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Association between dietary acid–base load and cardiometabolic risk factors in young Japanese women

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Mild metabolic acidosis, which can be caused by diet, may adversely affect cardiometabolic risk factors, possibly by increasing cortisol production. Methodologies for estimating diet-induced acid–base load using dietary-intake information have been established. To our knowledge, however, the possible association between dietary acid–base load and cardiometabolic risk factors has not been investigated. We cross-sectionally examined associations between dietary acid–base load and cardiometabolic risk factors in a free-living population. The subjects were 1136 female Japanese dietetic students aged 18–22 years. Dietary acid–base load was characterized as the potential renal acid load (PRAL), which was determined using an algorithm including dietary protein, P, K, Ca and Mg, as well as the ratio of dietary protein to K (Pro:K). Estimates of each nutrient were obtained from a validated comprehensive self-administered diet history questionnaire. Body height and weight, waist circumference and blood pressure were measured. Fasting blood samples were collected. After adjustment for potential confounding factors, higher PRAL and Pro:K (more acidic dietary acid–base loads) were associated with higher systolic and diastolic blood pressure (*P* for trend=0·028 and 0·035 for PRAL and 0·012 and 0·009 for Pro:K, respectively). PRAL was also independently positively associated with total and LDL-cholesterol (*n* 1121; *P* for trend=0·042 and 0·021, respectively). Additionally, Pro:K showed an independent positive association with BMI and waist circumference (*P* for trend=0·024 and 0·012, respectively). In conclusion, more acidic dietary acid–base load was independently associated with adverse profile of several cardiometabolic risk factors in free-living young Japanese women.

Acid–base balance: Potential renal acid load: Ratio of dietary protein to potassium: Blood pressure

The potential importance of acid–base homeostasis to cardiometabolic risk factors has been recently suggested in the literature[1,2]. Mild metabolic acidosis, which can be caused by diet[3–5], may adversely affect blood pressure[6–8], possibly by increasing cortisol production[3], increasing Ca excretion[9,10] or decreasing citrate excretion[11]. Increased cortisol production caused by mild metabolic acidosis[3–5] may also have a detrimental influence on other cardiometabolic risk factors, including obesity and cholesterol[12–14].

Since acid–base status is markedly influenced by diet[15,16], diet-dependent acid–base load can be calculated based on dietary intake information. Remer and colleagues developed an equation for estimating potential renal acid load (PRAL), an indicator of dietary acid–base load, using the dietary intake of five nutrients (protein, P, K, Ca and Mg)[15,17]. In addition, Frassetto and colleagues proposed the ratio of dietary protein to K (Pro:K) as an indicator of dietary acid–base load[16]. Both PRAL and Pro:K estimated from dietary

Abbreviations: DHQ, diet history questionnaire; MET, metabolic equivalents; NAE, net acid excretion; OA, organic acids; PRAL, potential renal acid load; Pro:K, ratio of dietary protein to K.

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intake information have been validated against objective measures of acid–base load determined from 24 h urine (i.e. PRAL and net acid excretion (NAE), respectively)[17]. Using these measures, an expected negative relationship of dietary acid–base load with bone health was demonstrated in several epidemiologic studies that relied on a dietary questionnaire for nutrient intake estimation[18–22].

Despite the potential influence of dietary acid–base load on cardiometabolic risk factors and the availability of dietary acid–base load measurements using dietary intake data, no study has examined the possible association between measures of dietary acid–base load and cardiometabolic risk factors. Here, we investigated the associations of measures of dietary acid–base load (i.e. PRAL and Pro:K), calculated using nutrient intake estimates obtained from a validated self-administered comprehensive diet history questionnaire (DHQ)[23–25] with several cardiometabolic risk factors including BMI, waist circumference, systolic and diastolic blood pressure, total, HDL, and LDL cholesterol, fasting TAG, fasting glucose and glycated Hb, using data gathered from a cross-sectional observational study of free-living young Japanese women.

Subjects and methods

Subjects

The present study was based on a cross-sectional multi-centre survey conducted from February to March 2006 and from January to March 2007 among female dietetic students from fifteen institutions in Japan. All measurements at each institution were conducted according to the survey protocol. Briefly, staff at each institution explained an outline of the survey to potential subjects. Those who responded positively were then provided detailed written and oral explanations of the survey's general purpose and procedure. The protocol of the study was approved by the Ethics Committee of the National Institute of Health and Nutrition, and written informed consent was obtained from each subject, and also from a parent for subjects aged <20 years.

