SUPPLEMENTARY INFORMATION

Human Metabolic Phenotypes Diversity and its Association with Diet and Blood Pressure

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Supplementary Information

INTERMAP exclusion criteria

Of 4,895 individuals initially surveyed, 215 were excluded as follows: 110 persons who did not attend all 4 study visits, 7 individuals for whom the diet data were considered unreliable, 37 persons for whom the calorie intake from any 24-hour dietary recall was less than 500 kcal/d (2,100 kJ/d) or was greater than 5,000 kcal/d (21,000 kJ/d) for women or greater than 8,000 kcal/d (33,600 kJ/d) for men, 37 individuals for whom 2 complete urine samples were unavailable, and 24 persons with other data that were incomplete, missing, or indicating a protocol violation. Therefore, 4,680 participants (2,359 men and 2,321 women) were included in INTERMAP. One urine specimen was lost and the ^{1}H NMR urinary spectra were unusable from one or both of the first and second urine specimens for 49 participants, leaving a total of 4,630 available for the metabonomic study (Supplementary Fig. 1a).

Sample Preparation for NMR spectroscopy

Urine samples were allowed to thaw at room temperature and centrifuged at 3000 rpm for 5 minutes to remove any debris. An aliquot of urine (500 µL) was mixed with phosphate buffer solution (250 µL of 0.2 M Na_2HPO_4/0.2M NaH_2PO_4, pH 7.4 ± 0.5) in an Eppendorf to minimise variations in chemical shift values in the acquired ^{1}H NMR spectra due to pH differences. The resulting solution was left to stand for 10 min. The buffered urine samples were then centrifuged at 6-8000 rpm for 5 min to remove any particulates. Supernatant (750 µL) was removed to which sodium 3-trimethylsilyl-($^{2}H_4$)-1-propionate (TSP) in D_2O (75 µL) (final concentration 0.1 mg/mL) was added. D_2O

provided a deuterium lock signal and TSP the chemical shift reference (δ 0.0). Each sample (825 µL) was placed into a 96-well plate for analysis and the remaining sample refrozen. Samples were mixed and the 96-well plate was left to stand for 10 minutes before centrifuging at 4,000 rpm for 10 minutes thus removing any precipitate from the solution.

^{1}H NMR spectroscopic analysis of urine samples and spectral quantification of selected metabolites

Conventional ^{1}H NMR spectra of the urine samples were acquired using a Bruker (Bruker Biospin, Rheinstetten, Germany) Avance 600 spectrometer operating at 600.29 MHz in flow-injection mode. Samples were automatically delivered to the spectrometer using a Gilson 215 robot incorporated into the BEST (Bruker Efficient Sample Transfer) system. The 1D ^{1}H NMR spectra of urine were acquired using a standard one-dimensional pulse sequence with water pre-saturation (NOSEYPR1D, recycle delay-90°-t_1-90°-t_m-90°-acquisition; XWIN-NMR 3.5) during both the recycle delay (2 s) and mixing time (t_m, 150 ms). The 90° pulse length was adjusted to approximately10 µs and t_1 was set to 3 µs, providing an acquisition time of 2.73 s and a total pulse repetition time of 4.88 s. For each sample, 64 free induction decays (FIDs) were collected into 32K data points using a spectral width of 20 ppm. The FIDs were multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz and data were zero-filled to 64K data points prior to Fourier Transformation (FT). Four metabolites (alanine, δ 1.48; formate, δ 8.45; hippurate, δ 7.87 and NMNA, δ 4.44) were quantified via integration of signals relative to the creatinine CH_2 signal (δ 4.06) using externally standardised values for creatinine measured using the Jaffé reaction[1]. Mean

T_1 values were measured for each analyte and appropriate relaxation saturation factors calculated and applied across the whole data set. Integrations were also corrected for the minor suppression of the creatinine CH_2 signal resulting from keto-enol mediated partial deuteration (measured empirically from standard creatinine measurements in D_20/H_20 solutions).

Technical errors and reliability estimates of quantified metabolites

Technical errors for the four quantified metabolites, alanine, formate, hippurate and *N*-methylnicotinate, estimated from 8% split specimens, ranged from 11-12% for *N*-methylnicotinate to 24-27% for hippurate (Supplementary Table 4). Reliability for mean of the two 24 h urinary excretion values, estimated as percentage of the theoretical univariate regression coefficient (**Methods**), was 78.0% for alanine, 70.2% formate, 67.3% hippurate, 72.0% *N*-methylnicotinate. Correlation analysis relating NMR and ion exchange chromatography data for alanine, and NMR and gas chromatography mass spectrometry data for hippurate, gave Spearmen-r_s correlation coefficients of 0.93 and 0.91 respectively (**Methods**).

Descriptive characteristics of East Asian and western countries

East Asian countries are characterized by low Keys dietary lipid score[2, 3], high dietary salt, low dietary K^+ and high dietary Na^+/K^+ ratio (China particularly); high prevalence of smoking (for men), low average serum total, non-HDL and LDL cholesterol, high upward slope of BP with age, high prevalence rates of adverse BP levels in middle and older age (despite normal average body mass index)[4]; low CHD and high stroke death rates

(markedly reduced in Japan in recent decades)[5]. In contrast, the UK and USA (like other western countries) have high Keys dietary lipid score, high intakes of total, saturated and trans fats and cholesterol; high average body mass index, high serum total, non-HDL and LDL cholesterol; high CHD mortality rates (persisting in the recent data despite substantial declines) with relatively low stroke death rates[5].

Data on serum cholesterol were obtained from the MONICA[6] and INTERLIPID/INTERMAP studies[7] (Supplementary Table 5). Data on mortality are published in Zhou *et al.*[6] and Rosamond *et al.*[8] (Supplementary Table 6). We used the extensive INTERMAP database to provide data on dietary intakes, 24 h urinary sodium and potassium excretion, and body mass index for China, Japan, UK and USA (Supplementary Figs. 8 and 9).

Definitions of multivariate model statistics R^2 and Q^2

R^2 is defined as the proportion of variance in the data explained by the model. Q^2 is defined as the proportion of variance in the data predictable by the model under cross validation. These quantities are derived in more detail below.

Each O-PLS-DA model regresses a set of X data (NMR spectra) against a response variable Y (dummy class matrix). For each model, the Residual Error Sum of Squares in the X space can be calculated as

$$RESS_X = \sum_i \left\| \hat{\mathbf{x}}_i - \mathbf{x}_i \right\|^2$$

where $\hat{\mathbf{x}}_i$ denotes the spectrum predicted by the model for sample *i*. The proportion of explained variance in the X space, R^2, is then calculated as

$$R_X^2 = 1 - \frac{RESS_X}{SS_X}$$

where SS_X is the total sum of squares of the centred X data, $SS_X = \sum_i \|\mathbf{x}_i - \bar{\mathbf{x}}\|^2$. An analogous quantity R_Y^2 can be calculated for the response Y

$$RESS_Y = \sum_i \|\hat{y}_i - y_i\|^2, \quad R_Y^2 = 1 - \frac{RESS_Y}{SS_Y}.$$

When cross-validation is used to estimate the predictive quality of the model, each spectrum or response may be predicted from the model built when it is left out, denoted by $\tilde{\mathbf{x}}$ and $\tilde{y}$. Similar quantities as above may be calculated to estimate proportion of variance predictable in cross-validation, Q^2, based on the Predicted Residual Error Sum of Squares (*PRESS*)

$$PRESS_X = \sum_i \|\tilde{\mathbf{x}}_i - \mathbf{x}_i\|^2, \quad Q_X^2 = 1 - \frac{PRESS_X}{SS_X},$$

and

$$PRESS_Y = \sum_i \|\tilde{y}_i - y_i\|^2, \quad Q_Y^2 = 1 - \frac{PRESS_Y}{SS_Y}.$$

Q^2 is therefore a measure of the robustness of a model to sub-sampling of the data and was used to choose the number of components for each model. For further information see Eriksson *et al.*[8]

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Supplementary Tables and Figures

Supplementary Table 1 Discriminatory metabolites† from population pairwise comparisons, first and second urine specimens

a regression coefficients (r^2) for each pairwise OPLS-DA comparison

	OPLS-DA of pairwise population comparisons											
	Ch/Jp		Ch/UK		Ch/US		Jp/UK		Jp/US		Jp/Jp-A	
Specimen	1	2	1	2	1	2	1	2	1	2	1	2
Metabolites												
2-aminoisobutyric acid			**0.28**	**0.26**	**0.26**	**0.23**	**0.17**	**0.19**				
2-hydroxyibuprofen									*0.09*	*0.10*		
2-oxoglutarate			**0.30**	**0.34**	**0.28**	**0.32**						
3-hydroxyisovalerate			**0.22**	**0.30**								
Acetylcarnitine	*0.40*	*0.41*	*0.36*	*0.38*	*0.15*	*0.15*						
Alanine			**0.27**	**0.32**	**0.21**	**0.28**	**0.17**	**0.14**				
Citrate	*0.18*	*0.20*	*0.38*	*0.32*	*0.17*	*0.16*			*0.09*	*0.09*		
Creatine	*0.33*	*0.37*			*0.14*	*0.17*	**0.18**	**0.13**				
Ethanol							**0.15**	**0.13**	**0.20**	**0.16**	**0.17**	**0.13**
Ethylglucoside							**0.14**	**0.14**	**0.15**	**0.13**		
Formate			**0.17**	**0.21**	**0.18**	**0.26**			**0.16**	**0.17**	**0.12**	**0.13**
Guanidinoacetate	*0.17*	*0.27*					**0.29**	**0.27**	**0.25**	**0.22**	**0.10**	**0.09**
Hippurate			*0.32*	*0.32*	*0.12*	*0.16*	*0.30*	*0.29*	*0.14*	*0.15*		
Lysine	*0.27*	*0.23*							**0.19**	**0.19**		
N-acetyl-glycoproteins	**0.26**	**0.30**	**0.31**	**0.28**	**0.30**	**0.35**	**0.21**	**0.17**	**0.14**	**0.12**	**0.12**	**0.19**
N-methyl-2-pyridone-5 carboxamide			*0.29*	*0.25*							*0.20*	*0.18*
N-methylnicotinate	*0.19*	*0.24*			*0.14*	*0.15*						
NNN-trimethylysine							**0.18**	**0.16**	**0.18**	**0.15**		
Phenylacetyglutamine			*0.31*	*0.26*			*0.18*	*0.16*				
Protein envelope (unresolved)‡	*0.33*	*0.35*			**0.21**	**0.23**	**0.23**	**0.21**	**0.32**	**0.24**	**0.17**	**0.17**
Sarcosine	**0.22**	**0.35**	**0.24**	**0.32**	**0.31**	**0.40**						
Suberate	**0.43**	**0.50**	**0.29**	**0.42**	**0.38**	**0.39**						
Succinate			*0.29*	*0.30*	*0.12*	*0.11*	*0.21*	*0.17*				
Taurine	*0.20*	*0.17*					***0.19***	***0.15***	**0.22**	**0.14**		
Trimethylamine-*N*-oxide	*0.29*	*0.31*					**0.22**	**0.18**	**0.21**	**0.22**	**0.09**	**0.11**
Unknown 1 (δ2.80,s)	**0.23**	**0.32**	**0.24**	**0.30**	**0.24**	**0.27**						
Unknown 2 (δ3.36,s)			*0.30*	*0.29*								
Unknown 3 (δ1.27)							*0.26*	*0.25*				
Unknown 4 (δ7.96, m)			*0.27*	*0.30*	*0.14*	*0.15*						
Unknown 5 (δ2.78,s)	**0.24**	**0.34**	**0.33**	**0.28**	**0.31**	**0.35**						
Q^2Y (%)	83.5	86.1	84.5	85.8	82.8	82.1	77.2	77.9	77.5	79.4	68.4	75.3
Number of orthogonal components	3	3	2	2	4	3	3	4	4	4	3	4

