Original Article

Vegetable Protein Intake was Inversely Associated with Cardiovascular Mortality in a 15-Year Follow-Up Study of the General Japanese Population

Ayako Kurihara[1], Tomonori Okamura[1], Daisuke Sugiyama[1], Aya Higashiyama[2], Makoto Watanabe[2], Nagako Okuda[3], Aya Kadota[5, 6], Naoko Miyagawa[5], Akira Fujiyoshi[5], Katsushi Yoshita[7], Takayoshi Ohkubo[8] , Akira Okayama[4], Katsuyuki Miura[5, 6], Hirotsugu Ueshima[5, 6]
for the NIPPON DATA90 Research Group

[1] Department of Preventive Medicine and Public Health, Keio University School of Medicine, Tokyo, Japan
[2] Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Osaka, Japan
[3] Department of Health and Nutrition, University of Human Arts and Sciences, Saitama, Japan
[4] Research Institute of Strategy for Prevention, Tokyo, Japan
[5] Department of Public Health, Shiga University of Medical Science, Shiga, Japan
[6] Center for Epidemiologic Research in Asia, Shiga University of Medical Science, Shiga, Japan
[7] Department of Food and Nutrition, Osaka City University, Osaka, Japan
[8] Department of Hygiene and Public Health, Teikyo University, Tokyo, Japan

Aim: To examine the relationship between the intake of dietary vegetable protein and CVD mortality in a 15-year follow-up study of a representative sample of the Japanese population.

Methods: A total of 7,744 participants aged 30 years or older (3,224 males and 4,520 females) who were free of CVD at baseline were included in this analysis. Vegetable protein intake (% energy) was assessed using a three-day semi-weighed dietary record at baseline. Multivariable-adjusted hazard ratios (HRs) were calculated using Cox's proportional hazards model after adjusting for confounding factors.

Results: The total person-years studied were 107,988 with a mean follow-up period of 13.9 years. There were 1,213 deaths during the follow-up period, among which 354 (29.2%) were due to CVD. Vegetable protein intake was associated inversely with CVD and cerebral hemorrhage mortality, with the HRs for a 1% energy increment in vegetable protein intake being 0.86 (95% CI, 0.75−0.99) and 0.58 (95% CI, 0.35−0.95), respectively. In the subgroup analysis of participants with or without hypertension, the inverse association between vegetable protein intake and CVD mortality was more evident in the nonhypertensive group, with the HRs for CVD and stroke being 0.68 (95% CI, 0.50−0.94) and 0.50 (95% CI, 0.30−0.84), respectively.

Conclusions: Vegetable protein intake may prevent future CVD, particularly in nonhypertensive subjects in the Japanese population. However, further studies are necessary to examine the biological mechanisms of this effect.

Key words: Vegetable protein, National nutrition survey, Hypertension, Cohort studies, Cardiovascular disease, Stroke

Introduction

A previous study reported that the prevalence of stroke and coronary heart disease (CHD) in East Asian countries was higher and lower than in Western countries, respectively[1]. The management of blood pressure appeared to be more important in East Asian populations than in Western populations. Some previ-

ous studies have suggested that there is an inverse association between total protein intake and blood pressure[2-4]. On the other hand, a recent meta-analysis of RCT and intervention studies reported an inverse association between vegetable protein and blood pressure levels[5, 6]. However, it remains unclear whether the intake of animal protein or vegetable protein is inversely associated with blood pressure levels[7-15]. The relationship between the types of dietary protein intake and cardiovascular disease (CVD) also remains unclear. Some epidemiological studies have shown no association between any kind of protein intake and CVD[16-19], whereas other studies reported inverse associations between animal protein intake and intracerebral hemorrhage[20, 21] or any type of protein and risk of cerebral infarction[22]. However, to our knowledge only a few reports have suggested an inverse association between vegetable protein intake and risk of CVD[23].

Aim

The objective of this study was to examine the relationship between the intake of dietary vegetable protein and CVD mortality in a 15-year follow-up study of a representative sample of the Japanese population (i.e., NIPPON DATA90).

