.8 8.5) 0.18
3-OH-glutaric acid	0.01	0.11	23.2	39.6	-12.4 (-28.2 6.2) 0.14
3-OH-isovaleric acid	0.17	0.28	7.9	10.7	-2.3 (-7.1 0.7) 0.16
3-OH-methylglutaric acid	0.01	0.18	430	389	56 (-139 319) 0.69
3-methyladipic acid	0.01	0.25	45.6	78.6	-23.3 (-65.8 17.7) 0.21
3-methylglutaconic acid	0.01	0.09	151	292	-159 (-306 216) 0.24
4-OH-butyric acid	0.17	0.04	3.5	7.3	-3.7 (-7.6 0.4) 0.07
4-OH-hippuric acid	0.01	0.22	3.7	397	-1.3 (-397 768) 0.62
5-OH-indoleacetic acid	0.05	NA	NA	NA	NA

Note: Urine data show the correlations between the renal excretion of the metabolite and eGFR. Blood data show the correlation between 1/plasma metabolite concentration and eGFR. Analysis includes both controls and CKD participants on placebo. Fractional excretion describes the effect of tubular reabsorption and secretion (e.g. FE 5% means that 95% of the filtered amount is reabsorbed). The median of the differences are displayed with 95% confidence intervals and p-values based on Wilcoxon rank sum test.

Supplemental Table 2: Fractional excretion (FE %) of metabolites in CKD patients on paricalcitol versus placebo treatment.

