

Urea nitrogen concentrations in spot urine, estimated protein intake and blood pressure levels in a Japanese general population

Running head: urea nitrogen in spot urine and blood pressure

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Abstract

Background. An inverse association between total protein intake and blood pressure has been reported in Western countries. Such evidence is limited in the Japanese population, however, whose major protein sources are plants and seafood.

Methods. We conducted a population-based cross-sectional study of 986 men and 1,636 women, aged 40 to 74 years, in a Japanese rural community to examine the association between blood pressure levels and urea nitrogen concentrations in spot urine.

Results. The concentration of urea nitrogen in spot urine, an indicator of total protein intake that was validated by 24-hour urea nitrogen excretion and 24-hour dietary research, was inversely associated with systolic blood pressure levels for men: a 238 mg/dl increase in urea nitrogen concentration was associated with a 2.1 mmHg lower mean systolic blood pressure. For women, a weaker and non-significant inverse association was observed. There was no association between urea nitrogen concentrations and diastolic blood pressure levels in either sex. Total protein intake estimated from spot urine was also inversely associated with systolic blood pressure levels for men: a 19.2 g/day increase in estimated protein intake was associated with a 1.5 mmHg lower mean systolic blood pressure.

Conclusion. A urinary biomarker for total protein intake was inversely associated with systolic blood pressure levels for men in a Japanese general population.

The relationship between protein intake and blood pressure levels has been investigated in large observational and interventional studies [1-7]. These studies showed that dietary intakes of total, animal, and vegetable protein were inversely associated with systolic [1,3-8] and diastolic blood pressure [1,2,5-7] levels. We have recently shown an inverse association between dietary total protein intake and diastolic blood pressure levels [7], while a previous study of 503 Japanese men and 617 Japanese women showed that the ratio of urine nitrogen to creatinine (a urinary biomarker of total protein intake) was positively associated with systolic blood pressure levels [9]. Another Japanese study of 885 men and 414 women showed no association between total protein intake and blood pressure levels, after adjustment for cardiovascular risk factors [10]. Because of these inconsistent findings, we conducted a cross-sectional study to examine the association of total protein intake and blood pressure levels among a larger sample of Japanese men and women.

The amount and composition of protein intake are different for Japanese compared to Westerners. In the Japan National Nutrition Survey of 2004, mean protein intake was 77 g/day for men and 66 g/day for women [11]. In the United States, mean protein intake was 97 g/day and 65 g/day, respectively [12]. The Japanese take 47 percent of their protein from plant foods, 23 percent from seafood, 17 percent from meat and poultry, and 6 percent from dairy foods [11], while Americans take 33 percent, 6 percent, 36 percent and 20 percent, respectively [13].

Our *a priori* hypothesis is that total protein intake is inversely associated with blood pressure levels in a Japanese population. To test our hypothesis, we examined the association by using a urinary biomarker for total protein intake.

Methods

Population and subjects

The survey population was drawn from residents of the Kyowa rural community in Chikusei city, Ibaraki Prefecture, Japan. The census population in the area was 16,778 (8,270 men and 8,508 women) in October 1, 2005. In this community, annual cardiovascular risk surveys have been conducted since 1981. There were 3,244 participants (1,247 men and 1,997 women) in the 2005 survey. Seven individuals were excluded from the study because they refused urinalysis, 32 individuals were excluded because they had high serum creatinine concentrations (≥ 1.4 mg/dL for men and ≥ 1.2 mg/dL for women) or had a history of renal disease, and two individuals were excluded because their height could not be measured due to scoliosis. Furthermore, we excluded 581 individuals because they were out of the age window of 40 to 74 years. Data from 2,622 subjects (986 men and 1,636 women) were used for analysis.

Population surveys

Individuals who participated in the survey collected their urine in 10 ml urine plastic containers. Concentrations of urine components, urine nitrogen, sodium, and potassium were analyzed using an electrolyte analyzer (Hitachi/Roche, Hitachi, Tokyo, Japan).

Arterial systolic blood pressure and fifth-phase diastolic blood pressure were measured by well-trained observers using standard mercury sphygmomanometers on the right arm in the cardiovascular risk survey. Participants were quietly seated at least 5 minutes before measurement. For every participant, blood pressure was measured twice. We used the second measurement values for the analysis.

