Aldosterone-to-Renin Ratio as a Predictor of Stroke Under Conditions of High Sodium Intake: The Ohasama Study

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BACKGROUND

Aldosterone is thought to have deleterious effects on the cardiovascular system. The aldosterone-to-renin ratio (ARR) is more reproducible than aldosterone levels alone and could be an index for inappropriate aldosterone secretion or activity. We previously reported the apparent relation between ARR and hypertension in subjects with high sodium intake. This prospective study investigated the risk of ARR for a first stroke in a general population stratified by sodium intake.

METHODS

We obtained plasma renin activity (PRA) and plasma aldosterone concentrations (PAC) for 883 participants aged ≥35 years not receiving antihypertensive treatment in the general population of Ohasama (mean age: 59.0 ± 11.3 years; 65.6% women).

RESULTS

Over a mean of 10.9 follow-up years, 45 strokes occurred. The median PRA, PAC, and ARR were 1.2 ng/ml/h, 6.4 ng/dl, and 5.3 ng/dl per ng/ml/h, respectively. Using Cox regression, we computed hazard ratios adjusted for sex, age, body mass index (BMI), and systolic blood pressure. No association between logARR and stroke was observed in subjects overall. However, in subjects with high sodium intake (≥median of 4,058 mg/day (salt equivalent, 10.5 g/day)), each 1 s.d. increase in logARR was associated with an increased hazard ratio for stroke (hazard ratio: 1.49, $P = 0.04$). No significant association was observed in subjects with low sodium intake ($P = 0.7$). When we repeated all the analyses using logPRA or logPAC, no significant associations were found.

CONCLUSION

These results suggest that high ARR, that is, relative aldosterone excess, is a predictor for stroke under conditions of high sodium intake.

Keywords: *aldosterone-to-renin ratio; blood pressure; hypertension; relative aldosterone excess; salt-sensitive hypertension; sodium intake; stroke*

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Aldosterone is thought to have deleterious effects on the cardiovascular system through several mechanisms, including cardiovascular remodeling, endothelial dysfunction, vascular inflammation, and atherosclerosis.[1,2] In fact, patients with primary aldosteronism have an increased prevalence of stroke compared with those with essential hypertension.[3] Recently, Tomashitz *et al.*[4] showed that, even in the absence of primary aldosteronism, a higher plasma aldosterone concentration (PAC) is strongly associated with an increased risk for stroke mortality. However, their study[4] was conducted only in a special population (patients referred for coronary angiography).

The aldosterone-to-renin ratio (ARR) is believed to be more reproducible than aldosterone levels alone and could be an index for inappropriate aldosterone secretion or activity and salt sensitivity.[5,6] Previously, we reported that not PAC but ARR was significantly associated with a high prevalence of hypertension.[7] Furthermore, the relation between

ARR and hypertension was strengthened for subjects with high sodium intake.[7] Therefore, it could be hypothesized that ARR may be a more accurate predictor of stroke than PAC and that the association between ARR and stroke risk may be more remarkable under conditions of high sodium intake.

The objective of this prospective study was to investigate the risk of ARR for stroke in a general population stratified by sodium intake.

METHODS

Design. This investigation was a part of the Ohasama study. Socioeconomic and demographic characteristics of this region and details of the study project have been described previously.[8,9] The institutional review boards of Tohoku University School of Medicine and the Department of Health of the Ohasama Municipal Government approved the study.

Study population. In Japan, annual health checkups are available for farmers, the self-employed, pensioners, and dependents aged >35 years. In 1997, the population of Ohasama included 7,318 subjects. Of those, 4,992 were ≥35 years, and 2,719 were eligible for a health checkup that year. Subjects (n = 888) who did not undergo a health checkup were ineligible. Of the 1,831 eligible individuals, 1,346 subjects (74%, mean age: 61.5 ± 11.2 years; 65% women) gave informed consent and participated in the present study. For the current analysis, we excluded 34 subjects due to insufficient data on nutrient intake and 42 subjects due to a previous history of stroke at entry. Furthermore, we excluded 339 subjects treated with antihypertensive drugs because of the effects of antihypertensive medications on the renin–angiotensin system.[10] To eliminate patients with primary aldosteronism completely, we further excluded 48 subjects who had ARR ≥20 ng/dl per ng/ml/h.[11] The remaining 883 subjects were followed.

