morning urine. Taken together with these studies, the data suggest that the current treatment by diabetologists along with administration of the usual hypoglycaemic and hypotensive drugs from the stage of normoalbuminuria or low microalbuminuria reduced the annual incidence of proteinuria to a level as low as 0.67/100 person-years. Ideally, however, the inclusion of a control group receiving placebo and matched to the drug-treated diabetic patients would be desirable in order to allow a firm conclusion to be drawn, although admittedly this would be ethically problematic. As the baseline UACR profoundly affected the cumulative incidence of proteinuria, it might be clinically useful to divide patients with microalbuminuria into low- and high-risk groups, i.e. those with low and high microalbuminuria, although the cut-off value remains to be determined.

In the present study, progression to proteinuria was independently associated with higher baseline HbA_{1c} and SBP levels in addition to an elevated baseline UACR. Furthermore, smoking was also a significant predictor of proteinuria. These results are consistent with previous studies [11, 15]. In the UKPDS, the risk factors most highly associated with proteinuria were reported to be urinary albumin, plasma creatinine, waist circumference, SBP, glycaemic control, LDL-cholesterol, and plasma triacylglycerol [15]. Indian-Asian ethnicity was also an independent risk factor for microalbuminuria and/or proteinuria [12, 15]. Smoking and male sex were reported to be independent predictors of proteinuria in addition to plasma cholesterol, mean blood pressure and HbA_{1c} [11]. Based on these epidemiological studies, tight glycaemic control has been reported to be effective for preventing the onset and/or progression of nephropathy in clinical trials such as the Diabetes Control and Complications Trial (DCCT), the Kumamoto study and the UKPDS [16–18]. Strict blood pressure control, especially with ACE inhibitors or ARBs, has also been demonstrated to be effective for delaying the progression of diabetic nephropathy [6, 7, 19–21]. However, in the present study, the initial usage of an ACE inhibitor and/or ARB, or statin was not significantly associated with the prevention of proteinuria. As this study was designed to clarify the effects of lifestyle intervention on subsequent occurrence of diabetic complications, it might have been difficult to recognise the effects of such drugs on the progression of diabetic nephropathy. In some studies, normalisation of microalbuminuria, i.e. remission/regression, has also been reported [6, 12]. In fact, in our study, 30.3% of 452 individuals with low microalbuminuria demonstrated normalisation.

However, following the advent of modern therapeutics, especially hypoglycaemic and antihypertensive agents, diabetic nephropathy is the most common cause of ESRD, and the number of patients being started on haemodialysis

is still increasing dramatically in many countries, particularly in Asia. Our data have major clinical relevance because we have demonstrated that the initiation of hypoglycaemic and antihypertensive treatment from the early stage of nephropathy might lower the rate of transition to proteinuria even in the Japanese, who are highly susceptible to diabetic nephropathy. To reduce the number of patients who require haemodialysis, it is very important to measure UACR, make a diagnosis of diabetic nephropathy, define the stage of nephropathy and initiate strict glycaemic and blood pressure control as early as at the normo- or low-microalbuminuria stage.

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SHORT COMMUNICATION

Low serum potassium levels and risk of type 2 diabetes: the Toranomon Hospital Health Management Center Study 1 (TOPICS 1)

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Abstract

Aims/hypothesis Evidence has suggested that low serum potassium concentrations decrease insulin secretion, leading to glucose intolerance, and that hypokalaemia induced by diuretics increases the risk for diabetes in hypertensive individuals. However, no prospective study has investigated the association between serum potassium and the development of type 2 diabetes in a healthy cohort comprised of Asian individuals not being administered antihypertensive medications. This study aimed to investigate whether low serum potassium is associated with increased risk of type 2 diabetes in apparently healthy Japanese men.

Methods We followed 4,409 Japanese men with no history of diabetes, use of antihypertensives, renal dysfunction or liver dysfunction (mean ± SD age, 48.4±8.4 years). Cox proportional hazards regression was used to estimate HRs for incident diabetes (fasting plasma glucose level ≥7.0 mmol/l, HbA_{1c} ≥6.5% or self-reported) including serum potassium concentration as either a categorical or a continuous variable.

