

Inverse relationship between urinary markers of animal protein intake and blood pressure in Chinese: results from the WHO Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study

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Background	This issue of the relationship between animal protein intake and blood pressure (BP) is unsolved. We examined the associations between urinary 3-methylhistidine (3MH) excretion (a biological marker of animal protein intake) and BP in 11 Chinese population samples (Urumqi, Altai, Lhasa, Tulufan, Hetian, Guiyang, Guangzhou, Shanghai, Beijing, Shijiazhuang and Taipei).
Methods	This was a multi-centre cross-sectional study. In each centre, 100 men and 100 women aged 48–56 years were selected randomly from the general population. 3-methylhistidine in 24-hour (24-h) urine collections was measured by an Amino Acid Analyzer (Hitachi 835, Ibaragi, Japan). The total study sample included 966 men and 1025 women. Subjects who failed to collect complete 24-h urine samples were excluded in data analyses regarding associations between 3MH and BP.
Results	The results showed that: (1) for within-centre analyses of individuals, the 3MH and 3MH to creatinine ratios (3MH:cre) were significantly and negatively associated with BP and hypertension. These associations remained significant after adjustment for age, sex, sodium to potassium ratio, body mass index, calcium and magnesium. The pooled regression coefficients (SE) of systolic blood pressure (SBP) and diastolic blood pressure (DBP) on 3MH were –0.020 (0.01) and –0.018 (0.01), and of SBP and DBP on 3MH:cre were –0.022 (0.01) and –0.016 (0.01), respectively. Subjects with lower 3MH excretion had higher relative risks of hypertension than those who had higher 3MH excretion. (2) In cross-centre analyses, mean SBP and DBP were significantly and negatively associated with the mean 3MH:cre across the 11 population samples ($R^2 = 0.56$, $P < 0.01$).
Conclusion	The results provide strong evidence that animal protein intake is associated inversely with BP in Chinese populations.
Keywords	Animal protein intake, blood pressure, Chinese
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There is continuing interest in links between dietary factors and blood pressure (BP) levels. The individual factors that have been the subject of most intensive investigation are salt (sodium), potassium, calcium, magnesium and alcohol. About 20 years ago, Japanese investigators found evidence of an inverse relationship between dietary protein (particularly animal protein) and BP. This came from two sources, namely feeding studies in spontaneously hypertensive rats, and cross-sectional epidemiological studies in Japanese farmers.^{1–4} At around the same time, the Honolulu Heart Disease Study reported an inverse

association between dietary protein and BP among Japanese-American men living in Hawaii.⁵ However, not all studies, observational or intervention, have supported this relationship.⁶ For instance, Sacks and colleagues reported that an increase in animal protein intake increased systolic blood pressure (SBP) in a 4-week intervention trial of 21 men and women aged 20–55 years.⁷ In the present study, we examined the association between BP and animal protein intake (assessed by measuring urinary excretion of 3-methylhistidine [3MH]) in 1991 individuals from 11 Chinese co-operative study centres; the data were collected from 1985 to 1999 with a standard study design and the same research methods.

Subjects and Methods

The research is a component part of the World Health Organization (WHO) Cardiovascular Disease and Alimentary Comparison (CARDIAC) Study.^{8–10} The standard protocol of the WHO-CARDIAC Study was used in all 11 Chinese study centres, located in the different regions across the country (Figure 1). Details of the WHO-CARDIAC Study design and methods have been published elsewhere.⁸ Briefly, it is a multi-centre cross-sectional study, and in each study centre, 100 men and 100 women aged 48–56 years (mean age close to 52 years) were selected randomly from the general population and invited to attend a free physical examination. A 15-ml blood sample and a 24-hour (24-h) urine sample were collected.

Blood pressure in the sitting position was measured using a standardized automated sphygmomanometer (Khi machine, VINE Co., Ltd., Tokyo).¹¹ The measurement was repeated three times. The lowest values of the three readings were taken as the

SBP and diastolic BP (DBP). A structured questionnaire was used for face-to-face interviews. It included items on demographic data, lifestyle factors and medical history. Twenty-four-hour urine samples were collected using a standard Aliquot Cup that allowed participants to collect an exact portion of each voiding of urine.¹² All blood and urine samples were analysed centrally at the WHO Collaborating Center for Research on Primary Prevention of Cardiovascular Diseases, Izumo, Japan. In 1993 this centre was transferred to the Graduate School of Human and Environmental Studies, Kyoto University, Japan, but all the methods and the main staff remained the same.

