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ORIGINAL ARTICLE

Lower vegetable protein intake and higher dietary acid load associated with lower carbohydrate intake are risk factors for metabolic syndrome in patients with type 2 diabetes: *Post-hoc* analysis of a cross-sectional study

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Keywords
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ABSTRACT

Aims/Introduction: A low-carbohydrate diet based on animal sources is associated with higher all-cause mortality, whereas a vegetable-based low-carbohydrate diet is associated with lower cardiovascular disease mortality. It has been suggested that acid/base imbalance might play an important role in some cardiometabolic abnormalities. The aims of the present study were to evaluate whether carbohydrate intake is associated with quality of dietary protein and acid load, and whether these are related to metabolic syndrome in patients with type 2 diabetes.

Materials and Methods: The present cross-sectional study involved 149 patients with type 2 diabetes. Dietary intake was assessed using a validated self-administered diet history questionnaire. Dietary acid load was assessed by potential renal acid load and net endogenous acid production.

Results: Mean daily total energy intake, carbohydrate intake, animal protein intake and vegetable protein intake were 1821.5 kcal, 248.8 g, 36.1 g and 31.1 g, respectively. Carbohydrate energy/total energy was negatively correlated with animal protein energy/total energy, potential renal acid load or net endogenous acid production score, and was positively correlated with vegetable protein energy/total energy. Logistic regression analyses showed that the subgroup of patients with a lower vegetable protein energy/total energy or higher potential renal acid load or net endogenous acid production score was significantly associated with the prevalence of metabolic syndrome.

Conclusions: The present study showed that carbohydrate intake was associated with the quality of dietary protein and dietary acid load. Furthermore, decreased vegetable protein intake and increased dietary acid load were associated with the prevalence of metabolic syndrome.

INTRODUCTION

Type 2 diabetes mellitus is a chronic condition that can lead to various complications over time, and it has developed into a public health issue. Type 2 diabetes mellitus is a multifactorial disease that is caused by environmental and genetic factors. In the environmental factors, medical nutrition therapy for the management of diabetes plays a crucial role in preventing diabetic complications, and especially in the management of

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metabolic control and optimal bodyweight[1,2]. Currently, controversy regarding the appropriate carbohydrate intake is in progress. The guidelines for the medical treatment of diabetes in Japan recommend a diet based on the following: carbohydrates comprising 50–60% of the total energy intake, protein 1.0–1.2 g/kg of the ideal bodyweight and fat ≤25% of the total energy intake. In the recommendations of the American Diabetes Association, which was published in 2012, it was reported that either low-fat calorie-restricted, low-carbohydrate or Mediterranean diets could be effective for bodyweight loss, and that glycemic control can be improved by regulating carbohydrate intake if followed for up to 2 years[3].

A recent study showed that high intakes of total and animal protein were associated with a moderate elevated risk of type 2 diabetes in a large cohort of European adults[4]. An animal-based low-carbohydrate diet was also associated with higher all-cause mortality, whereas a low-carbohydrate diet based on vegetable sources was associated with lower cardiovascular disease and all-cause mortality rates[5]. Besides, it has been suggested that acid/base imbalance might play a crucial role in some cardiometabolic abnormalities[6,7].

Nevertheless, little is known about the actual dietary habits of Japanese patients with type 2 diabetes. In particular, the association between carbohydrate intake and dietary animal or vegetable protein intake or dietary acid load still remains unclear in patients with type 2 diabetes. In addition, the association between dietary animal or vegetable protein intake or dietary acid load and metabolic syndrome (MetS), which increases the risk of developing cardiovascular disease, remains unclear. Therefore, we aimed to evaluate whether carbohydrate intake is associated with the quality of dietary protein and acid load, and whether these are related to the presence of MetS in patients with type 2 diabetes.