Data collection. Blood for measurement of PRA (ng/ml/h) and PAC (ng/dl) was drawn with subjects in a sitting position after ~30 min rest, between 9 and 11 AM or between 1 and 3 PM; most subjects had not fasted. Blood was collected in chilled EDTA tubes, and measured by radioimmunoassay (SRL, Tokyo, Japan) with the SPAC-S Aldosterone Kit (TFB, Tokyo, Japan) for PAC and with the plasma renin activity (PRA) "TFB" (TFB) for PRA. The interassay coefficients of variation were 10.5% at 1.5 ng/ml/h for PRA and 4.55% at 8.98 ng/dl for PAC. The intra-assay coefficients of variation were 7.78% at 1.25 ng/ml/h for PRA and 4.81% at 8.95 ng/dl for PAC. The lower limits of detections were 0.1 ng/ml/h for PRA and 1.0 ng/dl for PAC. If the values were less than the lower limits, PRA and PAC were considered equal to 0.1 ng/ml/h and 1.0 ng/dl, respectively. Hypercholesterolemia was defined as total cholesterol ≥5.68 mmol/l (≥220 mg/dl), use of medication for hypercholesterolemia, and/or a history of hypercholesterolemia. Diabetes mellitus was defined as a fasting blood glucose level ≥7.0 mmol/l (≥126 mg/dl), random blood glucose level ≥11.11 mmol/l (≥200 mg/dl), hemoglobin A_{1c} level ≥6.5%, use of medication for diabetes, and/or a history of diabetes mellitus. Blood pressure was measured twice consecutively during the health checkup. Measurements were taken by nurses or technicians at local medical centers using an automatic USM-700F sphygmomanometer[12] (UEDA Electronic Works, Tokyo, Japan) based on the Korotkoff sound technique. All measurements were taken with subjects in the sitting position, after a minimum 2-min rest. The mean of the two readings was defined as the blood pressure. Estimated glomerular filtration rate was estimated from the serum creatinine using a Japanese equation.[13]

Sodium and potassium intakes. A standardized method was used to calculate food consumption and related nutrients from data obtained in a Japanese version of the food-frequency questionnaire, which asked about the average frequency of consumption of each food during the previous year.[14] We previously confirmed that the food-frequency questionnaire is reasonably reproducible and comparable with diet records, taking into account seasonal variations in food consumption.[14,15] We then estimated sodium and potassium intakes, which were adjusted for total energy intake using the residual method. Sodium or potassium intakes were regressed on total energy, and residuals from the regression line were defined as sodium or potassium intakes adjusted by the residual method.[16] To assess the reproducibility of the food-frequency questionnaire in a previous study, we calculated correlation coefficients between two food-frequency questionnaires completed with a 1-year interval.[14] The correlation coefficient between the two assessments of sodium chloride was 0.70 after adjustment for age, gender, and total energy.[14]

Follow-up and outcome. Residence in Ohasama (as of 30 November 2010) was confirmed by the residents' registration cards. In Japan, these cards are considered accurate and reliable, because they are used for pensions and social security benefits. The incidence of stroke until 30 November 2010, was determined by reviewing the Stroke Registration System of Iwate Prefecture, death certificates, National Health Insurance receipts, and questionnaires sent to each household at the time of health-checkup. This information was then confirmed by checking the medical charts of Ohasama Hospital, which is the only hospital in the town and where ≥90% of subjects had regular checkups. Almost all stroke cases were admitted to Ohasama Hospital, where the diagnosis was confirmed by computed tomography and/or magnetic resonance imaging of the brain. The diagnostic criteria of stroke subtypes were based on the Classification of Cerebrovascular Disease III of the National Institute of Neurological Disorders and Stroke.[17] We defined "cerebral infarction" as ischemic stroke and defined "intracerebral hemorrhage" and "subarachnoid hemorrhage" as hemorrhagic stroke. Transient ischemic attacks were not included in stroke.