Measurements included in the present report were: age (years), weight (kg), height (cm), body mass index (BMI, kg/m^2), BP (mmHg), 24-h urinary excretion of 3MH ($\mu\text{mol}/\text{day}$), creatinine (cre, mg/day), sodium (Na, mmol/day), potassium (K, mmol/day), calcium (Ca, mg/day) and magnesium (Mg, mg/day). 3-methylhistidine, a biological marker of dietary animal protein intake,^{13–19} was measured using an Amino Acid Analyzer (Hitachi 835, Ibaragi, Japan).⁸ Sodium and K were measured by flame photometry, creatinine by Jaffe's method, Ca by the O-Cresolphthalein Complexone (OCPC) method and Mg by the Xylidyl Blue method.⁸ The 24-h urine collections were assessed for completeness using creatinine excretion in relation to weight (i.e. the creatinine coefficient = creatinine [mg/day]/body weight [kg]). Creatinine coefficients of 14.4 to 33.6 in men and 10.8 to 25.2 in women were classified as indicating an acceptable 24-h urine collection.⁸ Status of anti-hypertensive drug use was classified by the survey questionnaire. The 3MH values were also expressed in relation to urinary creatinine excretion (3MH:cre, $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$), to remove endogenous muscle degradation effects on 3MH excretion.^{15,18,19}



Figure 1 Location of the 11 co-operating centres

Statistical analysis

Pearson correlation analyses were initially used for an overall estimate of BP in relation to 3MH, 3MH:cre and other factors, with adjustment for age and sex. Subjects who failed to complete 24-h urine collections and who were on anti-hypertensive medications were excluded from the analyses of the associations between urinary variables and BP (when BP was taken as a continuous variable in Pearson correlation and linear regression analyses). This was done to minimize possible problems due to incorrect 24-h urine samples, or changes in BP values in subjects receiving anti-hypertensive treatment. Further analyses were done for all subjects by classifying them as two groups: normotensive and hypertensive subjects (when BP was taken as a binary variable in logistic regression models). This was done to generalize the results in the study populations. Hypertension was defined as those who had SBP \geq 140 mmHg, or DBP \geq 90 mmHg or those on anti-hypertensive medications.

In within-centre analysis of individuals, regression coefficients of BP on 3MH and 3MH:cre were estimated using multiple linear regression analysis for each centre, with adjustment for age- and sex (Model 1), and further adjustment for Na/K ratio (Model 2), BMI, Ca and Mg (Model 3). The regression coefficients of each centre were then averaged (pooled) to obtain an overall estimate for individuals of the 11 study centres. In the pooled computation, each centre coefficient was weighted by the inverse of its variance. This procedure was done to minimize the variance of the pooled coefficients.^{20–22} Relative risks of less protein intake associated with hypertension were estimated using logistic regression models, taking hypertension as a dependent variable (0, 1 for none and yes), and 3MH and other covariates as independent variables.

In cross-centre analysis, multi-adjusted mean BP and 3MH:cre values were estimated using general linear models.^{23,24} Simple linear regression analysis was then used to examine the across-centre associations between 3MH:cre and BP across the 11 centres.

All data analyses were done initially for men and women separately, but because associations of BP with 3MH and 3MH:cre were similar for men and women, the results presented here are for the two sexes combined after adjustment for age, sex and other factors. SPSS for PC software, version 8.0 (SPSS Inc., Chicago, IL) was used in data analyses.²⁴ Two-tailed *P*-values < 0.05 were considered to indicate statistical significance.

Results

Descriptive statistics

As shown in Table 1, 1991 participants (966 men and 1025 women) aged 48–56 years were included in the total sample. The mean age (SD) was 51.8 (1.7) years in men and 51.7 (1.5) years in women (*P* > 0.05). The proportion of completed 24-h urine collections was 64.9% in men and 62.1% in women (*P* > 0.05). In the sub-sample, subjects had slightly lower mean age, BP and BMI than those in the total sample. There were no significant differences in mean age, SBP, DBP, BMI or education levels between those with and without complete 24-h urine collection. Mean 24-h urinary creatinine and 3MH excretion levels were higher in men than in women. However, mean 3MH:cre was lower in men than in women. This was because men had higher creatinine excretion levels than women.