Metabolite	Median (IQR) FE (%) for CKD on paricalcitol	Median (IQR) FE (%) for CKD on placebo	Median difference in FE (%) (95% CI)	P-value
(R)-3-OH-butyric acid	0.1 (0.0-0.2)	0.1 (0.0-0.1)	0 (-0.1, 0)	0.95
(S)-3-OH-isobutyric acid	2.3 (1.7-2.6)	2.4 (1.6-2.7)	-0.1 (-0.6, 0.3)	0.63
2-Ethylhydracrylic acid	3.6 (2.3-4.5)	2.9 (2.5-4.7)	0.3 (-0.3, 0.9)	0.27
2-OH-3-Me-butyric acid	0.4 (0.3-0.5)	0.3 (0.2-0.5)	0 (0, 0.1)	0.076
2-OH-butyric acid	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0 (-0.1, 0)	0.15
2- Me-3-OH-butyric acid	10.7 (6.5-13.3)	10.0 (7.8-12.4)	0.1 (-1.9, 2)	0.97
2- Me-acetoacetic acid	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0 (0, 0)	0.86
2- Me-citric acid	24.8 (22.7-36.8)	27.8 (22.5-44.9)	-2.1 (-9.8, 3)	0.36
2-Octenedioic acid	15.0 (9.8-22.2)	8.3 (2.2-11.9)	10.1 (3.8, 22.9)	0.011
3-OH-adipic acid	18.7 (15.5-23.0)	13.4 (11.3-21.5)	2.4 (-0.8, 6.9)	0.16
3-OH-glutaric acid	15.1 (12.4-32.4)	23.2 (11.1-32.2)	-4.7 (-31.1, 2.6)	0.2
3-OH-isovaleric acid	7.7 (6.0-9.9)	7.9 (6.3-9.0)	-0.8 (-2.3, 0.8)	0.29
3-OH-Me-glutaric acid	294.7 (180.4-525.1)	429.8 (236.4-668.5)	-90.2 (-276.2, 31.9)	0.17
3- Me-adipic acid	38.3 (14.6-74.0)	45.6 (28.1-83.8)	-14.2 (-55.5, 23.8)	0.47
3- Me-glutaconic acid	179.1 (28.1-722.3)	151.5 (30.6-569.2)	2.9 (-75.2, 99.7)	0.81
4-OH-butyric acid	8.4 (4.1-15.5)	3.5 (1.2-8.7)	2.6 (-4.8, 7.5)	0.41
4-OH-hippuric acid	2.5 (0.0-531.8)	3.7 (0.0-1009.1)	-226.9 (-624.5, 1.3)	0.14
Acetoacetic acid	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0 (-0.1, 0)	0.19
Adipic acid	17.6 (15.6-25.6)	16.4 (15.4-26.5)	0.7 (-8.1, 6.9)	0.64
Azelaic acid	14.5 (6.1-34.0)	10.7 (6.8-22.2)	3.6 (-6.6, 18.7)	0.63
Benzoic acid	4.1 (3.1-5.5)	4.4 (2.7-6.9)	0.4 (-0.7, 1.4)	0.54
cis-Aconitic acid	31.6 (22.9-38.3)	32.0 (23.1-35.7)	0.6 (-6.8, 7.4)	0.89
Citric acid	5.8 (3.3-10.1)	4.5 (3.2-6.4)	0.8 (-1.7, 2.9)	0.49
Dodecanoic acid	1.3 (1.0-2.1)	1.5 (0.9-1.9)	0 (-0.2, 0.4)	0.66
Ethylmalonic acid	16.2 (14.2-18.9)	17.4 (13.7-20.6)	0.2 (-4.9, 4.2)	0.97
Fumaric acid	2.6 (2.1-7.4)	3.0 (1.9-6.6)	0 (-0.7, 1.2)	0.97
Glutaconic acid	28.9 (16.6-42.3)	34.8 (19.3-43.7)	-3.4 (-15.4, 11)	0.79
Glutaric acid	9.8 (7.3-16.7)	11.6 (7.0-16.7)	0.2 (-4.6, 4.2)	0.86
Glyceric acid	7.6 (6.1-16.9)	8.6 (6.4-14.9)	-1.4 (-3.3, 0.4)	0.11
Glycolic acid	11.5 (9.0-13.4)	10.0 (8.0-15.8)	-0.1 (-3, 2.3)	0.95
Hexanoylglycine	67.3 (39.1-128.7)	75.2 (36.0-98.9)	2 (-31.4, 76.7)	0.89
Hippuric acid	374.8 (283.7-725.4)	325.5 (190.2-594.9)	36.9 (-62, 126.1)	0.45
Homovanillic acid	7.4 (5.1-13.0)	5.5 (4.5-10.0)	3.1 (-0.8, 10.3)	0.14
OH-phenyllactic acid	2.8 (1.9-4.3)	3.0 (2.2-3.3)	0.2 (-0.3, 0.8)	0.45
OH-propionic acid	6.3 (5.4-10.0)	6.9 (5.4-8.1)	0.2 (-1.5, 1.7)	0.81
Isocitric acid	24.2 (19.8-33.3)	26.1 (19.3-30.9)	1.4 (-4.2, 6.5)	0.66
L-2-OH-glutaric acid	16.4 (12.1-27.3)	14.9 (13.1-19.9)	0.5 (-1.9, 5.4)	0.68

