aged, and we used this system to collect the data for this study. In each community, a municipal government office sent personal invitations to all the potential participants for the examination by letter or using public information services. The overall participation rate was 65.4%.

FI level was measured once at the baseline in three communities (Wara, Takasu and Sakuma) as an optional examination that included 3,100 subjects (1,338 men and 1,762 women).

In this study, we excluded individuals who had a past history of stroke or myocardial infarction (n = 50), who were undergoing treatment for diabetes mellitus (n = 61), who were diagnosed as indeterminable cases in the diagnosis of stroke (n = 1), whose blood samples could not be obtained (n = 26), who did not respond to questions about past history of stroke, myocardial infarction, diabetes mellitus, alcohol consumption or smoking habit (n = 251), and whose data about physical findings were incomplete (n = 101). Finally, 2,610 subjects (1,097 men and 1,513 women) remained and were analyzed as study participants.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a fully automated sphygmomanometer BP203RV-II (Nippon Colin, Komaki, Japan), placed on the right arm of a seated subject who had rested in the sitting position for five minutes before the measurement. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Triglycerides (TG) were measured by an enzymatic method (Wako, Osaka, Japan; interassay coefficient of variation (CV): 1.7%). HDL cholesterol was measured using the phosphotungstate precipitation method (Wako, Osaka, Japan; interassay CV: 1.9%). Fasting glucose (FG) was measured by an enzymatic method (Kanto Chemistry, Tokyo, Japan; interassay CV: 1.9%). FI levels were determined with a radioimmunoassay kit (Dainabot, Tokyo, Japan; interassay CV: 4.5 %). The lower detection limit was 2.5 mU/L, and insulin levels below this limit were taken as 2.0 mU/L.

Information about medical history and lifestyle was obtained with a questionnaire. Smoking status was classified as current smoker or non-smoker. Drinking status was classified as current drinker or non-drinker.

*Ethical issues*

Written informed consent for the study was obtained individually from all respondents of the mass screening. The study design and procedures were approved by each community government and the Ethical Committee of Epidemiologic Research at JMS.

*Follow-up system*

We asked the subjects directly whether they had a history of stroke and/or cardiovascular diseases after enrolling them in the present study by means of a health examination program in each community. If they had a history of such disease, we asked which hospital they visited and when the disease was diagnosed. Subjects who did not come to the screening examination were contacted by mail or phone. We also checked the medical records to see if the subjects had been hospitalized. Public health nurses also visited the subjects to obtain additional information. If an incident was suspected, forms for stroke incidence were filled out and duplicate computer tomography films or magnetic resonance imaging films were obtained for diagnostic confirmation. We collected death certificates to ascertain cause of death and date of death at the public health center of each community with permission from the Agency of General Affairs and the Ministry of Health, Labor, and Welfare. We were able to ascertain the endpoint of all participants who died between the date of their health examination and the end of 2002. Those who moved out of the communities during the observation period were followed until the date they left and data on these study subjects were obtained by each municipal government annually.

*Diagnostic criteria*

The diagnosis of stroke and stroke subtype were determined independently by the Diagnosis Committee, composed of one radiologist, one neurologist and two cardiologists. Diagnosis of stroke was determined by the presence of a focal and nonconvulsive neurological deficit lasting for more than 24 hours with a clear onset. Stroke subtype was determined by the criteria of the National Institute of Neurological Disorder and Stroke.

*Statistical analysis*

Values are expressed as the mean ± standard deviation (SD), except for TG and FG. The distributions of TG and FG were highly skewed; these data were expressed as the median and interquartile range and transformed into natural logarithms for statistical analysis. Data regarding proportions were expressed as a percentage.

Smoking status and drinking status were compared using the chi-square test. Multiple group comparisons were evaluated by the Kruskal-Wallis test. To investigate the risk of cerebral infarction associated with the FI, we divided the participants into five equal groups according to the quintiles of FI levels. Cutoffs of quartiles for the FI were 2.5, 3.7, 4.9 and 7.1. Crude incidence rates of cerebral infarction were calculated per 1,000 person-years. We used a Cox's proportional hazard model to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for cerebral infarction with FI quintiles. HRs and 95% CIs were first calculated after adjustment for age and then for age, SBP, smoking status, drinking status, HDL cholesterol, and BMI. The quintile with the lowest risk of cerebral infarction was defined as the reference category. A significant difference was defined as $p < 0.05$. Statistical analyses were performed using PASW Statistics 18 (SPSS Inc, Japan).

## RESULTS

During a mean follow-up of 11.1 years (Men, 10.9 years; Women, 11.2 years), 87 of 2,610 participants developed cerebral infarction (men, 46 cases; women, 41 cases).

### *Baseline characteristics*

Baseline characteristics of the study population in accordance with quintiles of the FI at the baseline are shown in Table 1. FI levels were positively associated with BMI, SBP, DBP, TG and FG. On the other hand, FI levels were inversely associated with age and HDL cholesterol.

### *Fasting insulin and risk of cerebral infarction*

Table 2 shows crude incidence rates and HRs for cerebral infarction by quintiles of FI. Crude incidence rates of FI quintiles 1-5 were 4.69, 2.35, 1.85, 2.77 and 3.30 per 1,000 person-years. The quintile with the lowest risk of cerebral infarction was Q3. Therefore, we defined Q3 as the reference category.

In age and sex-adjusted analysis (model 1), HRs for cerebral infarction were 2.11 (95% CI, 1.02 – 4.37) in Q1, 1.23 (95% CI, 0.54 – 2.77) in Q2, 1.70 (95% CI, 0.77 – 3.75) in Q4 and 2.29 (95% CI, 1.06 – 4.95) in Q5, using Q3 as the reference. In this analysis, HR in Q1 and Q5 were significantly higher than Q3. In multivariate-adjusted analysis (model 2), HRs for cerebral infarction were 2.33 (95% CI, 1.10 – 4.96) in Q1, 1.25 (95% CI, 0.55 – 2.84) in Q2, 1.68 (95% CI, 0.76 – 3.70) in Q4 and 2.06 (95% CI, 0.94 – 4.47) in Q5. In this analysis, HR in Q1 was significantly higher than Q3, and HR in Q5 was higher than Q3, but not significantly. A U-shaped relationship was seen between FI and risk of cerebral infarction and this relationship did not change after adjustment for several cardiovascular risk factors.