Statistical analysis. To analyze the relationship between tertiles of ARR and subjects characteristics, we compared means and proportions using analysis of variance and the χ^2 test for uni-

variate analysis. For computing sex- and age-adjusted *P* values, we used analysis of covariance and logistic regression analysis as appropriate. To explore the plausibility of the Cox model, we first plotted incidence rates by tertiles of PRA, PAC, and ARR, while standardizing by the direct method for sex and age (<50, 50–65, and ≥65 years). We calculated hazard ratios of PRA, PAC, and ARR using multiple Cox regression while adjusting for sex as a categorical variable, and age, body mass index (BMI), and systolic blood pressure as continuous variables. Because subgroup analyses by salt intake were performed in previous studies of ARR,[7,17–19] we divided participants into two groups based on median salt intake. SAS version 9.1 software (SAS Institute, Cary, NC) was used for statistical analysis.

RESULTS

Participant characteristics

The 883 participants included 579 females (65.6%). Mean values were 59.0 ± 11.3 years for age, 23.5 ± 3.1 kg/m^2 for BMI, 5.02 ± 0.86 mmol/l for total cholesterol level, and 126.2 ± 12.3/79.1 ± 7.7 mm Hg for systolic/diastolic blood pressure. At enrollment, 155 (17.6%) participants were current smokers, 343 (38.8%) were current drinkers, 218 (24.7%) had hypercholesterolemia, 59 (6.7%) had diabetes mellitus, and 34 (3.9%) had a history of cardiovascular disease. The median (25th–75th percentiles) PRA level was 1.2 (0.7–2.0) ng/ml/h, the median PAC level was 6.4 (4.9–8.1) ng/dl, and the median ARR level was 5.3 (3.4 to 8.6) ng/dl per ng/ml/h. BMI, diastolic blood pressure, serum sodium, and serum potassium levels significantly increased with increases in ARR after adjustment for sex and age (**Table 1**). No other variables showed a significant and consistent association with ARR.

Association of PRA, PAC, and ARR with stroke risk

The mean duration of follow-up was 10.9 ± 2.5 years. Of the 883 subjects, a first stroke occurred in 45 subjects, including cerebral infarction in 29, intracerebral hemorrhage in 10, and subarachnoid hemorrhage in 6.

Figure 1 shows incidence rates of stroke across tertiles of PRA, PAC, and ARR. In subjects with high sodium intake (≥median of 4,058 mg/day (salt equivalent, 10.5 g/day)), the incidence rate of stroke increased with increases in ARR, whereas PRA and PAC had no consistent relation with stroke. Next, Cox regression analyses were performed using ARR as a continuous variable (**Figure 2**). Because of their positively skewed distributions, PRA, PAC, and ARR were natural-log transformed. In the Cox regression model, male gender (hazard ratio: 1.86, $P = 0.047$), older age (hazard ratio: 2.22 per 10-year increase, $P < .0001$), and systolic blood pressure