† see **Methods**

Abbreviations: Ch, China; Jp, Japan; UK, United Kingdom; US, United States of America; Jp-A, Japanese Americans; δ, proton chemical shift; s, singlet

Values in bold denote metabolites higher in concentration in the first population of the pairwise OPLS-DA analyses.

Values in italics denote metabolites higher in concentration in the second population of the pairwise OPLS-DA analyses.

‡ protein envelope is overlapped with superimposed signals from isoleucine, leucine, valine

b ^{1}H NMR spectral parameters (chemical shift and peak appearance) for discriminatory metabolites from pairwise population sample comparisons

	NMR Spectroscopic Data	
Urinary Metabolites	**Moieties**	**Signal directly observed, δ ¥**
2-aminoisobutyric acid	CH; CH_2	1.20 (d); **2.61(m); 3.11(m)**
2-hydroxyibuprofen	CH_3	**1.12(s);** 1.52(d); 2.78(s); 3.56(t); 3.83(q); 3.98(q); 7.22-7.37(m)
2-oxoglutarate	CH_2	**2.45(t),** 3.00(t)
3-hydroxyisovalerate	CH_3	**1.27(s);** 2.37(s)
Acetylcarnitine	CH_3	2.17 (s); 2.52(dd); 2.65 (dd); **3.21(s);** 3.61 (d); 3.85(dd)
Alanine	CH_3	**1.48 (d)**; 3.79 (qt)
Citrate	CH_2	**2.54(d); 2.66(d);**
Creatine	CH_2	3.04(s); **3.94(s)**
Ethanol	CH_2; CH_3	**1.2(t); 3.65(q)**
Ethylglucoside	CH_3	**1.24(t)**;3.41(dd);3.55(dd);3.69(m);3.71(dd);3.77(dd);3.80(q);3.86(dd)
Formate	CH	**8.46(s)**
Guanidinoacetate	CH_2	**3.80(s)**
Hippurate	CH_2; CH; CH; CH	**3.97 (d); 7.55 (t); 7.64 (t); 7.84(d)**
Lysine	CH_2;CH_2	1.47(m); 1.72 (m); **1.89 (m); 3.01 (t)**; 3.77(t)
N-acetyl-glycoproteins	CH_3	**1.98 - 2.06**
N-methyl-2-pyridone-5 carboxamide	CH; CH	**8.34(m)**
N-methylnicotinate	CH_3;CH; CH;CH	**4.44(s); 8.08(t); 8.84(t); 9.13(s)**
NNN-trimethylysine	N-(CH_3)	**3.12(s)**; 3.35(m); 3.76(m)
Phenylacetyglutamine	CH; CH	**1.98 (m)**; 2.13 (m); 4.20(m); **7.37(t); 7.43(t)**
Protein envelope (unresolved)‡	-	**0.90 - 0.98**
Sarcosine	CH_3	**2.75 (s)**
Suberate	CH_2	**1.31(m); 1.55(m); 2.18(t)**
Succinate	CH_3	**2.41(s)**
Taurine	CH_2	3.26 (t); **3.43(t)**
Trimethylamine-*N*-oxide	N-(CH_3)$_3$	**3.27 (s)**
Unknown 1	-	**2.80 (s)**
Unknown 2	-	**3.36(s)**
Unknown 3	-	**1.27**
Unknown 4	-	**7.96 (m)**
Unknown 5	CH_3	**2.78 (s)**

¥ chemical shifts in bold indicated signals found to be significantly different in the OPLS-DA pairwise comparisons (Suppl Fig. 1A) and those not in bold were directly observed in 1D ^{1}H-NMR and their connectivities confirmed by statistical total correlation spectroscopy (STOCSY)

‡ protein envelope is overlapped with superimposed signals from isoleucine, leucine, valine

Supplementary Table 2 Partial Pearson-r correlation coefficients for quantified urinary metabolites (mmol/24 h)* with dietary and urinary variables, and body mass index, adjusted for age, gender, and sample

Variable	Metabolite			
	Alanine (n= 4,232)	Formate (n= 4,147)	Hippurate (n= 4,184)	*N*-methylnicotinate (n= 4,081)
Energy (kcal/24 hr)	0.17	0.14	0.03	0.05
Total protein (%kcal)	0.07	0.06	0.05	0.02
Animal protein (%kcal)	0.07	0.03	0.00	-0.02
Vegetable protein (%kcal)	-0.01	0.03	0.07	0.06
Total fat (%kcal)	0.09	0.03	-0.03	0.04
Monounsaturated fatty acids (%kcal)	0.08	0.02	-0.04	0.02
Polyunsaturated fatty acids (%kcal)	0.06	0.01	-0.02	-0.01
Saturated fatty acids (%kcal)	0.06	0.04	-0.02	0.07
Total available carbohydrate (%kcal)	-0.02	-0.01	0.07	-0.06
Starch (%kcal)	0.03	0.01	0.01	-0.01
Estimated total sugars (%kcal)	-0.06	-0.02	0.08	-0.06
Trans fatty acids (%kcal)	0.04	0.00	-0.04	0.00
Omega 3 fatty acids (%kcal)	0.04	0.00	0.00	-0.03
Omega 6 fatty acids (%kcal)	0.06	0.01	-0.02	0.00
Alcohol (%kcal)	-0.11	-0.06	-0.10	0.02
Cholesterol (mg/1,000kcal)	0.11	0.04	-0.03	0.00
Fibre (g/1,000kcal)	-0.05	0.05	0.17	0.08
Calcium (mg/1,000kcal)	-0.01	0.05	0.09	0.05
Magnesium (mg/1,000kcal)	-0.05	0.09	0.19	0.18
Phosphorus (mg/1,000kcal)	0.03	0.07	0.11	0.04
Urinary sodium (mmol/24 hr)	0.39	0.37	0.11	0.10
Urinary potassium (mmol/24 hr)	0.21	0.32	0.40	0.29
Urinary sodium to potassium ratio	0.13	0.05	-0.22	-0.13
Body mass index (kg/m^2)	0.28	0.03	-0.05	-0.04
Keys dietary lipid score†	0.08	0.04	-0.03	0.05

* Dietary data are from mean of four 24 h recalls. Urinary data are from mean of two 24 h urine collections. Body mass index is calculated from mean of two measurements of height and weight.
†Keys score: $1.35\times(2\times\mathrm{SFA}-\mathrm{PFA})+1.5\sqrt{\mathrm{Chol}}$ where SFA = saturated fatty acids (%kcal), PFA = polyunsaturated fatty acids (%kcal) and chol = cholesterol (mg/1,000kcal). $|r|>0.06$, $P<0.001$

Supplementary Table 3 Estimated mean differences* in systolic and diastolic BP (Z-scores‡), non-intervened persons§

	A				B			
	Not adjusted for BMI†		Adjusted for BMI†		Not adjusted for BMI†		Adjusted for BMI†	
Systolic BP								
Alanine (n= 2,018)	2.83	(4.27)	0.62	(0.94)	3.48	(4.84)	1.86	(2.63)
Formate (n= 1,976)	-1.22	(-1.97)	-1.39	(-2.34)	-1.78	(-2.59)	-0.83	(-1.25)
Hippurate (n= 1,936)	-2.09	#(-3.17)	-1.81	#(-2.85)	-1.21	#(-1.68)	-0.48	#(-0.70)
N-methylnicotinate (n= 1,876)	0.68	(1.08)	0.83	(1.37)	1.01	(1.54)	1.44	(2.28)
Diastolic BP								
Alanine (n= 2,018)	1.97	(4.30)	0.40	(0.87)	2.18	(4.35)	1.00	(2.03)
Formate (n= 1,976)	-0.89	(-2.10)	-0.98	(-2.44)	-1.50	(-3.19)	-0.85	(-1.88)
Hippurate (n= 1,936)	-1.01	(-2.23)	-0.84	(-1.91)	-0.76	(-1.53)	-0.29	(-0.61)
N-methylnicotinate (n= 1,876)	0.21	(0.47)	0.32	(0.73)	0.29	(0.61)	0.60	(1.30)

*Systolic and diastolic BP differences (mm Hg) per +2 standard deviation difference in each of four quantified urinary metabolites (mean of two 24 h urine values). Two standard deviation difference – based on total population with valid quantified urinary metabolite data (see Table 1 and Suppl. Fig. 1b) – for alanine = 0.34 mmol/24 h; formate = 0.29 mmol/24 h; hippurate = 3.55 mmol/24 h; *N*-methylnicotinate = 0.41 mmol/24 h. (Chemical shifts used for quantification: alanine, δ1.48; formate, δ8.45; hippurate, δ7.85 and *N*-methylnicotinate, δ4.44.) Regression coefficients for individuals are pooled across countries (**Methods**).