We performed the same analyses in other stroke subtypes (intracerebral hemorrhage, subarachnoid hemorrhage), but there were no significant differences between the quintiles in terms of FI (data not shown).

## DISCUSSION

The present prospective study found a positive relationship between the lowest quintile of FI and the risk of cerebral infarction, while a U-shaped relation was seen between FI and the risk of cerebral infarction in a Japanese general population. This association was not substantially altered by adjustment for established cardiovascular risk factors like BMI, SBP, HDL cholesterol, cigarette smoking and alcohol intake habits.

The relation between FI and stroke was examined in some previous studies. Folsom *et al.*[7], in the ARIC Study, reported there was positive association between high FI levels and the risk of stroke after adjustment for other cardiovascular risk factors. Nakamura *et al.*[3] reported that a positive relationship was shown between risk of stroke and FI in a prospective cohort study of middle-aged non-diabetic Japanese men. One nested case control study conducted in a nondiabetic population in northern Sweden reported that a high FI level was significantly positively associated with stroke.[11] Our results are consistent with the findings of these previous studies, although there was no statistically significant relation between the highest quintile of FI and risk of cerebral infarction after adjustment for other cardiovascular risk factors. An important finding of our study was that the lowest FI level increased the risk of cerebral infarction, but we found no reports investigating directly the association between a low FI level and cerebral infarction. The association of a low FI level with cerebral infarction has some indirect support from previously reported results. Wannamethee *et al.*[12], in the British Regional Heart Study, reported that risk of stroke increased in the lowest nonfasting insulin quintile. In the Bruneck Study, it was reported that not only high FI levels, but also

**Table 1: Baseline characteristics of the study population**

| | | Fasting insulin, mU/L | | | | | |
|---|---|---|---|---|---|---|---|
| | **Total** | **Q 1 (< 2.5)** | **Q 2 (2.5 – 3.6)** | **Q 3 (3.7 – 4.8)** | **Q 4 (4.9 – 7.0)** | **Q 5 (7.1 ≤)** | **P value** |
| **Subjects** | **2610** | **552** | **536** | **484** | **520** | **518** | |
| Age, years | 57.3 ± 11.9 | 59.0 ± 11.6 | 58.4 ± 11.0 | 56.7 ± 12.6 | 56.4 ± 12.0 | 55.7 ± 11.8 | < 0.001 |
| sex, men/women | 1097/1513 | 345/207 | 221/315 | 182/302 | 177/343 | 172/346 | < 0.001 |
| Current smoking, % | 22.5 | 36.6 | 20.5 | 19.0 | 19.4 | 16.0 | < 0.001 |
| Current alcohol drinking, % | 49.9 | 61.1 | 49.6 | 48.3 | 46.2 | 43.6 | < 0.001 |
| Body mass index, kg/m2 | 22.8 ± 3.0 | 21.0 ± 2.4 | 22.1 ± 2.5 | 22.8 ± 2.5 | 23.6 ± 2.9 | 24.5 ± 3.3 | < 0.001 |
| Systolic blood pressure, mm Hg | 130.2 ± 22.0 | 124.9 ± 21.8 | 128.9 ± 21.9 | 130.5 ± 21.8 | 132.4 ± 21.1 | 134.6 ± 22.2 | < 0.001 |
| Diastolic blood pressure, mm Hg | 77.3 ± 12.7 | 74.2 ± 11.9 | 76.7 ± 12.7 | 77.4 ± 12.9 | 78.2 ± 12.2 | 80.1 ± 13.0 | < 0.001 |
| HDL cholesterol, mg/dL | 49.8 ± 12.6 | 54.5 ± 13.7 | 50.6 ± 12.0 | 49.6 ± 12.3 | 48.5 ± 11.8 | 45.3 ± 11.3 | < 0.001 |
| Triglycerides, mg/dL | 89 (66 – 123) | 75 (59 – 102) | 84 (62– 115) | 86 (65 – 120) | 94 (70 – 132) | 108 (82 – 147) | < 0.001 |
| Fasting glucose, mg/dL | 92 (86 – 99) | 89 (84 – 95) | 91 (86 – 97) | 91 (86 – 99) | 93 (87 – 99) | 95 (89 – 104) | < 0.001 |

Values represent mean ± SD or percent except for triglycerides and fasting glucose, for which median and interquartile range are shown.
Q: quintile.



**Table 2: Risk of cerebral infarction and fasting insulin level**

| | | Fasting insulin, mU/L | | | | |
|---|---|---|---|---|---|---|
| | **Total** | **Q 1 (< 2.5)** | **Q 2 (2.5 – 3.6)** | **Q 3 (3.7 – 4.8)** | **Q 4 (4.9 – 7.0)** | **Q 5 (7.1 ≤)** |
| **Subjects** | **2610** | **552** | **536** | **484** | **520** | **518** |
| Number of events | 87 | 28 | 14 | 10 | 16 | 19 |
| Crude incidence rate[a] | 3.02 | 4.69 | 2.35 | 1.85 | 2.77 | 3.30 |
| HR (95% CI) in model 1[b] | | 2.11 (1.02 – 4.37) | 1.23 (0.54 – 2.77) | 1.00 (Reference) | 1.70 (0.77 – 3.75) | 2.29 (1.06 – 4.95) |
| HR (95% CI) in model 2[c] | | 2.33 (1.10 – 4.96) | 1.25 (0.55 – 2.84) | 1.00 (Reference) | 1.68 (0.76 – 3.70) | 2.06 (0.94 – 4.47) |

Q: quintile.
HR: Hazard ratio.
CI: Confidence interval.
a: per 1,000 person-years of follow-up.
b: Adjusted for age and sex.
c: Adjusted for age, sex, body mass index, systolic blood pressure, serum HDL cholesterol, cigarette smoking and alcohol intake category.

low FI levels were positively associated with risk of carotid atherosclerosis and coronary heart disease after adjustment for cardiovascular risk factors.[13,14] In this study, the authors suggested a hypothesis that hypoinsulinemia leads to insufficient insulinization, despite the expected higher insulin sensitivity.