Table 1 | Clinical characteristics among groups classified by tertiles of ARR

	Tertiles of ARR (ng/dl per ng/ml/h)				
	<4.1	4.1–7.1	≥7.1		Sex- and age-adjusted
Characteristic	*n* = 294	*n* = 294	*n* = 295	*P*	*P*
Women, %	50.7	69.4	76.6	<.0001	—
Age, years	59.5 ± 11.3	59.2 ± 11.2	58.3 ± 11.3	0.4	—
Body mass index, kg/m^2,[a]	23.1 ± 3.2	23.5 ± 3.1	23.9 ± 3.1	0.009	0.006
Past history of heart disease, %	3.4	4.4	3.7	0.8	0.7
Diabetes, %	8.5	5.8	5.8	0.3	0.1
Hypercholesterolemia, %	23.8	23.5	26.8	0.6	0.7
Smoking, %	22.4	16.7	13.6	0.02	0.5
Drinking, %	44.2	36.7	35.6	0.07	0.6
Systolic blood pressure, mm Hg	128.6 ± 14.7	128.2 ± 12.5	129.4 ± 13.6	0.5	0.08
Diastolic blood pressure, mm Hg	71.9 ± 9.9	72.0 ± 7.7	73.0 ± 9.5	0.2	0.02
HbA_{1c}, %	5.16 ± 0.66	5.08 ± 0.55	5.12 ± 0.57	0.3	0.3
Total cholesterol, mmol/l	4.97 ± 0.90	5.03 ± 0.81	5.06 ± 0.87	0.4	0.9
eGFR, ml/min/1.73 m^2	86.3 ± 19.2	84.9 ± 18.1	85.8 ± 17.5	0.6	0.4
Serum sodium, mEq/l	141.6 ± 1.9	142.0 ± 1.7	142.2 ± 1.9	0.0007	0.001
Serum potassium, mEq/l	4.29 ± 0.38	4.29 ± 0.36	4.34 ± 0.35	0.1	0.04
Dietary intake					
Energy, kcal/day	1,912 ± 855	1,750 ± 664	1,837 ± 996	0.07	0.2
Sodium, mg/day	4,379 ± 3,320	4,408 ± 2,693	4,558 ± 2,881	0.7	0.9
Salt, g/day	11.1 ± 8.4	11.1 ± 6.8	11.5 ± 7.3	0.7	0.9
Potassium, mg/day	2,362 ± 1,018	2,441 ± 850	2,437 ± 799	0.5	0.9

eGFR was estimated from the serum creatinine value using a Japanese equation: eGFR (ml/min/1.73 m^2) = 194 × serum creatinin$^{-1.094}$ × age $^{-0.287}$ (if female × 0.739).[13]
ARR, aldosterone-to-renin ratio; eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}.
[a]Among total study subjects, 866 had data of body mass index.

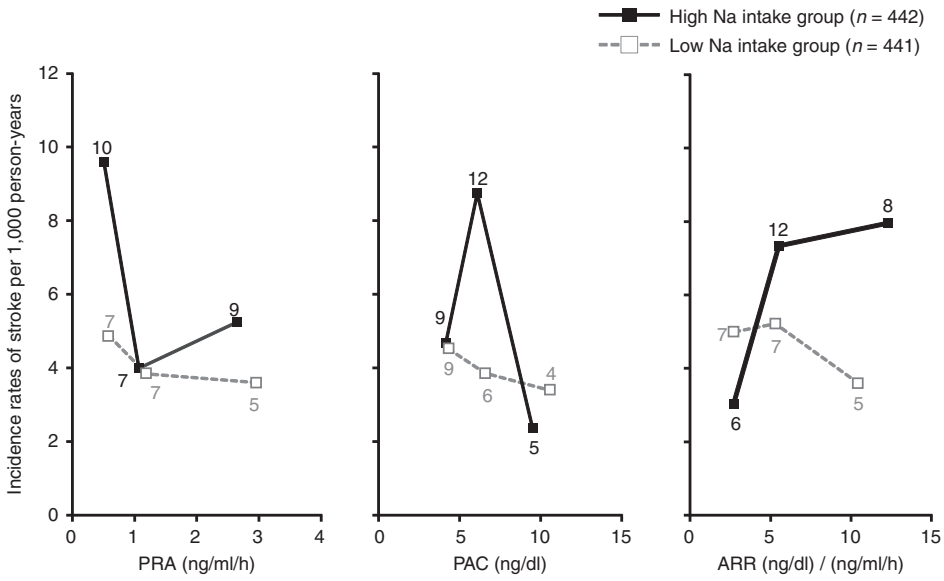

Figure 1 | Sex- and age-standardized incidence of stroke by median sodium intake. Incident rates of stroke across tertiles of plasma renin activity (PRA), plasma aldosterone concentration (PAC), and aldosterone-to-renin ratio (ARR) in all subjects (closed circles), in those with high sodium intake (≥median of 4,058 mg/day (salt equivalent, 10.5 g/day), closed squares), or in those with low sodium intake (<4,058 mg/day, open squares). Incidence rates were standardized by the direct method for sex and age (<50, 50–65, and ≥65 years). The number of events contributing to incidence rates is presented.