A: adjusted for age, sex, sample, physical activity (h/24 h moderate or heavy activity), family history of high blood pressure

B: A + 7-day alcohol (g/24 h) + urinary Na^+ (mmol/24 h) + urinary K^+ excretion (mmol/24 h)

‡ Z score: regression coefficient divided by standard error. Z-score ≥1.96, P<0.05; ≥2.58, P<0.01; ≥3.29, P<0.001; ≥3.89, P<0.0001.

§ Non-intervened: people without special diet/nutritional supplements or diagnosis/treatment for cardiovascular disease or diabetes

P for cross-country heterogeneity <0.05. Country-specific BP differences were inverse in Japan, China, and UK (systolic BP differences per +2 standard deviation difference in hippurate -2.16 to -4.79 mm Hg, Z-scores -1.36 to -3.15), direct in the USA (systolic BP differences +0.26 to +1.58 mm Hg, Z-scores +0.27 to +1.58).

† Body mass index, kg/m^2

Supplementary Table 4 Percent technical error* from split urinary specimens for alanine, formate, hippurate and *N*-methylnicotinate (mmol/24 h) quantified by ^{1}H NMR

Metabolite	First specimen		Second specimen	
	n†	Percent technical error*	n†	Percent technical error*
Alanine	308	17.3%	296	15.5%
Formate	293	11.9%	282	24.8%
Hippurate	292	24.0%	287	26.7%
N-methylnicotinate	289	11.7%	273	10.7%

* Percent technical error: 100 times technical error divided by mean value of split samples; technical error = $\Sigma d^2/2n^{\dagger}$, where d is the difference between a pair of measurements.

† number of split pairs

Supplementary Table 5 Serum total and LDL cholesterol, early 1990s (MONICA)* and late 1990s (INTERLIPID/INTERMAP)†, men and women, by country

Risk factor	China	Japan	UK		USA	
			Belfast	Glasgow	Stanford	Honolulu
Men						
Total cholesterol (mg/dl)						
MONICA*	174	–	228	236	209	–
INTERLIPID/INTERMAP†	–	198.6	–	–	–	211.6
LDL-cholesterol (mg/dl)						
INTERLIPID/INTERMAP†	–	120.4	–	–	–	137.9
Women						
Total cholesterol (mg/dl)						
MONICA*	174	–	228	236	205	–
INTERLIPID/INTERMAP†	–	202.1	–	–	–	210.2
LDL-cholesterol (mg/dl)						
INTERLIPID/INTERMAP†	–	123.7	–	–	–	136.1

* Zhou *et al*.[6]

† Okuda *et al*.[7]

MONICA: ages 35-64; INTERLIPID/INTERMAP: ages 40-59

Supplementary Table 6 Age-standardised* coronary heart disease and stroke mortality rates, 1970† and 1999/2004‡, men and women, ages 35-74 years by country

Cause of death by gender, year	China		Japan	UK		USA
	Rural	Urban		England & Wales	Scotland	
Men						
Coronary heart disease						
1970†	–	–	94	509	634	652
1999/2004‡	64	106	53	196	247	174
Stroke						
1970†	–	–	385	141	180	120
1999/2004‡	243	217	66	49	61	35
Women						
Coronary heart disease						
1970†	–	–	47	164	240	252
1999/2004‡	41	71	16	68	98	73
Stroke						
1970†	–	–	225	113	158	90
1999/2004‡	152	147	31	36	48	27

* Rate per 100,000 population adjusted to the standard European population

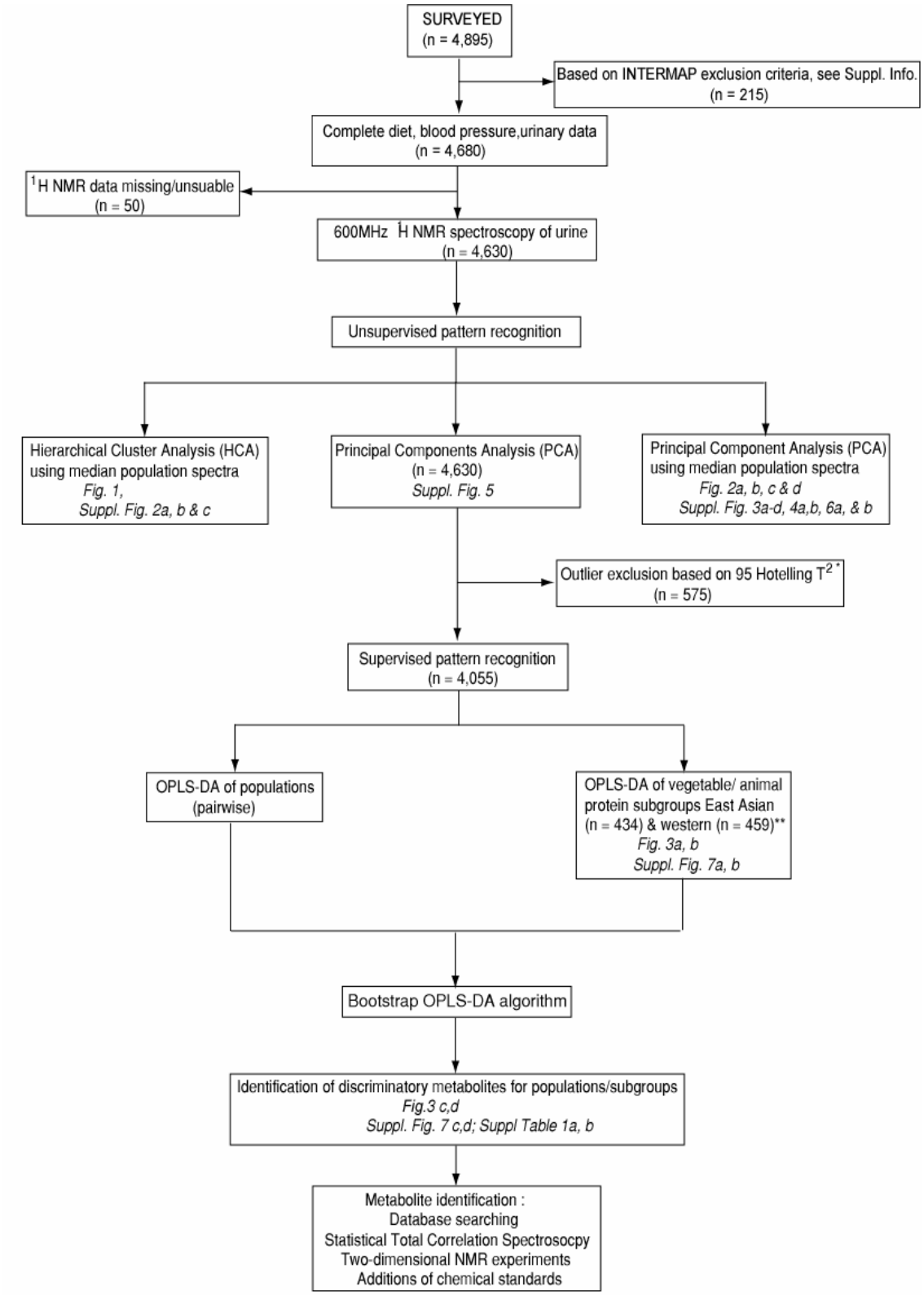
† Zhou *et al.*[6]

‡ China 1999; Japan 2003; UK 2002; USA 2004 (Rosamond *et al.*[8])

Mortality data not available for China in 1970

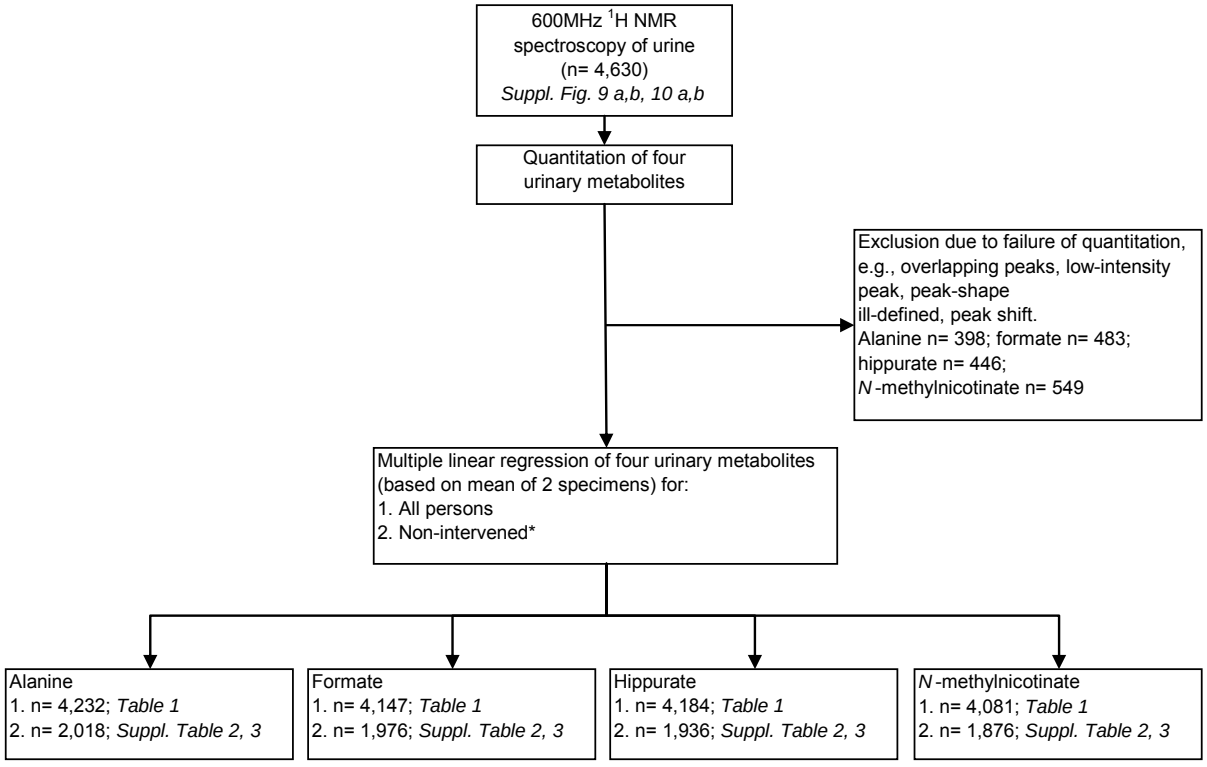
Supplementary Figure 1 Schematic of data analyses

a Participant numbers, exclusions, pattern recognition techniques and identification of discriminatory metabolites



