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Associations of plasma amino acid and acylcarnitine profiles with incident reduced glomerular filtration rate

Supplemental Information File

Supplementary Methods: Amino acid measurement.

Supplementary Table 1: Parameters for quantification of amino acids in human plasma by GC/MS platform.

Supplementary Table 2: Characteristics of metabolites in the Nutrition and Health of Aging Population in China study.

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Supplementary Figure 1: The correlations among baseline concentrations of biomarkers in the Nutrition and Health of Aging Population in China study.

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Supplementary Figure 3: Scree plot showing percentage of variance explained by each component in the Nutrition and Health of Aging Population in China study.

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Supplementary Methods

Amino Acid Measurement

Amino acids were detected by gas chromatography-mass spectrometer (GC/MS). Initially, a stock stable isotope-labeled internal standard (IS) solution was prepared with ultrapure water and included 0.1 μmol/ml of L-leucine-$^{13}C_6$, L-isoleucine-$^{13}C_6$-^{15}N, L-phenylalanine-$^{13}C9$-^{15}N, L-lysine-$^{13}C6$-$^{15}N_2$, 0.2 μmol/ml of L-alanine-$^{13}C_3$-^{15}N, 0.3 μmol/ml of L-valine-$^{13}C_5$-^{15}N, L-glutamic acid-$^{13}C_5$, L-glutamine-$^{13}C_5$, and L-arginine-$^{13}C_5$ (Cambridge Isotope Laboratories, Inc. Tewksbury, MA, USA). A mixture containing the stock IS solution, thawed plasma sample, and cold methanol was vortex-mixed for 30 seconds and held at -20°C for 1 hour. After centrifugation (15 minutes, 16000g, and 4°C), the supernatants were evaporated under nitrogen flow (45°C). Dried residues were re-dissolved in acetonitrile and N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) with 1% tert-Butyldimethylchlorosilane (TBDMCS) (Fluka/Sigma-Aldrich, St. Louis, MO, USA). The mixture was then vortex-mixed vigorously for 30 seconds and incubated at 100°C for 60 minutes prior to GC-MS analysis. A serial of diluted amino acid standard-mixture solutions and three QC samples were prepared and analyzed with the same protocol used for plasma samples. Chromatographic separation of amino acids was performed on an Agilent 7890A gas chromatography system (Agilent Technologies Inc., CA, USA) with a HP-5ms fused-silica capillary column (30 m × 0.25 mm × 0.25 μm; Agilent J&W Scientific, Folsom, CA). Mass spectrometric analysis of amino acids was performed on an Agilent 5975C inert MSD system (Agilent Technologies Inc., CA, USA). Due to the non-

negligible system variations introduced by the long-term GC/MS instrumental analysis, QC samples were included throughout the analysis, with ten random samples run between every two QC samples.

The acquired GC/MS data were first preprocessed to robustly extract mass fragment ions for quantification based on correlations of peak areas or abundance of all fragment ions among all samples at a range of chromatographic window (i.e., 6 seconds), in which all fragment ions belong to one clustered metabolite; thuinstrumental noise data and fragment ions from co-eluted metabolites could be discarded. Relative value of amino acids was obtained by correcting the raw peak area of a given plasma sample against the average values of two measured QC samples located before and after the sample, and then normalized against IS (see Supplementary Table 2). Absolute values of amino acids were calculated using the aforementioned relative values and the average concentration of three QC samples, which were quantified based on the calibration curves of amino acid standards.

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Supplementary Table 1. Parameters for quantification of amino acids in human plasma by GC/MS platform

Metabolite	Retention time (min)	Quantifier (m/z)	Internal standard	Quantifier (m/z) of internal standard	Calibration curves (x: μmol/ml)[a]	R^2 [b]	Linear range (μmol/l)	Lower limits of quantification		Repeatability (RSD,%)		Recovery (%)
								pmol on column	S/N	Intra-day	Inter-day	
L-Alanine	11.09	232	L-Alanine-$^{13}C_3$-^{15}N	235	y = 4.8727x - 0.0084	0.9945	1 - 1000	0.133	89.4	1.89	4.61	93.2
Glycine	11.38	218	L-Alanine-$^{13}C_3$-^{15}N	235	y = 5.3730x + 0.0951	0.9991	1 - 1000	0.133	79.3	3.31	6.73	90.5
Sarcosine	11.90	158	L-Alanine-$^{13}C_3$-^{15}N	235	y = 6.2963x + 0.0097	0.9985	1 - 200	0.133	10.7	8.33	12.95	119.3
2-Aminobutyric acid	12.05	246	L-Alanine-$^{13}C_3$-^{15}N	235	y = 3.8780x - 0.0019	0.9993	1 - 200	0.133	53.2	1.77	5.78	91.4
L-Valine	12.74	288	L-Valine-^{13}C5-^{15}N	294	y = 4.0606x + 0.0314	0.9979	5 - 1000	0.133	26.7	0.78	2.47	99.8
L-Leucine	13.30	302	L-Leucine-$^{13}C_6$	308	y = 8.8768x + 0.0885	0.9983	5 - 1000	0.133	24.0	3.23	2.27	102.1
L-Isoleucine	13.71	302	L-Isoleucine-$^{13}C_6$-^{15}N	309	y = 13.9230x + 0.0432	0.9995	1 - 1000	0.133	16.7	2.14	5.26	112.0
L-Proline	14.19	286	L-Isoleucine-$^{13}C_6$-^{15}N	309	y = 8.4385 + 0.0323	0.9976	5 - 500	0.667	46.6	4.59	6.29	112.8
L-Methionine	16.70	320	L-Phenylalanine-$^{13}C_9$-^{15}N	346	y = 8.6864x + 0.0121	0.9967	2 - 1000	0.266	22.7	2.80	3.52	99.4
L-Serine	16.99	390	L-Phenylalanine-$^{13}C_9$-^{15}N	346	y = 11.7510x + 0.0538	0.9994	1 - 1000	0.133	134.4	2.54	5.58	117.9
L-Threonine	17.37	404	L-Phenylalanine-$^{13}C_9$-^{15}N	346	y = 3.6442x - 0.0018	0.9992	1 - 200	0.133	11.2	1.34	4.20	112.8
L-Phenylalanine	18.14	336	L-Phenylalanine-$^{13}C_9$-^{15}N	346	y = 9.0945x - 0.0023	0.9978	2 - 1000	0.133	26.0	0.93	3.66	98.8
L-Aspartic acid	18.89	418	L-Phenylalanine-$^{13}C_9$-^{15}N	346	y = 4.8437x + 0.0161	0.9985	1 - 200	0.133	13.2	3.79	15.14	103.3
4-Hydroxyproline	19.21	314	L-Phenylalanine-$^{13}C_9$-^{15}N	346	y = 11.8920x - 0.0548	0.9955	1 - 500	0.133	16.1	5.38	14.55	111.5
L-Cysteine	19.45	406	L-Phenylalanine-$^{13}C_9$-^{15}N	346	y = 8.4205x - 0.0478	0.9979	1 - 500	0.133	16.6	3.49	3.99	119.9
L-Glutamic acid	20.16	432	L-Glutamic acid-$^{13}C_5$	437	y = 2.8873x + 0.0040	0.9992	1 - 500	0.133	109.6	2.15	2.92	89.3
L-Ornithine/Arginine	20.22	286	L-Arginine-$^{13}C_6$-^{15}N	292	y = 13.3300x - 0.1269	0.9991	1 - 1000	0.133	13.6	2.85	7.35	112.6
L-Lysine	21.28	300	L-Lysine-$^{13}C_6$-$^{15}N_2$	307	y = 6.8965x - 0.0021	0.9990	2 - 1000	0.266	18.9	2.44	3.81	104.3
L-Glutamine	21.72	431	L-Glutamine-$^{13}C_5$	436	y = 2.2933x + 0.0186	0.9960	1 - 1000	0.133	20.5	1.23	1.66	98.0
L-Histidine	23.32	440	L-Glutamine-$^{13}C_5$	436	y = 6.5610x - 0.0377	0.9996	10 - 500	1.333	25.5	2.35	7.08	108.2
L-Tyrosine	23.76	302	L-Glutamine-$^{13}C_5$	436	y = 19.0390x - 0.0225	0.9991	1 - 1000	0.133	139.6	1.25	6.53	104.6