MATERIALS AND METHODS

Patients

We carried out a cross-sectional study of 260 consecutive patients with type 2 diabetes who were recruited from the outpatient clinic at Kyoto Prefectural University of Medicine, Kyoto, Japan. The data were collected from June to September 2011. A self-administered diet history questionnaire (DHQ) was given to 260 patients (140 men and 120 women), and a total of 215 patients (112 men and 103 women) completed the questionnaire. We excluded patients due to the following criteria: under the age of 40 years (4 women), patients with type 1 diabetes (6 men and 7 women), chronic renal failure or hemodialysis patients (7 men and 4 women), incomplete information (12 men and 6 women) and patients in the ≥95 or ≤5 percentile for energy intake (10 males and 10 females). Finally, a total of 149 patients (77 men and 72 women) met the inclusion criteria. Patients were divided into two subgroups according to the median animal or vegetable protein energy/total energy. In addition, patients were divided into two subgroups according to the median dietary acid load scores, assessed by both the potential renal acid load (PRAL) or the net endogenous acid production (NEAP). Approval for the study was obtained from the local research ethics committee, and written informed consent was obtained from all patients.

Estimation and Assessment of Habitual Food and Nutrient Intake

The usual dietary habits of the patients were assessed by the validated DHQ system[8]. DHQ is a questionnaire that inquires about the dietary intake situation of the past 1 month, and consists of questions of the following four items: questions about eating behavior of day-to-day, questions about each amount and frequency of intake of 117 foods, questions about each amount and frequency of staple foods, and questions about each amount and frequency of intake of food other than the aforementioned. Using DHQ and the nutritional value calculation program, we calculated dietary total, carbohydrate, animal protein, vegetable protein, animal fat, vegetable fat and salt intake.

Assessment of Dietary Acid Load Scores

The PRAL and NEAP scores were derived from estimations of several nutrient intakes[9]. The PRAL and NEAP scores were calculated as estimates of dietary acid load using the following equation.

$$\begin{aligned}\text{PRAL (mEq/day)} = {} & (0.49 \times \text{protein [g/day]}) \\ & + (0.037 \times \text{phosphorus [mg/day]}) \\ & - (0.021 \times \text{potassium [mg/day]}) \\ & - (0.026 \times \text{magnesium [mg/day]}) \\ & - (0.013 \times \text{calcium [mg/day]}) \end{aligned} \quad (1)$$

$$\begin{aligned}\text{NEAP (mEq/day)} = {} & (54.5 \times \text{protein [g/day]} / \text{potassium} \\ & \text{[mEq/day]}) - 10.2 \end{aligned} \quad (2)$$

Data collection

All patients provided details of their demographics. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. After an overnight fast, venous blood was collected for the measurement of the levels of various factors, including fasting plasma glucose, total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, uric acid and creatinine. Hemoglobin A1c was assayed using high-performance liquid chromatography and was expressed as a National Glycohemoglobin Standardization Program unit. Glomerular filtration rate (GFR) was estimated using the Japanese Society of Nephrology equation: estimated GFR (eGFR) = $194 \times \text{Cre}^{-1.094} \times \text{age}^{-0.287}$ (mL/min/1.73 m^2). For women, the eGFR was multiplied by a correction factor of 0.739. Urinary albumin and creatinine concentrations were determined using early morning spot urine. A mean value for urine albumin excretion was determined from three urine collections.

Definition of Metabolic Syndrome

The diagnosis of MetS was determined by a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; the National Heart, Lung and Blood Institute; the American Heart Association; the World Heart Federation; the International Atherosclerosis Society; and the International Association for the Study of Obesity, using the criteria for Asians[10]. The patients were diagnosed with the presence of MetS when three or more of the following criteria were present: elevated blood pressure (systolic blood pressure ≥130 mmHg and diastolic blood pressure ≥85 mmHg and/or medication for hypertension, in both sexes); hyperglycemia (fasting plasma glucose ≥5.6 mmol/L and/or medication for diabetes, in both sexes); hypertriglyceridemia (serum triglycerides ≥1.70 mmol/L and/or medication for dyslipidemia, in both sexes); low HDL cholesterol levels (serum HDL cholesterol <1.03 mmol/L in men and <1.29 mmol/L in women); and abdominal obesity (waist circumference ≥90 cm in men and ≥80 cm in women). Because waist measurements were not available for the entire study sample, we submitted a BMI of ≥25 kg/m^2, which has been proposed as a cut-off for the diagnosis of obesity in Asian people[11], for all patients as an index of obesity.