The association of a high FI level with cerebral infarction is suggested by a lot of evidence from previously reported results. In a nested case control study, FI in patients with intracranial or extracranial atherosclerosis was significantly higher than FI in patients without atherosclerosis.[4] In a cross-sectional study, a positive correlation was seen between FI and carotid intimal-medial wall thickness.[15] In a basic study, there was a positive relation between long-term insulin injection and thickness of the aorta intima in rats.[16] Two cohort studies reported that carotid atherosclerosis was associated with the risk of cerebral infarction.[17,18] Therefore, we assume that high insulin levels cause carotid atherosclerosis and contribute to the development of cerebral infarction.

Previous studies reported that FI correlated positively with fibrinogen[19,20] and plasminogen activator inhibitor type 1 (PAI-1) activity.[19,21,22] Other previous studies reported that elevated fibrinogen levels[23,24] and PAI-1 activity levels[25,26] were associated with cerebral infarction. Therefore, we assume high FI levels induce high fibrinogen levels and PAI-1 activity levels and lead to the development of cerebral infarction. On the other hand, it is unknown why a low FI level is associated with cerebral infarction.

The present study has some limitations. Although the study subjects were selected from a population-based health check-up system, they were not selected at random and they lived in only 3 districts. Thus, the data may not be generalizable to other urban populations. In addition, single point data collection may have affected the results. There are some strong points in the present study. First, it was a longitudinal population-based study. Second, the subjects were followed for more than 10 years and the follow-up rate was quite high. Third, diagnosis of stroke was made by an independent committee using accepted diagnostic criteria. Fourth, the blood samples were analyzed at a single laboratory using the same measurement method, so we believe that the reliability of the data is high.

In conclusion, we showed that a low FI level is associated with increased risk of cerebral infarction and that the association between FI and risk of cerebral infarction appears to be U-shaped in a Japanese general population. However, it remains uncertain whether a low FI level is an independent risk of cerebral infarction in other general populations. Future studies are needed to confirm the relation between a low FI level and cerebral infarction events in other general populations.

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## DISCLOSURE

Conflict of interest: None

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Regular Article

# Inverse association between serum lipoprotein(a) and cerebral hemorrhage in the Japanese population

Shizukiyo Ishikawa [a,*,1], Kazuhiko Kotani [b,c,1], Kazuomi Kario [d], Kazunori Kayaba [e], Tadao Gotoh [a], Yosikazu Nakamura [c], Eiji Kajii [a]
and the Jichi Medical School (JMS) cohort study group

[a] Division of Community and Family Medicine, Center for Community Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-City, Tochigi 329-0498, Japan
[b] Department of Clinical Laboratory Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-City, Tochigi 329-0498, Japan
[c] Department of Public Health, Center for Community Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-City, Tochigi 329-0498, Japan
[d] Division of Cardiology, Department of Internal Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-City, Tochigi 329-0498, Japan
[e] School of Health and Social Services, Saitama Prefectural University, 820 San-nomiya, Koshigaya-City, Saitama 343-8540, Japan

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ABSTRACT

*Introduction:* Although lipoprotein(a) (Lp(a)) is involved in cardiometabolic disease processes, the association between serum Lp(a) and stroke and/or its subtypes has not yet been elucidated among Japanese people. This study investigated the association between Lp(a) and the incidence of stroke and/or its subtypes in the general Japanese population.
*Materials and Methods:* This population-based prospective cohort study included 10,494 community-dwelling participants (4,030 males/6,464 females). The incidence of stroke and its subtypes was the primary outcome. The subjects were divided into tertiles based on their Lp(a) levels, and the risk of all stroke and stroke subtypes was examined using Cox's proportional hazard model.
*Results:* A total of 393 subjects (199 males and 194 females) with stroke were identified during a follow-up duration of 10.7 years. The multivariate-adjusted hazard ratios for all stroke events were 0.55 (95% confidence interval: 0.38-0.81) and 0.69 (0.49-0.99) in the 2nd (9–19 mg/dl) and 3rd tertiles (≥20 mg/dl) of Lp(a) in reference to the 1st tertile (<9 mg/dl) in males, and 0.85 (0.59–1.24) and 0.76 (0.52–1.11) in 2nd (10–22 mg/dl) and 3rd tertiles (≥23 mg/dl) of Lp(a) in reference to the 1st tertile (<10 mg/dl) in females. The multivariate-adjusted hazard ratios for cerebral hemorrhage were 0.26 (0.10–0.67) and 0.34 (0.15–0.76) in the 2nd and 3rd tertiles of Lp(a) in reference to the 1st tertile in males, and were 0.48 (0.23–1.04) and 0.44 (0.21–0.96) in the 2nd and 3rd tertiles of Lp(a) in females.
*Conclusions:* Lp(a) was associated with the incidence of cerebral hemorrhage in the general Japanese population, particularly among males, while a similar trend was seen among females. A low Lp(a) level may be a marker of the risk of cerebral hemorrhage in this population.



## Introduction

Lipoprotein(a) (Lp(a)) is a unique lipoprotein particle consisting of a cholesterol-rich low-density lipoprotein (LDL) bound to apolipoprotein(a) (apo(a)) by a disulfide bridge [1]. High circulating Lp(a) levels are thought to be associated with the development of cardiovascular disease, via 1) intimal deposition of cholesterol of Lp(a), 2) the inhibition of thrombolysis and fibrin clearance because of competition between apo(a) (which has homology with plasminogen) with plasminogen for plasminogen receptors on vascular cells, or both [2]. However, the detailed mechanism(s) linking Lp(a) with atherogenesis has not yet been completely characterized [3].

Stroke is a health problem with a high prevalence in aging societies, including Japanese communities [4], which can severely impair the quality of life [5]. It is important to elucidate the pathophysiology of stroke. Therefore, many studies have explored the relationship between stroke and serum Lp(a).