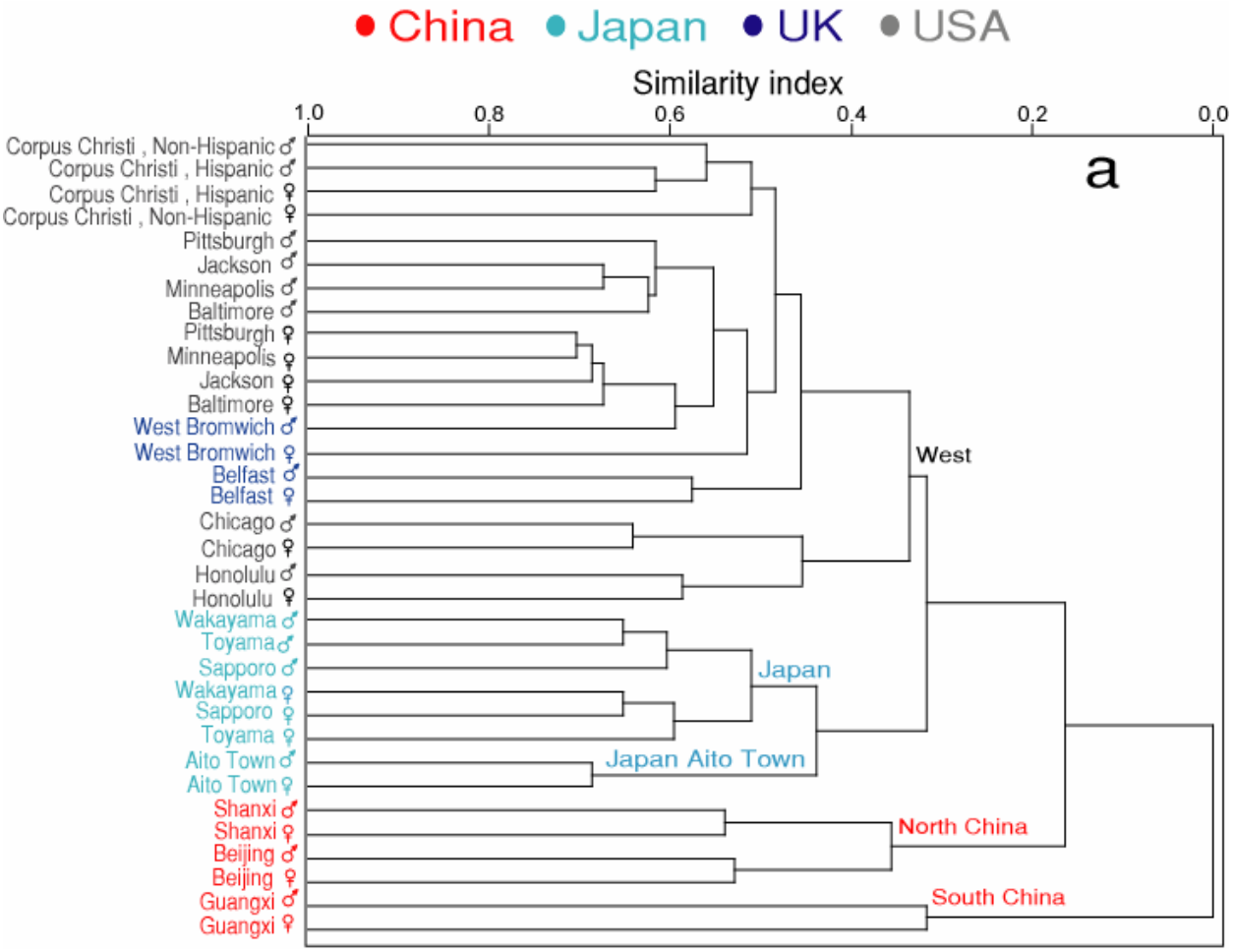
Key: * Spectra data normalised to total area, excluded 575 urine samples containing high glucose, trimethylamine N-oxide, paracetamol and alcohol. All other models normalised to probabilistic quotient method
OPLS-DA = orthogonal partial least square -discriminant analysis
** Sub-group analysis for high vegetable/low animal protein *vs* low vegetable/high animal protein for East Asian and Western populations

b Numbers included for multiple regression of four quantified metabolites

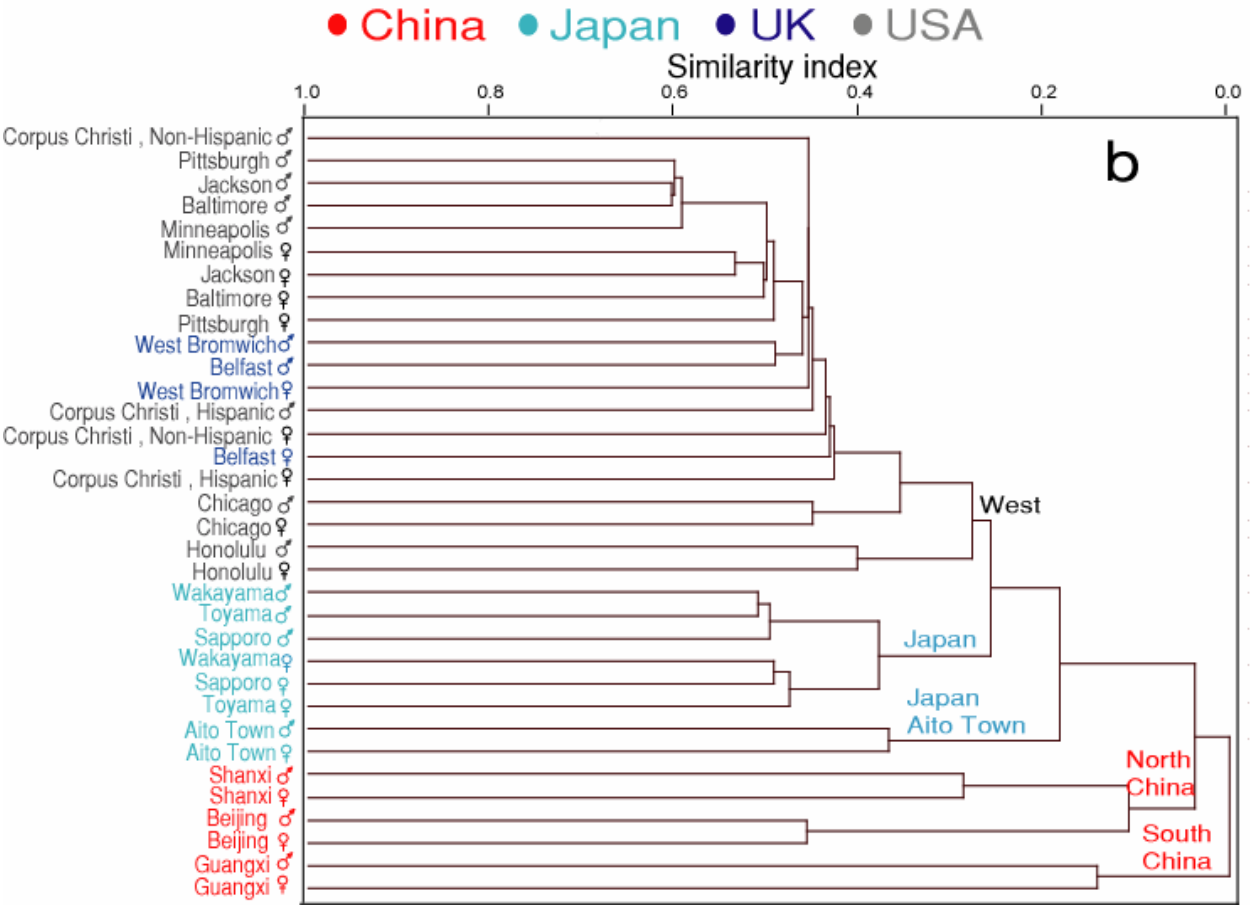


*Non-intervened: people without special diet/nutritional supplements or diagnosis/treatment for cardiovascular disease or diabetes

