Supplement to:

Serum metabolomic alterations associated with proteinuria in chronic kidney disease

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Running title: Metabolomics of proteinuria in chronic kidney disease

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Supplemental Table 1. Pathways and identifiers of metabolites significantly associated with proteinuria.

Metabolite	Super-pathway	Sub-pathway	PubChem ID	KEGG ID	HMDB ID	Blind duplicate CV in AASK	Blind duplicate CV in the MDRD Study
4-hydroxychlorothalonil	Xenobiotics	Chemical	34217			0.13	0.09
1,5-anhydroglucitol (1,5-AG)	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	64960	C07326	HMDB02712	0.04	0.09
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	Lipid	Phosphatidylethanolamine (PE)	5289133		HMDB09003	0.04	0.09
1-stearoyl-GPE (18:0)	Lipid	Lysophospholipid	9547068		HMDB11130	0.05	0.17
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	Lipid	Fatty Acid, Dicarboxylate	123979		HMDB61112	0.09	0.12
1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	Lipid	Phosphatidylinositol (PI)			HMDB09815	0.09	0.13
aspartate	Amino Acid	Alanine and Aspartate Metabolism	5960	C00049	HMDB00191	0.03	0.11
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	Lipid	Phosphatidylethanolamine (PE)	9546800		HMDB05323	0.06	0.10
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)	Lipid	Plasmalogen			HMDB11352	0.04	0.08
retinol (Vitamin A)	Cofactors and Vitamins	Vitamin A Metabolism	445354	C00473	HMDB00305	0.07	0.06
1-palmitoyl-GPE (16:0)	Lipid	Lysophospholipid	9547069		HMDB11503	0.06	0.17
4-acetamidobutanoate	Amino Acid	Polyamine Metabolism	18189	C02946	HMDB03681	0.09	0.27
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)	Lipid	Plasmalogen			HMDB11342	0.07	0.07
N6-carbamoylthreonyladenosine	Nucleotide	Purine Metabolism, Adenine containing	161466		HMDB41623	0.04	0.15
N-palmitoyl-sphingosine (d18:1/16:0)	Lipid	Ceramides	5283564		HMDB04949	0.09	0.10
N2,N5-diacetylornithine	Amino Acid	Urea cycle; Arginine and Proline Metabolism	10398396			0.12	0.15
3-hydroxy-5-cholestenoic acid	Lipid	Sterol	165511	C17333		0.12	0.18
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	Lipid	Phosphatidylethanolamine (PE)	5283496		HMDB05320	0.07	0.16

O-sulfo-L-tyrosine	Xenobiotics	Chemical	514186			0.09	0.16
1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4)	Lipid	Phosphatidylcholine (PC)	10747814		HMDB07982	0.04	0.07
6-hydroxyindole sulfate	Xenobiotics	Chemical				0.10	0.16
cholesterol	Lipid	Sterol	11025495	C00187	HMDB00067	0.07	0.11
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	Lipid	Phosphatidylethanolamine (PE)			HMDB08993	0.05	0.11
3-methylglutaconate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	1551553		HMDB00522	0.10	0.13
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	Lipid	Phosphatidylethanolamine (PE)	9546747		HMDB05322	0.05	0.07
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	Lipid	Phosphatidylethanolamine (PE)	9546749		HMDB08994	0.05	0.07
N-palmitoyl-sphinganine (d18:0/16:0)	Lipid	Sphingolipid Metabolism	5283572		HMDB11760	0.14	0.21
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	Lipid	Phosphatidylcholine (PC)	6436017		HMDB07972	0.05	0.07
N2,N2-dimethylguanosine	Nucleotide	Purine Metabolism, Guanine containing	92919		HMDB04824	0.06	0.10
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Lipid	Phosphatidylcholine (PC)	16219824		HMDB08048	0.04	0.09
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	Lipid	Phosphatidylcholine (PC)	5287971		HMDB07973	0.05	0.06
methylsuccinate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	10349		HMDB01844	0.10	0.19
1-arachidonoyl-GPE (20:4)	Lipid	Lysophospholipid	42607465		HMDB11517	0.05	0.10
N6-succinyladenosine	Nucleotide	Purine Metabolism, Adenine containing	165243		HMDB00912	0.11	0.22
kynurenate	Amino Acid	Tryptophan Metabolism	3845	C01717	HMDB00715	0.06	0.08
3-hydroxy-3-methylglutarate	Lipid	Mevalonate Metabolism	1662	C03761	HMDB00355	0.08	0.16
1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)	Lipid	Phosphatidylinositol (PI)			HMDB09789	0.10	0.48
N-acetyl-3-methylhistidine	Amino Acid	Histidine Metabolism	193270			0.33	0.18
2-stearoyl-GPE (18:0)	Lipid	Lysophospholipid			HMDB11129	0.15	0.34
pseudouridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	15047	C02067	HMDB00767	0.06	0.15
3-indoxyl sulfate	Amino Acid	Tryptophan Metabolism	10258		HMDB00682	0.08	0.12
glycosyl-N-palmitoyl-sphingosine	Lipid	Ceramides				0.09	0.08