A recent review that summarized prospective studies published prior to March 2009, showed that Lp(a) is a possible predictor of ischemic stroke (the adjusted risk ratio: 1.10 [95% confidential interval [CI]: 1.02–1.18]) [6]. However, conflicting results regarding the association between Lp(a) and stroke have been presented even after this review was published [6], and their association is still debated. One prospective study has shown no significant association between Lp(a) and stroke [7] and several, though not all [8,9], case-control studies have shown

* Corresponding author. Tel.: +81 285 58 7394; fax: +81 285 44 0628.
*E-mail address:* i-shizu@jichi.ac.jp (S. Ishikawa).
[1] These authors contributed equally to this work.

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higher Lp(a) levels in patients with ischemic stroke in comparison to controls [10–14].

In addition, while people with different types of stroke have differing clinical characteristics and prognostic outcomes [5], no prospective studies have so far focused on the association between Lp(a) and stroke subtypes, including cerebral hemorrhage. The review of the Emerging Risk Factors Collaboration only described no significant association between Lp(a) and hemorrhagic stroke (the adjusted risk ratio: 1.06 [95% CI: 0.90–1.26]) [6].

Circulating Lp(a) levels also vary widely by ethnicity [15–17]. This variation of Lp(a) may affect the results of the association between Lp(a) and stroke across ethnic groups. One prospective study suggested Lp(a) as a possible predictor of stroke in the Japanese people, but this result was obtained from Japanese hypercholesterolemic patients enrolled in a clinical trial, a special population that cannot always permit the generalization of the findings [18]. Furthermore, the influence of Lp(a) on stroke subtypes has not yet been characterized among the general Japanese population. Given these backgrounds, the objective of the present study was to investigate the association between the serum Lp(a) levels and the risk of stroke and/or its subtypes in a general Japanese population.

## Materials and Methods

### *Subjects*

The present study used the data of the Jichi Medical School (JMS) cohort study, a population-based prospective cohort study, with baseline data obtained between April 1992 and July 1995 in 12 communities in Japan. The details of the JMS cohort study have been described elsewhere [19]. There were 12,490 participants (4,911 males and 7,579 females; age, 19–93 years) in the JMS cohort study. The follow-up rate of this cohort population was 99.9% (only seven subjects were lost to follow-up). A total of 10,494 subjects (4,030 males and 6,464 females) were eligible for the present study, after excluding subjects without any data regarding serum Lp(a) and those with a past history of stroke.

### *Follow-up and Diagnosis of Stroke*

A routine mass screening examination system for cardiovascular disease was held in Japan by the Health and Medical Law Service in Japan and the system was utilized to collect the baseline data of the cohort study [19]. Most subjects were followed-up in repeated routine mass examinations every year. Subjects who did not come to the repeated examinations were directly contacted by mail and/or phone. All subjects were asked whether or not they had a history of new-onset stroke after participating in this cohort study, and those with a history of stroke were required to provide the information of hospital to which they were referred. The medical records at hospitals were checked to determine whether these subjects had been diagnosed and/or hospitalized for stroke. Public health nurses also visited these subjects to supplement information. The forms specific for recording the information of stroke were filled out by the investigators of this study, and computed tomography films and/or magnetic resonance imaging films for diagnosing a stroke were obtained from hospitals.

### *Diagnosis of Stroke*

The diagnosis of stroke, including transient ischemic attack or mini-stroke, was carried out independently by a diagnosis committee, of one radiologist, one neurologist and two cardiologists. The diagnosis was determined by the presence of a focal and nonconvulsive neurological deficit lasting for 24 hours and longer with a clear onset. Neuroimaging, such as computed tomography and/or magnetic resonance imaging, was used for 98% of all stroke cases, while only 2% of all stroke cases were diagnosed mainly based on the detailed information of the medical records at hospitals and by public health nurses. Stroke was classified into major subtypes according to the criteria of the National Institute of Neurological Disorder and Stroke [20].

### *Laboratory Examination*

The systolic blood pressure (SBP) and diastolic blood pressure were measured using an automated sphygmomanometer (BP203RV-II; Nippon Colin Co. Ltd., Komaki, Japan) placed on the right arm of a seated subject who had rested in the sitting position for 5 minutes before the measurement. The body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. The serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels were measured by enzymatic methods (Wako Co. Ltd., Osaka, Japan). Blood glucose was measured by an enzymatic method (Kanto Chemistry Co. Ltd., Tokyo, Japan). The serum Lp(a) levels were measured using an enzyme-linked immunosorbent assay kit (Biopool Co. Ltd., Uppsala, Sweden; intra- and inter-assay coefficients of variation <5%). The minimum detectable Lp(a) level was 1 mg/dl and if such levels are obtained, the undetectable Lp(a) value was recorded as 0.5 mg/dl. In addition, the medical history and lifestyle-related factors (smoking status: current-, ex- and non-smoking; alcohol drinking status: current-, ex- and non-drinking) were obtained by self-reported questionnaires.

### *Statistical Analysis*

Data are expressed as the means ± standard deviation (SD). The distribution of Lp(a) was skewed, and the data are expressed as the geometric means ± SD. Proportional data are expressed as percentages. Unpaired *t*-test for variables, Mann-Whitney U test for Lp(a) and chi-square test for categorical data were used to detect the difference in data between males and females. The crude incidence ratios were expressed as per 1000 person-years. Hazard ratios (HRs) and 95% CI for stroke and its subtypes were calculated using Cox's proportional hazard model with the 1st tertile of Lp(a) levels as a reference. Adjustment for age was used in calculating HRs, and full adjustment for age, smoking status and drinking status, BMI, SBP, TC, HDL-C and blood glucose was also used. These analyses were done using the SAS software package (version 8.2; SAS Institute, Inc., Cary, North Carolina, USA). A p-value<0.05 was considered to be significant.

## Results

A total of 393 subjects (199 males and 194 females) with new-onset stroke were identified over a mean follow-up duration of 10.7 years. The baseline characteristics of the cohort subjects are shown in Table 1. Males had significantly higher levels of blood pressure and blood glucose, and significantly a higher prevalence of current smokers and drinkers than females. Females had significantly higher levels of BMI, TC, HDL-C and Lp(a) than males.