L-Tryptophan	25.86	244	L-Glutamine-$^{13}C_5$	436	y = 15.9280x - 0.0404	0.9996	1 - 500	0.133	23.1	1.27	9.00	115.5
L-Cystine	27.86	348	L-Glutamine-$^{13}C_5$	436	y = 2.6065x - 0.0856	0.9966	10 - 500	1.333	10.4	1.47	3.88	118.3

[a]Quantification calibration curves, where x is the concentration of amino acids (µmol/ml), and y is the quantifier (m/z) peak area ratio between amino acid and internal standard.

[b]Correlation coefficient of quantification calibration curve.

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Supplementary Table 2. Characteristics of metabolites in the Nutrition and Health of Aging Population in China study

Metabolite, μmol/l	Median (interquartile range)
Plasma amino acids	
Valine	211 (180-244)
Leucine	112 (94.6-131)
Isoleucine	75.5 (62.5-91.4)
Alanine	380 (314-451)
Glycine	119 (98.0-146)
Phenylalanine	58.0 (51.7-65.3)
Tyrosine	49.3 (41.3-58.3)
Tryptophan	91.2 (75.2-109)
Aspartic acid	18.9 (15.2-24.0)
Glutamic acid	46.5 (33.8-63.0)
Glutamine	791 (719-869)
Histidine	43.2 (36.2-54.9)
Lysine	187 (166-214)
Ornithine	70.0 (61.0-80.9)
Methionine	21.8 (19.1-25.1)
Cystine	41.3 (39.3-43.6)
Cysteine	25.7 (20.6-31.8)
Threonine	225 (191-265)
Serine	122 (103-145)
Proline	173 (134-220)
Hydroxyproline	20.1 (14.2-28.8)
Aminobutyrate	17.5 (13.5-22.4)
Acylcarnitines	
3-dehydroxycarnitine	0.36 (0.24-0.54)
3-dehydrocarnitine	2.60 (1.72-3.87)
C0	58.1 (49.0-68.1)
C2	11.2 (8.95-13.9)
C3	0.47 (0.35-0.60)
C3DC (10^{-2})	4.29 (3.24-5.68)
C4	0.20 (0.15-0.27)
C5 (10^{-2})	6.21 (4.75-7.94)
C5OH (10^{-2})	0.52 (0.43-0.64)
C5:1 (10^{-2})	0.97 (0.74-1.30)
C6 (10^{-2})	3.45 (2.90-4.28)
C6OH (10^{-2})	1.25 (0.96-1.66)
C6DC (10^{-2})	0.51 (0.34-0.76)
C7DC (10^{-2})	0.24 (0.17-0.35)
C8	0.20 (0.15-0.28)
C8:1	0.51 (0.33-0.75)

C10	0.26 (0.18-0.37)
C10DC (10^{-2})	0.47 (0.41-0.55)
C12 (10^{-2})	7.78 (5.54-10.8)
C12OH (10^{-2})	0.72 (0.52-0.96)
C12:1 (10^{-2})	11.2 (7.50-16.2)
C12DC (10^{-2})	0.79 (0.34-1.30)
C14 (10^{-2})	2.15 (1.64-2.89)
C14OH (10^{-2})	0.85 (0.54-1.74)
C14:1OH (10^{-2})	1.13 (0.69-2.09)
C16 (10^{-2})	8.67 (7.10-10.5)
C16:1 (10^{-2})	3.92 (2.33-7.49)
C16:2 (10^{-2})	1.49 (0.88-3.23)
C18 (10^{-2})	4.34 (2.71-12.0)
C18OH (10^{-2})	0.18 (0.12-0.28)
C18:1 (10^{-2})	3.50 (1.98-16.0)
C18:2	0.13 (0.09-0.32)
C20 (10^{-2})	0.37 (0.24-0.85)
C20:4 (10^{-2})	0.37 (0.23-0.81)

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Supplementary Table 3. Associations of single metabolites and combined indexes with baseline eGFR in the Nutrition and Health of Aging Population in China study