A total of 1176 Japanese women took part. For the present analysis, women aged 18–22 years were selected (n 1154), not only because dietetic students outside this age range are rare in Japan but also because their dietary and cardiometabolic characteristics may differ from those of dietetic students aged 18–22 years. We then excluded from the 1154 women aged 18–22 years those not completing survey questionnaires (n 1), those with extremely low or high reported energy intakes (<2092 or $>16\,736$ kJ/d; n 2), those currently receiving dietary counselling from a doctor or dietitian (n 13), those with previously diagnosed diabetes, hypertension or CVD (n 1), and those without measurement of body height and weight (n 2). Additionally, women with missing information regarding cardiometabolic risk factors were excluded from the respective analyses (n 2 for BMI, n 2 for waist circumference, n 0 for systolic and diastolic blood pressure, n 16 for cholesterol (total, HDL and LDL), n 16 for TAG, n 15 for glucose, and n 16 for glycated Hb). Further, those providing non-fasting blood samples (n 34) were excluded from the fasting TAG and glucose analyses. Some women fell into more than one exclusion category. The final sample size was 1136 for BMI, waist circumference, and systolic and diastolic blood pressure, 1121 for cholesterol (total, HDL, and LDL) and glycated Hb, 1089 for fasting glucose, and 1088 for fasting TAG.

Dietary assessment

Dietary habits during the preceding month were assessed using a self-administered comprehensive DHQ[23–25]. Responses to the DHQ, as well as to a lifestyle questionnaire, were checked at least twice for completeness. When necessary, forms were reviewed with the subject to ensure the clarity of answers. The DHQ is a sixteen-page structured questionnaire that consists of the following seven sections: general dietary behaviour; major cooking methods; consumption frequency and amount of six alcoholic beverages; consumption frequency and semi-quantitative portion size of 118 selected food and nonalcoholic beverage items; dietary supplements; consumption frequency and semi-quantitative portion size of nineteen cereals (rice, bread, and noodles), soup consumed with noodles, and *miso* (fermented soyabean paste) soup; and open-ended items for foods consumed regularly ($\geq$once/week), but not appearing in the DHQ[23]. The food and beverage items were selected as foods commonly consumed in Japan, mainly from a food list used in the National Nutrition Survey of Japan, and standard portion sizes were derived mainly from several recipe books for Japanese dishes[23].

Estimates of dietary intake for a total of 150 food and beverage items (including five seasonings), energy, and nutrients were calculated using an *ad hoc* computer algorithm for the DHQ based on the Standard Tables of Food Composition in Japan[26]. Information on dietary supplements and data from the open-ended questionnaire items were not used in the calculation of dietary intake[23]. Nutrient and food intake was energy-adjusted using the residual method[27]. Detailed descriptions of the methods used to calculate dietary intake and the validity of the DHQ regarding nutrients have been published elsewhere[23–25]. The Pearson correlation coefficients between the DHQ and the 3 d estimated dietary records for forty-seven women were 0·48 for protein, 0·59 for P, 0·68 for K, and 0·49 for Ca (data not available for Mg)[23]. The Pearson correlation coefficients between the DHQ and the 16 d weighed dietary records for ninety-two women were 0·52 for protein, 0·55 for P, 0·55 for K, 0·56 for Ca, and 0·56 for Mg (S. Sasaki, unpublished results). In addition, the Spearman correlation coefficients were 0·66 for meats, 0·55 for fish and shellfish, 0·38 for eggs, 0·61 for dairy products, and 0·40 for fruits, 0·57 for vegetables, and 0·46 for cereals in ninety-two women (S. Sasaki, unpublished results). Furthermore, the Pearson correlation coefficient between the DHQ and the 24 h urinary excretion for K was 0·40 in sixty-nine women[24].

Calculation and validation of dietary acid–base load measures

Urinary NAE is an established index of net endogenous acid production[16,17], which is difficult to measure directly[28,29]. Because the sum of cations excreted in the urine equals the sum of anions, urinary NAE is also equal to the difference between the sum of the major urinary non-bicarbonate anions minus the sum of the non-titratable acid and non-ammonium cations[30]. The amounts of these non-bicarbonate

anions and mineral cations in urine (excluding organic acids (OA)) are primarily influenced by dietary nutrient intake[15,16]. OA are largely independent of dietary acid load or macronutrient composition[15,17,31,32], and can be reasonably estimated from body surface area[32]:

$$\text{OA (mEq/d)} = \text{body surface area (m}^2\text{)} \times 41/1{\cdot}73,$$

$$\text{where body surface area (m}^2\text{)} = 0{\cdot}0007484 \times \text{body height (cm)}^{0{\cdot}725} \times \text{body weight (kg)}^{0{\cdot}425}.$$

Thus, the estimate of the urinary difference in non-bicarbonate anions (without OA) and mineral cations can be considered an index of diet-induced acid load[17]. Remer and colleagues referred to this estimate as PRAL (i.e. NAE = PRAL + OA)[17], and developed the equation for estimating PRAL from dietary information[15,17,30]:

$$\text{PRAL (mEq/d)} = 0{\cdot}4888 \times \text{protein (g/d)} + 0{\cdot}0366 \times \text{P (mg/d)} - 0{\cdot}0205 \times \text{K (mg/d)} - 0{\cdot}0125 \times \text{Ca (mg/d)} - 0{\cdot}0263 \times \text{Mg (mg/d)}.$$

The validity of PRAL estimated from this equation has been established against PRAL measured from 24 h urine[15,17].