With regard to potential confounders, body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Height was measured in socks, and weight in light clothing. Every participant was interviewed to determine their usual weekly alcohol consumption in *go* units, a traditional Japanese unit of volume equivalent to 23 grams of ethanol. Smoking status was also determined in the interview together with smoking history and the present daily number of cigarettes smoked. History of hypertension, stroke, coronary heart disease, renal disease, and use of antihypertensive medication were also recorded during the interview.

Validation study

The validity of the estimated urea nitrogen was examined in a sub-sample of 85 men and 140 women who also completed 24-hour urine collection, and in another sub-sample of 80 men and 105 women who underwent 24-hour dietary recall. The concordance rate between quartiles of urinary nitrogen concentration in spot urine and 24-hour urinary nitrogen excretion was 30.5% for men, 36.4% for women, and 34.2% for all subjects. The concordance rate between quartiles of urinary nitrogen concentration in spot urine and total protein intake from dietary research was 32.1% for men, 31.7% for women, and 31.9% for all subjects.

The concordance rate between quartiles of the ratio of urine nitrogen to creatinine and 24-hour urinary nitrogen excretion were 30.5% for men, 28.6% for women and 29.3% for all subjects, and that between quartiles of the ratio of urine nitrogen to creatinine and total protein intake were 22.2%, 34.6% and 29.1%, respectively.

Statistical analysis

Previously, the ratio of urine nitrogen to creatinine was used as a marker of total protein intake [14]. In the present study, however, we found a stronger association of urinary nitrogen concentration with 24-hour urinary nitrogen excretion and total protein intake from dietary research than with the ratio of urinary nitrogen concentration to creatinine. For this reason, we used urinary nitrogen concentration as a marker of total protein intake.

The analysis was based on the sex-specific quartiles of urinary nitrogen concentrations in spot urine. Age-adjusted means and proportions of confounding variables and age-adjusted and multivariable-adjusted mean values of systolic and diastolic blood pressure (according to quartiles of urea nitrogen concentrations) were calculated and tested by analysis of covariance. The mean values of blood pressure levels associated with changes in one standard deviation (1-SD) of urea nitrogen concentrations were calculated using multivariate regression analysis. The confounding variables for adjustment included age (years), BMI (kg/m^2), current use of antihypertensive medication (yes or no), ethanol intake (g ethanol/day), current smoking (yes or no), and urinary concentrations of creatinine, sodium, and potassium (sex-specific quartiles).

Total protein intake was calculated by the Maroni method [15] as follows: [(estimated 24-hour urinary nitrogen excretion + $0.031 \times$ body weight) \times 6.25], where 24-hour urinary urea nitrogen excretion was calculated as follows: [(urinary urea nitrogen concentration/urinary creatinine concentration) \times (24-hour urinary creatinine excretion)]. The formula of 24-hour urinary creatinine excretion was as follows [16]: For men, [$15.1 \times$ (weight) + $7.4 \times$ (height) - $12.6 \times$ (age) - 80], for women, [$8.6 \times$ (weight) + $5.1 \times$ (height) - $4.7 \times$ (age) - 75]. This formula of 24-hour creatinine excretion was applied to 256 Japanese men and 231 Japanese women, and the multiple correlation coefficients between estimated 24-hour creatinine excretion by this formula and measured 24-hour creatinine excretion of 3-5 day urea samples were 0.87 for men and 0.73 for women [16]. We also calculated sodium

intake and potassium intake as follows: [(urinary sodium or potassium concentration/urinary creatinine concentration) \times (24-hour urinary creatinine excretion)].

We used SAS version 9.1.3 software (SAS Institute Inc., Cary) for all analyses. P-values less than 0.05 (two-tailed) were considered statistically significant, and p-values between 0.06 and 0.10 were regarded as being borderline significant.

Results

Mean age was 60.2 years for men and 58.6 for women. Mean systolic blood pressure was 130.2 mmHg for men and 124.8 mmHg for women, and mean diastolic blood pressure was 79.3 mmHg and 75.3 mmHg, respectively. The mean urinary nitrogen concentration was 536 mg/dL for men and 451 mg/dL for women. The mean estimated 24-hour protein intake was 52.9 g/day and 44.0 g/day, respectively (data not shown).