Statistical Analysis

The statistical analyses were carried out using the JMP version 10.0 software (SAS Institute Inc., Cary, NC, USA) and a *P*-value <0.05 was considered statistically significant. The mean or frequencies of potential confounding variables were calculated. Continuous variables were presented as the mean ± standard deviation. Because urine albumin excretion showed a skewed distribution, logarithmic transformation was carried out before carrying out unpaired Student's *t*-tests. The relationships between carbohydrate energy/total energy (C/E ratio) and animal protein energy/total energy (AP/E ratio), vegetable protein energy/total energy (VP/E ratio) or dietary acid load were examined by Pearson's correlation analyses. Differences in categorical and continuous variables across two subgroups according to the median of AP/E ratio, VP/E ratio or dietary acid load scores were assessed by Chi square test analyses and unpaired Student's *t*-tests. The associations between the two subgroups and prevalence of MetS were analyzed by logistic regression analyses. The logistic regression analyses were adjusted for age, sex, serum uric acid and creatinine, total energy intake, carbohydrate intake, and sodium intake, which were known to be risk factors of MetS. Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

RESULTS

The characteristics of patients are shown in Table 1. There were 149 patients, aged 65.7 ± 9.3 years. Mean total energy intake, carbohydrate intake, animal protein intake and vegetable protein intake were 1821.5 kcal, 248.8 g, 36.1 g and 31.1 g, respectively. The median of AP/E ratio, VP/E ratio, C/E ratio and PRAL and NEAP scores were 7.7%, 6.5%, 56.9%, 6.9 mEq/day or −8.7 mEq/day, respectively. Carbohydrate energy/total energy was negatively correlated with AP/E ratio, PRAL or NEAP score, and was positively correlated with VP/E ratio (Figure 1). The subgroup of patients with higher AP/E ratio was associated with age, sodium intake, C/E ratio, protein energy/total energy, AP/E ratio, fat energy/total energy, animal fat intake energy/total energy, PRAL or NEAP score (Table 2). The subgroup of patients with lower VP/E ratio was associated with sex, prevalence of MetS, total energy intake, C/E ratio, VP/E ratio, fat energy/total energy, animal fat intake energy/total energy, PRAL or NEAP score. In addition, the subgroup of patients with higher PRAL score was associated with sex, LDL cholesterol, triglycerides, eGFR, prevalence of MetS, total energy intake, sodium intake, C/E ratio, AP/E ratio, VP/E ratio, fat energy/total energy, animal fat intake energy/total energy or NEAP score (Table 2). The subgroup of patients with higher NEAP score was associated with sex, triglycerides, prevalence of

Table 1 | Clinical characteristics of patients

n	149
Age (years)	65.7 ± 9.3
Sex (male/female)	77/72
Body mass index (kg/m^2)	24.0 ± 4.2
Systolic blood pressure (mmHg)	129.6 ± 14.6
Diastolic blood pressure (mmHg)	72.4 ± 10.4
HbA1c (%)	6.9 ± 0.9
Total cholesterol (mmol/L)	4.8 ± 0.8
High-density lipoprotein cholesterol (mmol/L)	1.6 ± 0.4
Low-density lipoprotein cholesterol (mmol/L)	2.6 ± 0.8
Triglycerides (mmol/L)	1.5 ± 1.0
Uric acid (μmol/L)	298.4 ± 78.9
Estimated glomerular filtration rate (mL/min/1.73 m^2)	77.9 ± 21.7
Urinary albumin excretion (mg/g creatinine)	46.9 ± 94.1
No. metabolic risk factors	2.5 ± 2.0
Metabolic syndrome (–/+)	82/67
Insulin treatment (–/+)	116/33
Antihypertensive drugs (–/+)	79/70
Statins (–/+)	91/58
Total energy intake (kcal)	1821.5 ± 400.1
Carbohydrate intake (g)	248.8 ± 60.9
Protein intake (g)	67.2 ± 18.5
Animal protein intake (g)	36.1 ± 14.4
Vegetable protein intake (g)	31.1 ± 8.4
Fat intake (g)	53.3 ± 20.2
Animal fat intake (g)	23.3 ± 10.5
Vegetable fat intake (g)	29.9 ± 12.9
Sodium intake (g)	10.5 ± 3.5
Carbohydrate energy/total energy (%)	56.9 ± 7.7
Protein energy/total energy (%)	15.3 ± 2.4
Fat energy/total energy (%)	26.9 ± 6.4
Potential renal acid load score (mEq/day)	5.7 ± 11.8
Net endogenous acid production score (mEq/day)	−8.7 ± 0.3

