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

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**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, respectively1). 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\(^5, 6\). 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 NSCDJ. 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 participants, 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. A limited number of nutrients were calculated in the NNSJ 90, and vegetable protein intake was not included. We used the integrated food database developed for the international collaborative INTERMAP Study 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), 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.

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 mortality. There was an inverse association due to stroke (88 cerebral infarction cases, 28 intracerebral hemorrhage cases, and 28 other cases).

Table 3 shows the number of deaths and multi-variable-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 1. Baseline characteristics of participants according to quartiles of vegetable protein intake: NIPPON DATA90

| Stratum mean ± SD | Total vegetable protein intake | P-values |
|-------------------|-------------------------------|----------|
|                   | Q1 (Low) (6.2 ± 0.4% energy)  | Q2 (6.7-7.2 ± 0.2% energy) | Q3 (7.3-7.8 ± 0.2% energy) | Q4 (High) (7.9 ± 0.7% energy) |
| 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/m²)        | 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                   |
| DBP (mmHg)         | 131 ± 20                      | 134 ± 20 | 137 ± 21                      | 139 ± 20                      | < 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     | 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         | 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                     |
| Potassium (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 (%)        | 53.9                          | 60.3     | 61.9                          | 67.2                          | < 0.001                   |
| Neversmoked (%)    | 10.6                          | 10.8     | 12.0                          | 10.8                          | < 0.001                   |
| Current smoker (%) | 35.5                          | 29.0     | 26.1                          | 22.0                          | < 0.001                   |
| Drinking (%)       | 63.1                          | 68.8     | 69.5                          | 74.3                          | < 0.001                   |
| Nondrinker (%)     | 3.0                           | 2.6      | 3.0                           | 3.7                           | < 0.001                   |
| Current drinker (%)| 33.8                          | 28.6     | 27.5                          | 22.0                          | < 0.001                   |

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.

shippers 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.\(^\text{18}\) reported a multivariate
than half of protein intake in Western populations. 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 2. The number of deaths and multivariable-adjusted HRs (95% CIs) for CVD deaths according to vegetable protein intake: NIPPON DATA90 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Stratum mean ± SD | 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-adjusted 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 proportional hazard model.

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
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.\textsuperscript{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

| Stratum mean ± SD | Total vegetable protein intake | 1% energy increment of vegetable protein intake |
|-------------------|-------------------------------|-----------------------------------------------|
|                   | Q1 (Low) ≤ 6.6 | Q2 6.7-7.2 | Q3 7.3-7.8 | Q4 (High) 7.9 ≤ |                           |
|                   | (6.2 ± 0.4% energy) | (6.9 ± 0.2% energy) | (7.5 ± 0.2% energy) | (8.5 ± 0.7% energy) |                           |
| No. of participants | 7744 | 2201 | 1988 | 1814 | 1741 |
| Person-years Hypertension + | 4247 | 1406 | 1135 | 914 | 792 |
| Person-years Hypertension - | 3497 | 795 | 853 | 900 | 949 |
| Cardiovascular disease Hypertension + | 273 | 50 | 74 | 66 | 83 |
| Cardiovascular disease Hypertension - | 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) |
| Coronary heart disease Hypertension + | 273 | 50 | 74 | 66 | 83 |
| Coronary heart disease Hypertension - | 19 | 3 | 7 | 5 | 4 |
| Multivariable-adjusted HR | 1.00 | 2.37 (0.58-9.75) | 1.61 (0.34-7.72) | 0.95 (0.15-5.70) | 0.79 (0.42-1.50) |
| Stroke Hypertension + | 52 | 9 | 19 | 13 | 11 |
| Stroke Hypertension - | 19 | 3 | 7 | 5 | 4 |
| 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) |
| Cerebral infarction Hypertension + | 113 | 21 | 24 | 29 | 39 |
| Cerebral infarction Hypertension - | 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) |
| Cerebral hemorrhage Hypertension + | 69 | 11 | 14 | 20 | 24 |
| Cerebral hemorrhage Hypertension - | 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 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 proportional hazard model.
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, 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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to the conception or design of the study; AKu, TOk, DS, NM, AF, NO, AO, TOh, and HU contributed to the acquisition, analysis, or interpretation of data for the study; AKu, TOk, MW, NO, TOh, KM, and HU drafted the manuscript; AKu, TOk, DS, NO, KM, and HU critically revised the manuscript. All authors gave their final approval and agreed to be accountable for all aspects of work to ensure the integrity and accuracy of the study.

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Conflict of Interest

The authors have no conflicts of interest.

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