The geometric mean Lp(a) level was 12.4 mg/dl in males and 14.6 mg/dl in females, and thus the cut-off levels of Lp(a) were 9 and 20 mg/dl in males and 10 and 23 mg/dl in females in tertiles of Lp(a). Table 2 shows that the crude annual incidence rates of all stroke events were 4.05 per 1,000 person-years in the 1st tertile of Lp(a) levels, 3.14 in the 2nd tertile and 3.46 in the 3rd tertile among all subjects. There was a significantly lower risk of all stroke events in the 2nd and the 3rd tertiles than that in the 1st tertile by an age-adjusted model (2nd tertile; HR: 0.72, 95% CI: 0.56–0.91, 3rd tertile; 0.71, 0.56–0.89) or a fully adjusted model (2nd tertile; HR: 0.74, 95% CI: 0.57–0.96, 3rd tertile; 0.76, 0.58–0.98). A lower risk of all stroke events with increased Lp(a) tertile levels was clearly observed in males (2nd tertile: 0.55, 0.38–0.81, 3rd tertile: 0.70, 0.49–0.99 in a fully adjusted model), while this was similarly but insignificantly

**Table 1**
Baseline characteristics of the study population.

| | Males | | | Females | | | P-value |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | n | Mean | SD | |
| Age (years) | 4,030 | 55.2 | 12.0 | 6,464 | 55.2 | 11.4 | 0.92 |
| Body mass index ($kg/m^2$) | 3,840 | 22.9 | 2.9 | 6,230 | 23.2 | 3.2 | 0.001 |
| Systolic blood pressure (mmHg) | 3,852 | 131.1 | 20.5 | 6,269 | 127.7 | 21.0 | <0.001 |
| Diastolic blood pressure (mmHg) | 3,852 | 79.1 | 12.3 | 6,269 | 76.2 | 12.1 | <0.001 |
| Total cholesterol (mg/dl) | 4,026 | 185.0 | 34.0 | 6,458 | 195.9 | 34.6 | <0.001 |
| HDL-cholesterol (mg/dl) | 4,026 | 49.1 | 13.5 | 6,458 | 52.7 | 12.6 | <0.001 |
| Blood glucose (mg/dl) | 4,026 | 106.8 | 30.6 | 6,455 | 101.2 | 22.8 | <0.001 |
| Lipoprotein(a) (mg/dl) § | 4,030 | 12.4 | (4.5-33.9) | 6,464 | 14.6 | (5.7-37.4) | <0.001 |
| Smoking status† | | | | | | | |
| Current smoker | 1,905 | 49.9 | | 344 | 5.6 | | <0.001 |
| Ex-smoker | 1,095 | 28.7 | | 181 | 3.0 | | |
| Non-smoker | 815 | 21.4 | | 5,598 | 91.4 | | |
| Drinking status† | | | | | | | |
| Current drinker | 2,792 | 75.6 | | 1,516 | 25.5 | | <0.001 |
| Ex-drinker | 132 | 3.6 | | 96 | 1.6 | | |
| Non-drinker | 767 | 20.8 | | 4,327 | 72.9 | | |

SD: standard deviation, HDL: high-density lipoprotein. §: Geometric mean ± SD. †: Percentage for proportion. P-value: comparison between males and females by unpaired t-test, Mann-Whitney U test (§) or chi-square test (†).

observed in females (2nd tertile: 0.86, 0.59–1.24, 3rd tertile: 0.76, 0.52–1.11 in a fully adjusted model).

The HRs by stroke subtype are shown in Table 3. There was a significantly lower risk of cerebral hemorrhage in the 2nd and the 3rd tertiles than that in the 1st tertile among all subjects in an age-adjusted model (2nd tertile; HR: 0.46, 95% CI: 0.27–0.77, 3rd tertile; 0.38, 0.23–0.65) or fully adjustment model (2nd tertile; HR: 0.44, 95% CI: 0.25–0.77, 3rd tertile; 0.39, 0.22–0.70). The lower risk of cerebral hemorrhage with increased Lp(a) tertile levels was clearly observed in males (2nd tertile: 0.25, 0.10–0.63, 3rd tertile: 0.30, 0.13–0.71 in a fully adjusted model), while a similar trend in the 2nd tertile and a significantly lower risk of cerebral hemorrhage in the 3rd tertile was observed in females (2nd tertile: 0.50, 0.23–1.06, 3rd tertile: 0.46, 0.21–0.99 in a fully adjusted model). No significant differences were seen in the risks of cerebral infarction and subarachnoid hemorrhage in males and females, except for a significantly lower risk of cerebral infarction in the 2nd tertile in males (2nd tertile: 0.63, 0.41–0.98, 3rd tertile: 0.81, 0.54–1.23 in a fully adjusted model).

## Discussion

The present study showed that Lp(a) was significantly, independently and inversely associated with the incidence of stroke, and cerebral hemorrhage in particular, in a general Japanese population. The association was significantly observed in males, while females had a similar trend. These results suggest that low Lp(a) levels may be a predictive marker of future cerebral hemorrhage especially in Japanese males.

The association between Lp(a) and stroke is still debatable and there is little available data on the association between Lp(a) and stroke subtypes. Furthermore, there is paucity of data on the association between Lp(a) and stroke and its subtypes among the general Japanese population. Therefore, the current JMS cohort study of a general Japanese population offers valuable data on the association between Lp(a) and stroke subtypes. Moreover, cerebral hemorrhage often shows serious outcomes in comparison to other stroke events [21], and a deeper understanding of the pathophysiology of cerebral hemorrhage is thus a crucial issue. The finding of the inverse relationship between Lp(a) and cerebral hemorrhage may lead to an understanding of the mechanism of the association of Lp(a) with cerebral hemorrhage.

A common cause of hemorrhage is an imbalance in hemostasis (i.e. coagulopathy) [22]. Lp(a) can compete with plasminogen for plasminogen receptors because of a homology with plasminogen, presumably regulating *in vitro* hemostasis [2], and therefore low Lp(a) levels may be potentially associated with a bleeding trend. We think that low Lp(a) levels may hypothetically indicate some physiological dysfunction, because Lp(a) can function as a transporter of oxidized lipids in the circulation [23]. There has also been a recent study showing a significant association between low Lp(a) levels and all-cause deaths [24]. Meanwhile, the explanation for the finding of the clearer association between Lp(a) and cerebral hemorrhage in males, relative to females, observed in the present study, is further unknown. While a recent review of Emerging Risk Factors Collaboration [6] did not describe a gender-difference of the association between Lp(a) and stroke, an earlier review of the association between Lp(a) and vascular disease, including stroke, in older people has found that Lp(a) might predict stroke in males, but not in females [25]. It is necessary to establish the etiobiological mechanism for the current results.