pyridoxate	Cofactors and Vitamins	Vitamin B6 Metabolism	6723	C00847	HMDB00017	0.06	0.14
3-acetylphenol sulfate	Xenobiotics	Chemical				0.02	0.21
arabitol/xylitol	Carbohydrate	Pentose Metabolism	6912	C01904		0.10	0.15
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	Lipid	Phosphatidylinositol (PI)			HMDB09784	0.06	0.26
indolin-2-one	Xenobiotics	Food Component/Plant	321710	C12312		0.12	0.24
1-palmitoleoyl-GPC (16:1)	Lipid	Lysophospholipid	24779461		HMDB10383	0.04	0.17
1-methylimidazoleacetate	Amino Acid	Histidine Metabolism	75810	C05828	HMDB02820	0.06	0.10
xanthurenate	Amino Acid	Tryptophan Metabolism	5699	C02470	HMDB00881	0.19	0.17
N-acetylphenylalanine	Amino Acid	Phenylalanine Metabolism	74839	C03519	HMDB00512	0.05	0.10
tiglylcarnitine	Amino Acid	Leucine, Isoleucine and Valine Metabolism	22833596		HMDB02366	0.11	0.12
pantothenate	Cofactors and Vitamins	Pantothenate and CoA Metabolism	6613	C00864	HMDB00210	0.06	0.13
serine	Amino Acid	Glycine, Serine and Threonine Metabolism	5951	C00065	HMDB00187	0.03	0.11
N1-methylinosine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	65095		HMDB02721	0.10	0.21
C-glycosyltryptophan	Amino Acid	Tryptophan Metabolism	10981970			0.06	0.19
oxalate (ethanedioate)	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism	971	C00209	HMDB02329	0.05	0.28
N-acetylleucine	Amino Acid	Leucine, Isoleucine and Valine Metabolism	70912	C02710	HMDB11756	0.09	0.21

AASK, the African-American Study of Kidney Disease and Hypertension. CV, coefficient of variation. HMDB, Human Metabolome Database. KEGG, Kyoto Encyclopedia of Genes and Genomes. MDRD, the Modification of Diet in Renal Disease Study.

Supplemental Table 2. Associations of significant metabolites with cross-sectional eGFR in AASK and the MDRD Study, and eGFR change over time in AASK.