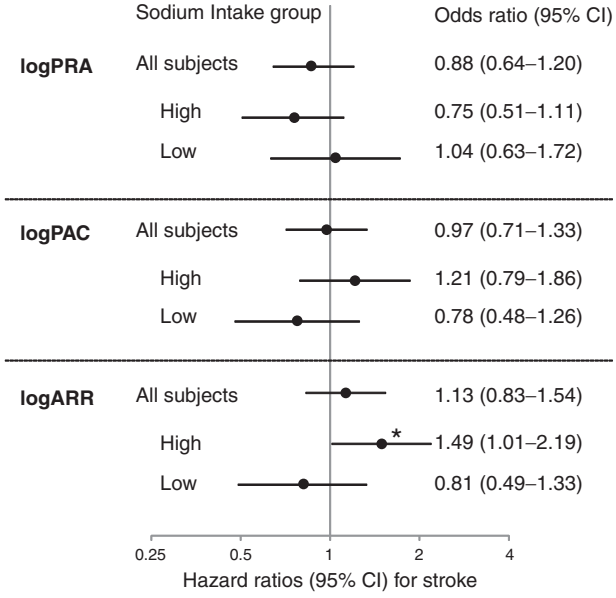

Figure 2 | Adjusted hazard ratios (95% CIs) for stroke by median sodium intake. Hazard ratios (95% CIs) indicated the stroke risk associated with each 1 s.d. increase in logPRA, logPAC, or logARR by median sodium intake (4,058 mg/day (salt equivalent, 10.5 g/day)) after adjusting for sex, age, body mass index, and systolic blood pressure. Stroke incidence occurred in 26 and 19 subjects with high and low sodium intake, respectively. *$P < 0.05$. ARR, aldosterone-to-renin ratio; CI, confidence interval; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

(hazard ratio: 1.25 per 10 mm Hg increase, $P<0.038$) were significantly associated with stroke risk. However, logARR ($P = 0.4$) and BMI ($P = 0.2$) were not significant predictors for stroke after adjustments in all subjects. Then, we stratified subjects by median sodium intake (4,058 mg/day (salt equivalent, 10.5 g/day)). In subjects with high sodium intake, each 1 s.d. increase in logARR (hazard ratio: 1.49, $P = 0.04$), male gender (hazard ratio: 3.26, $P = 0.005$), older age (hazard ratio: 2.58 per 10-year increase, $P \leq .0001$), and systolic blood pressure (hazard ratio: 1.41 per 10 mm Hg increase, $P = 0.034$), but not BMI ($P = 0.07$), were significantly associated with an increased hazard ratio for stroke. The interaction between logARR and sodium intake on stroke was not statistically significant ($P = 0.2$).

For the sensitivity analyses, we adjusted diastolic blood pressure or pulse pressure instead of systolic blood pressure, and we additionally adjusted for estimated glomerular filtration rate, serum sodium and serum potassium, because these variables were significantly associated with ARR as shown in **Table 1**. Even after applying these adjustments, the hazard ratio of logARR was almost consistent (hazard ratio: ≥1.46, $P \leq 0.057$). When logPRA and logPAC were simultaneously included in the same Cox regression model, similar results were observed; the hazard ratios were 0.67 ($P = 0.051$) for logPRA and 1.47 ($P = 0.1$) for logPAC. The association between ARR and stroke was not observed in subjects with low sodium intake ($P = 0.7$). When we repeated all of the analyses using logPRA or logPAC instead of logARR, we did not find any significant associations.