A threshold level of Lp(a) (i.e. 25-30 mg/dl) for the development of cardiovascular disease and stroke has been suggested [26]. The cut-off level of the reference group of tertiles was approximately 10 mg/dl in the present study, and this is similar to that of the reference group in an earlier study showing a possible prediction of ischemic stroke by Lp(a) [27]. However, this cut-off level may not necessarily be valid [26]. Moreover, a review of the Emerging Risk Factors Collaboration [6] did not find the threshold effect of Lp(a) on vascular disease, including stroke. Further studies are necessary to clarify the cut-off value of Lp(a) for predicting the development of stroke.

**Table 2**
HRs of all stroke events according to the tertiles of lipoprotein(a).

| | Lipoprotein(a) | n | case | Incidence# | Age-adjusted HRs† | 95%CIs | Multivariate-adjusted HRs‡ | 95%CIs |
|---|---|---|---|---|---|---|---|---|
| All | 1st (<10) | 3,357 | 143 | 4.05 | 1.00 | | 1.00 | |
| | 2nd (10-21) | 3,525 | 119 | 3.14 | 0.72 | 0.56–0.91 | 0.74 | 0.57–0.96 |
| | 3rd (≥22) | 3,612 | 135 | 3.46 | 0.71 | 0.56–0.89 | 0.76 | 0.58–0.98 |
| Males | 1st (<9) | 1,273 | 76 | 5.79 | 1.00 | | 1.00 | |
| | 2nd (9-19) | 1,351 | 52 | 3.64 | 0.57 | 0.40–0.81 | 0.55 | 0.38–0.81 |
| | 3rd (≥20) | 1,406 | 71 | 4.73 | 0.67 | 0.49–0.93 | 0.70 | 0.49–0.99 |
| Females | 1st (<10) | 1,928 | 62 | 3.02 | 1.00 | | 1.00 | |
| | 2nd (10-22) | 2,319 | 68 | 2.71 | 0.84 | 0.59–1.18 | 0.86 | 0.59–1.24 |
| | 3rd (≥23) | 2,217 | 68 | 2.82 | 0.77 | 0.55–1.09 | 0.76 | 0.52–1.11 |

HRs: Hazard ratios, CIs: confidence intervals. #: Crude incidence rates are calculated per 1,000 person-years. †: HRs and 95%CIs are calculated with adjustment for age. ‡: HRs and 95% CIs are calculated with adjustment for age, smoking status, drinking status, body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol and blood glucose.

**Table 3**
HRs of stroke subtypes according to the tertiles of lipoprotein(a).

| Subtypes | | Lipoprotein(a) | case | Incidence# | Age-adjusted HRs† | 95%CIs | Multivariate-adjusted HRs‡ | 95%CIs |
|---|---|---|---|---|---|---|---|---|
| Cerebral hemorrhage | All | <10 | 41 | 1.16 | 1.00 | | 1.00 | |
| | | 10–21 | 22 | 0.58 | 0.46 | 0.27–0.77 | 0.44 | 0.25–0.77 |
| | | ≥22 | 21 | 0.54 | 0.38 | 0.23–0.65 | 0.39 | 0.22–0.70 |
| | Males | <9 | 22 | 1.68 | 1.00 | | 1.00 | |
| | | 9–19 | 8 | 0.56 | 0.31 | 0.14–0.69 | 0.25 | 0.10–0.63 |
| | | ≥20 | 10 | 0.67 | 0.33 | 0.16–0.70 | 0.30 | 0.13–0.71 |
| | Females | <10 | 19 | 0.93 | 1.00 | | 1.00 | |
| | | 10–22 | 13 | 0.52 | 0.52 | 0.26–1.05 | 0.50 | 0.23–1.06 |
| | | ≥23 | 12 | 0.50 | 0.45 | 0.22–0.92 | 0.46 | 0.21–0.99 |
| Cerebral infarction | All | <10 | 84 | 2.38 | 1.00 | | 1.00 | |
| | | 10–21 | 80 | 2.11 | 0.81 | 0.60–1.10 | 0.85 | 0.61–1.19 |
| | | ≥22 | 96 | 2.46 | 0.84 | 0.63–1.13 | 0.93 | 0.67–1.29 |
| | Males | <9 | 51 | 3.88 | 1.00 | | 1.00 | |
| | | 9–19 | 40 | 2.80 | 0.65 | 0.43–0.99 | 0.63 | 0.41–0.98 |
| | | ≥20 | 56 | 3.73 | 0.78 | 0.54–1.15 | 0.81 | 0.54–1.23 |
| | Females | <10 | 29 | 1.41 | 1.00 | | 1.00 | |
| | | 10–22 | 41 | 1.63 | 1.07 | 0.66–1.72 | 1.13 | 0.67–1.92 |
| | | ≥23 | 43 | 1.78 | 1.03 | 0.64–1.65 | 1.03 | 0.60–1.76 |
| Subarachnoid hemorrhage | All | <10 | 17 | 0.48 | 1.00 | | 1.00 | |
| | | 10–21 | 17 | 0.45 | 0.89 | 0.46–1.75 | 0.99 | 0.49–1.99 |
| | | ≥22 | 18 | 0.46 | 0.86 | 0.44–1.67 | 0.86 | 0.42–1.74 |
| | Males | <9 | 3 | 0.23 | 1.00 | | 1.00 | |
| | | 9–19 | 4 | 0.28 | 1.14 | 0.25–5.08 | 1.64 | 0.30–9.08 |
| | | ≥20 | 5 | 0.33 | 1.25 | 0.30–5.24 | 1.90 | 0.36-10.2 |
| | Females | <10 | 13 | 0.63 | 1.00 | | 1.00 | |
| | | 10–22 | 14 | 0.56 | 0.84 | 0.39–1.79 | 0.92 | 0.42–1.99 |
| | | ≥23 | 13 | 0.54 | 0.76 | 0.35–1.64 | 0.73 | 0.32–1.64 |

HRs: Hazard ratios, CIs: confidence intervals. #: Crude incidence rates are calculated per 1,000 person-years. †: HRs and 95%CIs are calculated with adjustment for age. ‡: HRs and 95% CIs are calculated with adjustment for age, smoking status, drinking status, body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol and blood glucose.