	MDRD		AASK			
Metabolite	Cross-sectional β coefficient (95% CI)	p-value	Cross-sectional β coefficient (95% CI)	p-value	Random slope coefficient (95% CI)	p-value
4-hydroxychlorothalonil	**3.31 (1.96, 4.65)**	**1.90E-06**	**3.82 (2.67, 4.98)**	**1.40E-10**	**0.06 (0.04, 0.07)**	**1.10E-12**
1,5-anhydroglucitol (1,5-AG)	**7.77 (6.47, 9.07)***	**5.70E-29**	**8.68 (6.99, 10.38)***	**1.00E-22**	0.03 (0, 0.05)	4.90E-02
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	**-4.03 (-6.26, -1.8)**	**4.20E-04**	**-5.92 (-7.98, -3.85)**	**2.50E-08**	**-0.08 (-0.11, -0.05)**	**9.80E-07**
1-stearoyl-GPE (18:0)	-1.57 (-4.65, 1.52)	0.32	**-4.6 (-7.15, -2.05)**	**4.20E-04**	**-0.06 (-0.1, -0.03)**	**8.30E-04**
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	**-3.48 (-4.38, -2.57)***	**1.50E-13**	**-2.42 (-3.26, -1.58)***	**1.80E-08**	0.01 (0, 0.02)	0.1
1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	-0.64 (-3.6, 2.32)	0.67	-1.31 (-4.29, 1.68)	0.39	**-0.07 (-0.12, -0.03)**	**8.10E-04**
Aspartate	-3.46 (-8, 1.08)	0.14	-0.76 (-4.31, 2.8)	0.68	-0.06 (-0.11, 0)	3.70E-02
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	**-4.48 (-6.58, -2.38)**	**3.20E-05**	**-5.59 (-7.39, -3.78)**	**1.90E-09**	**-0.07 (-0.1, -0.04)**	**1.60E-07**
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)	-1.35 (-4.16, 1.47)	0.35	0.44 (-1.64, 2.51)	0.68	-0.01 (-0.04, 0.02)	0.38
Retinol (Vitamin A)	**-12.62 (-15.81, -9.44)***	**3.10E-14**	**-12.84 (-14.82, -10.85)***	**3.20E-34**	**-0.07 (-0.1, -0.03)**	**4.40E-05**
1-palmitoyl-GPE (16:0)	-3.72 (-6.53, -0.91)	9.50E-03	**-6.09 (-8.39, -3.8)**	**2.20E-07**	**-0.07 (-0.1, -0.03)**	**8.80E-05**
4-acetamidobutanoate	**-17.22 (-18.26, -16.18)***	**<1.00E-39**	**-16.06 (-17.24, -14.88)***	**<1.00E-39**	-0.03 (-0.05, 0)	2.70E-02
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)	-0.53 (-2.96, 1.9)	0.67	0.58 (-1.25, 2.42)	0.53	-0.01 (-0.04, 0.02)	0.47
N6-carbamoylthreonyladenosine	**-22.31 (-23.62, -21.01)***	**<1.00E-39**	**-28.09 (-29.71, -26.47)***	**<1.00E-39**	-0.05 (-0.09, -0.01)	1.20E-02
N-palmitoyl-sphingosine (d18:1/16:0)	-5.75 (-9.48, -2.02)	2.60E-03	-3.14 (-6.02, -0.26)	3.20E-02	-0.05 (-0.1, -0.01)	1.30E-02
N2,N5-diacetylornithine	**-11.17 (-12.14, -10.19)***	**<1.00E-39**	**-10.93 (-11.85, -10.01)***	**<1.00E-39**	**-0.03 (-0.05, -0.01)**	**6.80E-04**
3-hydroxy-5-cholestenoic acid	**5.08 (2.69, 7.46)**	**3.40E-05**	3.14 (1.29, 4.99)	8.90E-04	0.03 (0.01, 0.06)	1.60E-02
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	**-3.37 (-5, -1.73)**	**6.10E-05**	**-3.6 (-5.14, -2.06)**	**4.90E-06**	**-0.06 (-0.08, -0.03)**	**6.90E-07**
O-sulfo-L-tyrosine	**-19.64 (-21.06, -18.22)***	**<1.00E-39**	**-22.9 (-24.38, -21.42)***	**<1.00E-39**	**-0.07 (-0.1, -0.03)**	**4.90E-05**
1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4)	-0.04 (-1.76, 1.69)	0.97	-3.67 (-7.43, 0.08)	5.50E-02	**-0.12 (-0.17, -0.06)**	**2.40E-05**
6-hydroxyindole sulfate	**-9.93 (-10.9, -8.95)***	**<1.00E-39**	**-10.99 (-12.12, -9.86)***	**<1.00E-39**	**-0.03 (-0.05, -0.01)**	**7.00E-04**
Cholesterol	-2.27 (-6.03, 1.48)	0.24	-3.37 (-6.22, -0.52)	2.10E-02	-0.03 (-0.08, 0.01)	0.11
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	-2.93 (-4.69, -1.18)	1.10E-03	**-2.97 (-4.51, -1.43)**	**1.70E-04**	**-0.04 (-0.07, -0.02)**	**1.60E-04**