Table 1 Descriptive statistics for the total and sub-samples in the 11 study centres

	Men	Women
	Mean (SD)	Mean (SD)
Total sample (No.)	966	1025
Age (years)	51.8 (1.7)	51.7 (1.5)
SBP ^a (mmHg)	123.1 (20.3)	122.1 (22.3)
DBP ^b (mmHg)	74.4 (13.4)	72.4 (13.9)
BMI ^c (kg/m ²)	22.7 (4.1)	22.8 (4.0)
Anti-HT ^d drug therapy (%)	8.1	10.5
Completed 24-h urine ^e (%)	64.9	62.1
Sub-sample (No.)	572	563
Age (years)	51.7 (1.6)	51.6 (1.6)
SBP (mmHg)	121.9 (17.9)	121.7 (21.2)
DBP (mmHg)	73.1 (12.4)	71.6 (13.1)
BMI (kg/m ²)	22.3 (3.6)	22.3 (3.6)
Creatinine (mg/day)	1.3 (0.3)	0.8 (0.2)
3MH ^f (μmol/day)	222.5 (95.8)	171.8 (79.4)
3MH:cre ^g	177.2 (64.3)	204.0 (84.5)
Na ^h (mmol/day)	205.1 (84.8)	175.4 (83.9)
K ⁱ (mmol/day)	38.0 (21.8)	32.0 (19.8)
Ca ^j (mg/day)	194.0 (123.5)	151.1 (92.5)
Mg ^k (mg/day)	121.8 (72.0)	90.8 (49.4)

Sub-sample included those with complete 24-h urine collections and without anti-hypertensive drug therapy.

^a Systolic blood pressure.

^b Diastolic blood pressure.

^c Body mass index.

^d Subjects with anti-hypertensive drug therapy

^e Subjects with complete 24-h urine collections.

^f 3-methylhistidine.

^g Ratio of 3-methylhistidine (μmol/day) to creatinine (g/day).

^h Sodium.

ⁱ Potassium.

^j Calcium.

^k Magnesium.

Table 2 shows that age- and sex-adjusted SBP and DBP were significantly negatively correlated with 3MH and 3MH:cre (*P* < 0.05 or *P* < 0.01). The SBP and DBP were positively associated with Na and BMI (*P* < 0.01). Significant positive associations between SBP and Ca, and between DBP and K were observed (*P* < 0.05).

Within-centre analysis of individuals

Table 3 shows that after adjustment for age and sex (model 1), the pooled regression coefficients (βs) of SBP and DBP on 3MH and 3MH:cre were −0.019 (0.01) and −0.017 (0.01), and −0.021 (0.01) and −0.015 (0.01), respectively. These negative βs remained significant after adjustment for Na/K ratio (Model 2), and for BMI, Ca and Mg (Model 3).

Table 4 shows that subjects who had lower 3MH excretion and 3MH:cre ratios had a higher risk of hypertension as compared to those with higher 3MH excretion and 3MH:cre ratios. After adjustment for age, sex, Na/K ratio, BMI, Ca and Mg (Model 3), these relative risks were 1.68 (95% CI: 1.11–2.53) in those who had urinary excretion of 3MH < 253 μmol/day, and 2.63 (95% CI: 1.69–4.09) in those with 3MH:cre < 224 μmol/day, respectively.

Table 2 Pearson correlation coefficients matrix (adjusted for age and sex) of blood pressure in relation to 3-methylhistidine (3MH), 3MH:creatinine ratio (3MH:cre), and other factors in subjects with completed 24-h urine collection and without anti-hypertensive drug therapy^{a,b}

	DBP	3MH	3MH:cre	Na	K	Ca	Mg	BMI
SBP	0.79**	-0.10*	-0.16**	0.19**	0.04	0.09*	0.01	0.20**
DBP	1	-0.19**	-0.27**	0.22**	0.11*	0.05	-0.03	0.30**
3MH		1	0.78**	0.07	0.03	0.19**	0.06	-0.09*
3MH:cre			1	0.02	0.22**	0.09**	-0.04	-0.18**
Na				1	0.35**	0.39**	0.37**	0.17**
K					1	-0.01	0.33**	0.30**
Ca						1	0.52**	0.02
Mg							1	0.10**

^a SBP and DBP, systolic and diastolic blood pressure (mmHg). 3MH: 3-methylhistidine ($\mu\text{mol/day}$). 3MH:cre, ratio of 3MH to creatinine (mg/day). BMI, body mass index (kg/m^2). Na, sodium excretion (mmol/day). K, potassium (mmol/day). Ca, calcium (mg/day). Mg, magnesium (mg/day).