The rate of H_2SO_4 production from protein metabolism and the rate of bicarbonate generation from the metabolism of intestinally absorbed K salts are major and highly variable components of net endogenous acid production[29]. Based on this, Frassetto and colleagues proposed the ratio of dietary protein (g/d) to K (mEq/d) (i.e. Pro:K) as an index of diet-induced acid load[16]. The validity of Pro:K has been established against NAE measured in 24 h urine[17].

In the present study PRAL and Pro:K were used as measures of dietary acid–base load. PRAL and Pro:K were calculated according to the equations described above using crude nutrient intake data estimated from the DHQ. Higher values of PRAL and Pro:K mean more acidic dietary acid–base load. Calculated PRAL and Pro:K were then energy-adjusted using the residual method[27]. Prior to the present analysis, the relative validity of PRAL and Pro:K estimated from the DHQ was examined against that from the 16 d weighed dietary records in ninety-two women aged 31–69 years. The Pearson correlation coefficient between the two methods was 0·35 for PRAL and 0·37 for Pro:K (S. Sasaki, unpublished results).

Cardiometabolic risk factors

Cardiometabolic risk factors were measured 1–3 d after completion of the questionnaires. Body height was measured to the nearest 0·1 cm with the subject standing without shoes. Body weight in light indoor clothes was measured to the nearest 0·1 kg. BMI was calculated as body weight (kg) divided by the square of body height (m). Waist circumference was measured at the level of the umbilicus to the nearest 0·1 cm. The measurement was taken at the end of a normal expiration while the subject was standing erect with her arms at her side and feet together. Systolic and diastolic blood pressure was measured on the left arm with an automatic device (Omron model HEM-770A; Omron Health Care, Kyoto, Japan) after the subject had been sitting quietly for ≥3 min. A second measurement was carried out about 1 min after the first, and the mean value of the two was used. Peripheral blood samples were obtained from subjects after an overnight fast. Blood was collected in evacuated tubes containing no additives, allowed to clot, and centrifuged at 3000 ***g*** for 10 min at room temperature to separate the serum. Blood samples for glycated Hb measurements were also collected in evacuated tubes containing no additives. In accordance with the survey protocol, blood samples were transported at −20°C by car or airplane to ensure delivery to a laboratory in Tokyo, Japan (SRL, Inc. in the 2006 survey and Mitsubishi Kagaku Bio-Clinical Laboratories (MBCL), Inc. in the 2007 survey). Biochemical variables were assayed at SRL in the 2006 survey and MBCL in the 2007 survey within 1–2 d of collection to avoid significant degradation, as follows. Serum total cholesterol concentration was measured enzymically using a kit from Wako Junyaku Co. Ltd (Tokyo, Japan) at SRL and using a kit from Daiya Shiyaku Co. Ltd (Tokyo, Japan) at MBCL. Serum LDL- and HDL-cholesterol concentrations were measured enzymically using a kit from Daiichi Kagaku Co. Ltd at SRL and using a kit from Daiichi Kagaku Co. Ltd at MBCL. Serum TAG concentration was measured enzymically using a kit from Daiichi Kagaku Co. Ltd at SRL and using a kit from Kyowa Medex Co. Ltd (Tokyo, Japan) at MBCL. Serum glucose concentration was measured enzymically using a kit from Shino Tesuto Co. Ltd (Tokyo, Japan) at SRL and using a kit from Kanto Kagaku Co. Ltd (Tokyo, Japan) at MBCL. Glycated Hb was measured using whole blood by latex agglutination-turbidimetric immunoassay (Fuji Revio Co. Ltd (Tokyo, Japan) at SRL and Kyowa Medex Co. Ltd (Tokyo, Japan) at MBCL). In-house quality-control procedures for all assays were conducted at SRL in the 2006 survey and MBCL in the 2007 survey.

Other variables

In the lifestyle questionnaire, the subject reported her residential area, which was grouped into one of three regions (residential block: north (Kanto, Hokkaido, and Tohoku); central (Tokai, Hokuriku, and Kinki) or south (Kyushu and Chugoku)). The residential areas were also grouped into three categories according to population size (size of residential area: city with population ≥1 million; city with population <1 million or town and village). Current smoking (yes or no) was self-reported in the lifestyle questionnaire. Physical activity was computed as the average metabolic equivalents (MET)-hours per day[33] on the basis of the frequency and duration of five different activities (sleeping, high- and moderate-intensity activities, walking, and sedentary activities) over the preceding month, as reported in the lifestyle questionnaire.