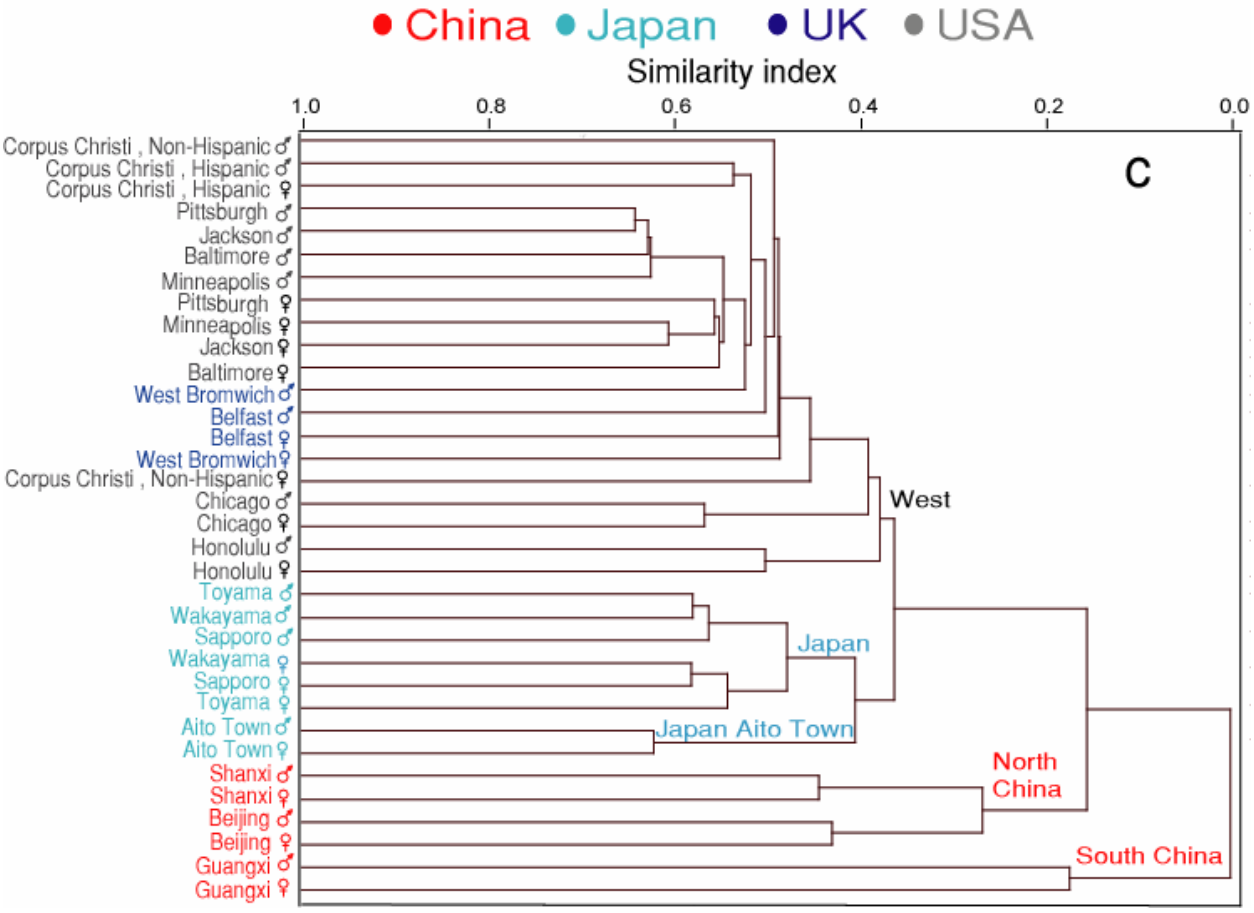
Supplementary Figure 2 Hierarchical cluster analysis (HCA) based on median ^{1}H NMR urine spectra by population sample and gender (n= 4,630)



a group average linkage method, second urine specimens



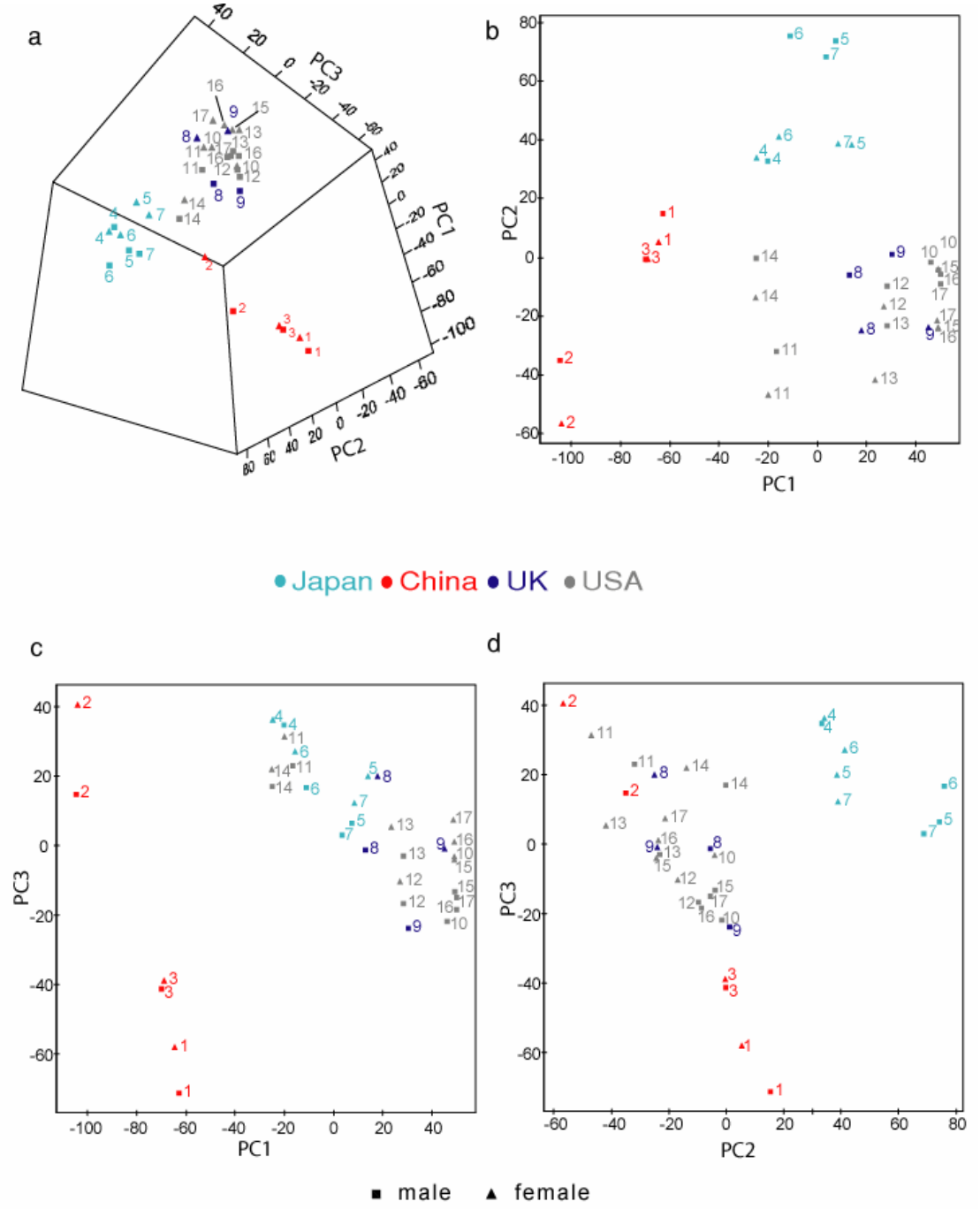
b single linkage method, first urine specimens



c single linkage method, second urine specimens

The similarity index measures the multivariate distance between clusters. A similarity of one indicates zero distance between clusters; a value of zero indicates the maximum intercluster separation seen in the data.

Supplementary Figure 3 Cross validated Principal Components Analysis (PCA) scores plots based on a 7 component model (n= 4,630)

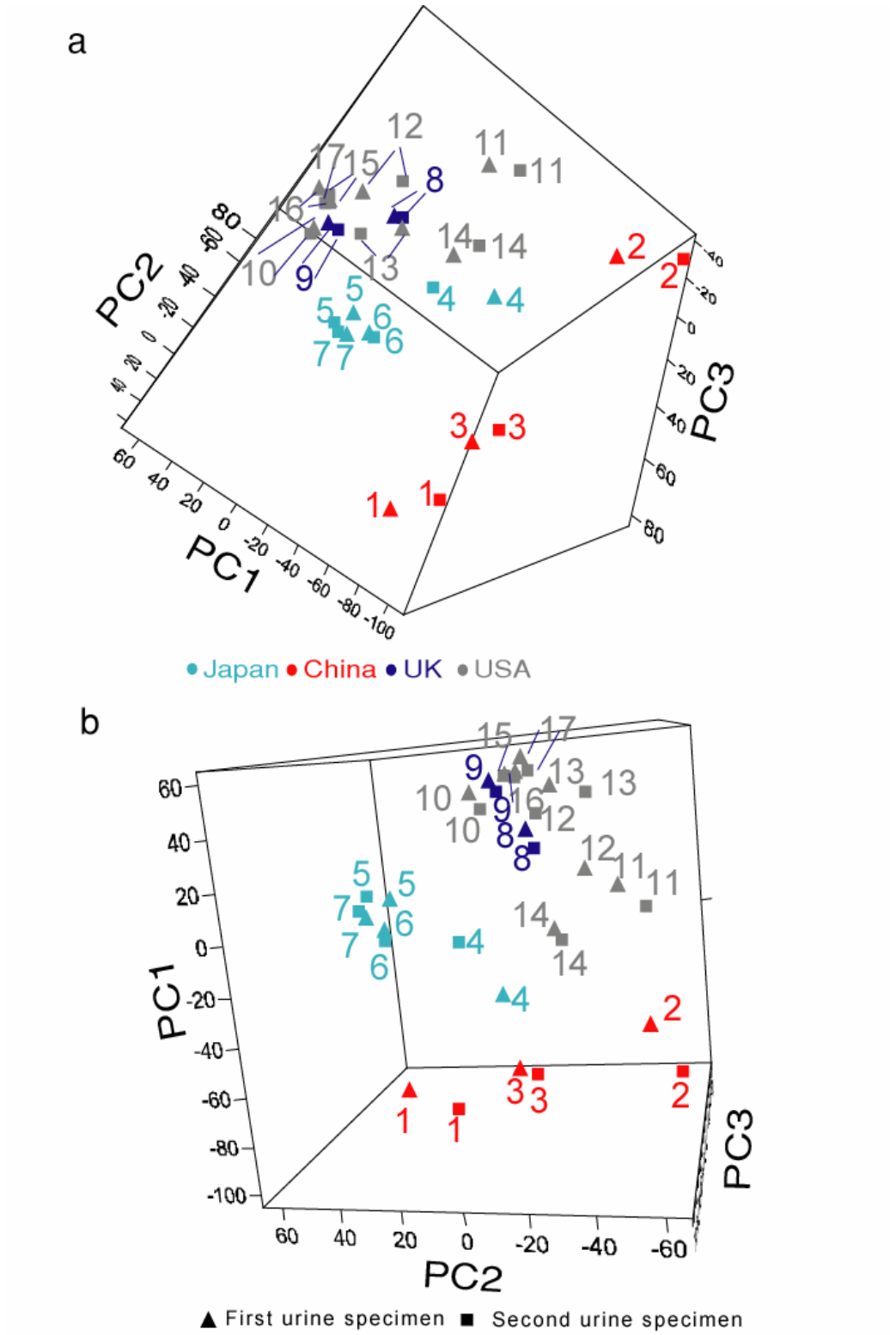


Key: 1, Beijing; 2, Guangxi; 3, Shanxi; 4, Aito Town; 5, Sapporo; 6, Toyama; 7, Wakayama; 8, Belfast; 9, West Bromwich; 10, Baltimore; 11, Chicago; 12, Corpus Christi Hispanic; 13, Corpus Christi non-Hispanic; 14, Honolulu; 15, Jackson; 16, Minneapolis; 17, Pittsburgh

a 3-dimensional plot for Principal Components (PC) 1-3; **b** PC2 *vs* PC1; **c** PC3 *vs* PC1; **d** PC3 *vs* PC2