Among 442 subjects with high sodium intake, 356 (80.5%) had intermediate follow-up data regarding hypertensive status based on blood pressure and antihypertensive medication use after 8.0 ± 1.7 years of the baseline examination. Among these 356 patients, 134 patients became hypertensive, defined as a blood pressure of 140 mm Hg systolic or 90 mm Hg diastolic or the use of antihypertensive drugs. Then hypertensive status was further adjusted in the Cox regression analysis using two design variables: (1) hypertensive status (present = 1, absent = 0) and (2) missing value for hypertensive status (missing = 1, present = 0). With this analysis, the association of 1 s.d.

increase in logARR with stroke in subjects with high sodium intake was slightly weakened to the nonsignificant level (hazard ratio: 1.37, $P = 0.1$).

Although we also stratified subjects according to sex (men/women), age (<65/≥65 years), BMI (<25/≥25 kg/m^2), total energy intake (below or above median of 1,656 kcal/day), or potassium intake (below or above median of 2,366 mg/day) instead of sodium intake, we did not find any statistically significant association of logPRA, logPAC, and logARR with stroke (all $P \geq 0.2$). When we reanalyzed the association of ARR with stroke after changing the ARR cutoff for exclusion of patients with primary aldosteronism from 20 to 30 ng/dl per ng/ml/h, the significant association of logARR with stroke remained in subjects with high sodium intake (hazard ratio: 1.49, $P = 0.03$, $n = 458$).

DISCUSSION

The key novel finding of our study was that higher ARR, even within normal ranges, can be a predictor for stroke in a general population with high sodium intake, independent of sex, age, BMI, and systolic blood pressure. It is noteworthy that this finding was observed in a general population in whom primary aldosteronism had been ruled out by a standardized criterion of ARR ≥20 ng/dl per ng/ml/h. It has been reported that patients with primary aldosteronism have an increased prevalence of stroke compared with those with essential hypertension.[3] Our findings extend the validation of the relationship between aldosterone levels and cerebrovascular disease from primary aldosteronism patients to a general population of an Asian cohort.

Several studies have investigated the association of aldosterone alone[4] or renin alone[20] with stroke or cardiovascular disease. However, in terms of the relation with ARR, only the Ludwigshafen Risk and Cardiovascular Health (LURIC) study has reported this in terms of prognostic significance of cardiovascular mortality.[4] Their results indicated an inverse association between ARR and cardiovascular mortality; among 3,153 Caucasian patients with New York Heart Association I–IV heart failure who were referred for coronary angiography, patients who died from cardiovascular disease had a lower ARR than survivors at baseline.[4] Unlike their study, we observed a positive association between ARR and stroke incidence in subjects with high sodium intake. The inconsistency in the results between the LURIC study and our present study could be explained by differences in study populations (patients with heart failure vs. general population), antihypertensive treatment use (with antihypertensive treatment vs. without), or salt intake. Salt intake is higher in East Asia, including Japan, compared with Western countries[21] and is considered to strengthen the association of ARR with cardiovascular disease, which might lead to the positive relation of ARR with stroke that we found.

Our results regarding the adverse prognostic value of high ARR in combination with high sodium intake suggest an involvement of salt-sensitivity hypertension. So far, previous studies reported that salt sensitivity is caused by insulin resistance,[22] nonmodulating,[23] circadian clock-deficient,[24] or relative aldosterone excess.[7,25,26] Among them, relative aldosterone excess is most likely to be accountable for our present results, as relative aldosterone excess is a clinical entity of hypertension characterized by low renin combined with "normal" aldosterone level, leading to high ARR. In this clinical condition, aldosterone does not decrease despite low renin suppressed by sodium-volume overload, which is responsible for salt-sensitive hypertension due to inappropriate sodium and fluid retention.[7,18,25,26] In fact, our group previously demonstrated the possible contribution of relative aldosterone excess to salt-sensitivity in the general population.[7,18] In addition, we recently reported that relative aldosterone excess was related to a nondipping pattern, especially in subjects with high sodium excretion. The nondipping pattern is also considered an independent risk factor for cardiovascular events in both hypertensive and normotensive subjects.[27] In line with our previous studies,[7,18,27] our present results raise the hypothesis that relative aldosterone excess may adversely affect the cardiovascular system under chronic high-salt dietary conditions. Although some studies indicate that high ARR individuals are more common among hypertensive patients,[7,18,28,29] results of the present study highlight that relative aldosterone excess in a general population, including normotensive subjects, should not be ignored. However, the association of logARR with stroke was slightly weakened after adjustment for hypertensive status during follow-up. Thus, the hypertensive condition during follow-up might represent the pathogenetic link between an increased ARR and stroke.