The baseline characteristics according to quartile of urea nitrogen concentration and quartile of estimated daily protein intake are shown in Tables 1 and 2, respectively. The median ranges of urea nitrogen concentration and dietary protein intake were larger in men than in women. For urea nitrogen concentration, both men and women showed an inverse association with age ($p < 0.001$ for both sexes). BMI was positively associated with urea nitrogen concentration in men ($p = 0.04$), but this association was not significant in women. Ethanol intake, current smoking, and use of antihypertensive medication were not significantly associated with urea nitrogen concentration. For estimated protein intake, both men and women showed an inverse association with age ($p < 0.001$ and $p = 0.007$, respectively), and positive association with BMI ($p < 0.001$ for both sexes). Ethanol intake was positively associated ($p = 0.046$) and smoking was inversely associated ($p = 0.002$) with estimated protein intake in men, but not in women ($p = 0.12$ and $p = 0.49$, respectively).

Table 3 shows age-adjusted and multivariate adjusted blood pressure levels according to quartiles of urea nitrogen concentrations in spot urine, as well as blood pressure levels associated with changes in a 1-SD increment of urea nitrogen concentrations in men (238 mg/dl), women (212 mg/dl), and total subjects (226 mg/dl). The concentrations of urea nitrogen in spot urine were inversely associated with systolic blood pressure levels for both men and total subjects. The multivariable-adjusted mean value of systolic blood pressure was 5.0 mmHg and 2.8 mmHg lower in the highest versus lowest quartiles of urea nitrogen

concentration for men and total subjects, respectively. The result in men is illustrated in Figure 1. When we excluded the subjects on antihypertensive medication, the results did not differ materially. The multivariable-adjusted mean value of systolic blood pressure was 6.8 mmHg ($p=0.007$) and 4.1 mmHg ($p=0.008$) lower in the highest versus lowest quartiles of urea nitrogen concentration for men and total subjects, respectively (data not shown). There was no significant association between urea nitrogen concentration and diastolic blood pressure. For women, a similar inverse trend was found between urea nitrogen concentrations in spot urine and systolic blood pressure levels, but the trend did not reach statistical significance. The inverse association between urea nitrogen concentrations and systolic blood pressure levels was similarly observed for age groups <60 and ≥ 60 years old in men and women combined. The multivariable-adjusted mean systolic blood pressure was 4.8 mmHg lower for age groups <60 years old ($p<0.01$) and 3.4 mmHg lower for age groups ≥ 60 years old ($p=0.07$), in the highest versus lowest quartiles of urea nitrogen concentrations (data not shown).

Table 4 shows age-adjusted and multivariable-adjusted blood pressure levels according to quartiles of estimated daily protein intake, and blood pressure levels associated with changes in a 1-SD increment of estimated daily protein intake among men (19.2 g/day), women (14.9 g/day), and total subjects (17.2 g/day). Estimated total protein intake was inversely associated with systolic blood pressure for men and total subjects. The multivariable-adjusted mean systolic blood pressure was 4.5 mmHg and 2.1 mmHg lower in the highest versus lowest quartiles of estimated protein intake for men and total subjects, respectively. The 1-SD increment of estimated protein intake was associated with 1.5 mmHg and 0.7 mmHg lower systolic blood pressures for men and total subjects, respectively. There was no significant association between protein intake and diastolic blood pressure levels. For women, an age-adjusted 1-SD increment of protein intake was positively associated with

diastolic blood pressure levels, but the association was no longer statistically significant after adjustment for confounding factors. The association between estimated protein intake and systolic blood pressure levels was not significant either in age group (data not shown).

We repeated the analyses using the mean of the first and second measurements of blood pressures and analyzed the results. For example, the multivariable-adjusted mean values (SE) of systolic blood pressure for the lowest versus highest quartiles of urea nitrogen concentrations in spot urine among men were 135.0(1.3) mmHg versus 129.9(1.2) mmHg ($p=0.02$ for difference), respectively, and those for the lowest versus highest quartiles of estimated protein intake among men were 135.4(1.1) mmHg versus 131.0(1.1) mmHg ($p<0.01$ for difference) (data not shown).