The strength of this study is the use of a population-based large sample from multiple communities. Previous reports have described a significant association between the Lp(a) and ischemic stroke [6,26] and no association between Lp(a) and hemorrhagic stroke [6]. The reasons for the inconsistency of these results between the previous reports and the present study are unknown; however, in addition to the differences in ethnic groups with regard to the Lp(a) levels [15–17] and in the assays used for detecting Lp(a) [26], the different characteristics of the study populations (e.g. the health consciousness may have been relatively high in participants recruited from a mass screening examination for cardiovascular disease in the present study setting [19]) may have partly affected the data. The present study has also limitations. The incidence of stroke, cerebral hemorrhage in particular, might have been relatively low to fully detect an association between Lp(a) levels and the risk of stroke. The subjects with transient ischemic attack or mini-strokes, who were not referred to hospitals, might not have been identified as stroke cases in this study. The follow-up period may have been too short to sufficiently evaluate the incidence of stroke. Serum Lp(a) levels were measured only one time at baseline, while Lp(a) levels are not significantly affected by external factors, such as nutrition, smoking, drinking status or the use of drugs and are known to remain unchanged during long periods of life [6]. On the other hand, genetic components of Lp(a) were not examined. These limitations will therefore need to be addressed in future studies.

In conclusion, the present cohort study on a general Japanese population showed that Lp(a) was significantly, independently and inversely associated with the incidence of cerebral hemorrhage among males in particular, while females had a similar trend. A low Lp(a) level may be a risk marker for cerebral hemorrhage in this population. The current data are therefore considered to merit further investigation.

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## Conflict of Interest Statement

The authors declare that there are no conflicts of interest associated with this research.

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# The effect of periodontal status and occlusal support on masticatory performance: the Suita study

Takayuki Kosaka[1], Takahiro Ono[1,2], Yoko Yoshimuta[1,2], Momoyo Kida[1,2], Miki Kikui[1], Takashi Nokubi[2], Yoshinobu Maeda[1], Yoshihiro Kokubo[3], Makoto Watanabe[3], Yoshihiro Miyamoto[3]

[1]Department of Prosthodontics and Oral Rehabilitation, Osaka University Graduate School of Dentistry, [2]Development Center for Evaluating Masticatory Function, Venture Business Laboratory, Office for University-Industry Collaboration, Osaka University

[3]Department of Preventive Cardiology, National Cerebral and Cardiovascular Center

Corresponding author: Takahiro Ono

Associate Professor, Department of Prosthodontics and Oral Rehabilitation, Osaka Univ. Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan.

Phone: +81-6-6879-2954; Fax: +81-6-6879-2957, E-mail: ono@dent.osaka-u.ac.jp

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## Abstract

**Aim:** In this study, we investigated the effect of differences in periodontal status in the masticatory performance of dentate subjects with the same occlusal supporting area

**Materials and Methods:** The subjects of the analysis were classified into those of Eichner A1–3 (n = 1094) and Eichner B1–4 (n = 529). Subjects' periodontal status was evaluated on the basis of the Community Periodontal Index (CPI). The number of functional teeth and occlusal support were investigated, the latter on the basis of the Eichner Index. Furthermore, masticatory performance was investigated by means of test gummy jelly. For each group, periodontal status was classified in two different ways, either with/without moderate periodontitis (CPI Code ≤2/≥3) or with/without severe periodontitis (CPI Code ≤3/4), and masticatory performance was compared between the various groups.

**Results:** In subjects who were Eichner A1 and B3, masticatory performance was significantly lower in subjects with moderate periodontitis compared with those without them, and in subjects with severe periodontitis compared with those without severe periodontitis.

**Conclusion:** Periodontal disease affects masticatory ability not only if occlusion is established by natural dentition with no tooth loss, but also if occlusal support has decreased.

## Clinical Relevance

**Scientific rationale for study:** Little has been known about the effect of periodontal status on masticatory ability in patients with a reduced number of teeth or occlusal support.

**Principal findings:** Periodontal disease affects masticatory ability not only if occlusion is established by natural dentition with no tooth loss, but also if occlusal support has decreased and is now being established by dentures.

**Practical implications:** These findings should provide important information for the provision of dietary guidance to elderly people with tooth loss or periodontal disease.

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## Introduction

Reduced masticatory ability as a result of tooth loss has an adverse effect on nutritional intake (Joshipura et al. 1996, Krall et al. 1998, Papas et al. 1998, Sheiham et al. 2001, Kanmori et al. 2003, Nowjack-Raymer et al. 2003, Sahyoun et al. 2003), which causes a decline in overall health, reportedly causing activities of daily living and quality of life to decrease (Miura et al. 1998, Marshall et al. 2002, Takata et al. 2004, Takata et al. 2006, Ikebe et al. 2007). Previous studies have identified factors such as number of residual teeth (Tatematsu et al. 2004, Ikebe et al. 2011), occlusal support (Ikebe et al. 2010), and maximum bite force (Tumrasvin et al. 2006, Lepley et al. 2011) as factors related to masticatory ability, and should these decrease or decline, then masticatory ability is reported to decrease.

Periodontal disease is a chronic inflammatory disease that causes swelling and pain in the gingiva and destruction of alveolar bone, and together with caries is a major cause of tooth loss in Japanese adults (Morita et al. 1994). The destruction of periodontal tissue due to chronic periodontitis adversely affects the sensory function of the periodontal ligament, reduces mechanical support of the teeth, and ultimately results in reduced masticatory ability (Johhansson et al. 2006), suggesting that periodontal tissue destruction as a result of advanced periodontal disease may affect masticatory ability. Previous studies have found that the loss of periodontal supporting structures for remaining molars due to periodontal disease has an adverse effect on masticatory ability (Borges et al. 2013), and that masticatory ability improved in patients with chronic periodontitis when they were given periodontal treatment (Pereira et al. 2012). These studies, however, addressed patients with natural dentitions, and there have been very

few studies investigating the effect of periodontal status on masticatory ability in patients with a reduced number of teeth or occlusal support. In particular, there has been no research on elderly people who have lost a large number of teeth, and the effect of periodontal status on masticatory ability in such individuals is unknown.