Statistical analysis

Measures of dietary acid–base load, i.e. PRAL and Pro:K, were examined in relation to ten cardiometabolic risk factors, namely BMI, waist circumference, systolic and diastolic blood

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pressure, cholesterol (total, HDL, and LDL), fasting TAG, fasting glucose, and glycated Hb. All statistical analyses were performed using SAS statistical software (version 8.2; SAS Institute Inc., Cary, NC, USA). Linear regression models were constructed using the PROC GLM procedure to examine the association between dietary acid–base load measures with cardiometabolic risk factors. For the analyses, subjects were categorized into quintiles according to the dietary acid–base load measures. The mean metabolic risk factor values (with standard errors) were calculated by quintiles of dietary acid–base load measures after multivariate adjustment for potential confounding factors. Confounding factors included residential block, size of residential area, survey year (2006 or 2007; because of the different laboratories with different kits used for blood analyses for the 2006 and 2007 surveys, even though there were no differences in the assay methods), current smoking, and physical activity (continuous). BMI (continuous) was added as a confounding factor in all analyses except for that for BMI itself. Waist circumference (continuous) was also added as a confounding factor in the analyses except for those for BMI and waist circumference. We initially intended to include estimated excretion of OA (the diet-independent acid–base load) as a confounding factor to investigate diet-dependent acid–base load (PRAL and Pro:K) and cardiometabolic risk factors. However, OA was strongly correlated with body height and weight and hence BMI (Pearson correlation coefficient with OA = 0·70, 0·95, and 0·68, respectively), as OA is just a function of the combination of height and weight as mentioned earlier. To avoid over-adjustment, we consequently did not include OA as a confounding factor. Since alcohol intake was extremely low (mean = 1·5 g/d), it was not considered as a confounding factor. Because the inclusion of measures of obesity (BMI, waist circumference, or both) as confounding factors did not influence the results materially, we present the full-adjustment models only. Linear trends with increasing levels of dietary acid–base load measures were tested for by assigning each participant a median value for the category and modelling this value as a continuous variable. All reported P values are two-tailed, and a value of $P<0{\cdot}05$ was considered statistically significant.

Results

Basic characteristics of all subjects (n 1136; those included in the analyses of BMI, waist circumference, and systolic and diastolic blood pressure) are shown in Table 1. Mean PRAL was 10·4 mEq/d, and mean Pro:K was 1·23 g/mEq. There was a strong correlation between these variables (Pearson correlation coefficient = 0·84). The potential confounding variables for all subjects are shown in Table 2 according to quintile of dietary acid–base load measure. Both PRAL and Pro:K were associated negatively with physical activity and positively with waist circumference. There was also a positive association between Pro:K and BMI. The dietary intakes of all subjects are shown in Table 3 according to quintile of dietary acid–base load measures. PRAL was associated positively with protein and negatively with K, Ca and Mg as well as P, while Pro:K was positively associated with K as well as protein. For foods, both PRAL and Pro:K showed a positive association with meats, eggs, cereals, and fish and shellfish, and a negative association with fruits, vegetables and dairy products. According to the quintiles of the dietary acid–base load measures, similar patterns were observed for potential confounding factors and dietary intake among those subjects included in the analyses of cholesterol (total, HDL, and LDL) and glycated Hb (n 1121), fasting TAG (n 1088), and fasting glucose (n 1089) (data not shown).

The multivariate-adjusted mean values for cardiometabolic risk factors across quintiles of dietary acid–base load

Table 1. Basic characteristics of subjects (n 1136)*
(Mean values and standard deviations)

	Mean	SD
Age (years)	19·6	1·1
Body height (cm)	158·4	5·5
Body weight (kg)	53·6	7·7
BMI (kg/m^2)	21·3	2·7
Waist circumference (cm)	72·9	7·1
Systolic blood pressure (mmHg)	106·4	10·6
Diastolic blood pressure (mmHg)	69·3	8·2
Total cholesterol (mg/l)	1889	318
HDL-cholesterol (mg/l)	706	127
LDL-cholesterol (mg/l)	1070	272
Fasting TAG (mg/l)	611	288
Fasting glucose (mg/l)	840	64
Glycated Hb (%)	4·87	0·26
Residential block (%)		
North (Kanto, Hokkaido, and Tohoku)	56	
Central (Tokai, Hokuriku, and Kinki)	24	
South (Kyushu and Chugoku)	20	
Size of residential area (%)		
City with population ≥1 million	16	
City with population <1 million	78	
Town and village	6	
Survey year (%)		
2006	41	
2007	59	
Current smoking (%)		
No	97	
Yes	3	
Physical activity (total MET-h/d)	33·9	3·1
Estimated urinary excretion of organic acid (mEq/d)	36·3	2·7
Energy intake (kJ/d)	7376	1874
Nutrient intake†		
Protein (g/d)	59·7	8·8
P (mg/d)	915	169
K (mg/d)	1971	471
Ca (mg/d)	502	171
Mg (mg/d)	213	49
Food intake (g/d)		
Meats	60·1	31·7
Fish and shellfish	50·3	28·6
Eggs	35·6	21·4
Dairy products	145·6	127·2
Fruits	57·9	57·3
Vegetables	206·9	121·2
Cereals	380·3	92·1
Measures of dietary acid–base load‡		
Potential renal acid load (mEq/d)	10·4	7·6
Pro:K (g/mEq)	1·23	0·22

MET, metabolic equivalents; Pro:K, ratio of dietary protein to K.
* n 1121 for cholesterol (total, HDL, and LDL) and glycated Hb; 1088 for fasting TAG; 1089 for fasting glucose.
† Energy-adjusted using the residual method.
‡ Calculated using crude nutrient intake values and then energy-adjusted using the residual method.