Discussion

The main finding of this study was that urea nitrogen concentrations in spot urine were inversely associated with systolic blood pressure in men in a Japanese rural community. A similar but weak association was observed in women, with no statistical significance. The weak association in women may in part be due to the smaller range of urea nitrogen excretion and the lower mean systolic blood pressure compared to men.

A previous Japanese study showed that men whose protein intake as estimated by spot urine was ≥ 1.0 g/kg/day (median value), showed a 2.2 mmHg lower mean diastolic blood pressure compared to those whose estimated total protein intake was < 1.0 g/kg/day. In that study, however, the total protein intake was not associated with systolic blood pressure levels in either men or women, or with diastolic blood pressure levels in women [10].

We found an inverse relationship between a urinary biomarker of total protein intake and systolic blood pressure in a Japanese general population. This finding suggests that total protein intake may have a hypotensive effect among the Japanese population, as well as Western populations, although the sources of protein are quite different. However, the present study cannot guarantee a causal relationship due to its cross-sectional design.

Several mechanisms may be involved in the inverse association between urea nitrogen and blood pressure levels. Firstly, several amino acids influence vascular regulation [17-19]. Nitric oxide, for example, which is produced by the amino acid substrate L-arginine [17], reduces vascular smooth muscle tension and causes arterial dilatation. L-arginine infusion in the peripheral circulation lowers both systolic and diastolic blood pressure in men [18]. A clinical trial demonstrated that 7-day taurine supplementation (6 g/day) lowered systolic and diastolic blood pressures by 9.0 mmHg and 4.1 mmHg, respectively [19]. Secondly, amino acids generally act as diuretics [20], which reduce cardiac output and lower blood pressure.

The limitations of the present study warrant discussion. Firstly, we used the second blood pressure measurements because we assumed that they were more representative of blood pressure levels at rest. When we used the means of two blood pressure measurements, the observed associations did not differ materially. Secondly, we have no information about education status, income, physical activity, or macronutrients as potential confounding factors. Because of this, it is uncertain whether the association between blood pressure levels and urea nitrogen concentrations and total protein intake are independent of these confounding variables. Thirdly, we used urea nitrogen concentration, a marker of total protein intake, but we could not examine what sources of protein intake were associated with lower blood pressure levels. Our previous study showed that animal protein intake (estimated by 24-hour dietary recall) was inversely associated with blood pressure levels, while plant protein intake was not [7]. Finally, as stated above, the present study does not prove a causal relationship due to its cross-sectional design.

In conclusion, the present study showed that urea nitrogen concentration in spot urine, a biomarker for total protein intake, was inversely associated with systolic blood pressure levels for men in a Japanese general population.

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Figure legend

Figure 1. Multivariable-adjusted mean systolic blood pressure levels according to quartiles of urea nitrogen concentrations in spot urine and quartiles of estimated total protein intake in men.