Many studies have investigated methods of evaluating masticatory ability. These can be broadly divided into methods based on the patient's own subjective evaluations, and objective methods that use some sort of objective index for evaluation. Subjective evaluation methods include methods of judging masticatory ability on the basis of food acceptability, determined by means of medical history-taking or questionnaires on food intake (Wayler et al. 1982, Sato et al. 1989, Slagter et al. 1992). Objective evaluation methods include the following techniques. One is the sieve method, which uses peanuts (Manly & Braley 1950, Kapur et al. 1964, Yamashita et al. 2000), hydrocolloid material (Ohara et al. 2003), or silicone impression blocks (Slaqter et al. 1993) and passes them through sieves that separate different degrees of grinding in order to measure masticatory performance. Other methods measure changes in ingredients eluted from within test materials, such as dye eluted from capsules containing pigment-coated granules (Nakashima et al. 1989) and the optical density method, which utilizes glucose eluted from gummy jelly as an indicator (Okiyama et al. 2003, Ikebe et al. 2005). Evaluation methods that focus on mixing ability include the mastication of cubes of paraffin wax (Sato et al. 2003) or color-changeable chewing gum (Hayakawa et al. 1998) and the evaluation of the mixing of the test material. Of all these evaluation methods, the glucose elution method using test gummy jelly is a simple, clinically applicable method of measuring masticatory performance, and has been shown to provide high accuracy and reproducibility provided that the method of measurement is

strictly controlled (Ikebe et al. 2005). Numerous epidemiological studies utilizing test gummy jelly have been performed, and contraction of the occlusal supporting area and reductions in occlusal force and salivary flow rate have been shown to be associated with reduced masticatory performance (Ikebe et al. 2012).

In this study, we carried out a large-scale survey of the general population of a Japanese city in the form of participants in the Suita Study, a prospective cohort study of cardiovascular diseases (Kokubo 2011), using test gummy jelly to measure masticatory performance in order to ascertain the effect of periodontal status on masticatory ability. Previous studies have shown that a decrease in occlusal support reduces masticatory ability (Ikebe et al. 2010), and in this study we investigated the effect of differences in periodontal status in the masticatory performance of dentate subjects with the same occlusal supporting area.

## Methods

1. Subjects

The subjects of this study were 1839 members of the general public (817 men, 1022 women, mean age 67.2 ± 7.9 years) residing in Suita City, Osaka Prefecture, who underwent a health checkup as part of the Suita Study, a prospective cohort study of cardiovascular diseases, implemented by the Department of Preventive Cardiology of the National Cerebral and Cardiovascular Center between June 2008 and June 2012. The Suita Study is a cohort study of a random sample of Suita residents that was started in 1989 by the Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Osaka, Japan and the Suita Medical Association with the aim of

promoting measures to prevent cardiovascular disease in Japan. The primary cohort comprised 6485 individuals who underwent basic health checkups at the National Cerebral and Cardiovascular Center from among 12,200 individuals selected randomly by sex and age group from the register of residents of Suita City in 1989, and the secondary cohort similarly comprised 1329 individuals out of 3000 selected in 1996, with the addition of 546 volunteers to make a total sample size of 8360 who in principle underwent a basic health checkup every other year.

Before the study started, the study protocol was approved by the Ethics Committee of the National Cerebral and Cardiovascular Center (M19-62), and only individuals who provided informed consent after receiving a full explanation of the study purpose and methods both in writing and orally were included among the subjects.

2. Oral examination

Subjects lay on a bed in the supine position, and an oral examination was performed under sufficiently bright artificial lighting. The number of functional teeth and occlusal support were investigated as factors associated with masticatory ability, the latter on the basis of the Eichner Index. Whether or not subjects wore dentures was also surveyed.

Subjects' periodontal status was evaluated on the basis of the Community Periodontal Index (CPI) by means of partial 10 index teeth recording (Ainamo et al. 1982).

a) Number of functional teeth, occlusal support

The number of functional teeth was defined as the total number of natural and treated teeth involved in masticatory function, including pontics in fixed partial dentures and implant-supported dental prostheses, but excluding wisdom teeth that were impacted or

had a high degree of torsion or slant. Occlusal support was evaluated using the Eichner Index, which is widely used for clinical prostheses.

b) Periodontal tissue examination

The periodontal tissue examination was performed by five dentists who had undergone calibration in advance. A total of 10 teeth were examined, comprising the maxillary and mandibular left and right first and second molars, the maxillary right central incisor, and the mandibular left central incisor, and if this test could not be performed because of loss of one or both of the central incisors concerned, the same tooth on the opposite side was examined. No evaluation was performed if all the relevant teeth were missing. Periodontal status was examined using a CPI probe (YDM, Tokyo, Japan) to evaluate each tooth with respect to six periodontal pockets according to the following criteria, and the highest-value code was recorded. The CPI codes were as follows. Code 0: No signs of inflammation of the gingiva; Code 1: Bleeding was evident after probing; Code 2: Dental calculus deposits (including those detected by probing up to 4 mm beneath the gingival margin); Code 3: Periodontal pocket of depth ≥4 mm but <6 mm; Code 4: Periodontal pocket of depth ≥6 mm. Cohen's κ value for the consistency between the periodontal tissue examinations of the five dentists was 0.78.

3. Masticatory performance examination

The subjects were first instructed to masticate a piece of test gummy jelly (20 mm × 20 mm × 10 mm, 5.5 ± 0.5 g, UHA Mikakuto, Osaka, Japan) freely 30 times without swallowing, after which they expectorated the comminuted jelly onto a piece of cotton gauze spread over the top of a paper cup, without leaving anything in their mouths. The