Results During a 5 year follow-up, 250 individuals developed type 2 diabetes. The lowest tertile of serum potassium (2.8–3.9 mmol/l) was independently associated with the development of diabetes after adjustment for known predictors (HR 1.57 [95% CI, 1.15–2.15]) compared with the highest tertile (4.2–5.4 mmol/l). Every 0.5 mmol/l lower increment in the baseline serum potassium level was associated with a 45% (12–87%) increased risk of diabetes.

Conclusions/interpretation Mild to moderately low serum potassium levels, within the normal range and without frank hypokalaemia, could be predictive of type 2 diabetes in apparently healthy Japanese men.

Abbreviations
FPG   Fasting plasma glucose
TBK   Total body potassium
eGFR   Estimated glomerular filtration rate

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Introduction

Evidence has suggested that hypokalaemia or decreased serum potassium induced by diuretics is related to impaired glucose tolerance and increased risk of diabetes in hypertensive individuals [1, 2]. Low serum potassium approximates body potassium depletion, which is difficult to assess clinically [3]. In a small number of healthy individuals experimentally placed on a low-potassium diet, significant falls in serum potassium and total body potassium (TBK) were suggested to induce glucose intolerance associated with impaired insulin secretion [4,

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5], which is controlled by ATP-sensitive potassium channels [6]. A large cohort study showed an inverse association between dietary potassium intake and risk of type 2 diabetes [7]. More recently and concomitantly with the performance of our study, an inverse association of serum potassium and increased risk of type 2 diabetes independent of diuretic use was reported in white Americans and African-Americans [8]. However, until the present report, this issue was not studied in Asians. We examined whether low serum potassium was associated with an increased risk of type 2 diabetes in apparently healthy Japanese men without dietary restrictions.

Methods

The Toranomon Hospital Health Management Center Study (TOPICS) included apparently healthy Japanese government employees who underwent annual multiphasic health screening examinations. Each examination included biochemical tests and standard questionnaires on demographic characteristics, medical history and health-related habits. The study attempted to elucidate the incidence of and risk factors for various diseases among the Japanese population. Data were collected on 21,362 men and 8,222 women who had baseline examinations from 1997 to 2002. For our purpose, we analysed data on 5208 men who received four or more annual examinations during a 5 year follow-up. There were too few incident cases of diabetes among women for a meaningful analysis (27/1513 women after exclusions).

Diabetes was defined according to ADA criteria of fasting plasma glucose (FPG) ≥ 7.0 mmol/l, self-reported clinician-diagnosed diabetes or HbA_{1c} level $\geq 6.5\%$ [9]. Reasons for exclusion were diabetes at the baseline examination ($n=278$), taking antihypertensive medication ($n=453$), renal dysfunction (serum creatinine level >177 μmol/l, $n=3$), liver dysfunction (under outpatient treatment, $n=116$) or missing data on baseline characteristics ($n=59$). Data from 4,409 men aged 25–80 years were eligible for analysis.

Overnight fasting blood samples were immediately placed into Vacutainer tubes and spun in a cold centrifuge. Serum potassium concentration was measured by the ion-selective electrode method (Hitachi 008, Tokyo, Japan). Individuals were categorised into tertiles of serum potassium distribution. For comparison, data were analysed in four groups according to equal intervals of 0.3 mmol/l serum potassium (<3.7, 3.7–3.9, 4.0–4.2 and ≥ 4.3 mmol/l) based on our institute's normal range (3.7–4.8 mmol/l). The association of change in serum potassium during follow-up with risk of type 2 diabetes was also investigated.

Statistical analysis HRs and 95% CIs were estimated by Cox regression for each serum potassium category using the highest tertile (4.2–5.4 mmol/l) as the reference or potassium as a continuous variable. Follow-up time was calculated from the first examination to the date of confirmed diabetes or last follow-up examination. Tests for linear trends across increasing categories of serum potassium concentrations treated categories as continuous variables in a model. Changes in serum potassium from baseline were calculated only when data for 5 years of follow-up were available (2,594 men and 176 incident cases). SPSS version 17.0 (SPSS, Chicago, IL, USA) was used for statistical analysis; $p<0.05$ indicated statistical significance.

Informed consent was obtained from all participants. The study protocol followed the Japanese Government's Ethical Guidelines Regarding Epidemiological Studies in accordance with the Declaration of Helsinki and was reviewed by the Institutional Review Board at Toranomon Hospital.