Methods and Population

Population

The National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged 1990 (NIPPON DATA90) is a cohort study based on the National Nutrition Survey in Japan (NNSJ) and the National Survey on Circulatory Disorders (NSCDJ) of the Japanese government. The baseline survey was conducted in 1990. The details of the study population have been described previously[24-27]. Participants in NIPPON DATA90 were community dwellers living in 300 randomly selected districts throughout Japan who participated in the NNSJ. The subjects were enrolled automatically as participants in the NSCDJ, which was performed at the same time. A dietary survey was conducted for three consecutive days in each household by using the semi-weighed record method. The details of this method are described elsewhere[28].

A total of 8,383 community dwellers aged 30 years or older (3,504 males and 4,879 females) participated in the survey and were followed until November 15, 2015. The number of participants aged 30 years or older in all districts was 10,956. The participation rate in the survey was 76.5%. Among the 8383 partic-

ipants, 639 were excluded because of the following reasons: previous history of CVD ($n=248$), missing baseline information ($n=120$), and communication failure due to incomplete residential information at the follow-up survey ($n=271$). The remaining 7,744 participants (3,224 males and 4,520 females) were included in the analysis.

Follow-Up Survey

The participants in this study were followed for 15 years. The procedure for endpoint determination has been reported previously[24-27, 29]. Briefly, the causes of death were identified every five years by searching the National Vital Statistics database. The underlying causes of death identified by the National Vital Statistics were coded according to the 9th International Classification of Disease (ICD-9) for deaths up to the end of 1994 and the 10th International Classification of Disease (ICD-10) for deaths from 1995 onwards. Deaths from CVD included ICD-9 codes $393-459$ and ICD-10 codes $I00-I99$. Deaths from CHD included ICD-9 codes $410-414$ and ICD-10 codes $I20-I25$. Deaths from heart failure included ICD-9 code 428 and ICD-10 code I50. Deaths from stroke included ICD-9 codes $430-438$ and ICD-10 codes $I60-I69$. Deaths from cerebral infarction included ICD-9 codes 433, 434, 437.8a, and 437.86 and ICD-10 codes $I63-I69.3$. Deaths from cerebral hemorrhage included ICD-9 codes $431-432$ and ICD-10 codes $I61-I69.1$. Permission to use the National Vital Statistics was obtained from the Management and Coordination Agency, Government of Japan. Approval for this study was obtained from the Institutional Review Board of Shiga University of Medical Science (No. 12-18, 2000; No. 17-21-3, 2010, 2017).

Dietary Survey

We used the NIPPON DATA90 dataset integrated with the results of NNSJ90. The details of the dietary survey, calculation of nutrient intakes for individual participants, and the integration of the data of NIPPON DATA90 and NNSJ90 are described elsewhere[28]. Semi-weighed dietary records for three consecutive days per household were implemented for NNSJ until 1994, and only average food/nutrient intakes per capita were reported. Since 1995, NNSJ has collected information about food distribution among household members within the family and has reported the average food/nutrient intakes by sex and age categories. The results of NNSJ95 show that the food/nutrients intakes of each household in NNSJ90 were distributed to each household member proportionally according to his/her age and sex. We used the distributed food/nutrient intake data of each individ-

ual in this analysis[28, 30-32].

A limited number of nutrients were calculated in the NNSJ 90, and vegetable protein intake was not included[28]. We used the integrated food database developed for the international collaborative INTER-MAP Study[28, 33-35] to calculate animal and vegetable protein intakes. In the INTERMAP food table, the protein content of each food product was total-animal, total-vegetable, or a mix of animal and vegetable (i.e., manufactured foods using both animal and vegetable foods)[28, 35], and the contents of animal and vegetable protein were calculated. We calculated the intakes of the animal and vegetable protein of the current participants by using the INTERMAP food table and food intake data obtained in NNSJ90. The details of the calculation were described elsewhere[28, 35].