Data are number of patients or mean ± standard deviation. HbA1c, hemoglobin A1c.

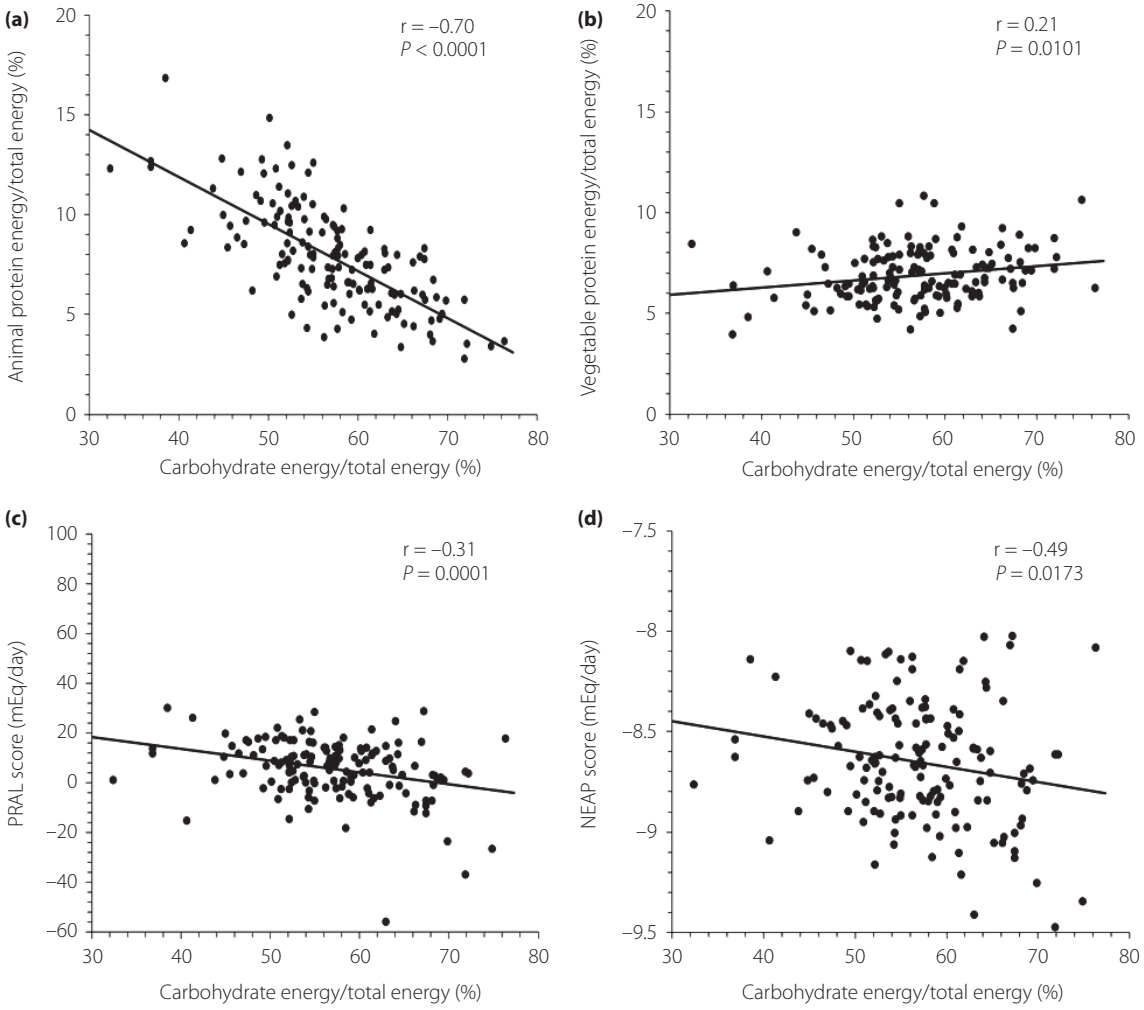

Figure 1 | Relationships of carbohydrate energy/total energy with (a) animal or (b) vegetable protein energy/total energy, or (c) potential renal acid load (PRAL) or (d) net endogenous acid production (NEAP) score.

MetS, VP/E ratio or PRAL score (Table 2). Logistic regression analyses showed that the subgroup of patients with lower VP/E ratio, or higher PRAL or NEAP score was significantly associated with the prevalence of MetS (Table 3). In a multivariate approach, including age, sex, serum uric acid and creatinine, total energy intake, carbohydrate intake and sodium intake, the subgroup of patients with lower VP/E ratio or higher PRAL or NEAP score showed an increased OR for the prevalence of MetS. In logistic regression analyses, animal fat energy/total energy was not a statistically significant risk factor for the prevalence of MetS.

DISCUSSION

In the present study, we found that C/E ratio was negatively correlated with AP/E ratio, PRAL or NEAP score, and was positively correlated with VP/E ratio in patients with type 2 diabetes. Furthermore, the decreased VP/E ratio and increased acid load scores, assessed by PRAL and NEAP scores, were associated with the prevalence of MetS after adjusting for confounding variables.

Evidence has been accumulating to suggest that low carbohydrate diets and their combination with high-protein diets are potent in bodyweight loss[12], and might have favorable effects on the risk markers of cardiovascular disease in the short term[13]. However, recent reports suggested that low-carbohydrate diets were associated with a significantly higher risk of all-cause mortality in the long term[14]. One of the possible explanations for the association might be increased intake of protein based on animal sources and reduced intake of protein based on vegetable sources[15]. In addition, a low-carbohydrate diet has a tendency to result in a reduced intake of fruits and fiber, and an increased intake of protein based on cholesterol, saturated fat and animal sources[16,17]. The present study also provided equally suggestive evidence that low-carbohydrate intake was associated with increased animal protein intake and decreased vegetable protein intake in patients with type 2 diabetes.

Table 2 | Clinical characteristics of patients divided into four subgroups according to the median of animal or vegetable protein energy/total energy or potential renal acid load or net endogenous acid production score