Difference from the value in the lowest quartile: * $p < 0.05$, ** $P < 0.01$

Figure.1

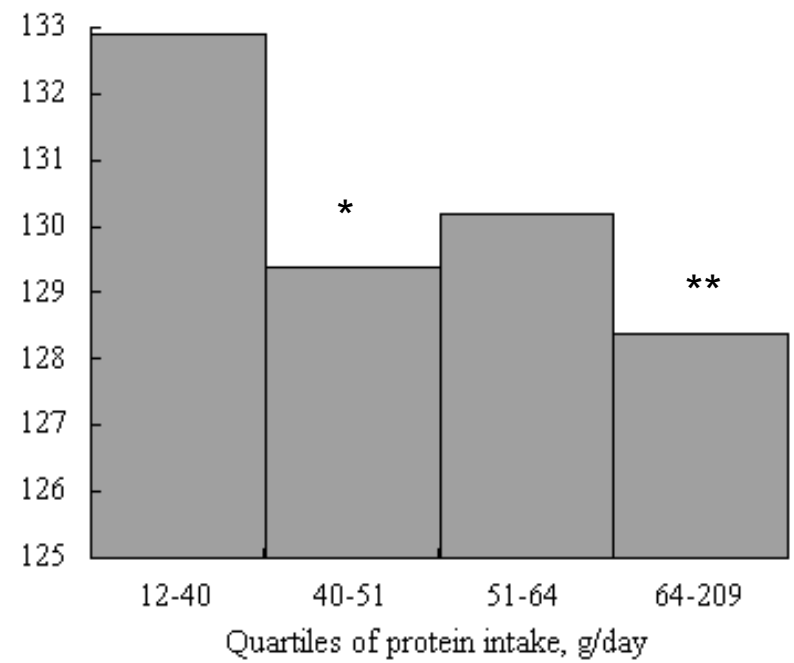
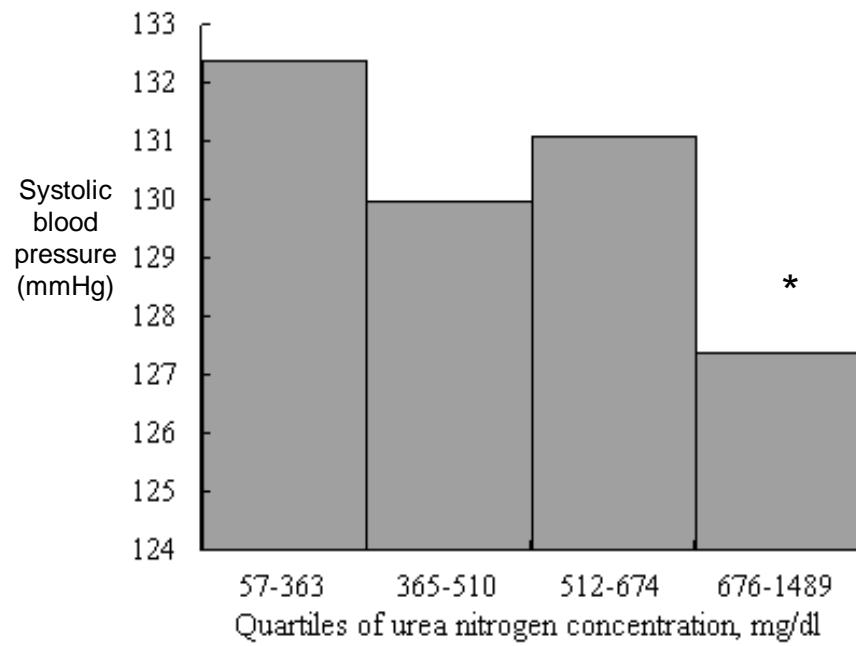


Table 1. Age, sex-adjusted characteristics according to quartiles of urea nitrogen concentrations in spot urine samples for men and women aged 30-74 years.

	Quartiles of nitrogen concentration in spot urine				P for trend
	1(low)	2	3	4(high)	
Men					
n	249	243	247	247	
Urea nitrogen concentrations in spot urine (mg/dl)					
Range	57-363	365-510	512-674	676-1489	
Median	267	441	596	815	
Age, years	61.9	61.5	60.2	57.2	<0.001
Body mass index, kg/m ²	23.5	23.7	24.5	23.9	0.04
Ethanol intake, g/day	8.3	10.3	8.8	9.0	0.77
Antihypertensive medication, %	31	23	28	26	0.45
Current smoker, %	37	40	31	38	0.81
Concentrations in spot urine samples					
Sodium, mmol/l	98	148	159	158	<0.001
Potassium, mmol/l	29	47	55	60	<0.001
Creatinine, mg/dl	45	82	107	144	<0.001
Women					
n	413	406	407	410	
Urea nitrogen concentrations in spot urine (mg/dl)					
Range	67-294	296-422	424-567	569-1465	
Median	227	363	489	693	
Age, years	59.6	60.3	58.8	56.0	<0.001
Body mass index, kg/m ²	23.1	23.5	23.2	23.5	0.13
Ethanol intake, g/day	0.8	0.5	0.3	0.8	0.89
Antihypertensive medication, %	25	23	21	22	0.39
Current smoker, %	5	3	4	4	0.89
Concentrations in spot urine samples					
Sodium, mmol/l	86	120	141	151	<0.001
Potassium, mmol/l	27	44	52	64	<0.001
Creatinine, mg/dl	30	53	71	104	<0.001
Total					
n	662	649	654	657	
Urea nitrogen concentrations in spot urine (mg/dl)					
Range	56-363	296-510	424-674	569-1489	
Median	237	386	525	746	
Age, years	60.5	60.7	59.3	56.4	<0.001
Body mass index, kg/m ²	23.3	23.6	23.7	23.7	0.02
Ethanol intake, g/day	3.6	4.2	3.5	3.9	0.83
Antihypertensive medication, %	27	23	24	24	0.28
Current smoker, %	17	17	15	17	0.89
Concentrations in spot urine samples					
Sodium, mmol/l	90	130	148	154	<0.001
Potassium, mmol/l	28	45	53	62	<0.001
Creatinine, mg/dl	35	64	85	119	<0.001