Statistical Analysis

The intake of animal protein, vegetable protein, and fat were expressed as % energy. Sodium and potassium were expressed in mg, whereas other nutrients were calculated as g/1000 kcal. The participants were divided into four categories according to the quartile of vegetable protein intake: vegetable protein intake ≤ 6.6% energy, 6.7%−7.2% energy, 7.3%−7.8% energy, and ≥ 7.9% energy. The analysis of variance for the means or the chi square test for proportions was used to compare the risk factors across quartiles of vegetable protein intake. A Cox proportional hazards model was used to examine the association of vegetable protein intake with CVD mortality. The multivariable-adjusted hazard ratio (HR) and 95% CIs of each vegetable protein intake quartile for CVD were calculated after adjusting for sex, age, body mass index (BMI), animal protein intake, animal fat intake, vegetable fat intake, sodium, potassium, total dietary fiber, smoking (never smoked, ex-smoker, current smoker, ≤ 20 cigarettes/day, and ≥ 21 cigarettes/day), and alcohol drinking (nondrinker, ex-drinker, current drinker). The HRs from the second to top quartiles of the vegetable protein intakes were then expressed in comparison with the lowest quartile (the reference). A model with continuous vegetable protein intake values instead of quartiles was used to clarify the linear trend between vegetable protein intake and mortality. Stratified analyses with or without hypertension were also performed. Hypertension was defined as systolic blood pressure (SBP) ≥ 140, diastolic blood pressure (DBP) ≥ 90, and/or intake of antihypertensive medications.

All confidence intervals were estimated at the 95% level; a *p*-value < 0.05 is considered significant. The Statistical Package for the Social Sciences version 22 (SPSS Japan Inc., Tokyo, Japan) was used for the analyses.

Results

The mean age ± standard deviation of the participants at the baseline survey was 52.6 ± 13.8 years (males 52.9 ± 13.5 years and females 52.4 ± 14.0 years). The mean amount of vegetable protein intake was 7.1 ± 0.9% energy/day for males, 7.3 ± 1.0% energy/day for females, and 7.2 ± 1.0% energy/day for males and females.

Table 1 shows the baseline characteristics of the participants. There were significant differences in the mean values for the percentage of females, age, BMI, prevalence of hypertension, total protein intake, sodium, potassium, and total dietary fiber intake. These variables were increased in the higher vegetable protein intake quartiles. On the other hand, the intakes of total energy, animal protein, total fat, animal fat, and vegetable fat were lower in the higher vegetable protein quartiles. The prevalence of never smokers and nondrinkers was higher in the higher vegetable protein intake quartiles.

The total person-years studied were 107,988 (43,952 for males, 64,035 for females), and the mean follow-up period was 13.9 years. During follow-up, there were 1,213 deaths (649 for males and 594 females), among which 29.2% (*n* = 354) were due to CVD. There were 71 deaths due to CHD, and 144 due to stroke (88 cerebral infarction cases, 28 intracerebral hemorrhage cases, and 28 other cases).

Table 2 shows the number of deaths and the multivariable-adjusted HRs with their 95% CIs for CVD mortality. There was an inverse association between vegetable protein intake and CVD mortality. However, with the exception of the third quartile for cerebral hemorrhage, no quartile showed statistical significance. The analysis using continuous values showed significant inverse relationships among vegetable protein intake, CVD, and cerebral hemorrhage mortality (HRs, 0.86 (95% CI, 0.75−0.99) and 0.58 (95% CI, 0.35−0.95), respectively). Sex-specific analysis showed almost similar results. The HR for CVD was 0.78 (95% CI, 0.63−0.96) for males and 0.95 (95% CI, 0.78−1.15) for females. The HR for cerebral hemorrhage was 0.70 (95% CI, 0.37−1.34) for males and 0.43 (95% CI, 0.18−1.06) for females (table not shown).

Table 3 shows the number of deaths and multivariable-adjusted HRs (95% CIs) for CVDs according to vegetable protein intake quartiles after the stratification of the data according to the presence or absence of hypertension. Vegetable protein intake (continuous value) showed inverse relationships with mortality for almost all CVD subtypes irrespective of the presence of hypertension. We also observed significant relation-

Table 1. Baseline characteristics of participants according to quartiles of vegetable protein intake: NIPPON DATA90