	Animal protein energy/total energy			Vegetable protein energy/total energy		
	≤7.7%	≥7.8%	*P*	≤6.5%	≥6.6%	*P*
n	74	75	–	74	75	–
Age (years)	63.9 ± 8.8	67.5 ± 9.5	0.0183	66.4 ± 10.2	65.0 ± 8.4	0.3687
Sex (male/female)	40/34	37/38	0.5642	48/26	29/46	0.0014
Body mass index (kg/m^2)	24.1 ± 3.9	23.9 ± 4.5	0.7010	24.5 ± 4.0	23.5 ± 4.3	0.1203
Systolic blood pressure (mmHg)	131.5 ± 14.4	127.7 ± 14.5	0.1107	130.8 ± 14.6	128.4 ± 14.5	0.3191
HbA1c (%)	7.0 ± 1.1	6.7 ± 0.7	0.0935	7.0 ± 1.1	6.8 ± 0.7	0.2008
Low-density lipoprotein cholesterol (mmol/L)	2.6 ± 0.7	2.6 ± 0.8	0.6041	2.7 ± 0.7	2.5 ± 0.8	0.3556
Triglycerides (mmol/L)	1.5 ± 0.9	1.5 ± 1.0	0.7747	1.7 ± 1.0	1.4 ± 0.9	0.0511
Estimated glomerular filtration rate (mL/min/1.73 m^2)	79.8 ± 22.6	75.9 ± 20.8	0.2718	75.7 ± 22.4	80.0 ± 20.9	0.2288
Metabolic syndrome (–/+)	40/34	42/33	0.8113	30/44	52/23	0.0004
Total energy intake (kcal)	1810.5 ± 438.6	1832.4 ± 360.7	0.7397	1902.2 ± 360.9	1741.9 ± 422.7	0.0139
Carbohydrate energy/total energy (%)	61.3 ± 6.2	52.7 ± 6.7	<0.0001	55.5 ± 7.5	58.4 ± 7.8	0.0247
Protein energy/total energy (%)	13.6 ± 1.6	16.9 ± 1.9	<0.0001	15.0 ± 2.3	15.6 ± 2.5	0.1039
Animal protein energy/total energy (%)	5.8 ± 1.2	9.9 ± 1.8	<0.0001	8.2 ± 2.5	7.5 ± 2.4	0.0809
Vegetable protein energy/total energy (%)	7.0 ± 1.3	6.7 ± 1.2	0.1729	5.8 ± 0.6	7.9 ± 0.9	<0.0001
Fat energy/total energy (%)	24.3 ± 5.5	29.5 ± 6.2	<0.0001	28.2 ± 6.2	25.6 ± 6.4	0.0122
Animal fat energy/total energy (%)	8.7 ± 2.7	14.1 ± 3.7	<0.0001	12.5 ± 4.4	10.3 ± 3.6	0.0013
Vegetable fat energy/total energy (%)	14.1 ± 4.0	14.9 ± 4.9	0.2864	14.1 ± 4.2	15.0 ± 4.7	0.2615
Sodium intake (g)	9.6 ± 3.2	11.5 ± 3.5	0.0009	10.3 ± 3.0	10.7 ± 3.9	0.4543
Potential renal acid load score (mEq/day)	3.1 ± 11.2	8.2 ± 11.9	0.0079	9.4 ± 8.8	2.0 ± 13.2	0.0001
Net endogenous acid production score (mEq/day)	–8.7 ± 0.3	–8.6 ± 0.3	0.0235	–8.6 ± 0.3	–8.7 ± 0.3	0.0049