Table 2. Selected characteristics according to quintile of measures of dietary acid–base load (*n* 1136)
(Mean values and standard deviations)

	Quintile of measures of dietary acid–base load										
	1 (*n* 227)		2 (*n* 227)		3 (*n* 228)		4 (*n* 227)		5 (*n* 227)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P**
Potential renal acid load (mEq/d)†	−0·8	6·5	7·8	1·2	11·1	0·8	14·2	0·9	19·5	3·4	
Residential block (%)											0·69
North (Kanto, Hokkaido, and Tohoku)	57		55		54		57		58		
Central (Tokai, Hokuriku, and Kinki)	24		27		24		22		25		
South (Kyushu and Chugoku)	19		19		23		21		17		
Size of residential area (%)											0·036
City with population ≥1 million	16		15		13		15		22		
City with population <1 million	76		80		79		79		74		
Town and village	7		5		9		6		3		
Survey year (%)											0·86
2006	44		36		42		41		41		
2007	56		64		58		59		59		
Current smoking (%)											0·11
No	96		98		97		98		98		
Yes	4		2		3		2		2		
Physical activity (total mEq-h/d)	34·4	3·5	33·8	2·5	33·8	3·4	33·6	2·4	33·8	3·3	0·021
BMI (kg/m^2)	21·1	2·6	21·2	3·2	21·5	2·5	21·6	2·6	21·3	2·7	0·11
Waist circumference (cm)	72·3	7·0	72·0	7·6	73·2	6·6	73·9	6·8	73·2	7·1	0·019
Pro:K (g/mEq)†	0·94	0·09	1·11	0·03	1·22	0·03	1·33	0·04	1·54	0·13	
Residential block (%)											0·14
North (Kanto, Hokkaido, and Tohoku)	56		57		60		55		51		
Central (Tokai, Hokuriku, and Kinki)	24		26		22		22		27		
South (Kyushu and Chugoku)	19		17		18		23		22		
Size of residential area (%)											0·084
City with population ≥1 million	15		14		16		18		18		
City with population <1 million	78		78		77		78		78		
Town and village	7		8		7		5		4		
Survey year (%)											0·31
2006	44		38		41		41		38		
2007	56		62		59		59		62		
Current smoking (%)											0·59
No	96		98		98		97		97		
Yes	4		2		2		3		3		
Physical activity (total MET-h/d)	34·4	3·6	34·0	2·6	33·7	2·6	34·1	3·9	33·4	2·2	0·002
BMI (kg/m^2)	21·0	2·5	21·2	3·1	21·4	2·5	21·6	2·7	21·5	2·8	0·030
Waist circumference (cm)	72·1	7·0	72·2	7·4	72·8	6·3	73·8	7·0	73·6	7·4	0·002

MET, metabolic equivalents; Pro:K, ratio of dietary protein to K.
* For categorical variables, a Mantel–Haenszel χ^2 test was used; for continuous variables, a linear trend test was used with the median value in each quintile as a continuous variable in linear regression.
† Calculated using crude nutrient intake values and then energy-adjusted using the residual method.

measures are shown in Table 4. After adjustment for potential confounding factors, higher PRAL and Pro:K (more acidic dietary acid–base loads) were associated with higher systolic and diastolic blood pressure (mean difference between the lowest and highest quintiles = 2·1 mmHg (*P* for trend = 0·028) and 1·6 mmHg (*P* for trend = 0·035) for PRAL, and 2·5 mmHg (*P* for trend = 0·012) and 2·3 mmHg (*P* for trend = 0·009) for Pro:K, respectively). In addition, PRAL showed an independent positive association with total and LDL cholesterol (mean difference = 59 mg/l (*P* for trend = 0·042) and 60 mg/l (*P* for trend = 0·021), respectively). Pro:K was positively associated with BMI and waist circumference independently of potential confounding factors (mean difference = 0·5 kg/m^2 (*P* for trend = 0·024) and 0·8 cm (*P* for trend = 0·012), respectively). No significant associations were observed between PRAL or Pro:K and any of the other cardiometabolic risk factors examined.

Discussion

In a group of free-living young Japanese women, we found that higher PRAL and Pro:K (more acidic dietary acid–base loads) were associated with higher systolic and diastolic blood pressure after adjustment for possible confounding factors. We also found independent positive associations between PRAL and total and LDL-cholesterol, as well as between Pro:K and BMI and waist circumference. To our knowledge, this is the first study to examine the relationships between dietary acid–base load measures and cardiometabolic risk factors.