Metabolite and combined index	Model 1		Model 2	
	Beta (95% CI)	*P*	Beta (95% CI)	*P*
Metabolite				
C6DC	-2.90 (-3.51, -2.29)	3.9E-20[a]	-2.56 (-3.18, -1.95)	6.0E-16[a]
C7DC	-2.06 (-2.73, -1.40)	1.3E-09[a]	-2.08 (-2.73, -1.42)	6.9E-10[a]
C3DC	-2.09 (-2.72, -1.46)	1.1E-10[a]	-1.86 (-2.49, -1.23)	6.9E-09[a]
Glutamic Acid	1.90 (1.20, 2.59)	1.0E-07[a]	2.04 (1.30, 2.78)	7.0E-08[a]
C4	-1.97 (-2.60, -1.34)	1.2E-09[a]	-1.73 (-2.36, -1.09)	1.1E-07[a]
C5OH	-1.72 (-2.36, -1.08)	1.5E-07[a]	-1.60 (-2.23, -0.96)	8.5E-07[a]
C5:1	-1.50 (-2.13, -0.87)	3.4E-06[a]	-1.54 (-2.16, -0.92)	1.3E-06[a]
Valine	1.60 (0.93, 2.26)	2.7E-06[a]	1.81 (1.08, 2.54)	1.3E-06[a]
Tyrosine	1.62 (0.98, 2.27)	9.0E-07[a]	1.55 (0.89, 2.22)	4.5E-06[a]
Tryptophan	1.19 (0.54, 1.84)	4.0E-04[a]	1.46 (0.82, 2.11)	9.6E-06[a]
Glutamine	1.51 (0.88, 2.14)	2.6E-06[a]	1.33 (0.71, 1.96)	3.2E-05[a]
Serine	1.92 (1.30, 2.54)	1.7E-09[a]	1.34 (0.71, 1.98)	3.7E-05[a]
Lysine	1.60 (0.97, 2.23)	7.2E-07[a]	1.25 (0.61, 1.89)	1.0E-04[a]
Alanine	1.15 (0.51, 1.79)	4.0E-04[a]	1.28 (0.58, 1.97)	3.0E-04[a]
C12OH	-0.94 (-1.57, -0.31)	3.4E-03[a]	-1.10 (-1.72, -0.48)	5.0E-04[a]
Aminobutyrate	1.12 (0.45, 1.80)	1.2E-03	1.14 (0.46, 1.81)	1.0E-03
Leucine	1.19 (0.47, 1.91)	1.2E-03	1.23 (0.45, 2.02)	2.2E-03
Aspartic Acid	0.86 (0.19, 1.53)	1.2E-02	0.96 (0.29, 1.63)	4.8E-03
C12:1	-0.64 (-1.28, 0.01)	5.2E-02	-0.90 (-1.53, -0.26)	5.6E-03
Histidine	1.44 (0.82, 2.06)	6.2E-06[a]	0.89 (0.26, 1.53)	5.9E-03
Threonine	0.75 (0.12, 1.39)	2.0E-02	0.88 (0.26, 1.51)	5.9E-03
C10	-1.06 (-1.69, -0.43)	1.0E-03	-0.86 (-1.48, -0.24)	6.8E-03
C16	0.69 (0.06, 1.32)	3.1E-02	0.79 (0.15, 1.42)	1.6E-02
Isoleucine	1.16 (0.47, 1.85)	1.1E-03	0.91 (0.15, 1.67)	1.9E-02
C18OH	0.63 (-0.03, 1.28)	6.0E-02	0.77 (0.12, 1.42)	2.0E-02
Hydroxyproline	-0.83 (-1.46, -0.20)	1.0E-02	-0.73 (-1.35, -0.10)	2.3E-02
Phenylalanine	-0.63 (-1.28, 0.01)	5.5E-02	-0.73 (-1.38, -0.08)	2.7E-02
C12	-0.60 (-1.24, 0.03)	6.2E-02	-0.69 (-1.32, -0.07)	2.9E-02
C8	-0.77 (-1.39, -0.14)	1.7E-02	-0.66 (-1.29, -0.04)	3.6E-02
C10DC	0.65 (0.03, 1.28)	4.0E-02	0.65 (0.03, 1.27)	4.0E-02
Ornithine	0.93 (0.27, 1.58)	5.4E-03	0.59 (-0.06, 1.24)	7.3E-02
Cystine	-0.72 (-1.36, -0.07)	3.0E-02	-0.59 (-1.24, 0.06)	7.4E-02
Glycine	-0.37 (-1.01, 0.27)	2.6E-01	-0.55 (-1.19, 0.09)	8.9E-02
C14:1OH	-0.77 (-1.41, -0.12)	1.9E-02	-0.48 (-1.12, 0.16)	1.4E-01
C8:1	-0.76 (-1.38, -0.13)	1.8E-02	-0.47 (-1.10, 0.17)	1.5E-01
C2	-0.53 (-1.17, 0.11)	1.1E-01	-0.45 (-1.08, 0.18)	1.6E-01
C6OH	-0.12 (-0.75, 0.51)	7.1E-01	-0.44 (-1.07, 0.18)	1.7E-01
C12DC	0.70 (0.07, 1.33)	3.0E-02	0.42 (-0.21, 1.05)	1.9E-01

C6	-0.49 (-1.12, 0.13)	1.2E-01	-0.39 (-1.01, 0.23)	2.1E-01
C20	0.08 (-0.58, 0.75)	8.0E-01	0.40 (-0.26, 1.06)	2.4E-01
C16:1	0.05 (-0.59, 0.69)	8.8E-01	0.32 (-0.31, 0.96)	3.2E-01
C14	0.40 (-0.23, 1.04)	2.1E-01	0.31 (-0.31, 0.94)	3.3E-01
C5	-0.59 (-1.26, 0.07)	8.2E-02	-0.33 (-1.02, 0.35)	3.4E-01
3-dehydrocarnitine	0.61 (-0.02, 1.24)	5.6E-02	0.30 (-0.32, 0.93)	3.4E-01
Cysteine	-0.80 (-1.44, -0.16)	1.4E-02	-0.26 (-0.90, 0.39)	4.4E-01
C3	-0.49 (-1.15, 0.16)	1.4E-01	-0.26 (-0.93, 0.41)	4.5E-01
C0	-0.43 (-1.07, 0.21)	1.9E-01	0.23 (-0.43, 0.89)	4.9E-01
Proline	0.43 (-0.22, 1.07)	2.0E-01	0.19 (-0.47, 0.85)	5.8E-01
C18:2	-0.24 (-0.90, 0.41)	4.7E-01	0.15 (-0.50, 0.80)	6.5E-01
3-dehydroxycarnitine	0.41 (-0.21, 1.04)	1.9E-01	0.14 (-0.48, 0.76)	6.5E-01
Methionine	0.25 (-0.42, 0.91)	4.7E-01	0.14 (-0.52, 0.80)	6.7E-01
C18:1	-0.06 (-0.69, 0.58)	8.6E-01	0.13 (-0.49, 0.76)	6.8E-01
C18	-0.39 (-1.05, 0.28)	2.5E-01	0.11 (-0.56, 0.78)	7.5E-01
C20:4	-0.38 (-1.03, 0.27)	2.5E-01	0.08 (-0.57, 0.73)	8.1E-01
C16:2	-0.35 (-1.00, 0.30)	2.9E-01	-0.07 (-0.71, 0.58)	8.4E-01
C14OH	-0.30 (-0.95, 0.35)	3.6E-01	-0.06 (-0.71, 0.59)	8.5E-01
Combined index				
Serine/Glycine	1.90 (1.27, 2.53)	3.9E-09	1.62 (0.99, 2.25)	4.9E-07
Tyrosine/Phenylalanine	2.13 (1.51, 2.76)	2.9E-11	2.03 (1.41, 2.65)	1.8E-10
Short-chain AcylCN	-0.88 (-1.51, -0.25)	5.5E-11	-0.91 (-1.54, -0.29)	2.8E-09
Medium-chain AcylCN	-0.06 (-0.70, 0.59)	6.1E-03	0.25 (-0.39, 0.89)	4.1E-03
Long-chain AcylCN	-0.06 (0.33, 0.00)	8.6E-01	0.25 (0.33, 0.00)	4.5E-01