	PRAL score			NEAP score		
	≤6.9 mEq/day	≥7.0 mEq/day	*P*	≤–8.7 mEq/day	≥–8.6 mEq/day	*P*
n	74	75	–	74	75	–
Age (years)	66.2 ± 8.8	65.2 ± 9.8	0.5479	66.4 ± 9.1	65.0 ± 9.6	0.3324
Sex (male/female)	29/45	48/27	0.0024	31/44	46/29	0.0176
Body mass index (kg/m^2)	23.7 ± 4.3	24.3 ± 4.1	0.3590	23.8 ± 4.3	24.2 ± 4.1	0.4742
Systolic blood pressure (mmHg)	130.7 ± 16.0	128.4 ± 13.0	0.3358	129.9 ± 15.7	129.2 ± 13.4	0.7556
HbA1c (%)	6.9 ± 0.9	6.8 ± 0.9	0.8540	6.9 ± 0.9	6.9 ± 0.9	0.9612
Low-density lipoprotein cholesterol (mmol/L)	2.5 ± 0.7	2.7 ± 0.8	0.0474	2.5 ± 0.7	2.7 ± 0.8	0.1437
Triglycerides (mmol/L)	1.3 ± 0.7	1.7 ± 1.1	0.0257	1.3 ± 0.7	1.7 ± 1.2	0.0046
Estimated glomerular filtration rate (mL/min/1.73 m^2)	81.5 ± 21.4	74.3 ± 21.6	0.0429	79.8 ± 19.6	76.0 ± 23.6	0.2902
Metabolic syndrome (–/+)	49/25	33/42	0.0064	50/24	32/43	0.0023
Total energy intake (kcal)	1706.9 ± 405.8	1934.5 ± 362.7	0.0004	1786.8 ± 424.2	1855.7 ± 374.4	0.2949
Carbohydrate energy/total energy (%)	59.0 ± 8.0	54.9 ± 6.9	0.0009	58.0 ± 7.8	55.9 ± 7.6	0.0998
Protein energy/total energy (%)	14.9 ± 2.6	15.6 ± 2.2	0.0703	15.4 ± 2.5	15.2 ± 2.4	0.7260
Animal protein energy/total energy (%)	7.3 ± 2.5	8.4 ± 2.4	0.0069	7.7 ± 2.5	8.0 ± 2.5	0.5116
Vegetable protein energy/total energy (%)	7.1 ± 1.2	6.5 ± 1.3	0.0046	7.1 ± 1.2	6.5 ± 1.3	0.0061
Fat energy/total energy (%)	25.6 ± 6.4	28.2 ± 6.2	0.0127	26.3 ± 6.3	27.5 ± 6.5	0.2689
Animal fat energy/total energy (%)	10.2 ± 3.9	12.6 ± 4.1	0.0004	10.9 ± 3.9	11.9 ± 4.4	0.1780
Vegetable fat energy/total energy (%)	14.6 ± 4.5	14.5 ± 4.4	0.9066	14.6 ± 4.6	14.5 ± 4.3	0.9866
Sodium intake (g)	9.9 ± 3.9	11.1 ± 2.9	0.0417	10.6 ± 3.9	10.4 ± 3.0	0.7821
Potential renal acid load score (mEq/day)	–3.0 ± 10.0	14.2 ± 5.5	<0.0001	–2.7 ± 10.3	13.9 ± 6.0	<0.0001
Net endogenous acid production score (mEq/day)	–8.9 ± 0.2	–8.4 ± 0.2	<0.0001	–8.9 ± 0.2	–8.4 ± 0.2	<0.0001