Table 2. Age, sex-adjusted characteristics according to quartiles of estimated dietary protein intake for men and women aged 30-74 years.

	Quartiles of estimated protein intake				P for trend
	1(low)	2	3	4(high)	
Men					
n	246	247	247	246	
Estimated protein intake (g/day)					
Range	12-40	40-51	51-64	64-209	
Median	32	45	57	74	
Age, years	63.3	60.9	59.5	57.2	<0.001
Body mass index, kg/m ²	23.2	23.7	24.1	24.6	<0.001
Ethanol intake, g/day	7.9	8.7	10.3	9.5	0.05
Antihypertensive medication, %	26	23	31	27	0.38
Current smoker, %	43	40	35	28	0.002
Estimated daily intake					
Sodium, mmol/day	157	223	273	339	<0.001
Potassium, mmol/day	58	73	82	98	<0.001
Creatinine, mg/day	1287	1330	1362	1396	<0.001
Women					
n	409	409	409	409	
Estimated protein intake (g/day)					
Range	7-34	34-43	43-53	53-109	
Median	28	38	48	61	
Age, years	59.6	58.8	58.6	57.5	0.007
Body mass index, kg/m ²	22.8	23.1	23.4	24.0	<0.001
Ethanol intake, g/day	0.5	0.4	0.7	0.7	0.12
Antihypertensive medication, %	24	21	19	27	0.31
Current smoker, %	3	4	6	4	0.49
Estimated daily intake					
Sodium, mmol/day	136	193	233	296	<0.001
Potassium, mmol/day	57	69	77	86	<0.001
Creatinine, mg/day	863	878	891	905	<0.001
Total					
n	655	656	656	655	
Estimated protein intake (g/day)					
Range	7-40	34-51	43-64	53-209	
Median	29	40	51	67	
Age, years	61.0	59.6	58.9	57.4	<0.001
Body mass index, kg/m ²	22.9	23.3	23.7	24.3	<0.001
Ethanol intake, g/day	3.3	3.5	4.3	4.0	0.02
Antihypertensive medication, %	25	22	23	27	0.13
Current smoker, %	18	18	17	13	0.01
Estimated daily intake					
Sodium, mmol/day	144	204	248	313	<0.001
Potassium, mmol/day	58	71	79	90	<0.001
Creatinine, mg/day	1018	1046	1070	1094	<0.001

Table 3. Age,sex-adjusted and multivariable-adjusted blood pressure levels according to quartile of urea nitrogen concentrations in spot urine samples.