Median ^{1}H NMR spectra of the second 24 h urine specimen stratified by country and by gender; female (▲) and male (■), R^2_X= 74.9% (percent variation in the NMR data explained by the model); Q^2_X= 52.1% (percent variation in the NMR data predictable by the model from cross validation). Data for the first three components available in an Excel file in **Supplementary Information**.

Supplementary Figure 4 Cross validated Principal Components Analysis (PCA) scores plots based on median ^{1}H NMR urine spectra stratified by country

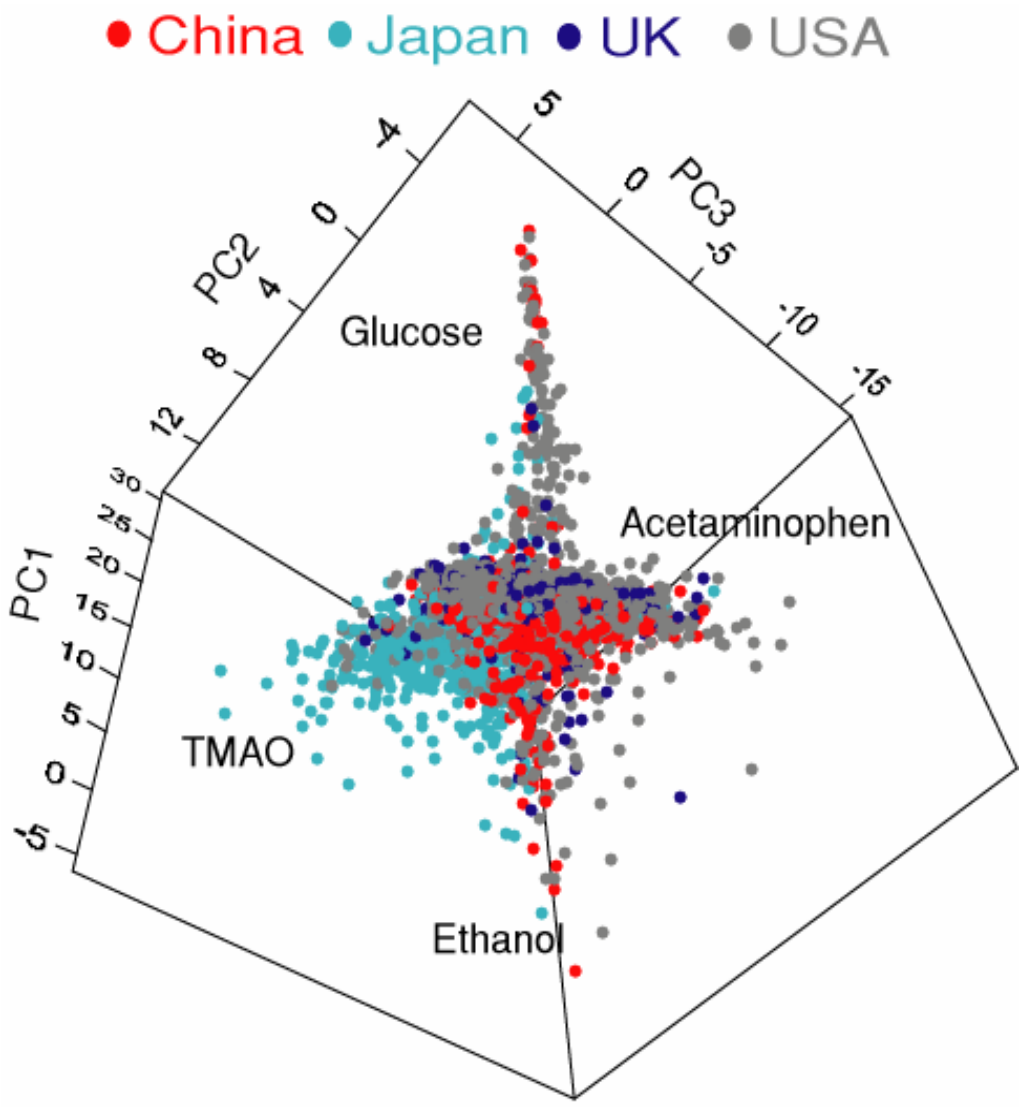


Key: 1, Beijing; 2, Guangxi; 3, Shanxi; 4, Aito Town; 5, Sapporo; 6, Toyama; 7, Wakayama; 8, Belfast; 9, West Bromwich; 10, Baltimore; 11, Chicago; 12, Corpus Christi Hispanic; 13, Corpus Christi non-Hispanic; 14, Honolulu; 15, Jackson; 16, Minneapolis; 17, Pittsburgh

a first (▲) and second (■)24 h urine specimens (n= 4,630). R^2_X= 82.4% (percent variation in the NMR data explained by the model); Q^2_X= 63.1% (percent variation in the NMR data predictable by the model from cross validation), based on an 8 component model. **b** first (▲) and second (■) 24 h urine specimens (R^2_X= 70.2%, Q^2_X= 48.8%, based on a 6 component model), for normal weight†, non-diabetic participants (n= 2,063).

† Body mass index 18.50-24.99 kg/m^2

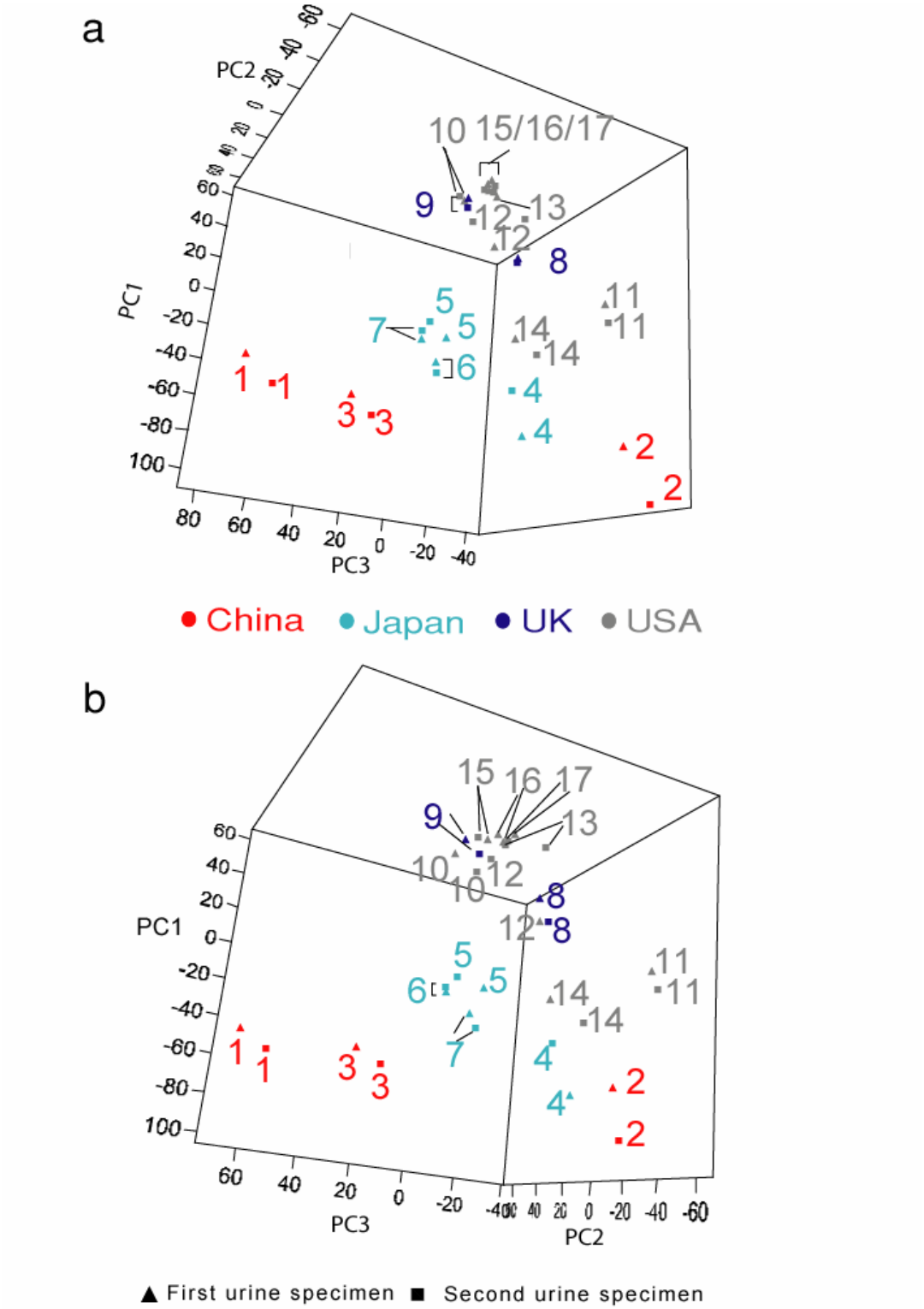
Supplementary Figure 5 PCA scores plot for individuals, first three principal components derived from the ^{1}H NMR spectra of first urine specimens (n= 4,630)



Key: PC, principal component; TMAO, trimethylamine *N*-oxide

Co-ordinates are coloured according to country of residence of participants. The plot reveals outlying groups due to high levels of urinary glucose, trimethylamine-*N*-oxide, ethanol, acetaminophen and their metabolites. R^2_X=80.9% (percent variation in the NMR data explained by the model); Q^2_X=76.5 % (percent variation in the NMR data predictable by the model from cross validation), based on a 7 component model.