Data are number of patients or mean ± standard deviation. HbA1c, hemoglobin A1c; NEAP, net endogenous acid production; PRAL, potential renal acid load.

Table 3 | Odds ratios for prevalence of metabolic syndrome (logistic regression) according to the median of animal or vegetable protein energy/total energy or potential renal acid load or net endogenous acid production score

		OR (95% CI)	*P*
Animal protein energy/total energy			
	≤7.7% (*n* = 74)	≥7.8% (*n* = 75)	
Unadjusted OR	1 (Reference)	0.92 (0.48–1.76)	0.8113
Adjusted OR*	1 (Reference)	1.17 (0.52–2.60)	0.6978
Vegetable protein energy/total energy			
	≤6.5% (*n* = 74)	≥6.6% (*n* = 75)	
Unadjusted OR	1 (Reference)	0.30 (0.15–0.59)	0.0005
Adjusted OR*	1 (Reference)	0.28 (0.12–0.66)	0.0038
PRAL score			
	≤6.9 mEq/day (*n* = 74)	≥7.0 mEq/day (*n* = 75)	
Unadjusted OR	1 (Reference)	2.49 (1.28–4.84)	0.0083
Adjusted OR*	1 (Reference)	2.22 (1.04–4.83)	0.0384
NEAP score			
	≤–8.7 mEq/day (*n* = 74)	≥–8.6 mEq/day (*n* = 75)	
Unadjusted OR	1 (Reference)	2.79 (1.43–5.46)	0.0029
Adjusted OR*	1 (Reference)	2.61 (1.25–5.55)	0.0098

*Adjusted for age, sex, serum uric acid and creatinine, total energy intake, carbohydrate intake and sodium intake. CI, confidence interval. NEAP; net endogenous acid production; OR, odds ratio; PRAL, potential renal acid load.