3-methylglutaconate	**-12.93 (-14.06, -11.8)***	**<1.00E-39**	**-18.2 (-19.56, -16.84)***	**<1.00E-39**	-0.03 (-0.06, 0)	3.50E-02
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	**-4.35 (-6.33, -2.38)**	**1.80E-05**	**-4.04 (-5.74, -2.34)**	**3.60E-06**	**-0.06 (-0.08, -0.03)**	**8.80E-06**
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	**-3.84 (-5.93, -1.74)**	**3.40E-04**	**-3.83 (-5.63, -2.04)**	**3.10E-05**	**-0.06 (-0.08, -0.03)**	**4.60E-05**
N-palmitoyl-sphinganine (d18:0/16:0)	-0.61 (-2.81, 1.59)	0.58	-0.43 (-1.92, 1.06)	0.57	-0.03 (-0.05, -0.01)	5.20E-03
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	-2.73 (-6.3, 0.84)	0.13	-4.06 (-7.65, -0.47)	2.70E-02	-0.07 (-0.13, -0.02)	6.10E-03
N2,N2-dimethylguanosine	**-21.94 (-23.46, -20.42)***	**<1.00E-39**	**-24.83 (-26.5, -23.16)***	**<1.00E-39**	-0.04 (-0.08, -0.01)	1.40E-02
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	-1.75 (-4.51, 1)	0.21	-1.89 (-5.7, 1.92)	0.33	-0.08 (-0.13, -0.02)	6.00E-03
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	-5.3 (-10.18, -0.42)	3.30E-02	-3.74 (-8.73, 1.25)	0.14	-0.12 (-0.19, -0.05)	1.40E-03
Methylsuccinate	**-11.26 (-12.75, -9.77)***	**<1.00E-39**	**-17.44 (-19.43, -15.44)***	**<1.00E-39**	0 (-0.03, 0.03)	0.99
1-arachidonoyl-GPE (20:4)	-1.43 (-4.6, 1.74)	0.38	**-4.83 (-7.47, -2.19)**	**3.50E-04**	-0.03 (-0.07, 0)	8.70E-02
N6-succinyladenosine	**-15.08 (-16.56, -13.59)***	**<1.00E-39**	**-19.15 (-20.5, -17.79)***	**<1.00E-39**	-0.03 (-0.05, 0)	4.00E-02
Kynurenate	**-14.41 (-15.52, -13.3)***	**<1.00E-39**	**-17.14 (-18.62, -15.65)***	**<1.00E-39**	-0.03 (-0.06, 0)	4.70E-02
3-hydroxy-3-methylglutarate	**-17.22 (-18.77, -15.67)***	**<1.00E-39**	**-18.88 (-20.71, -17.05)***	**<1.00E-39**	-0.02 (-0.05, 0.02)	0.3
1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)	-0.62 (-2.42, 1.18)	0.5	-1.95 (-4.05, 0.15)	6.80E-02	-0.05 (-0.08, -0.02)	2.70E-03