Results

During 5 years of follow-up (median 4.98 years, 21,094 person-years), we documented 250 incident cases of type 2 diabetes (32% by self-report; 68% by FPG and/or HbA_{1c}). No correlation between serum potassium at baseline and BMI gain during follow-up was revealed ($p=0.928$). Older age was associated with higher serum potassium. The association between serum potassium and glucose or HbA_{1c} was attenuated after adjustment for age or a stratified analysis (Table 1).

Lower tertiles compared with the highest tertile of serum potassium trended to be associated with a higher HR for incident type 2 diabetes, but without statistical significance in the age-adjusted model (Table 2). The HR in the lowest tertile (2.8–3.9 mmol/l) became statistically significant after adjustment for HbA_{1c}, and the lowest tertile of serum potassium was associated with an increased risk independent of traditional predictors. A similar association was observed for categories of equal intervals of 0.3 mmol/l of serum potassium. Results for lower values (<3.7 mmol/l) within the first tertile (2.8–3.9 mmol/l) were associated with even greater risk. The association between serum potassium and risk of diabetes was fundamentally the same when HbA_{1c} values were not included among criteria for type 2 diabetes. Among the entire group (4,423 men) there were 230 incident cases of diabetes. Multivariate-adjusted HR for the lowest compared with the highest tertile was 1.74 (1.25–2.41).

Cox analysis with potassium as a continuous variable showed that every 0.5 mmol/l lower serum potassium level at baseline was associated with a 45% (12–87%) higher risk of type 2 diabetes in the entire group (multivariate + HbA_{1c} and FPG model). When individuals were stratified according to baseline prediabetic state, low potassium levels were signifi-

Table 1 Baseline characteristics of the study participants by tertiles of serum potassium levels

Variable	Serum potassium (mmol/l)			p value for trend
	Tertile 1 (2.8–3.9)	Tertile 2 (4.0–4.1)	Tertile 3 (4.2–5.4)	
n	1,296	1,402	1,711	
Serum potassium (mmol/l)	3.8±0.1	4.1±0.1	4.4±0.2	<0.001
Age (years)	47.2±7.9	48.1±8.2	49.7±8.8	<0.001
BMI (kg/m^2)	23.1±2.6	23.2±2.7	23.1±2.6	0.935
FPG (mmol/l)	5.3±0.4	5.3±0.5	5.3±0.4	0.017
HbA$_{1c}$ (%)[a]	5.2±0.3	5.3±0.3	5.3±0.4	<0.001
Systolic blood pressure (mmHg)	127±15	124±14	124±14	<0.001
Diastolic blood pressure (mmHg)	77±11	76±10	76±10	0.162
Prevalence of hypertension (%)[b]	18.6	14.1	14.6	0.002
Total cholesterol (mmol/l)	5.17±0.82	5.22±0.83	5.27±0.83	0.001
HDL-cholesterol (mmol/l)	1.31±0.36	1.30±0.35	1.31±0.36	1.000
Triacylglycerol (mmol/l)	1.21 (0.85–1.73)	1.15 (0.85–1.67)	1.19 (0.86–1.75)	0.633
Uric acid (μmol/l)	355.4±70.5	361.9±74.3	365.3±72.0	<0.001
eGFR (ml min^{-1} 1.73 m^{-2})[c]	76.4±12.2	75.8±11.9	74.1±12.4	<0.001
<60 ml min^{-1} 1.73 m^{-2} (%)	7.1	8.4	11.9	<0.001
Smoking (never/former/current) (%)	47.4/28.5/24.1	42.2/29.0/28.7	34.8/30.4/34.8	<0.001
Parental diabetes (%)	13.7	13.1	13.3	0.894

Data are n (%), mean±SD, % or median (interquartile ranges) unless otherwise indicated

[a] HbA$_{1c}$ determined as the National Glycohemoglobin Standardization Program: value (%)=0.0981×(10.39×[Japan Diabetes Society Committee for the Standardization value (%)]−16.8) + 1.95

[b] Hypertension defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg

[c] eGFR = 194 × creatinine$^{-1.094}$ × age$^{-0.287}$

p values for trend were tested by ANOVA for continuous variables and χ^2 test for categorical variables

eGFR, estimated glomerular filtration rate

cantly associated with an increased risk of type 2 diabetes, particularly among prediabetic individuals at baseline.

During follow-up, 315 individuals began antihypertensive therapy, but results differed little from those not taking antihypertensives. There was a 50% (15–96%) adjusted increased risk for each 0.5 mmol/l lower increment of serum potassium at baseline.