Model 1: adjusted for age, sex, region, residence;

Model 2: further adjusted for education attainment, physical activity, current smoking, current drinking, BMI, lipid-lowering medication use, HDL, LDL, cardiovascular disease, hypertension and type 2 diabetes.

AcylCN: acylcarnitines.

[a]*P* values were significant after Bonferroni correction (*P* <0.05/56=8.9E-04).

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Supplementary Table 4. Associations of single metabolites and combined indexes with annual eGFR change in the Nutrition and Health of Aging Population in China study

Metabolite and combined index	Model 1		Model 2		Model 3	
	Beta (95% CI)	*P*	Beta (95% CI)	*P*	Beta (95% CI)	*P*
Metabolite						
Cysteine	-0.18 (-0.28, -0.09)	1.0E-04[a]	-0.25 (-0.34, -0.15)	3.5E-07[a]	-0.25 (-0.33, -0.17)	2.7E-09[a]
C3DC	-0.04 (-0.13, 0.05)	3.9E-01	-0.06 (-0.15, 0.03)	2.1E-01	-0.19 (-0.27, -0.11)	5.8E-06[a]
C10	-0.09 (-0.18, 0.00)	4.8E-02	-0.12 (-0.21, -0.02)	1.4E-02	-0.17 (-0.25, -0.10)	1.6E-05[a]
C20	-0.17 (-0.27, -0.07)	6.0E-04[a]	-0.20 (-0.30, -0.10)	5.0E-05[a]	-0.18 (-0.26, -0.09)	3.8E-05[a]
C14:1OH	-0.11 (-0.20, -0.01)	2.3E-02	-0.13 (-0.22, -0.04)	7.0E-03	-0.17 (-0.25, -0.09)	4.5E-05[a]
C18	-0.12 (-0.22, -0.03)	1.2E-02	-0.17 (-0.27, -0.08)	5.0E-04[a]	-0.17 (-0.26, -0.09)	5.6E-05[a]
C18:2	-0.14 (-0.24, -0.05)	3.6E-03	-0.17 (-0.27, -0.08)	4.0E-04[a]	-0.17 (-0.25, -0.09)	1.0E-04[a]
C16:2	-0.13 (-0.22, -0.03)	7.4E-03	-0.15 (-0.25, -0.06)	1.9E-03	-0.16 (-0.24, -0.07)	2.0E-04[a]
Phenylalanine	-0.11 (-0.20, -0.01)	2.9E-02	-0.11 (-0.20, -0.01)	3.2E-02	-0.16 (-0.24, -0.07)	2.0E-04[a]
Glutamine	-0.26 (-0.35, -0.16)	5.7E-08[a]	-0.24 (-0.33, -0.15)	4.5E-07[a]	-0.15 (-0.23, -0.07)	3.0E-04[a]
C8	-0.09 (-0.18, 0.01)	6.8E-02	-0.10 (-0.19, -0.01)	3.3E-02	-0.14 (-0.22, -0.06)	4.0E-04[a]
C20:4	-0.12 (-0.21, -0.02)	1.6E-02	-0.16 (-0.25, -0.06)	1.6E-03	-0.15 (-0.23, -0.07)	4.0E-04[a]
C5OH	-0.05 (-0.15, 0.04)	2.8E-01	-0.07 (-0.16, 0.03)	1.7E-01	-0.15 (-0.23, -0.06)	4.0E-04[a]
Threonine	-0.17 (-0.27, -0.08)	3.0E-04[a]	-0.18 (-0.28, -0.09)	1.0E-04[a]	-0.13 (-0.21, -0.05)	1.3E-03
C14OH	-0.11 (-0.20, -0.01)	2.7E-02	-0.13 (-0.22, -0.03)	9.9E-03	-0.13 (-0.21, -0.05)	2.0E-03
Ornithine	-0.21 (-0.30, -0.11)	2.0E-05[a]	-0.19 (-0.28, -0.09)	2.0E-04[a]	-0.13 (-0.21, -0.05)	2.2E-03
C8:1	-0.08 (-0.17, 0.02)	1.1E-01	-0.10 (-0.19, -0.01)	3.6E-02	-0.12 (-0.21, -0.04)	2.3E-03
C6DC	0.07 (-0.02, 0.17)	1.1E-01	0.04 (-0.06, 0.13)	4.3E-01	-0.12 (-0.20, -0.04)	2.4E-03
Cystine	-0.09 (-0.18, 0.01)	7.5E-02	-0.10 (-0.20, -0.01)	3.9E-02	-0.12 (-0.20, -0.04)	4.0E-03
Tyrosine	-0.23 (-0.32, -0.13)	3.1E-06[a]	-0.23 (-0.33, -0.13)	5.3E-06[a]	-0.12 (-0.21, -0.04)	5.0E-03
C16:1	-0.13 (-0.22, -0.03)	8.2E-03	-0.14 (-0.24, -0.05)	2.8E-03	-0.11 (-0.19, -0.03)	5.6E-03
C12	-0.07 (-0.17, 0.02)	1.1E-01	-0.07 (-0.16, 0.02)	1.3E-01	-0.11 (-0.19, -0.03)	6.4E-03
Aspartic Acid	-0.18 (-0.27, -0.08)	4.0E-04[a]	-0.19 (-0.29, -0.09)	2.0E-04[a]	-0.11 (-0.20, -0.03)	9.4E-03
C12OH	-0.05 (-0.14, 0.04)	2.7E-01	-0.04 (-0.13, 0.05)	4.2E-01	-0.10 (-0.18, -0.02)	1.2E-02
C16	-0.16 (-0.25, -0.06)	1.0E-03	-0.17 (-0.27, -0.08)	3.0E-04[a]	-0.10 (-0.18, -0.02)	1.3E-02
Proline	-0.13 (-0.23, -0.04)	5.6E-03	-0.13 (-0.23, -0.03)	1.0E-02	-0.10 (-0.19, -0.02)	1.8E-02
C14	-0.14 (-0.23, -0.05)	3.3E-03	-0.14 (-0.23, -0.04)	3.9E-03	-0.10 (-0.18, -0.02)	1.8E-02
Tryptophan	-0.17 (-0.26, -0.07)	6.0E-04[a]	-0.20 (-0.29, -0.10)	1.0E-04[a]	-0.10 (-0.18, -0.01)	2.4E-02
Methionine	-0.11 (-0.21, -0.01)	2.6E-02	-0.10 (-0.20, 0.00)	4.2E-02	-0.09 (-0.18, -0.01)	2.8E-02
Valine	-0.19 (-0.28, -0.09)	2.0E-04[a]	-0.24 (-0.35, -0.13)	1.8E-05[a]	-0.10 (-0.20, -0.01)	3.2E-02
Glycine	-0.05 (-0.14, 0.04)	2.9E-01	-0.04 (-0.13, 0.06)	4.4E-01	-0.09 (-0.17, -0.01)	3.7E-02
C5	-0.06 (-0.16, 0.04)	2.2E-01	-0.09 (-0.19, 0.01)	7.5E-02	-0.09 (-0.18, 0.00)	4.1E-02
C18:1	-0.08 (-0.17, 0.01)	1.0E-01	-0.09 (-0.18, 0.00)	5.9E-02	-0.08 (-0.16, 0.00)	4.2E-02
Leucine	-0.16 (-0.26, -0.05)	3.8E-03	-0.19 (-0.31, -0.08)	1.1E-03	-0.10 (-0.20, 0.00)	6.0E-02
3-dehydroxycarnitine	0.04 (-0.05, 0.13)	4.3E-01	0.06 (-0.04, 0.15)	2.3E-01	0.07 (0.00, 0.15)	6.4E-02
Isoleucine	-0.15 (-0.25, -0.05)	4.1E-03	-0.16 (-0.27, -0.05)	5.7E-03	-0.08 (-0.18, 0.01)	9.0E-02
C7DC	0.07 (-0.03, 0.17)	1.8E-01	0.07 (-0.03, 0.17)	1.7E-01	-0.07 (-0.16, 0.01)	1.1E-01
C4	0.06 (-0.03, 0.15)	2.0E-01	0.04 (-0.06, 0.13)	4.2E-01	-0.07 (-0.15, 0.02)	1.1E-01