N-acetyl-3-methylhistidine	**-5.81 (-6.43, -5.19)***	**<1.00E-39**	**-4.37 (-4.94, -3.79)***	**<1.00E-39**	-0.01 (-0.02, 0)	5.80E-02
2-stearoyl-GPE (18:0)	0.5 (-1.6, 2.59)	0.64	-2.1 (-4.02, -0.17)	3.30E-02	-0.04 (-0.06, -0.01)	1.20E-02
Pseudouridine	**-22.9 (-24.47, -21.33)***	**<1.00E-39**	**-32.54 (-34.29, -30.8)***	**<1.00E-39**	-0.06 (-0.11, -0.02)	3.10E-03
3-indoxyl sulfate	**-11.75 (-12.88, -10.63)***	**<1.00E-39**	**-11.05 (-12.34, -9.77)***	**<1.00E-39**	**-0.04 (-0.06, -0.02)**	**6.90E-04**
Glycosyl-N-palmitoyl-sphingosine	0.89 (-2.71, 4.5)	0.63	-2.07 (-4.76, 0.62)	0.13	-0.03 (-0.07, 0.01)	0.21
Pyridoxate	**-5.65 (-6.57, -4.73)***	**4.90E-30**	**-7.13 (-8.21, -6.04)***	**4.80E-35**	-0.03 (-0.04, -0.01)	2.10E-03
3-acetylphenol sulfate	**-6.48 (-7.38, -5.58)***	**8.80E-39**	**-5.48 (-6.58, -4.37)***	**6.30E-21**	-0.01 (-0.03, 0.01)	0.39
Arabitol/xylitol	**-18.31 (-19.91, -16.71)***	**<1.00E-39**	**-19.44 (-21.17, -17.71)***	**<1.00E-39**	-0.05 (-0.09, -0.02)	9.70E-04
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	-1.83 (-3.6, -0.06)	4.20E-02	-1.6 (-4.02, 0.82)	0.19	-0.04 (-0.08, -0.01)	1.80E-02
Indolin-2-one	**-9.92 (-10.94, -8.9)***	**<1.00E-39**	**-11.25 (-12.75, -9.75)***	**<1.00E-39**	-0.03 (-0.06, 0)	2.00E-02
1-palmitoleoyl-GPC (16:1)	**7.14 (3.94, 10.35)***	**1.40E-05**	3.65 (0.93, 6.37)	8.70E-03	-0.01 (-0.05, 0.03)	0.54
1-methylimidazoleacetate	**-18.93 (-20.4, -17.46)***	**<1.00E-39**	**-20.5 (-22.02, -18.99)***	**<1.00E-39**	-0.05 (-0.08, -0.02)	1.50E-03
Xanthurenate	**-7.81 (-8.82, -6.81)***	**<1.00E-39**	**-5.48 (-6.43, -4.53)***	**5.10E-28**	-0.02 (-0.04, -0.01)	6.00E-03
N-acetylphenylalanine	**-14.62 (-16.27, -12.98)***	**<1.00E-39**	**-8.41 (-10.09, -6.74)***	**7.50E-22**	-0.01 (-0.04, 0.01)	0.35
Tiglylcarnitine	**-16.81 (-18.3, -15.32)***	**<1.00E-39**	**-11.3 (-12.6, -10.01)***	**<1.00E-39**	**-0.05 (-0.07, -0.02)**	**5.60E-05**