Quintile distribution of serum potassium changes suggested that larger decreases in serum potassium after baseline were associated with increasingly higher incident rates regardless of baseline serum potassium (quintile [Q] 1 less than −0.1 mmol/l, 9.0%; Q2 −0.1–0.0 mmol/l, 8.6%; Q3 0.1 mmol/l, 6.7%; Q4 0.2 mmol/l, 5.6%; and Q5 ≥0.3 mmol/l, 4.7%; p=0.001 for trend).

Discussion

This prospective study showed an association between lower serum potassium and increased risk of type 2 diabetes in Japanese men not using antihypertensive medications. Also, the results suggested hypokalaemia as a possible factor in progression from a prediabetic state to type 2 diabetes.

Our results coincide with studies of both hypertensive and healthy individuals [1, 2, 4, 5, 8]. Among hypertensive patients, every 0.5 mmol/l decrease induced by diuretics was associated with a 45% higher risk of diabetes [2]. Maintaining serum potassium above 4.0 mmol/l was reported to be useful in preventing thiazide-induced diabetes [1].

Glucose intolerance associated with decreased insulin secretion was induced in healthy individuals placed in a hypokalaemic state (2.4–3.6 [4] or 2.4–3.5 mmol/l by dietary restrictions [5]). The Atherosclerosis Risk in Communities (ARIC) study of white Americans and African-Americans in the USA [8] reported an independent association of low-normal serum potassium (2.5–5.0 mmol/l) with development of type 2 diabetes compared with high-normal levels (5.0–5.5 mmol/l), while our results showed an independent association of values <4.0 mmol/l with an increased risk of type 2 diabetes. Our results for an Asian study population agree with the results from the US study that a mild-to-moderate decrease in serum potassium, even within

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Table 2 HRs of incident type 2 diabetes for tertiles of serum potassium and for every 0.5 mmol/l lower serum potassium level according to baseline diabetic status

Variable	HR (95% CI) for tertile of serum potassium (mmol/l)				HR (95% CI) for each 0.5 mmol/l lower levels of serum potassium				
						Normoglycaemia	Pre-diabetes		
	Tertile 1 (2.8–3.9)	Tertile 2 (4.0–4.1)	Tertile 3 (4.2–5.4)	p value for trend	Total	FPG <5.6 mmol/l and HbA$_{1c}$ <5.7%	HbA$_{1c}$ ≥5.7%	FPG ≥5.6 mmol/l	HbA$_{1c}$ ≥5.7% and/or FPG ≥5.6 mmol/l
n	1,296	1,402	1,711		4,409	2,853	603	1,267	1,556
Incident cases/person-years	70/6,204	84/6,678	96/8,211		250/21,094	34/13,880	140/2,665	188/5,833	216/7,214
Age-adjusted	1.06 (0.78–1.45)	1.15 (0.85–1.54)	1.00 (Ref.)	0.660	1.12 (0.89–1.42)	1.12 (0.59–2.14)	1.38 (0.99–1.93)	1.31 (1.00–1.72)	1.30 (1.01–1.68)*
Multivariate[a]	1.10 (0.80–1.50)	1.17 (0.87–1.57)	1.00 (Ref.)	0.504	1.16 (0.92–1.48)	1.21 (0.63–2.33)	1.36 (0.96–1.93)	1.34 (1.02–1.77)*	1.33 (1.03–1.73)*
Multivariate[a]+FPG	1.30 (0.95–1.78)	1.14 (0.85–1.53)	1.00 (Ref.)	0.097	1.29 (1.02–1.64)*	1.20 (0.63–2.30)	1.31 (0.91–1.88)	1.43 (1.08–1.88)*	1.33 (1.03–1.72)*
Multivariate[a]+HbA$_{1c}$	1.58 (1.15–2.16)**	1.28 (0.95–1.71)	1.00 (Ref.)	0.004	1.51 (1.17–1.94)**	1.35 (0.69–2.65)	1.47 (1.04–2.07)*	1.57 (1.17–2.10)**	1.55 (1.18–2.03)**
Multivariate[a]+HbA$_{1c}$ and FPG	1.57 (1.15–2.15)**	1.17 (0.87–1.58)	1.00 (Ref.)	0.006	1.45 (1.12–1.87)**	1.34 (0.69–2.61)	1.32 (0.92–1.90)	1.56 (1.16–2.11)**	1.48 (1.12–1.95)**

Ref., reference

*$p<0.05$; **$p<0.01$

[a] Multivariate models were adjusted for age, parental history of diabetes, BMI, hypertension (SBP≥140 and/or DBP≥90), HDL cholesterol, log-transformed triacylglycerol and smoking habit (never/former/current)

normal range and without frank hypokalaemia, could influence the development of type 2 diabetes. Lower serum potassium appears important in the risk of type 2 diabetes across various ethnic groups.