C12DC	-0.02 (-0.11, 0.08)	7.5E-01	0.01 (-0.08, 0.10)	8.1E-01	0.06 (-0.02, 0.14)	1.5E-01
C2	-0.04 (-0.14, 0.05)	3.7E-01	-0.05 (-0.14, 0.05)	3.5E-01	-0.06 (-0.14, 0.02)	1.6E-01
C18OH	-0.09 (-0.19, 0.00)	5.3E-02	-0.11 (-0.21, -0.01)	2.5E-02	-0.06 (-0.14, 0.03)	1.8E-01
3-dehydrocarnitine	0.00 (-0.09, 0.09)	9.5E-01	0.02 (-0.08, 0.11)	7.3E-01	0.05 (-0.03, 0.13)	1.9E-01
C12:1	-0.02 (-0.12, 0.07)	6.1E-01	0.00 (-0.10, 0.09)	9.7E-01	-0.05 (-0.13, 0.03)	2.2E-01
Hydroxyproline	0.02 (-0.08, 0.11)	7.3E-01	0.01 (-0.09, 0.10)	9.1E-01	-0.05 (-0.13, 0.03)	2.3E-01
C6OH	-0.05 (-0.15, 0.04)	2.6E-01	-0.02 (-0.12, 0.07)	6.1E-01	-0.04 (-0.12, 0.03)	2.7E-01
C0	-0.02 (-0.11, 0.07)	6.6E-01	-0.08 (-0.18, 0.02)	1.1E-01	-0.04 (-0.13, 0.04)	3.0E-01
Histidine	-0.16 (-0.25, -0.07)	6.0E-04[a]	-0.11 (-0.21, -0.02)	1.8E-02	-0.04 (-0.12, 0.04)	3.2E-01
C3	0.05 (-0.04, 0.15)	2.9E-01	0.03 (-0.07, 0.13)	5.4E-01	0.04 (-0.05, 0.12)	3.7E-01
C6	-0.01 (-0.10, 0.08)	7.8E-01	-0.02 (-0.12, 0.07)	6.1E-01	-0.03 (-0.11, 0.04)	4.0E-01
Alanine	-0.10 (-0.19, -0.01)	3.6E-02	-0.13 (-0.23, -0.02)	1.7E-02	-0.04 (-0.13, 0.05)	4.1E-01
C10DC	-0.09 (-0.18, 0.01)	6.7E-02	-0.09 (-0.18, 0.00)	4.8E-02	-0.03 (-0.11, 0.05)	4.5E-01
Aminobutyrate	-0.09 (-0.19, 0.01)	8.0E-02	-0.10 (-0.20, 0.00)	5.2E-02	-0.02 (-0.11, 0.06)	6.0E-01
Glutamic Acid	-0.13 (-0.23, -0.02)	1.5E-02	-0.15 (-0.26, -0.04)	9.5E-03	0.02 (-0.07, 0.12)	6.2E-01
C5:1	0.06 (-0.03, 0.15)	2.0E-01	0.06 (-0.03, 0.16)	1.8E-01	-0.01 (-0.09, 0.06)	7.2E-01
Serine	-0.14 (-0.23, -0.05)	2.6E-03	-0.09 (-0.19, 0.00)	5.9E-02	-0.01 (-0.09, 0.08)	9.0E-01
Lysine	-0.11 (-0.21, -0.02)	1.6E-02	-0.09 (-0.18, 0.01)	7.4E-02	0.00 (-0.08, 0.08)	9.9E-01
Combined index						
Serine/Glycine	-0.08 (-0.17, 0.01)	8.7E-02	-0.06 (-0.15, 0.04)	2.4E-01	0.07 (-0.02, 0.15)	1.1E-01
Tyrosine/Phenylalanine	-0.15 (-0.24, -0.06)	1.2E-03	-0.14 (-0.24, -0.05)	2.5E-03	0.00 (-0.08, 0.08)	9.3E-01
Short-chain AcylCN	0.00 (-0.09, 0.09)	9.7E-01	-0.02 (-0.12, 0.07)	6.4E-01	-0.12 (-0.21, -0.04)	2.5E-03
Medium-chain AcylCN	-0.08 (-0.17, 0.02)	1.1E-01	-0.08 (-0.17, 0.02)	1.1E-01	-0.12 (-0.20, -0.05)	2.1E-03
Long-chain AcylCN	-0.15 (-0.24, -0.06)	1.7E-03	-0.18 (-0.27, -0.08)	2.0E-04	-0.16 (-0.24, -0.08)	1.0E-04