	Quartiles of urea nitrogen concentration				
	1(low)	2	3	4(high)	1SD
Men					
n	249	242	248	247	238mg/dl
SBP					
Age-adjusted(SE)	130.7(1.1)	130.0(1.1)	132.0(1.0)	128.2(1.1) †	-1.0(0.5) †
Multivariable-adjusted(SE)+	132.4(1.3)	130.0(1.0)	131.1(1.0)	127.4(1.2) *	-2.1(0.7) **
DBP					
Age-adjusted(SE)	79.4(0.7)	78.1(0.7)	80.5(0.7)	79.3(0.7)	0.0(0.3)
Multivariable-adjusted(SE)+	80.0(0.8)	78.1(0.7) †	80.1(0.7)	79.1(0.8)	-0.4(0.5)
Women					
n	413	406	407	410	212mg/dl
SBP					
Age-adjusted(SE)	126.2(0.8)	124.1(0.8) †	124.7(0.8)	124.4(0.8)	-0.3(0.4)
Multivariable-adjusted(SE)+	125.8(1.1)	124.0(0.8)	125.2(0.8)	124.4(1.0)	0.1(0.6)
DBP					
Age-adjusted(SE)	75.3(0.5)	75.4(0.5)	74.9(0.5)	75.6(0.5)	0.2(0.3)
Multivariable-adjusted(SE)+	74.5(0.6)	75.2(0.5)	75.4(0.5)	76.1(0.6)	0.6(0.4)
Total					
n	662	648	655	657	226mg/dl
SBP					
Age,sex-adjusted(SE)	127.9(0.7)	126.3(0.7) †	127.4(0.7)	125.8(0.7) *	-0.5(0.3)
Multivariable-adjusted(SE)+	128.3(0.8)	126.4(0.6) †	127.3(0.6)	125.5(0.8) *	-0.7(0.5)
DBP					
Age,sex-adjusted(SE)	76.7(0.4)	76.4(0.4)	77.0(0.4)	77.0(0.4)	0.1(0.2)
Multivariable-adjusted(SE)+	76.5(0.5)	76.4(0.4)	77.1(0.4)	77.2(0.5)	0.2(0.3)

Difference from the lowest quartile from zero: †p<0.10, *p<0.05, **p<0.01

+: Adjusted for age (years), body mass index (kg/m²), current use of antihypertensive medication (yes or no), ethanol intake (g ethanol/day), current smoking (yes or no), concentrations of creatinine, sodium, and potassium (sex-specific quartiles)

Table 4. Age-adjusted and multivariable-adjusted blood pressure levels according to quartiles of estimated dietary protein intake

	Quartiles of				1SD
	dietary protein intake				
	1(low)	2	3	4(high)	
Men					
n	246	247	247	246	19.2g/day
SBP					
Age-adjusted(SE)	130.6(1.1)	128.7(1.1)	131.4(1.1)	130.2(1.1)	0.2(0.5)
Multivariable-adjusted(SE)+	132.9(1.1)	129.4(1.0) *	130.2(1.0) †	128.4(1.1) **	-1.5(0.6) *
DBP					
Age-adjusted(SE)	78.8(0.7)	79.0(0.7)	80.3(0.7)	79.2(0.7)	0.4(0.3)
Multivariable-adjusted(SE)+	79.9(0.7)	79.2(0.6)	79.9(0.7)	78.3(0.7)	-0.5(0.4)
Women					
n	409	409	409	409	14.9g/day
SBP					
Age-adjusted(SE)	125.0(0.8)	124.5(0.8)	123.7(0.8)	126.2(0.8)	0.5(0.4)
Multivariable-adjusted(SE)+	125.6(0.9)	125.0(0.8)	124.0(0.8)	124.8(0.8)	-0.2(0.5)
DBP					
Age-adjusted(SE)	74.9(0.5)	75.0(0.5)	74.9(0.5)	76.4(0.5) *	0.6(0.3) *
Multivariable-adjusted(SE)+	75.0(0.5)	75.2(0.5)	75.1(0.5)	75.9(0.5)	0.5(0.3)
Total					
n	655	656	656	655	17.2g/day
SBP					
Age-adjusted(SE)	127.0(0.7)	126.0(0.7)	126.7(0.7)	127.8(0.7)	0.5(0.3)
Multivariable-adjusted(SE)+	128.3(0.7)	126.6(0.6) †	126.2(0.6) *	126.2(0.7) *	-0.7(0.4) †
DBP					
Age-adjusted(SE)	76.2(0.4)	76.4(0.4)	77.0(0.4)	77.6(0.4) *	0.6(0.2) **
Multivariable-adjusted(SE)+	76.8(0.4)	76.7(0.4)	76.8(0.4)	77.0(0.4)	0.2(0.2)

Difference from the lowest quartile from zero: †p<0.10, *p<0.05, **p<0.01

+: Adjusted for age (years), body mass index (kg/m²), current use of antihypertensive medication (yes or no), ethanol intake (g ethanol/day), current smoking (yes or no), concentrations of creatinine, estimated sodium intake, and potassium intakes (sex-specific quartiles)