^b * $P < 0.05$, ** $P < 0.01$.

Table 3 Within-centre regression analyses of blood pressure in relation to urinary 3-methylhistidine (3MH) excretion: the pooled results for the 11 study centres

Pooled associations ^{a,b}	Systolic blood pressure			Diastolic blood pressure		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
3MH ($\mu\text{mol/day}$)						
Pooled (average) β	-0.019	-0.021	-0.020	-0.017	-0.017	-0.018
Pooled (average) SE	0.011	0.011	0.012	0.008	0.009	0.007
Z-score	1.73	1.91	1.67	2.13	1.89	2.57
P	0.042	0.028	0.048	0.02	0.03	0.01
3MH:cre^c						
Pooled (average) β	-0.021	-0.021	-0.022	-0.015	-0.015	-0.016
Pooled (average) SE	0.010	0.011	0.010	0.006	0.007	0.006
Z-score	2.02	1.91	2.13	2.43	2.14	2.48
P	0.02	0.03	0.02	0.01	0.02	0.01

^a The pooled regression coefficients (β) were the average values for individuals from all 11 samples. In this computation, the coefficient for each sample was weighted by the inverse of its variance.

^b Model 1: Adjusted for age (years), sex (0 and 1 for men and women). Model 2: Adjusted for age (years), sex (0 and 1 for men and women) and ratio of sodium to potassium (Na/K). Model 3: Adjusted for age (years), sex (0 and 1 for men and women), Na/K, body mass index (kg/m^2), calcium (mg/day) and magnesium (mg/day).

^c Ratio of 3MH to creatinine (mg/day).

Table 4 Relative risk of subjects with less 3-methylhistidine (3MH) excretion associated with hypertension^{a,b}

	Model 1			Model 2			Model 3		
	OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P
3MH ($\mu\text{mol/day}$)									
≥ 253	1			1			1		
< 253	1.33	(0.91–1.95)	0.14	1.35	(0.93–2.04)	0.11	1.68	(1.11–2.53)	0.01
3MH:cre ratio^c									
≥ 224	1			1			1		
< 224	2.72	(1.78–4.12)	< 0.01	2.81	(1.84–4.30)	< 0.01	2.63	(1.69–4.09)	< 0.01

^a Relative risks were estimated by odds ratios (OR) using logistic regression analysis. In the analysis, hypertension (0,1 for none and yes) was the dependent variable, and 3MH and 3MH:cre were independent variables.

^b Model 1: Adjusted for age (years), sex (0 and 1 for men and women). Model 2: Adjusted for age (years), sex (0 and 1 for men and women) and ratio of sodium to potassium (Na/K). Model 3: Adjusted for age (years), sex (0 and 1 for men and women), Na/K, body mass index (kg/m^2), calcium (mg/day) and magnesium (mg/day).

^c Ratio of 3MH to creatinine (mg/day).

Cross-centre analysis of 11 study centres

By using linear regression analyses, Figure 2 shows a significant inverse correlation between BP (SBP and DBP) and 3MH:cre across the 11 study centres (Altai [AT], Urumqi [UR], Tulufan

[TL], Hetian [HT], Lhasa [LS], Guiyang [GY], Guangzhou [GZ], Beijing [BJ], Shijiazhuang [SJ], Shanghai [SH] and Taipei [TP]). The 56% variance in SBP and 51% in DBP could be explained by the variance in 3MH:cre (both, $P = 0.01$).

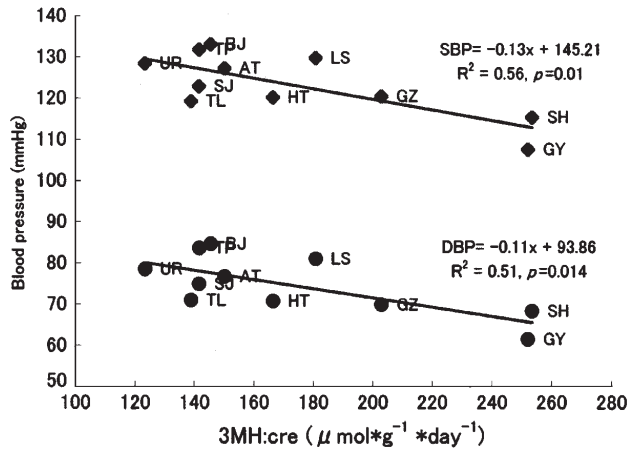


Figure 2 Regression of systolic blood pressure (SBP) and diastolic blood pressure (DBP) on 3-methylhistidine:creatinine (3MH:cre) across the 11 centres (values adjusted for age, sex, sodium/potassium, body mass index, calcium and magnesium). (For abbreviations of centres see text)