Stratum mean ± SD	Total vegetable protein intake				
	Q1 (Low) ≤ 6.6 (6.2 ± 0.4% energy)	Q2 6.7-7.2 (6.9 ± 0.2% energy)	Q3 7.3-7.8 (7.5 ± 0.2% energy)	Q4 (High) 7.9 ≤ (8.5 ± 0.7% energy)	P-values
No. of participants	2201	1988	1814	1741	
Male (%)[‡]	45.8	42.8	42.1	34.5	< 0.001
Age (years)	47.8 ± 12.9	51.8 ± 13.7	54.0 ± 13.6	58.3 ± 12.7	< 0.001
BMI (kg/m^2)	22.6 ± 3.1	22.8 ± 3.2	23.0 ± 3.1	23.2 ± 3.3	< 0.001
Hypertension (%)[†‡]	36.1	42.9	49.6	54.5	< 0.001
SBP (mmHg)	131 ± 20	134 ± 20	137 ± 21	139 ± 20	< 0.001
DBP (mmHg)	80 ± 12	81 ± 12	82 ± 12	82 ± 12	< 0.001
Anti-hypertensive medication (%)[‡]	8.2	11.6	14.8	18.7	< 0.001
Total energy (kcal)	2171 ± 462	2085 ± 462	2032 ± 457	1906 ± 441	< 0.001
Protein intake					
Total protein intake (% energy)	15.7 ± 2.0	15.7 ± 1.9	15.7 ± 1.9	16.1 ± 2.0	< 0.001
Animal (% energy)	10.7 ± 2.1	9.7 ± 2.1	9.1 ± 2.0	8.4 ± 2.2	< 0.001
Fat intake					
Total fat intake (% energy)	26.3 ± 4.6	23.8 ± 4.3	22.3 ± 4.4	21.2 ± 4.7	< 0.001
Animal (% energy)	13.2 ± 3.3	11.4 ± 3.0	10.3 ± 2.8	9.3 ± 3.1	< 0.001
Vegetable (% energy)	13.1 ± 3.9	12.4 ± 3.4	12.1 ± 3.4	11.9 ± 3.4	< 0.001
Sodium (mg)	5246 ± 1620	5323 ± 1654	5370 ± 1804	5467 ± 1809	0.001
Pottasium (mg/1000 kcal)	1295 ± 243	1379 ± 258	1437 ± 281	1583 ± 338	< 0.001
Total dietary fiber (g/1000 kcal)	6.5 ± 1.5	7.5 ± 1.8	8.1 ± 1.9	9.4 ± 2.5	< 0.001
Smoking[‡]					
Neversmoked (%)	53.9	60.3	61.9	67.2	< 0.001
Ex-smoker (%)	10.6	10.8	12.0	10.8	
Current smoker (%)	35.5	29.0	26.1	22.0	
Drinking[‡]					
Nondrinker (%)	63.1	68.8	69.5	74.3	< 0.001
Ex-drinker (%)	3.0	2.6	3.0	3.7	
Current drinker (%)	33.8	28.6	27.5	22.0	

Values are means ± standard deviation (SD) unless specified otherwise.
One-way analysis of variance was used to compare means of continuous variables.
[†] SBP ≥ 140 and/or DBP ≥ 90 and/or taking anti-hypertensive medication.
[‡] Chi-square test was used to compare prevalences.

ships in the nonhypertensive group between vegetable protein intake and CVD (HR, 0.68; 95% CI, 0.50−0.94) and stroke mortality (HR, 0.50; 95% CI, 0.30−0.84). In the nonhypertensive group, mortality was significantly lower in the following groups than in the reference: the HRs for stroke were 0.18 (95% CI, 0.09−0.62) in the third quartile and 0.18 (95% CI, 0.05−0.60) in the top quartile, whereas the HR for cerebral infarction was 0.19 (95% CI, 0.04−0.93) in the top quartile.

Discussion

We found a significant inverse association between vegetable protein intake and CVD mortality in a 15-year cohort study of a representative sample of the Japanese population. These findings were independent of other nutritional factors, such as fat, sodium intake, and BMI. Vegetable protein intake was also associated inversely with mortality because of cerebral hemorrhage. Sex-specific analysis showed similar results. Furthermore, the abovementioned association was evident in nonhypertensive participants at baseline (i.e., a significant inverse association of vegetable protein intake with CVD and stroke mortality).