Data are annual change in eGFR between baseline and 6-year follow-up per SD increase in metabolite concentration.
Model 1: adjusted for age, sex, region, residence;
Model 2: further adjusted for education attainment, physical activity, current smoking, current drinking, systolic blood pressure, BMI, lipid-lowering medication use, HDL, LDL, cardiovascular disease, hypertension and type 2 diabetes;
Model 3: further adjusted for eGFR.
AcylCN: acylcarnitines.
[a]*P* values were significant after Bonferroni correction ($P < 0.05/56 = 8.9E-04$).

Supplementary Table 5. Sensitivity analysis of associations between annual eGFR change associated metabolites and incident eGFR decline assessed by other definitions in the Nutrition and Health of Aging Population in China study

Metabolite	Incident eGFR decline (Compostie definition)[a]		Incident eGFR decline (CKD-EPI)[b]	
	Relative risk (95% confidence interval)	*P*	Relative risk (95% confidence interval)	*P*
Cysteine	1.30 (1.15, 1.47)	2.3E-05[c]	1.24 (1.15, 1.34)	1.1E-08[c]
C20:4	1.27 (1.14, 1.42)	1.0E-05[c]	1.14 (1.05, 1.22)	8.0E-04[c]
C18:2	1.26 (1.13, 1.40)	2.7E-05[c]	1.15 (1.07, 1.24)	1.0E-04[c]
C18	1.25 (1.12, 1.40)	1.0E-04[c]	1.13 (1.05, 1.22)	9.0E-04[c]
C14:1OH	1.22 (1.09, 1.36)	6.0E-04[c]	1.13 (1.05, 1.22)	8.0E-04[c]
C16:2	1.20 (1.07, 1.35)	1.4E-03[c]	1.14 (1.06, 1.22)	6.0E-04[c]
C3DC	1.22 (1.08, 1.39)	1.9E-03[c]	1.13 (1.05, 1.22)	1.5E-03[c]
Phenylalanine	1.17 (1.05, 1.31)	5.4E-03	1.07 (1.00, 1.15)	4.8E-02
C20	1.17 (1.04, 1.31)	7.1E-03	1.13 (1.05, 1.21)	1.2E-03[c]
C10	1.17 (1.04, 1.31)	1.1E-02	1.13 (1.05, 1.22)	9.0E-04[c]
Glutamine	1.13 (1.00, 1.28)	5.4E-02	1.12 (1.03, 1.20)	4.3E-03
C8	1.09 (0.97, 1.23)	1.4E-01	1.11 (1.03, 1.19)	3.8E-03
C5OH	1.07 (0.96, 1.19)	2.3E-01	1.05 (0.98, 1.11)	1.6E-01

Model included age, sex, region, residence, education, current smoking, current drinking, physical activity, BMI, lipid-lowering medication use, HDL, LDL, hypertension, cardiovascular disease, type 2 diabetes and eGFR at baseline.

[a]Incident eGFR decline (composite definition) was defined as both eGFR decline >3% per year and follow-up eGFR <60 ml/min/1.73 m^2.

[b]Incident eGFR decline (CKD-EPI) was defined as eGFR <60 ml/min/1.73 m^2 in which eGFR was calculated using the CKD-EPI equation.

[c]Significant association with incident eGFR decline with P <0.05/13=3.8E-03.

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Supplementary Table 6. Relative risks of incident eGFR decline by stratified analysis in the Nutrition and Health of Aging Population in China study

	n	Cysteine	*P*	C3DC	*P*	C10	*P*	C14:1OH	*P*
Age, y			*0.68*		*0.67*		*0.92*		*0.13*
50-59	1042	1.38 (1.15, 1.65)	0.001	1.12 (0.93, 1.34)	0.23	1.21 (1.01, 1.43)	0.03	1.30 (1.11, 1.53)	0.001
60-70	723	1.23 (1.08, 1.40)	0.002	1.22 (1.07, 1.40)	0.003	1.18 (1.04, 1.34)	0.01	1.07 (0.95, 1.21)	0.24
Sex			*0.06*		*0.16*		*0.35*		*0.22*
Men	745	1.47 (1.22, 1.78)	<0.001	1.28 (1.08, 1.51)	0.004	1.25 (1.08, 1.44)	0.003	1.23 (1.06, 1.43)	0.01
Women	1020	1.14 (1.00, 1.30)	0.05	1.10 (0.95, 1.27)	0.20	1.14 (0.98, 1.34)	0.09	1.13 (0.99, 1.29)	0.07
Region			*0.90*		*0.41*		*0.42*		*0.73*
Beijing	721	1.26 (1.05, 1.51)	0.01	1.12 (0.93, 1.36)	0.24	1.17 (0.96, 1.42)	0.11	1.14 (0.97, 1.34)	0.11
Shanghai	1044	1.22 (1.07, 1.39)	0.003	1.22 (1.07, 1.38)	0.005	1.22 (1.08, 1.37)	0.001	1.16 (1.02, 1.30)	0.02
BMI, kg/m^2			*0.69*		***0.03***		***0.02***		*0.18*
<24	828	1.23 (1.05, 1.44)	0.01	1.07 (0.91, 1.26)	0.39	1.06 (0.91, 1.23)	0.47	1.07 (0.93, 1.24)	0.33
≥24	937	1.26 (1.10, 1.46)	0.001	1.33 (1.15, 1.54)	<0.001	1.35 (1.18, 1.54)	<0.001	1.25 (1.09, 1.43)	0.001
Hypertension			*0.36*		*0.20*		*0.40*		*0.62*
Yes	850	1.38 (1.17, 1.64)	<0.001	1.06 (0.88, 1.27)	0.56	1.13 (0.95, 1.35)	0.16	1.22 (1.02, 1.45)	0.03
No	915	1.16 (1.01, 1.33)	0.03	1.26 (1.10, 1.43)	0.001	1.23 (1.08, 1.40)	0.002	1.15 (1.02, 1.29)	0.02
Type 2 diabetes			*0.67*		*0.18*		*0.21*		*0.48*
No	1599	1.23 (1.10, 1.38)	<0.001	1.21 (1.08, 1.36)	0.001	1.23 (1.10, 1.36)	<0.001	1.18 (1.06, 1.30)	0.002
Yes	166	1.51 (1.03, 2.21)	0.03	1.01 (0.67, 1.51)	0.97	1.15 (0.71, 1.86)	0.58	1.21 (0.78, 1.89)	0.39
eGFR, ml/min/1.73 m^2			***<0.001***		*0.80*		*0.84*		*0.55*
60–90	1093	1.14 (1.02, 1.27)	0.02	1.21 (1.08, 1.36)	0.001	1.19 (1.07, 1.33)	0.002	1.16 (1.04, 1.28)	0.006
>90	672	2.71 (1.96, 3.75)	<0.001	1.15 (0.77, 1.72)	0.50	1.29 (0.88, 1.89)	0.20	1.27 (0.93, 1.73)	0.13