Discussion

The present results provide strong evidence of an inverse association between BP and dietary animal protein intake, assessed by the measurement of 3MH excretion. Generally, these findings are consistent with previous observations.^{4–6,25–28} However, the present study advanced previous analysis methods and demonstrated this inverse association in a representative sample of 11 Chinese population samples. The two main strengths of the study were (1) a standardized study design was used in all 11 centres.⁸ To collect 24-h urine, a new device, the Aliquot Cup, was used. This has been confirmed as a reliable device and used all over the world in the WHO-CARDIAC Study.^{8,9,12} (2) All urine samples were analysed centrally, with a standard quality control system, at the WHO-CARDIAC Study Center in Japan. This centralized analysis has the advantage of avoiding inter-laboratory differences.

Detailed methodological assessment of 3MH as an index of animal protein has been reported in several studies.^{13–19} In brief, 3MH is a special component of actin and myosin in muscles. Upon breakdown of the muscle proteins, 3MH is not re-utilized for protein synthesis or metabolized oxidatively, but is rapidly and quantitatively excreted in the urine.^{17–19} Urinary 3MH has been recognized as a reliable marker of protein catabolism.^{8,13–19}

Epidemiological observation studies showing an inverse association between animal protein and BP were reported in the early 1980s.^{1,2,4–6} Since then, in addition to the preliminary reports of the WHO-CARDIAC Study in 1990 and 1995,^{9,15} another 10 observation studies have examined associations between dietary protein (total or animal) and BP.^{6,25–30} Eight of the 10 found an inverse association. For example, the Intersalt study of 10 020 men and women, aged 20–59 years from 52 centres in 32 countries worldwide, reported an inverse association between total protein and BP.²⁵ On the other hand, 2 of the 10 studies reported either a positive association of BP with total protein intake,²⁹ or no association.⁶ Some intervention studies in humans reported no associations between dietary protein (vegetable or animal) and BP.⁶ These studies in

humans, however, had several limitations. First, none of them were designed to specifically test the hypothesis that higher dietary protein intake lowers BP. Second, they all were small sample studies, ranging from 13 to 69 subjects, with a wide range of age groups in each study. Third, most of these studies were not randomized trials.⁶

In addition to our reports, two other cross-sectional studies have been carried out in Chinese populations.^{26–28} Initially, Zhou and colleagues found an inverse association between BP and animal protein intake (assessed using 24-h dietary intake recall) in an analysis of a small number of samples (18–20 subjects).²⁷ They followed this up with a larger study of 705 men and women aged 40–59 (farmers and fishermen), and protein intake was assessed using 3-day dietary recall, ratios of urea nitrogen and sulphate to creatinine, and methylhistidine in serum and 24-h urine samples; they again found an inverse association between BP and protein intake.²⁸ He *et al.* also observed an inverse association between BP and protein (assessed using 3-day dietary intake recall) in a sample of 827 men.²⁶ Their study was carried out in three population samples living in a poorer economic area of China (two samples were from ethnic minorities, and the third was of Han people, the ethnic majority in China). It is not clear, however, in either of these papers whether or not subjects without complete 24-h urine collections²⁸ or those with anti-hypertensive medication were included in their data analyses.^{26–28}