L-lactic acid	0.2 (0.1-0.2)	0.2 (0.1-0.3)	0 (-0.1, 0)	0.47
L-Malic acid	2.0 (1.4-8.5)	1.9 (1.4-6.7)	0.1 (-1.3, 2.1)	0.81
L-Octanoylcarnitine	2.7 (2.2-3.3)	2.5 (1.7-3.0)	0.4 (-0.1, 0.7)	0.15
Leucinic acid	0.8 (0.5-1.0)	0.8 (0.6-2.1)	-0.2 (-0.8, 0)	0.082
Me-lglutaric acid	149.3 (81.2-315.2)	151.3 (80.6-238.7)	11.1 (-36.9, 60.8)	0.66
Me-malonic acid	16.2 (3.6-43.4)	6.8 (2.9-33.2)	4 (-7.9, 22.5)	0.29
Me-succinic acid	3.4 (1.9-6.0)	4.8 (2.4-6.7)	0.1 (-2.2, 1.5)	0.92
Mevalonic acid	4.4 (3.6-6.3)	2.7 (1.7-5.4)	1.5 (-0.1, 4.4)	0.059
Myristic acid	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0 (0, 0)	0.84
N-Acetyl-L-aspartic acid	11.5 (7.2-18.6)	12.6 (5.3-20.3)	0.4 (-1.8, 2.4)	0.71
Oleic acid	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0 (0, 0)	0.97
o-OH-phenylacetic acid	1.0 (0.0-2.5)	0.0 (0.0-2.4)	0.2 (-1.3, 1.9)	0.71
Oxoglutaric acid	2.1 (1.1-3.6)	2.0 (1.3-4.8)	0.3 (-0.9, 1.7)	0.66
p-OH-phenylacetic acid	83.2 (71.1-125.6)	71.5 (62.6-93.6)	12.1 (-11.3, 42.2)	0.34
Palmitic acid	0.1 (0.0-0.1)	0.1 (0.0-0.1)	0 (0, 0)	0.89
Phenylpyruvic acid	1.4 (0.6-2.2)	1.8 (0.5-3.0)	-0.2 (-1.1, 0.9)	0.68
Pyroglutamic acid	8.1 (4.5-11.0)	7.7 (4.9-9.6)	0.2 (-1.2, 1.4)	0.81
Pyruvic acid	0.3 (0.3-0.4)	0.3 (0.2-0.6)	0 (-0.1, 0.1)	0.89
Stearic acid	0.0 (0.0-0.2)	0.1 (0.0-0.1)	0 (0, 0)	0.76
Suberic acid	111.6 (67.7-235.7)	77.3 (24.6-182.7)	25.5 (-19.2, 75)	0.14
Succinic acid	3.3 (2.3-6.9)	3.5 (1.9-6.3)	0.3 (-1.8, 1.7)	0.66
Tiglylglycine	78.6 (16.4-138.9)	106.4 (37.6-133.7)	-24.9 (-78.5, 40.6)	0.39
Vanillylmandelic acid	90.7 (71.6-114.8)	75.8 (57.3-111.6)	4.6 (-16.2, 25.3)	0.73

Supplemental Table 3. Precision of citric acid cycle metabolites

	CV (%)
2-Ketoglutaric acid	7.6
Cis-Aconitate	3.0
Citric Acid	4.3
Fumaric acid	8.8
Isocitric acid	11.4
Malic Acid	4.5
Pyruvic acid	4.2
Succinic acid	2.3

Supplemental table 4. Metabolites measured in primary study population (Paricalcitol trial).

HMDB00220	Palmitic acid
HMDB00115	Glycolic acid
HMDB00754	3-Hydroxyisovaleric acid
HMDB00193	Isocitric acid
HMDB00622	Ethylmalonic acid
HMDB00959	Tiglylglycine
HMDB00694	L-2-Hydroxyglutaric acid
HMDB00379	2-Methylcitric acid
HMDB00665	Leucinic acid
HMDB00011	(R)-3-Hydroxybutyric acid
HMDB00714	Hippuric acid
HMDB00207	Oleic acid
HMDB00060	Acetoacetic acid
HMDB00827	Stearic acid
HMDB00345	3-Hydroxyadipic acid
HMDB00008	2-Hydroxybutyric acid
HMDB00139	Glyceric acid
HMDB03771	2-Methylacetoacetic acid
HMDB00396	2-Ethylhydracrylic acid
HMDB00156	L-Malic acid
HMDB01844	Methylsuccinic acid
HMDB00700	Hydroxypropionic acid
HMDB00020	p-Hydroxyphenylacetic acid
HMDB00522	3-Methylglutaconic acid
HMDB00428	3-Hydroxyglutaric acid
HMDB00118	Homovanillic acid
HMDB00755	Hydroxyphenyllactic acid
HMDB13678	4-Hydroxyhippuric acid
HMDB00205	Phenylpyruvic acid
HMDB01870	Benzoic acid
HMDB00134	Fumaric acid
HMDB00448	Adipic acid
HMDB00701	Hexanoylglycine
HMDB00555	3-Methyladipic acid
HMDB00355	3-Hydroxymethylglutaric acid
HMDB00291	Vanillylmandelic acid
HMDB00094	Citric acid
HMDB00254	Succinic acid
HMDB00710	4-Hydroxybutyric acid
HMDB00072	cis-Aconitic acid