	n	C18	*P*	C18:2	*P*	C20:4	*P*
Age, y			*0.30*		*0.20*		*0.21*
50-59	1042	1.30 (1.10, 1.53)	0.002	1.31 (1.12, 1.53)	0.001	1.32 (1.13, 1.55)	0.001
60-70	723	1.08 (0.96, 1.22)	0.22	1.09 (0.97, 1.23)	0.16	1.10 (0.98, 1.24)	0.12
Sex			*0.75*		*0.87*		*0.21*
Men	745	1.18 (1.01, 1.39)	0.04	1.18 (1.02, 1.38)	0.03	1.27 (1.09, 1.47)	0.002
Women	1020	1.16 (1.01, 1.33)	0.03	1.19 (1.05, 1.35)	0.01	1.15 (1.01, 1.31)	0.04
Region			*0.44*		*0.40*		*0.82*
Beijing	721	1.20 (1.03, 1.40)	0.02	1.23 (1.06, 1.44)	0.008	1.17 (0.99, 1.37)	0.06
Shanghai	1044	1.11 (0.98, 1.26)	0.10	1.12 (1.00, 1.26)	0.06	1.17 (1.04, 1.31)	0.007
BMI, kg/m2			*0.22*		*0.32*		*0.28*
<24	828	1.08 (0.93, 1.26)	0.34	1.11 (0.96, 1.28)	0.17	1.11 (0.96, 1.28)	0.17
≥24	937	1.25 (1.09, 1.43)	0.002	1.24 (1.09, 1.41)	0.001	1.26 (1.10, 1.44)	0.001
Hypertension			*0.49*		*0.29*		*0.60*
Yes	850	1.25 (1.06, 1.48)	0.008	1.27 (1.09, 1.48)	0.002	1.23 (1.04, 1.45)	0.01
No	915	1.13 (1.00, 1.28)	0.05	1.12 (1.00, 1.27)	0.06	1.16 (1.03, 1.31)	0.02
Type 2 diabetes			*0.12*		*0.43*		*0.22*
No	1599	1.19 (1.07, 1.32)	0.001	1.19 (1.08, 1.32)	0.001	1.21 (1.09, 1.34)	<0.001
Yes	166	0.85 (0.53, 1.35)	0.49	1.06 (0.67, 1.69)	0.79	0.90 (0.58, 1.39)	0.64
eGFR, ml/min/1.73 m2			*0.24*		*0.20*		*0.18*
60–90	1093	1.14 (1.02, 1.27)	0.02	1.15 (1.03, 1.27)	0.01	1.14 (1.02, 1.27)	0.02
>90	672	1.47 (1.11, 1.95)	0.01	1.44 (1.07, 1.95)	0.02	1.48 (1.14, 1.93)	0.003

Relative risks were adjusted for age, sex, region, residence, education, current smoking, current drinking, physical activity, BMI, lipid-lowering medication use, HDL, LDL, cardiovascular disease, hypertension, type 2 diabetes and eGFR at baseline, except stratifying factors. *P* values of

interaction test were shown in *Italic* and significant *P* values were shown in bold.