Consistent with previous studies[19–21], the correlation between PRAL and Pro:K was quite high (Pearson correlation coefficient = 0·84–0·93), which indicates that these measures capture similar, but not the same, elements of dietary acid–base load. Given that only blood pressure was

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Table 3. Nutrient and food intake according to quintile of measures of dietary acid–base load (*n* 1136)
(Mean values and standard deviations)

	Quintile of measures of dietary acid–base load										
	1 (*n* 227)		2 (*n* 227)		3 (*n* 228)		4 (*n* 227)		5 (*n* 227)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P* for trend*
Potential renal acid load (mEq/d)†	−0·8	6·5	7·8	1·2	11·1	0·8	14·2	0·9	19·5	3·4	
Nutrient intake‡											
Protein (g/d)	58·5	10·8	59·0	8·4	58·8	7·2	59·4	7·3	62·9	9·1	<0·0001
P (mg/d)	942	203	922	154	896	154	890	150	924	173	0·043
K (mg/d)	2446	528	2067	361	1895	315	1771	318	1678	368	<0·0001
Ca (mg/d)	577	202	530	149	489	161	470	151	442	154	<0·0001
Mg (mg/d)	249	58	220	39	207	37	199	40	193	47	<0·0001
Food intake (g/d)‡											
Meats	49·8	28·7	55·6	24·6	59·7	27·6	61·3	29·4	73·8	41·1	<0·0001
Fish and shellfish	47·9	31·5	49·9	26·8	48·4	22·7	49·0	24·1	56·2	35·3	0·008
Eggs	31·5	21·9	32·3	18·1	34·5	19·3	37·3	20·1	42·7	25·0	<0·0001
Dairy products	156·4	142·9	167·1	132·0	142·3	128·8	138·6	118·7	123·3	107·5	0·001
Fruits	91·7	89·2	62·4	41·8	54·4	45·4	45·8	36·7	35·2	38·9	<0·0001
Vegetables	306·2	176·2	218·6	95·9	190·4	81·3	169·8	77·2	149·6	77·4	<0·0001
Cereals	344·8	94·5	376·0	79·9	380·9	83·6	394·4	89·4	405·5	100·8	<0·0001
Pro:K (g/mEq)†	0·94	0·09	1·11	0·03	1·22	0·03	1·33	0·04	1·54	0·13	
Nutrient intake‡											
Protein (g/d)	59·5	9·8	60·8	8·1	60·5	8·4	59·8	9·3	58·0	8·1	0·028
P (mg/d)	2492	468	2151	310	1949	270	1761	287	1504	273	<0·0001
Food intake (g/d)‡											
Meats	48·3	23·3	58·7	28·1	63·0	29·4	64·4	33·3	65·8	39·4	<0·0001
Fish and shellfish	50·2	28·7	52·1	28·0	51·9	27·4	51·4	30·1	45·8	28·3	0·088
Eggs	33·6	21·5	33·9	19·3	36·7	22·1	36·1	19·9	37·8	23·7	0·019
Dairy products	173·9	147·3	186·4	140·9	152·1	132·3	125·9	102·4	89·5	75·0	<0·0001
Fruits	95·0	87·5	64·6	47·2	53·0	44·2	48·1	41·6	28·8	22·4	<0·0001
Vegetables	308·4	163·9	232·8	110·7	195·6	79·6	170·2	78·6	127·8	58·2	<0·0001
Cereals	348·4	92·4	362·5	78·2	374·3	89·8	395·0	86·4	421·4	95·3	<0·0001

Pro:K, ratio of dietary protein to K.
* A linear trend test was used with the median value in each quintile as a continuous variable in linear regression.
† Calculated using crude nutrient intake values and then energy-adjusted using the residual method.
‡ Energy-adjusted using the residual method.

significantly associated with both PRAL and Pro:K in the present study, the finding on blood pressure may be more reliable than those on other cardiometabolic risk factors that are significantly associated with only one, either PRAL or Pro:K (i.e. total and HDL-cholesterol and BMI and waist circumference). Although both PRAL and Pro:K are established and reasonably valid measures of dietary acid–base load[17], further methodological research may be helpful for clarifying which is the better indicator of diet-induced acid–base load.

Both PRAL and Pro:K were independently and positively associated with systolic and diastolic blood pressure in the present study. Several previous experimental studies have also indicated a significant rise in blood pressure caused by mild metabolic acidosis[6–8], which can be caused by diet[3–5]. Although not established, several plausible mechanisms have been proposed, including increased cortisol production[3] increased Ca excretion[9,10] and reduced citrate excretion[11]. Further research to elucidate the apparent influence of dietary acid–base load on blood pressure using a range of free-living populations is required.

We found an independent and positive association of total and HDL-cholesterol with PRAL, but not with Pro:K. Likewise, an independent and positive association was also found between BMI and waist circumference and Pro:K, but not with PRAL. No significant association with any of the other cardiometabolic risk factors was observed, including fasting TAG, fasting glucose, and glycated Hb. Increasing cortisol production caused by mild metabolic acidosis[3–5] might have a detrimental influence on cardiometabolic risk factors[12–14]. Further investigation of this poorly understood field is warranted.

In the present study, PRAL showed a strong negative association with K (as well as Ca and Mg), but a weak association with protein and P. For foods, the negative association with fruits and vegetables was quite strong compared with that for other foods. Similar trends were observed for Pro:K. Thus, in Japanese populations, dietary acid–base load may be primarily determined by foods rich in K such as fruits and vegetables. This is somewhat different from Western populations, where PRAL was strongly associated not only negatively with fruits and vegetables, but also positively with meats[21].