Previous reports did not show a clear negative trend in vegetable protein intake and the risk of CVD. For example, Haring *et al.*[18] reported a multivariate

Table 2. The number of deaths and multivariable-adjusted HRs (95%CIs) for CVD deaths according to vegetable protein intake: NIPPON DATA90

| Stratum mean ± SD | Total vegetable protein intake | | | | 1% energy increment of vegetable protein intake |
	Q1 (Low) ≤ 6.6 (6.2 ± 0.4% energy)	Q2 6.7-7.2 (6.9 ± 0.2% energy)	Q3 7.3-7.8 (7.5 ± 0.2% energy)	Q4 (High) 7.9 ≤ (8.5 ± 0.7% energy)	
No. of participants	2201	1988	1814	1741	
Person-years	31428	27601	25111	23848	
Cardiovascular disease					
No. of deaths	69	97	86	102	
Age and sex adjusted HR	1.00	1.14 (0.84-1.55)	0.91 (0.66-1.25)	0.85 (0.62-1.15)	0.89 (0.80-0.99)
Multivariable-adjunted HR	1.00	1.10 (0.80-1.52)	0.88 (0.62-1.24)	0.80 (0.55-1.16)	0.86 (0.75-0.99)
Coronary heart disease					
No. of deaths	12	26	18	15	
Age and sex adjusted HR	1.00	1.87 (0.94-3.72)	1.18 (0.57-2.45)	0.80 (0.37-1.73)	0.83 (0.65-1.06)
Multivariable-adjusted HR	1.00	1.89 (0.93-3.84)	1.18 (0.53-2.60)	0.76 (0.31-1.86)	0.79 (0.58-1.09)
Stroke					
No. of deaths	33	31	33	47	
Age and sex adjusted HR	1.00	0.77 (0.47-1.26)	0.73 (0.45-1.18)	0.81 (0.52-1.28)	0.92 (0.78-1.10)
Multivariable-adjusted HR	1.00	0.68 (0.41-1.14)	0.60 (0.36-1.02)	0.61 (0.35-1.05)	0.81 (0.65-1.01)
Cerebral infarction					
No. of deaths	19	17	24	28	
Age and sex adjusted HR	1.00	0.72 (0.37-1.39)	0.88 (0.48-1.62)	0.79 (0.44-1.42)	0.90 (0.72-1.12)
Multivariable-adjusted HR	1.00	0.69 (0.35-1.37)	0.86 (0.44-1.67)	0.77 (0.38-1.57)	0.88 (0.66-1.16)
Cerebral hemorrhage					
No. of deaths	7	9	4	8	
Age and sex adjusted HR	1.00	1.13 (0.42-3.06)	0.47 (0.14-1.60)	0.78 (0.28-2.19)	0.89 (0.60-1.31)
Multivariable-adjusted HR	1.00	0.75 (0.26-2.15)	0.26 (0.07-0.98)	0.29 (0.08-1.06)	0.58 (0.35-0.95)

HR means hazard ratio and 95% CIs means 95% confidence interval.
The HR was adjusted for sex, age, BMI, animal protein intake, animal fat intake, vegetable fat intake, sodium, potassium, total dietary fiber, cigarette smoking category and alcohol intake category by a Cox propotional hazard model.

analysis that showed no association between vegetable protein intake and stroke mortality. Haring *et al.*[17] also reported that the CHD risk in the highest vegetable protein intake group was lower than that in the lowest intake group, although this difference did not reach statistical significance after adjusting for sex, age, race, participation area, and total energy intake. The range of median vegetable protein intake in previous studies was approximately 18 g/day to 31 g/day or approximately 5% energy from Western studies[14, 16, 18]. In the present study and in a previous study in Japan[23], the range of median vegetable protein intake was over 30 g/day or over 7% energy, which was higher than in Western populations; however, the method used to estimate the intake of vegetable protein was different. In the present study, rice (38.9%), soy and soy products (21.8%), and flour products (14.5%) contributed largely to the total vegetable protein intake (data not shown). For the ratio of animal to vegetable protein intake in previous studies, more than half of protein intake in Western popultaions[14, 16, 18] was animal protein; by contrast, more than half of vegetable protein was consumed in the Japanese population[23]. A relatively high intake of vegetable protein might be associated with a negative association between vegetable protein intake and CVD death in Japanese cohort studies.

For the relationship between animal protein intake and CVD mortality, Iso *et al.*[20, 21] reported a significantly negative association between animal protein intake and cerebral hemorrhage among women in the United States and men and women in Japan. In our study, vegetable protein intake was associated with low mortality for cerebral hemorrhage but animal protein was not. However, the number of deaths due to cerebral hemorrhage was small in the present study, and it is difficult to examine further the differences in the relations of vegetable protein intake and animal protein intake with cerebral hemorrhage.