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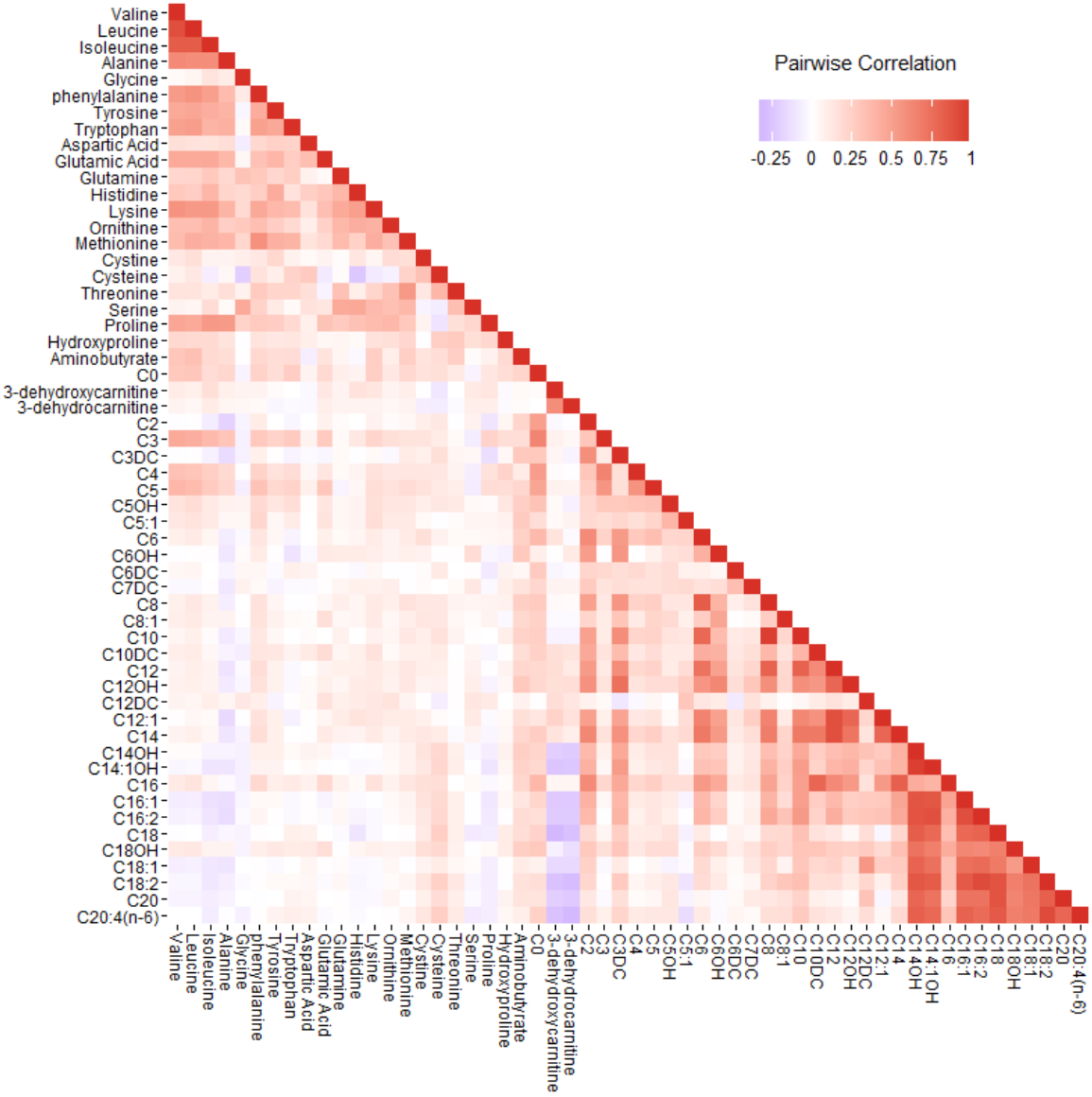
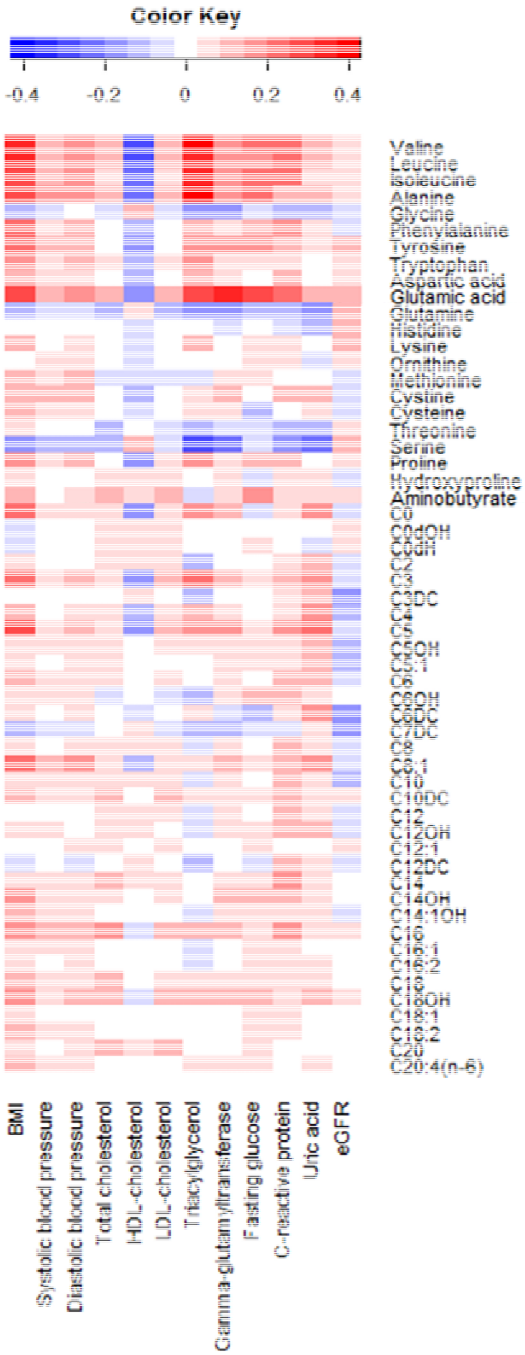
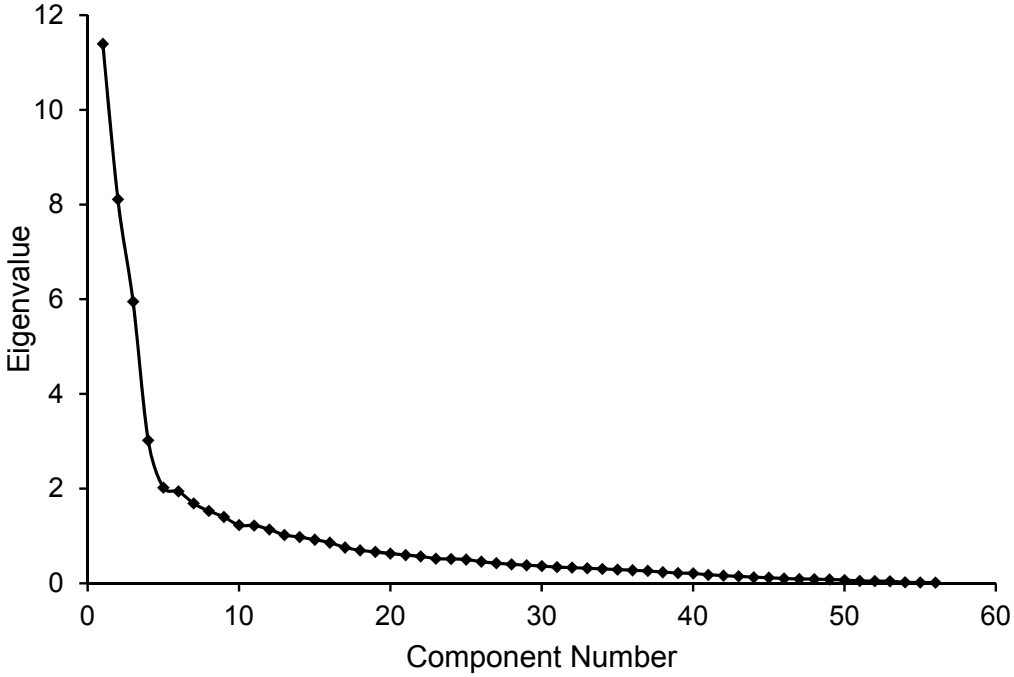
Supplementary Figure 1. The correlations among baseline concentrations of biomarkers in the Nutrition and Health of Aging Population in China study. Analyses were adjusted for age, sex, region and residence.



Supplementary Figure 2. The correlations between baseline metabolites and covariates in the Nutrition and Health of Aging Population in China study.
Analyses were adjusted for age, sex, region and residence.



Supplemental material is neither peer-reviewed nor thoroughly edited by CJASN. The authors alone are responsible for the accuracy and presentation of the material.

Supplementary Figure 3. Scree plot showing percentage of variance explained by each component in the Nutrition and Health of Aging Population in China study.
This scree plot indicates that six of those factors explain most of the variability because the line starts to straighten after factor 6.