Pantothenate	**-10.63 (-12.76, -8.49)***	**4.50E-21**	**-5.56 (-7.47, -3.65)***	**1.50E-08**	**-0.05 (-0.08, -0.02)**	**6.20E-04**
Serine	**18.18 (11.73, 24.63)***	**4.60E-08**	**9.54 (5.28, 13.81)***	**1.20E-05**	0.01 (-0.05, 0.08)	0.66
N1-methylinosine	**-14.81 (-16.06, -13.56)***	**<1.00E-39**	**-18.01 (-19.6, -16.42)***	**<1.00E-39**	-0.03 (-0.06, 0)	3.70E-02
C-glycosyltryptophan	**-18.97 (-20.44, -17.5)***	**<1.00E-39**	**-27.12 (-28.8, -25.44)***	**<1.00E-39**	-0.06 (-0.09, -0.02)	3.10E-03
Oxalate (ethanedioate)	-1.72 (-4.36, 0.92)	0.2	-0.72 (-2.58, 1.13)	0.44	0 (-0.03, 0.02)	0.9
N-acetylleucine	**-18.82 (-21.24, -16.41)***	**<1.00E-39**	**-13.81 (-16.08, -11.53)***	**1.50E-30**	-0.03 (-0.07, 0.01)	0.13

Statistically significant associations are bold-fonted. Cutoff for statistical significance = 0.05/58 =8.6 x10^{-4};

Cross-sectional β coefficients were estimated using linear regression; Random slope coefficients were estimated using mixed effects models;

Results were adjusted for baseline (AASK) or 12-month visit (MDRD) age, sex, trial arms, history of cardiovascular disease, history of smoking, body mass index, serum albumin, and in the MDRD Study, race. Asterisks (*) mark coefficients that remained statistically significant after additional adjustment for urine protein-to-creatinine ratio;

AASK, the African-American Study of Kidney Disease and Hypertension. CI, confidence interval. eGFR, estimated glomerular filtration rate (calculated using the CKD-EPI equation). MDRD, the Modification of Diet in Renal Disease Study.

Supplemental Appendix 1. Metabolite profiling procedures.

Samples were sent to Metabolon, Inc. (Morrisville, North Carolina) on dry ice. On receipt, samples were inventoried and stored immediately at -80°C until processing. Samples were processed by first adding several recovery standards, then using methanol to precipitate proteins. The extract was divided into five fractions: two for separate reverse phase ultraperformance liquid chromatography tandem mass-spectrometry (RP/UPLC-MS/MS) with a positive ion mode electrospray ionization (ESI), one for RP/UPLC-MS/MS with negative ion mode ESI, one for hydrophilic interaction ultra-performance liquid chromatography (HILIC-UPLC-MS/MS) with negative ion mode ESI, and one for back-up. These platforms are designed to provide complementary information regarding metabolites. Controls were analyzed concomitantly with the experimental samples, including a pooled matrix sample serving as a technical replicate, extracted water samples for negative controls, and certain quality control (QC) standards that do not interfere with endogenous compounds. The latter were added to each analyzed sample to provide monitoring for instruments and chromatographic alignment. Instrument variability was assessed as the median relative standard deviation for the QC standards (6% for AASK and 5% for MDRD) and process variability was assessed as the median relative standard deviation for the endogenous metabolites in the pooled matrix samples (10% for AASK and 11% for MDRD). Both the estimates of instrument variability and total process variability met Metabolon's internal acceptance criteria.

After analyzing via the four methods, each of which used a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution, data were extracted and processed using internal hardware and software. Peaks were identified through comparison to the Metabolon library, which catalogs purified, authenticated standards (currently >3300 compounds) and recurrent unknown entities. Experimental peaks were compared to the library with respect to retention time/index, mass to charge ratio, and chromatographic data; only compounds that matched on all three criteria are identified. The area under the curve was then used to quantify peaks, with correction for run-day blocks, since samples required more than one day of analysis.

For the present analysis, 1228 and 1193 biochemicals in AASK and MDRD respectively were matched with compounds in the Metabolon library, including 833 and 766 known biochemicals in AASK and MDRD respectively, that were authenticated using purified standards, and 395 and 427 recurrent unknown biochemicals in AASK and MDRD respectively. Biochemicals were classified into 89 pathways in AASK and 79 pathways in MDRD (see table, next page). For each study, 20 blind duplicate pairs were analyzed. The median metabolite correlation between duplicates was 0.94 in AASK and 0.91 in MDRD, 88% and 72% of all metabolites had a correlation >0.80 in AASK and MDRD, respectively.

Supplemental Appendix 2. Evaluated pathways and number of named, non-drug metabolites with missing <80%.