Supplementary Figure 6 Cross validated Principal Components Analysis (PCA) scores plots based on median ^{1}H NMR urine spectra stratified by country after the removal of outliers using 95% Hotellings T^2 statistic

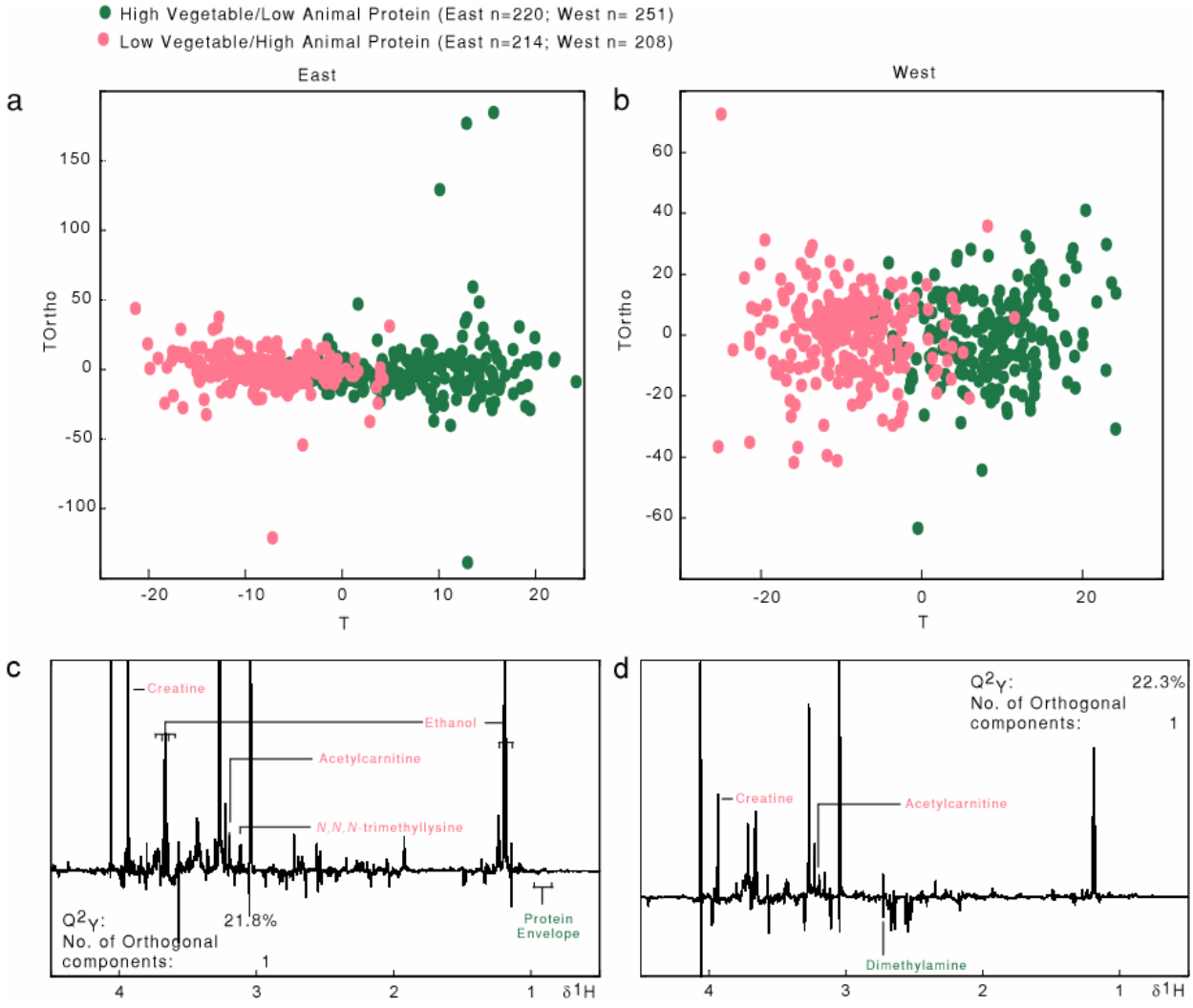


Key: 1, Beijing; 2, Guangxi; 3, Shanxi; 4, Aito Town; 5, Sapporo; 6, Toyama; 7, Wakayama; 8, Belfast; 9, West Bromwich; 10, Baltimore; 11, Chicago; 12, Corpus Christi Hispanic; 13, Corpus Christi non-Hispanic; 14, Honolulu; 15, Jackson; 16, Minneapolis; 17, Pittsburgh

a first (▲) and second (■)24 h urine specimens (n= 4,055). R^2_X=86.1% (percent variation in the NMR data explained by the model); Q^2_X=64.3 (percent variation in the NMR data predictable by the model from cross validation), based on a 10 component model). **b** first (▲) and second (■) 24 h urine specimens for normal weight†, non-diabetic participants (n= 1,810, R^2X= 69.4%, Q^2X= 46.7%, based on a 6 component model).

† Body mass index 18.50-24.99 kg/m^2

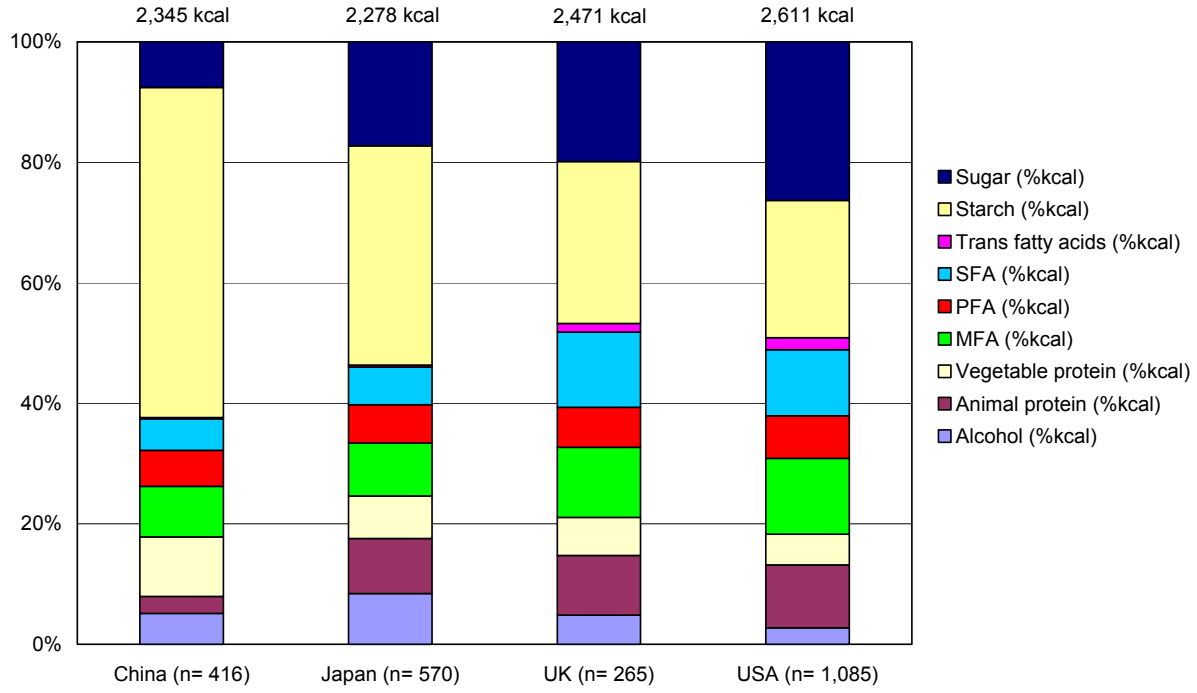
Supplementary Figure 7 O-PLS-DA scores and loadings plots (bootstrap analyses) for participants reporting high vegetable/low animal protein and low vegetable/high animal protein intakes, second 24 h urinary specimens