It should be noted that, first, few studies gave reports on how they assessed the completeness of 24-h urine collection, although it is well known that complete collection is necessary to obtain reliable measurements of urinary excretions of sodium, potassium, amino acids, etc.^{31–34} Two objective methods have been suggested for this assessment: the creatinine and p-amino benzoic acid check tests.³³ The use of creatinine is based on the assumption that excretion per kg body mass is constant, and this method is often used in clinical and population-based studies because the sample collection procedure and laboratory measurement are easy. The constancy of daily creatinine excretion is, however, still a matter of debate, because several studies indicated that the coefficient of variation of day-to-day creatinine excretion in well-motivated healthy subjects ranged from 5% to 13%.^{32,33} A new method, the p-amino benzoic acid (PABA) test was developed by Bingham *et al.*^{33,34} This test is based on recovery in the urine of three oral 80-mg capsules or tablets of PABA taken with meals. The use of the PABA check test was suggested to be a more sensitive and reliable verification of the completeness of 24-h urine collection than creatinine. We were unable to compare these two methods, since there was no PABA test in our study. Nevertheless, results from our studies and others indicate that if urine is conscientiously collected, creatinine excretion is remarkably constant, and can be used as a reliable marker for the assessment of 24-h urine collection.^{8,12,31,32} In our study, full information on the technique for 24-h urine collections was given in oral and written instructions, and demonstrated carefully to each participant. Second, it is known that subjects with anti-hypertensive medication may have lower BP values because of their treatment. Therefore, the association between BP and risk factors may be underestimated if those with lower, or even 'normal' BP values are not excluded from data analyses. On the other hand, this exclusion may limit the results for generalization in the study

populations, and reduce the power to assess the hypothesis of protein-BP associations. Therefore, the present study further advanced previous analyses by using logistic regression models to estimate the relative risks of subjects associated with hypertension (those taking anti-hypertensive medications were included). It is most interesting that consistent results in the present study were found using different analytical methods (Tables 2,3,4, and Figure 2). Third, covariates and co-linearity issues should be considered in epidemiological studies, because most nutrients are inter-correlated with each other. For example in the present study, increases in potassium, calcium and decreases in BMI were associated with increases in 3MH and/or 3MH:cre, and were also associated with BP (Table 2). This suggests the effects of multi-factorials on BP. To emphasize the importance of 3MH, however, independent associations between 3MH and BP were examined by adjustment for these covariates (Tables 3 and 4). Fourth, we noticed that different studies used different biological markers of protein intake, either specifying animal, vegetable or total proteins.^{6,16,25-30} In the present study, we focused the analyses on the association between animal protein and BP, because this association was much stronger than others (such as total protein-BP association). Furthermore, the findings were consistent with our previous studies and others in Japanese and Chinese population samples.^{1-6,10,15,16,26-28} Whether a particular protein or group of proteins is related to BP needs to be confirmed in further studies and in different populations.

One important limitation of the present study was the relatively lower proportions of subjects who completed 24-h urine collections successfully (64.9% in men and 62.1% in women). Although there were non-significant differences in mean age, BMI and education levels between subjects with and without complete 24-h urine collections, the proportions smoking and alcohol consumption (data available in seven centres only) were lower in subjects with complete 24-h urine collections. However, the main findings of the present study did not change by either adjustment for smoking and alcohol (for a sub-sample of seven centres, data not shown) or without adjustment for the two factors (Tables 3 and 4). Also, as is the case with other cross-sectional studies, a cause-effect association between animal protein and BP cannot be derived from the present study because of the nature of the study design. Nevertheless, keeping these limitations in mind, the observations in this study support the hypothesis of increases in dietary animal protein intake in relation to lower BP.

The mechanisms underlying the inverse associations between protein intake and BP are largely unknown and highly speculative. At present, it is suggested that (1) an increase in protein intake induces increases in renal plasma flow, the glomerular filtration rate and sodium excretion in the short term, and increases in renal size, renal plasma flow and the glomerular filtration rate in the long term.^{6,26,32} (2) Dietary protein makes up cellular ion (sodium, potassium and calcium) channels, which may indirectly influence BP regulation pathways.^{25,30}

Finally, there is a question as to whether an inverse relationship between protein intake and BP is operative over the whole range of protein intakes, or is found more frequently in people whose intake of animal protein is fairly low. The latter includes at least some past studies in Japanese and Chinese, including the present study. For example, our preliminary observations indicated that mean excretions of 3MH and urine nitrogen were lower in the Chinese sample than that in the Caucasian population samples of the CARDIAC Study.^{9,10} To date, there is no comparable report on the protein-BP associations by ethnic population, concerning possible practical and aetiological implications; it will be important to extend the observations in different ethnic groups and in those with different dietary habits in future studies.

In conclusion, the present study provides strong confirmatory evidence that dietary animal protein intake is associated inversely with BP in the Chinese population. Further studies, particularly prospective and intervention studies, are required.

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KEY MESSAGES

- Inverse association.
- Animal protein intake and blood pressure.
- Chinese.
- WHO-CARDIAC Study.

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