Mean PRAL and Pro:K were much more acidic in this study of young Japanese women (10·4 mEq/d and 1·2 g/mEq, respectively) than in other two studies of British middle-aged women (3·7 and −7·6 mEq/d and 1·0 and 1·0 g/mEq, respectively)[19,21]. The intake of K, Ca, and Mg as well as

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Table 4. Cardiometabolic risk factors according to quintile of measures of dietary acid–base load (*n* 1136)*
(Mean values with their standard errors)

	Quintile of measures of dietary acid–base load										
	1 (*n* 227)		2 (*n* 227)		3 (*n* 228)		4 (*n* 227)		5 (*n* 227)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P* for trend†
Potential renal acid load (mEq/d)‡	1·3		7·8		11·2		14·0		18·7		
BMI (kg/m^2)§	21·1	0·2	21·2	0·2	21·5	0·2	21·6	0·2	21·4	0·2	0·099
Waist circumference (cm)§,‖	72·7	0·3	72·4	0·3	72·8	0·3	73·3	0·3	73·2	0·3	0·058
Systolic blood pressure (mmHg)§,‖,¶	105·2	0·6	106·5	0·6	106·4	0·6	106·4	0·6	107·3	0·6	0·028
Diastolic blood pressure (mmHg)§,‖,¶	68·1	0·5	69·8	0·5	69·5	0·5	69·5	0·5	69·7	0·5	0·035
Total cholesterol (mg/l)§,‖,¶	1866	21	1854	21	1924	21	1874	21	1925	21	0·042
HDL-cholesterol (mg/l)§,‖,¶	706	8	696	8	711	8	708	8	707	8	0·68
LDL-cholesterol (mg/l)§,‖,¶	1043	18	1048	18	1097	18	1056	18	1103	18	0·021
Fasting TAG (mg/l)§,‖,¶	603	19	614	19	606	19	610	19	622	19	0·56
Fasting glucose (mg/l)§,‖,¶	841	4	837	4	847	4	839	4	838	4	0·76
Glycated Hb (%)§,‖,¶	4·85	0·02	4·88	0·02	4·88	0·02	4·85	0·02	4·86	0·02	0·99
Pro:K (g/mEq)‡	0·96		1·11		1·22		1·33		1·51		
BMI (kg/m^2)§	21·0	0·2	21·2	0·2	21·4	0·2	21·6	0·2	21·5	0·2	0·024
Waist circumference (cm)§,‖	72·6	0·3	72·5	0·3	72·7	0·3	73·2	0·3	73·4	0·3	0·012
Systolic blood pressure (mmHg)§,‖,¶	104·7	0·6	106·8	0·6	106·7	0·6	106·6	0·6	107·2	0·6	0·012
Diastolic blood pressure (mmHg)§,‖,¶	67·9	0·5	70·0	0·5	69·2	0·5	69·5	0·5	70·2	0·5	0·009
Total cholesterol (mg/l)§,‖,¶	1878	21	1906	21	1877	21	1907	21	1876	21	0·93
HDL-cholesterol (mg/l)§,‖,¶	712	8	707	8	705	8	711	8	693	8	0·19
LDL-cholesterol (mg/l)§,‖,¶	1049	18	1080	18	1060	18	1086	18	1072	18	0·38
Fasting TAG (mg/l)§,‖,¶	589	19	650	19	593	19	598	19	624	19	0·66
Fasting glucose (mg/l)§,‖,¶	840	4	839	4	840	4	841	4	842	4	0·75
Glycated Hb (%)§,‖,¶	4·87	0·02	4·87	0·02	4·88	0·02	4·88	0·02	4·84	0·02	0·31

Pro:K, ratio of dietary protein to K.

* *n* 1121 for cholesterol (total, HDL, and LDL) and glycated Hb (224 in the first, second, fourth, and fifth and 225 in the third quintiles); 1088 for fasting TAG (217 in the first and fifth and 218 in the second, third, and fourth quintiles); and 1089 for fasting glucose (217 in the first and 218 in the second, third, fourth, and fifth quintiles). For potential renal acid load, median value in each quintile is almost the same (within $<0{\cdot}1$ mEq/d difference) in all analyses; for pro:K, median value in each quintile is the same.

† A linear trend test was used with the median value in each quintile as a continuous variable in linear regression.

‡ Calculated using crude nutrient intake values and then energy-adjusted using the residual method. Values are median.

§ Adjusted for residential block (north (Kanto, Hokkaido, and Tohoku), central (Tokai, Hokuriku, and Kinki), or south (Kyushu and Chugoku)), size of residential area (city with population $\geq$1 million, city with population with $<$1 million, or town and village), survey year (2006 or 2007), current smoking (yes or no), and physical activity (total metabolic equivalent-h/d, continuous).

‖ Additionally adjusted for BMI (kg/m^2, continuous).

¶ Additionally adjusted for waist circumference (cm, continuous).

P was 37–50 % lower, although protein intake was 27 % lower in the Japanese subjects than in the British population[21], which seems to explain the more acidic dietary acid–base load in the present study.