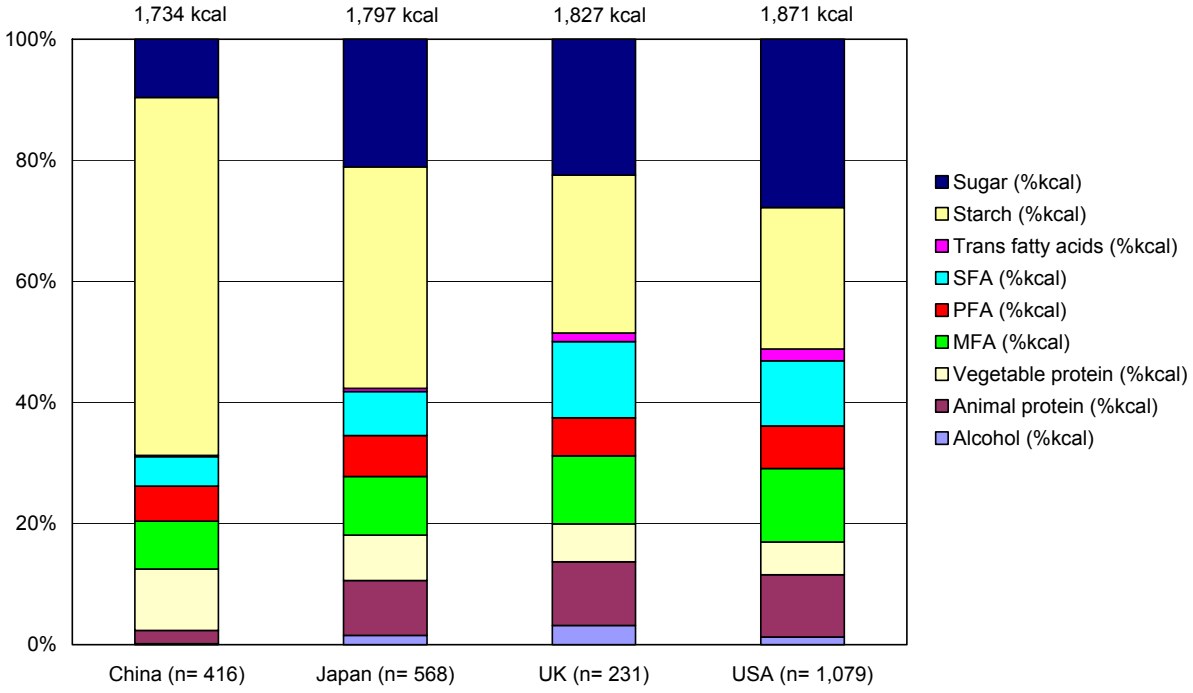
O-PLS-DA scores plots (1 orthogonal component) comparing high vegetable/low animal protein intake with low vegetable/high animal protein intake (top and bottom quartiles), adjusted for sample, age and sex, **a** eastern and **b** western population samples. Loadings plots from the O-PLS-DA bootstrap analyses are shown with discriminatory

metabolites labelled (see **Methods** for metabolite selection criteria) for **c** East Asian and **d** western participants. Analyses are after removal of metabolic outliers using the 95% Hotelling's T^2 statistic in the initial PCA. The plots show the number of participants, the number of components used in each model and the Q^2_Y values (percent variation in the protein subgroup assignment explained by the model, and predictable by the model from cross validation).

Supplementary Figure 8 Macronutrient intake (% kcal) by country (n= 4,630)

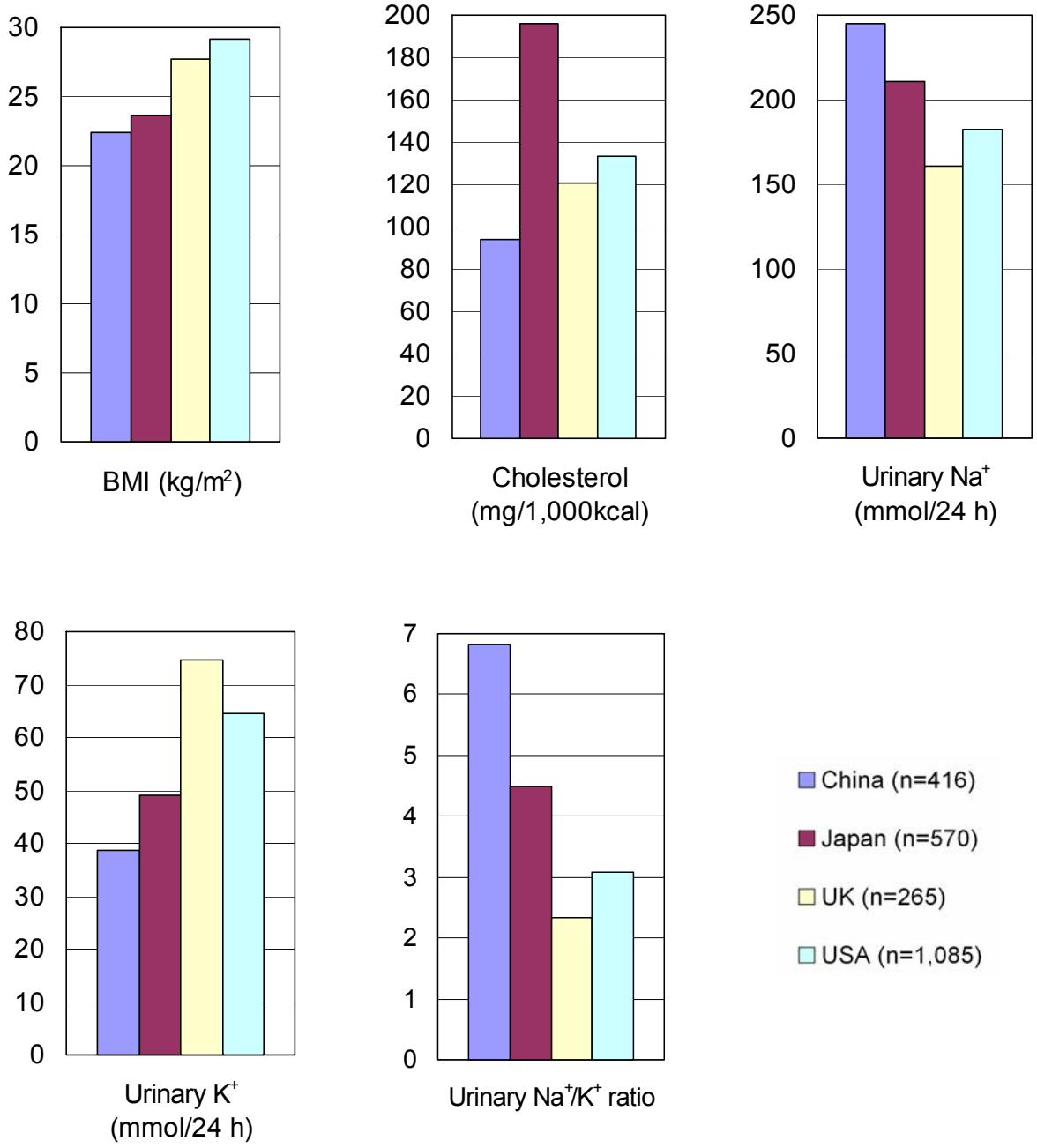


a men (n= 2,336)

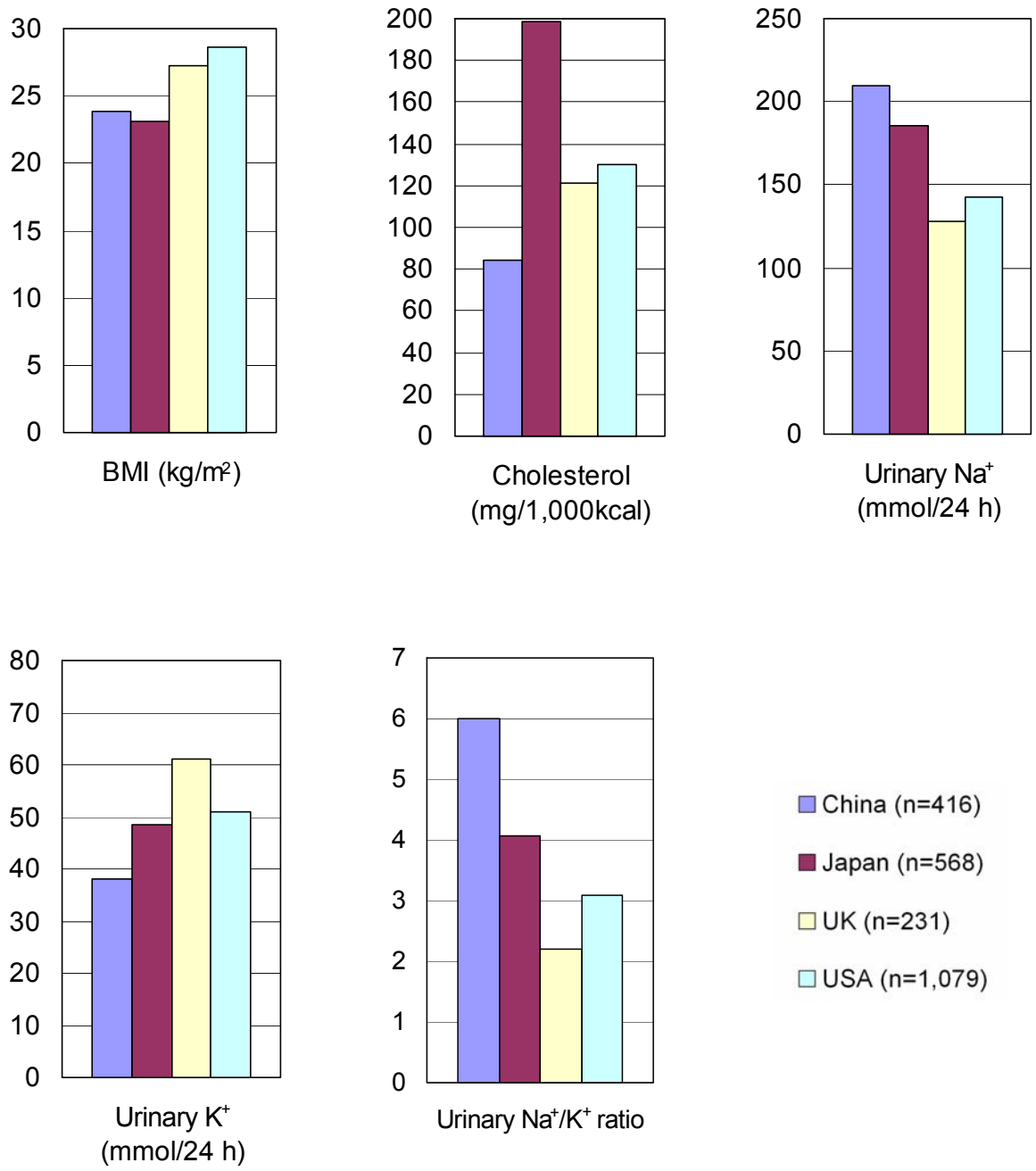


b women (n= 2,294)

Supplementary Figure 9 Mean body mass index (BMI, kg/m^2), dietary cholesterol (mg/1000kcal), urinary Na^+ excretion (mmol/24 h), urinary K^+ excretion (mmol/24 h) and urinary Na^+/K^+ ratio, by country (n= 4,630)



a men (n= 2,336)



b women (n= 2,294)