Super-pathway	Sub-pathway	Total N of metabolites In both studies
Amino acids	Alanine and Aspartate Metabolism	6
	Creatine Metabolism	3
	Glutamate Metabolism	6
	Glutathione Metabolism	5
	Glycine, Serine and Threonine Metabolism	8
	Guanidino and Acetamido Metabolism	3
	Histidine Metabolism	12
	Leucine, Isoleucine and Valine Metabolism	27
	Lysine Metabolism	9
	Methionine, Cysteine, SAM and Taurine Metabolism	18
	Phenylalanine and Tyrosine Metabolism	-
	Phenylalanine Metabolism	6
	Tyrosine Metabolism	18
	Polyamine Metabolism	5
	Tryptophan Metabolism	21
	Urea cycle; Arginine and Proline Metabolism	15
Carbohydrates	Advanced Glycation End-product	1
	Aminosugar Metabolism	4
	Disaccharides and Oligosaccharides	1
	Fructose, Mannose and Galactose Metabolism	3
	Glycogen metabolism	0
	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	5
	Pentose Metabolism	3
Cofactors and vitamins	Ascorbate and Aldarate Metabolism	3
	Hemoglobin and Porphyrin Metabolism	6
	Nicotinate and Nicotinamide Metabolism	5
	Pantothenate and CoA Metabolism	1
	Tocopherol Metabolism	5
	Vitamin A Metabolism	1
	Vitamin B6 Metabolism	1
Energy	Oxidative Phosphorylation	1
	TCA Cycle	9
Lipids	Carnitine Metabolism	2
	Diacylglycerol	2
	Eicosanoid	1
	Endocannabinoid	5
	Fatty Acid Metabolism (Acyl Choline)	6
	Fatty Acid Metabolism (Acyl Glutamine)	0
	Fatty Acid Metabolism (also BCAA Metabolism)	3
	Fatty Acid Metabolism (Acyl Carnitine)	18
	Fatty Acid Metabolism (Acyl Glycine)	2
	Fatty Acid Synthesis	1
	Fatty Acid, Amino	2
	Fatty Acid, Branched	2
	Fatty Acid, Dicarboxylate	16
	Fatty Acid, Dihydroxy	2
	Fatty Acid, Keto	0
	Fatty Acid, Monohydroxy	12
	Glycerolipid Metabolism	2

	Inositol Metabolism	2
	Ketone Bodies	1
	Long Chain Fatty Acid	14
	Lysophospholipid	25
	Lysoplasmalogen	4
	Medium Chain Fatty Acid	5
	Mevalonate Metabolism	1
	Monoacylglycerol	14
	Phospholipid Metabolism	-
	Ceramides	4
	Phosphatidylcholine (PC)	13
	Phosphatidylethanolamine (PE)	6
	Phosphatidylinositol (PI)	5
	Others	6
	Plasmalogen	11
	Polyunsaturated Fatty Acid (n3 and n6)	12
	Primary Bile Acid Metabolism	9
	Secondary Bile Acid Metabolism	15
	Sphingolipid Metabolism	22
	Steroid	-
	Androgenic Steroids	20
	Corticosteroids	2
	Pregnenolone Steroids	3
	Progestin Steroids	8
	Sterol	4
Nucleotides	Purine Metabolism, (Hypo)Xanthine/Inosine containing	7
	Purine Metabolism, Adenine containing	6
	Purine Metabolism, Guanine containing	2
	Pyrimidine Metabolism, Cytidine containing	2
	Pyrimidine Metabolism, Orotate containing	3
	Pyrimidine Metabolism, Thymine containing	2
	Pyrimidine Metabolism, Uracil containing	7
Peptides	Acetylated Peptides	3
	Dipeptide	7
	Dipeptide Derivative	1
	Fibrinogen Cleavage Peptide	1
	Gamma-glutamyl Amino Acid	15
	Polypeptide	1
Chemicals	Bacterial/Fungal	1
	Benzoate Metabolism	21
	Chemical	16
	Food Component/Plant	30
	Tobacco Metabolite	4
	Xanthine Metabolism	15
Total		637