MetS is defined by the clustering of cardiovascular risk factors, including hyperglycemia, hypertension, visceral obesity and dyslipidemia. MetS is also related to cardiovascular disease, which is the main cause of morbidity and mortality[18]. We showed that decreased vegetable protein intake was associated with the prevalence of MetS. In the PREMIER study of 810 participants, intake of vegetable protein was inversely associated with blood pressure in cross-sectional analyses[19]. In addition, a previous study reported that dietary vegetable protein intake improved metabolic features and reduced the pro-inflammatory status in obese subjects[20]. It is difficult to assess the effect of vegetable protein interventions compared with animal protein interventions, because the former includes more polyunsaturated fatty acid and fiber, whereas the latter includes more saturated fatty acid, and these of course are fundamental components of these two different protein sources. Although no studies, to our knowledge, have examined just the effect of the protein *per se*, this is not relevant to normal consumption of these protein sources.

In general, a high intake of fat has been shown to predict development of glucose intolerance in a group of healthy subjects[21,22]. Then, we analyzed the relationship between animal fat energy/total energy and the prevalence of MetS. However, in logistic regression analyses, animal fat energy/total energy was not a statistically significant risk factor for the prevalence of MetS in the present study.

The PRAL and NEAP scores are frequently used to estimate dietary acid load in epidemiological studies. The PRAL score considers the intestinal absorption rates of contributing nutrient ionic balances for protein, potassium, calcium and magnesium, and the dissociation of phosphate at pH 7.4. A positive PRAL value reflects an acid-forming potential, whereas a negative PRAL value reflects a base (or alkaline)-forming potential. The PRAL score was calculated using protein, potassium, calcium, magnesium and phosphorus intake from the diet. The NEAP score was developed by using chemical analysis of experimentally controlled diets and diet composition tables. The NEAP score was calculated from the dietary acid load from dietary intakes of protein and potassium, but did not contain the acid load of other nutrients. Therefore, dietary acid load was assessed by PRAL and NEAP scores in the present study. Our present finding, that increased dietary acid load was associated with the prevalence of MetS, was in line with the results of previous studies. Previous studies reported that high dietary acid load was associated with hypertension and type 2 diabetes[6,23]. This mechanism was supported by cross-sectional studies showing that dietary acid load was positively associated with insulin resistance[24,25]. Furthermore, chronic metabolic acidosis, which could lead to reduced insulin sensitivity, might be caused by an acidogenic diet over time[26]. Other possible mechanisms are as follows. A high dietary acid load causes compensatory increases in renal ammoniagenesis and acid excretion[27]. Although this is favorable in the short term for controlling acid-based homeostasis, it causes a decline in renal function and might elevate blood pressure in the long term[28]. In addition, diet-induced metabolic acidosis[29–31] could elevate blood pressure[32,33], probably by elevating cortisol production[29], elevating calcium excretion[34] or declining citrate excretion[35].

The present study had several limitations that require consideration. First, this study was a cross-sectional design, which did not permit the determination of causality. Thus, further studies are required to better assess the relationships between vegetable protein intake or dietary acid load and MetS. Second, to collect dietary data, other reports used a semiquantitative dietary assessment questionnaire (i.e. DHQ). The misreporting of dietary intake, especially by obese individuals, is an important problem related to self-report dietary assessment methods[36]. However, at least for dietary protein, energy adjustment seems to cancel BMI-dependent misreporting[37]. Third, the lack of waist circumference data weakens the definition of MetS. However, BMI of ≥25 kg/m^2 was reported as a cut-off for the diagnosis of obesity in Asian people[11], and was validated previously[38]. Finally, the study population consisted of Japanese men and women, therefore, it is uncertain whether these findings are generalized in other ethnic groups.

In conclusion, the present study showed that carbohydrate intake was negatively correlated with animal protein intake or dietary acid load, and was positively correlated with vegetable protein intake in patients with type 2 diabetes. Furthermore,

decreased vegetable protein intake and increased dietary acid load were associated with the prevalence of MetS. Further research to elucidate the apparent influence of vegetable protein intake and dietary acid load associated with low carbohydrate intake on metabolic risk factors is required in patients with type 2 diabetes.

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