HMDB00202	Methylmalonic acid
HMDB00354	2-Methyl-3-hydroxybutyric acid
HMDB00812	N-Acetyl-L-aspartic acid
HMDB00661	Glutaric acid
HMDB00620	Glutaconic acid
HMDB00669	Ortho-Hydroxyphenylacetic acid
HMDB00784	Azelaic acid
HMDB00208	Oxoglutaric acid
HMDB00267	Pyroglutamic acid
HMDB00763	5-Hydroxyindoleacetic acid
HMDB00023	(S)-3-Hydroxyisobutyric acid
HMDB00190	L-Lactic acid
HMDB00893	Suberic acid
HMDB00638	Dodecanoic acid
HMDB00752	Methylglutaric acid
HMDB00791	L-Octanoylcarnitine
HMDB00243	Pyruvic acid
HMDB00407	2-Hydroxy-3-methylbutyric acid
HMDB00226	Orotic acid
HMDB00341	2-Octenedioic acid
HMDB00227	Mevalonic acid
HMDB00806	Myristic acid
HMDB00792	Sebacic acid

Supplemental methods

Replication study population, the SUGAR study

For replication of TCA cycle findings, 45 participants with non-diabetic CKD and 15 matched controls from the SUGAR (StUdy of Glucose And insulin in Renal disease) study were used. Urine metabolites were quantified in 24-hour urine samples using GC-MS analysis at Northwest Metabolomics Research Center, University of Washington, Seattle. A 100 µL aliquot of each urine sample was transferred to a clean 2 mL Eppendorf microcentrifuge tube followed by addition of 100 U urease to remove urea from the sample. After incubation at 37 °C for 1 hour, the urease was precipitated with 850 µL methanol and mixed for 5 min at room temperature. Subsequently, samples were centrifuged for 10 min at 14000 rpm, 750 µL of the supernatant was transferred to a clean 2.0 mL Eppendorf tube and centrifuged under vacuum until dry. For derivatization, 25 µL of methoxyamine in pyridine (20 mg/mL) was added to each sample and incubated for 90 min at 30 °C. Metabolites were silylated with 75 µL MSTFA (1% TMCS v/v) for 30 min at 37 °C and 2 µL of FAMEs (fatty acid methyl-esters) containing myristic acid – d27 were added to provide a retention time index. GC-MS experiments were conducted using an Agilent 7890A gas chromatograph equipped with an Agilent DB-5ms column 30 m in length and 0.25 mm i.d. column with an additional integrated 10 m guard column coupled to an Agilent 5975C MSD. A total of 1 µL of each sample was injected into a single tapered, split less injector liner with glass wool heated at 230 °C. Metabolites were separated using a temperature gradient consisting of a 60 °C hold for 1 min, ramp at 10 °C/min until a final temperature of 325 °C and a holding for 5 min, for a total run time of 32.5 min. The method was retention time locked using the myristic acid – d27 with a targeted elution time of 16.75 min. Spectral deconvolution and metabolite identification were conducted using AMDIS software (Agilent) and the NIST library, respectively. Typically, relative quantitation of approximately 150 metabolites, along with approximately 200 unidentified metabolite signals are observed for each urine sample. The retention times on the LC-MS platform for TCA cycle metabolites citrate, isocitrate, succinate, and cis-aconitate were 16.62, 16.88, 10.58 and 13.56 minutes, respectively. The retention times using the GC-MS platform for these metabolites were as follows: citrate 13.84, isocitrate 13.10, succinate 7.93, 2-oxoglutarate 15.36, and cis-aconitate 13.10 minutes.

Gene expression in human kidney tissue

Total RNA was isolated from micro-dissected glomerular and tubulointerstitial compartments from kidney biopsies of 155 patients from the ERCB cohort with biopsy-proven, non-diabetic CKD. RNA was reverse-transcribed and amplified, fragmented and hybridized to Affymetrix GeneChip Human Genome U133A 2.0 and U133 Plus 2.0 Array. The Cel files were uploaded on Gene Omnibus website (http://www.ncbi.nlm.nih.gov/geo/) under reference numbers GSE32591, GSE37455,[1] GSE35488,[2] and GSE47185.[3] Gene expression data for 20 TCA cycle enzymes, and 11 genes regulating mitochondrial biogenesis was extracted from above datasets for further analysis

References

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