Regarding the association between protein intake

Table 3. The number of deaths and multivariable-adjusted (95%CIs) for CVD deaths according to vegetable protein intake stratified by the existence of Hypertension: NIPPON DATA90

Stratum mean ± SD		n|total	Q1 (Low) ≤ 6.6 (6.2 ± 0.4% energy)	Q2 6.7-7.2 (6.9 ± 0.2% energy)	Q3 7.3-7.8 (7.5 ± 0.2% energy)	Q4 (High) 7.9 ≤ (8.5 ± 0.7% energy)	1% energy increment of vegetable protein intake
No. of participants		7744	2201	1988	1814	1741	
Hypertention −		4247	1406	1135	914	792	
Hypertension +		3497	795	853	900	949	
Person-years		107988	31428	27601	25111	23848	
Hypertension −		4247	20485	16333	13043	11348	
Hypertension +		3497	10942	11267	12068	12499	
Cardiovascular disease							
Hypertension −	No. of deaths	81	19	23	20	19	
	Multivariable-adjusted HR		1.00	0.95 (0.50-1.80)	0.76 (0.38-1.54)	0.50 (0.22-1.11)	0.68 (0.50-0.94)
Hypertension +	No. of deaths	273	50	74	66	83	
	Multivariable-adjusted HR		1.00	1.16 (0.80-1.69)	0.89 (0.59-1.32)	0.91 (0.60-1.38)	0.92 (0.78-1.08)
Coronary heart disease							
Hypertension −	No. of deaths	19	3	7	5	4	
	Multivariable-adjusted HR		1.00	2.37 (0.58-9.75)	1.61 (0.34-7.72)	0.95 (0.16-5.70)	0.79 (0.42-1.50)
Hypertension +	No. of deaths	52	9	19	13	11	
	Multivariable-adjusted HR		1.00	1.75 (0.77-3.99)	1.01 (0.40-2.53)	0.69 (0.25-1.94)	0.79 (0.54-1.14)
Stroke							
Hypertension −	No. of deaths	31	12	7	4	8	
	Multivariable-adjusted HR		1.00	0.40 (0.15-1.07)	0.18 (0.09-0.62)	0.18 (0.05-0.60)	0.50 (0.30-0.84)
Hypertension +	No. of deaths	113	21	24	29	39	
	Multivariable-adjusted HR		1.00	0.87 (0.48-1.60)	0.83 (0.45-1.54)	0.88 (0.47-1.66)	0.92 (0.72-1.17)
Cerebral infarction							
Hypertension −	No. of deaths	19	8	3	4	4	
	Multivariable-adjusted HR		1.00	0.33 (0.08-1.31)	0.34 (0.08-1.36)	0.19 (0.04-0.93)	0.53 (0.27-1.02)
Hypertension +	No. of deaths	69	11	14	20	24	
	Multivariable-adjusted HR		1.00	1.04 (0.40-2.35)	1.25 (0.56-2.78)	1.27 (0.55-2.95)	1.04 (0.75-1.42)
Cerebral hemorrhage							
Hypertension −	No. of deaths	7	2	3	0	2	
	Multivariable-adjusted HR		1.00	0.75 (0.10-5.60)	-	0.22 (0.01-3.62)	0.47 (0.15-1.46)
Hypertension +	No. of deaths	21	5	6	4	6	
	Multivariable-adjusted HR		1.00	0.73 (0.21-2.55)	0.35 (0.09-1.43)	0.31 (0.07-1.37)	0.62 (0.36-1.09)

Hypertension was defined as SBP ≥ 140 and/or DBP ≥ 90 and/or taking anti-hypertensive medication.
HR means hazard ratio and 95% CI means 95% confidence interval. The HR was adjusted for sex, age, BMI, animal protein intake, animal fat intake, vegetable fat intake, sodium, potassium, total dietary fiber, cigarette smoking category and alcohol intake category by a Cox propotional hazard model.