Several limitations of the present study warrant mention. First, the cross-sectional nature of the study does not permit the assessment of causality owing to the uncertain temporality of the association. Nevertheless, there are biologically plausible mechanisms for the relationship between dietary acid–base load and the cardiometabolic risk factors, particularly blood pressure[1,2,6–11], although while the premise for the hypothesis examined is that the acidosis is associated with increased cortisol production[3–5], no measurements for cortisol are unfortunately available, which would help back up findings. Second, our subjects were selected female dietetic students, not a random sample of Japanese women. In addition, because of our recruitment procedure, the exact response rate was unknown, which might have produced recruitment bias. Thus, these results may not apply to the general Japanese population, although our population was on average comparable with a representative sample of Japanese women with similar age range (18–29 years for dietary intake and 20–29 years for cardiometabolic risk factors), at least with regard to the intake of energy (mean: 7117 kJ/d), protein (64·0 g/d), P (894 mg/d), K (1965 mg/d), Ca (459 mg/d), and Mg (210 mg/d) and several cardiometabolic risk factors including BMI (20·9 kg/m^2), systolic blood pressure (108·8 mmHg), diastolic blood pressure (67·0 mmHg), total cholesterol (1806 mg/l), HDL-cholesterol (689 mg/l), and glycated Hb (4·91 %) (data not available for other cardiometabolic risk factors)[34]. Further, because the study population consisted of generally healthy persons, the clinical relevance of our findings remains to be elucidated. Nevertheless, our results should provide valuable insight from a prevention perspective. Third, as with other studies on dietary acid–base load[18,20,21], a semi-quantitative dietary assessment questionnaire (i.e. DHQ) was used to collect dietary data[23–25]. As actual dietary habits were not observed, and the relative validity of the PRAL and Pro:K values derived from the DHQ against the 16 d weighed dietary records was also modest, the results should be interpreted with caution. It should be noted, however, at least for conventional nutrients, that the applicability of the DHQ is comparable to that of dietary questionnaires used in previous studies, in which the relative validity of PRAL and Pro:K was not examined[18,20,21]. Additionally, the misreporting of dietary intake, particularly by overweight subjects, is a serious problem associated with self-report dietary assessment methods[35]. However, at least for dietary protein, K, and Na, BMI-dependent misreporting seems to be cancelled by energy-adjustment[36]. To minimize the influence of dietary misreporting as much as possible[36], we used energy-adjusted values[27]. Actually, the ratio of reported energy intake to estimated basal metabolic rate, a surrogate measure of the general quality of dietary data[35] was not associated with PRAL or Pro:K (data not shown), which suggests that it is unlikely that dietary underreporting had a major effect on the observed associations. Further, because of the lack of a reliable composition table for dietary supplements in Japan, nutrient intake from dietary supplements was not included in the analysis. However, the percentage of subjects who consumed dietary supplements containing mostly protein and minerals during the preceding month was only about 2 %, and the exclusion of these supplement users did not change the results materially (data not shown). This suggests that it is unlikely that dietary supplements had a major effect on the findings. Moreover, although adjustments were attempted to compensate for a variety of potential confounding variables, residual confounding could not be ruled out. In particular, physical activity was assessed relatively roughly from only five activities, which may not have been sufficient. Finally, because the associations between diet-dependent acid load and cardiometabolic risk factors were examined using PRAL and Pro:K estimated from dietary intake, it is not known whether the observed associations were caused by a change in acid–base balance due to dietary intake or by some other mechanism. Additionally, the observed associations between PRAL and Pro:K and cardiometabolic risk factors may be simply due to the effects on cardiometabolic risk factors of intakes of some nutrients or foods associated with PRAL and Pro:K. For example, K and vegetable intake, of which not only the favourable effect on blood pressure is broadly recognized[37–39] but which was also most strongly correlated with PRAL and Pro:K in the present study (Pearson correlation coefficients with PRAL or Pro:K = −0·50 to −0·74), was negatively associated with BMI and waist circumference (P for trend ≤ 0·001), although there was no association with blood pressure (systolic and diastolic) or cholesterol (total and LDL). However, it is not possible to understand what food sources or nutrients are driving the observed associations between dietary acid–base load and cardiometabolic risk factors, because dietary acid–base load used in the present study was derived from estimates of dietary intakes, and hence was associated with these dietary intakes. Thus the results of the present study should be cautiously interpreted without oversimplification.

In conclusion, after adjusting for possible confounding factors, we found that higher PRAL and Pro:K (more acidic dietary acid–base loads) were associated with higher systolic and diastolic blood pressure in free-living young Japanese women. We also found independent positive associations between PRAL and total and LDL-cholesterol, as well as between Pro:K and BMI and waist circumference. Because the cross-sectional nature of the present study does not allow causal inferences, any firm conclusions regarding the effects of dietary acid–base load on cardiometabolic risk factors require additional observational and experimental studies.

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data, and wrote the manuscript. S. S. is a principal researcher of this project and contributed to the concept and design of the study, the study protocol, and data collection and management, and the writing and editing of the manuscript. Y. T. and K. U. contributed to the concept and design of the study, the study protocol, and data collection. All authors contributed to the preparation of the manuscript and approved the final version submitted for publication.

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