Experimental studies also have reported direct adverse effects of aldosterone on the cardiovascular system such as cardiac hypertrophy, fibrosis, or inflammation,[30] which can be increased under conditions of high sodium intake.[1] Recently, Rigsby *et al.* reported that a mineralocorticoid receptor antagonist improved cerebrovascular structure after remodeling developed in rats by preventing fibrosis or inflammation in spite of no change in blood pressure.[31,32] Thus, treatment with a mineralocorticoid receptor antagonist might be potentially effective for preventing cardiovascular disease in a population with high sodium intake. However, before initiation of antihypertensive treatment, lifestyle modifications, including restricting salt intake, should be considered first. Salt reduction is an important component of standard therapy for hypertension, has antihypertensive effects without any side effects, and is more cost-effective than antihypertensive medications.[33] Reducing salt intake should be a target not only for hypertensive patients but also for the general population. A series of our studies based on a general population[7,18] provides further evidence of the importance of salt reduction for public health.

In **Table 1**, more women were seen in higher tertiles of ARR. The Framingham study also showed higher ARR in women than in men.[29] Estrogen is one of the regulators of angiotensinogen synthesis, which can increase aldosterone synthesis and suppress renin secretion.[34] Thus, sex differences or sex hormone balances might affect the association of ARR with stroke. In the present study, subjects with higher ARR had a relatively

high serum potassium level (Table 1). In typical patients with primary aldosteronism, aldosterone excess induces hypokalemia. However, most patients with a mild form of primary aldosteronism do not show typical clinical symptoms or hypokalemia. High serum potassium in a general population without apparent primary aldosteronism may be affected by not only ARR but also other factors. In contrast, elevated serum potassium levels might stimulate aldosterone excretion,[35] which can cause a high level of ARR. However, the results shown in Table 1 were based on a cross-sectional analysis, and it is thus difficult to discuss any cause–effect relationship.

The present study has several limitations. First, the study population predominantly included middle-aged, elderly, and female individuals. These imbalances might, to some extent, limit the external validity of the findings. Second, subjects underwent blood sampling between 9 and 11 AM or between 1 and 3 PM and most often had not fasted. The nonstandardized conditions of blood sampling could affect both PRA and PAC levels through circadian variations and dietary salt intake before the health checkup. However, it is also important to emphasize that, even when measured under nonstandardized conditions, ARR levels are clinically useful indices for stroke.[36] Third, we collected data on nondipping patterns in only a small fraction of study subjects; thus, we could not take into account the effect of nondipping patterns. Fourth, we obtained data on sodium intake from the 1-year food-frequency questionnaire, which was previously validated against a 3-day diet record method as a gold standard method of dietary assessment.[14,15] However, the validation was not confirmed by direct comparison with urinary sodium or potassium excretion levels. This might weaken the interaction between logARR and sodium intake on stroke incidence observed in this study. Fifth, the number of stroke events was too few to allow definitive conclusions to be drawn. The limited number of events might lead to a nonsignificant interaction between sodium intake and logARR ($P = 0.2$). Further prospective studies including a larger sample size are needed to support the associations among ARR, salt intake, and the future risk of stroke.

In conclusion, the present study provides perhaps the first prospective evidence that high ARR, even within normal ranges, may predict stroke in a general population with high sodium intake. These results raise the hypothesis that relative aldosterone excess may have a deleterious effect on stroke mediated by salt-sensitivity and suggest the possibility that salt reduction may relieve the deleterious effects of relative aldosterone excess.

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