and blood pressure, it has been suggested that vegetable protein intake may decrease blood pressure. In the observational study, Kuil et al.[14] reported that vegetable protein intake was associated with a decrease in blood pressure levels, whereas both total and animal protein intake was not associated with changes in blood pressure levels in a Dutch population aged 20 to 65 years old. In that study, the mean vegetable protein intake was 25 g/day for the bottom quintile and 39 g/day for the top quintile. Elliott et al.[13] also observed an inverse association between vegetable protein intake and blood pressure levels in randomly selected men and women aged 40−59 years old living in Japan, China, United States, and United Kingdom during 1996 to 1997. By contrast, a cross-sectional study of community dwellers in Japan conducted by Umesawa et al.[15] showed that blood pressure level has an inverse association with total protein and animal protein intake but no association with vegetable protein intake. However, they performed their survey

over a long period from 1973 to 1997 and included a larger proportion of elderly people over 60 years old.

In a meta-analysis, Yokoyama *et al.*[5] reported that the vegetarian diet group had decreases of 4.8 and 2.2 mmHg for SBP and DBP in intervention trials, respectively, and had lower mean SBP and DBP at 6.9 and 4.7 mmHg in observational studies, respectively, than the usual diet group. Furthermore, Tielemans *et al.*[6] reported that an intake of 41 g per day of protein had a negative effect of 2.11 mmHg on pooled SBP in a meta-analysis of randomized controlled trials.

It is likely that the blood pressure lowering effect of vegetable protein intake may not be sufficiently large to reduce the risk of CVD in subjects whose blood pressure has already increased to the level of clinical diagnosed hypertension. The prevalence of hypertension, mean SBP and DBP levels, and the proportion of subjects taking antihypertensive medication in our study were relatively higher in the higher vegetable protein intake quartiles. We consider that the high proportion of elderly subjects in the higher vegetable protein intake quartile was one reason for this higher prevalence of hypertension. However, vegetable protein intake may prevent future CVD by suppressing future increases in blood pressure for nonhypertensive people. This may be the reason why we could clearly show an inverse association between vegetable protein intake and CVD mortality, particularly among participants without hypertension at baseline.

Vegetable protein intake might decrease the CVD risk of Japanese community dwellers, particularly those without hypertension. The result of this study may suggest that the intake of vegetable protein rich foods, such as soybeans or soybean products, should be recommended for the primary prevention of hypertension via a population strategy[36]; we believe that this approach can prevent CVD.

One of the strengths of this study was that we could regard participants as representative samples of the Japanese population because of the following: First, we randomly selected participants from 300 districts throughout Japan. Second, the number of total participants was more than 8,000. Third, both participation rate and follow-up rate were high at over 70% and 90%, respectively. Furthermore, we used baseline dietary data obtained from three-day weighed dietary records, which is a standardized record applied for NNS.

This study has some limitations. First, the vegetable protein intake and blood pressure levels were only evaluated at the baseline survey, and regression dilution bias might have been caused by the false classification because the changes during the follow-up period was not considered. Second, given that the

individual dietary intake used in this study was estimated from household base data for three days by using the weighted average method, the estimated values may be over- or underestimated without regard to personal preferences. However, we thought that this was not a large problem because this was not a systematically occurring error, and a large enough number of study subjects were included in the comparison of quartiles or analysis. Third, we were not aware of the physical activity, socioeconomic status, and alcohol consumption of the participants; thus, we could not adjust for these potential confounding factors. Fourth, information on supplementation was not available in the baseline survey, although supplement use was not common among the Japanese people in 1990. Fifth, although the number of cardiovascular deaths was relatively small, we could not adjust enough confounding factors that are related to CVD mortality in a statistical model. We added potential confounders, such as vitamin C, in our statistical model one by one for further analysis; however, the major findings were not altered (data not shown). Finally, we could not inspect whether the inverse association between vegetable protein intake and CVD mortality depends on the suppressive effect of blood pressure elevation after baseline survey. To clarify this issue, we have to frequently observe the changes in blood pressure from baseline to the end of follow-up, particularly for individuals without hypertension. We would like to examine this issue in the future.

Conclusion

In a 15-year cohort study of a representative sample of the Japanese population, we found a significant inverse association between vegetable protein intake and CVD mortality. Furthermore, the result was evident in nonhypertensive participants at baseline. This finding indicates that a higher amount of vegetable protein intake might prevent future CVD, particularly in people without hypertension. However, further studies that examine this biological mechanism are needed.

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Conflict of Interest

The authors have no conflicts of interest.

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