Being normoglycaemic, the majority of our study participants were assumed to have normal insulin secretion at baseline. However, that early-phase insulin decreased progressively when FPG exceeded 5.6 mmol/l indicates an important role for early-phase insulin secretion in the development of glucose intolerance, specifically in Asians [10]. Therefore, hypokalaemia could influence the development of type 2 diabetes among prediabetic individuals, that is, those having metabolic abnormalities of reduced TBK and diminished insulin secretion. In addition, as HbA$_{1c}$ reflects chronic hyperglycaemia rather than acute dysglycaemia, robust results might be shown after adjustment for HbA$_{1c}$.

Low dietary potassium intake of 40 mmol/day resulted in a significant fall in serum potassium and TBK, leading to glucose intolerance in normal individuals [4]. Theoretically, a 0.3 mmol/l decrease in serum potassium corresponds to a 100 mmol reduction in TBK on average [3]. However, because redistribution between extracellular and intracellular spaces also influences serum potassium, hypokalaemia should be considered to coexist with normal body potassium levels. As TBK values were unavailable, we could not address the role of TBK. However, we found that in Japanese men without dietary restrictions, serum potassium, a widely available measurement in clinical practice, was associated with incident type 2 diabetes.

The study strengths were the large sample size, strict exclusion criteria, especially excluding those taking diuretics at baseline, and the availability of HbA$_{1c}$ data. The limitations were that all participants were men, neither serum magnesium nor insulin was measured and information on potassium supplementation or dietary intake was unavailable. Whether poor dietary potassium intake affected serum potassium and subsequently influenced our results could not be determined, although no independent association between dietary potassium intake and risk of diabetes has been shown [8]. Factors such as lifestyle might have influenced results. That managing serum potassium is effective in preventing diabetes cannot be confirmed from the present results. Further research is required. Also, the association between changes in serum potassium and risk of diabetes in healthy individuals should be studied.

In conclusion, low serum potassium even within the normal range could be predictive of type 2 diabetes in healthy Japanese men not using antihypertensive medications. This association might be universal, regardless of ethnicity.

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Heart Rhythm Disorders

Alcohol Consumption and Risk of Atrial Fibrillation

A Meta-Analysis

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Objectives	The purpose of this meta-analysis is to summarize the estimated risk of atrial fibrillation (AF) related to alcohol consumption.
Background	Results from observational studies examining the relationship between alcohol consumption and AF are inconsistent.
Methods	A systematic electronic search of Medline (January 1966 to December 2009) and Embase (January 1974 to December 2009) databases was conducted for studies using key words related to alcohol and AF. Studies were included if data on effect measures for AF associated with habitual alcohol intake were reported or could be calculated. The effect measures for AF for the highest versus lowest alcohol intake in individual studies were pooled with a variance-based method. Linear and spline regression analyses were conducted to quantify the relationship between alcohol intake and AF risk.
Results	Fourteen eligible studies were included in this meta-analysis. The pooled estimate of AF for the highest versus the lowest alcohol intake was 1.51 (95% confidence interval: 1.31 to 1.74). A linear regression model showed that the pooled estimate for an increment of 10 g per day alcohol intake was 1.08 (95% confidence interval: 1.05 to 1.10; $R^2 = 0.43$, p < 0.001). A spline regression model also indicated that the AF risk increased with increasing levels of alcohol consumption.
Conclusions	Results of this meta-analysis suggest that not consuming alcohol is most favorable in terms of AF risk reduction. (J Am Coll Cardiol 2011;57:427–36) © 2011 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is the most common sustained arrhythmia, representing a growing epidemic, and is accompanied by serious complications. Atrial fibrillation accounts for 45% of all embolic strokes and has a deleterious impact on longevity, with an approximate doubling of all-cause mortality (1). Although the etiology of AF is not fully understood, many epidemiological associations with AF, including both cardiac (e.g., valvular disease, cardiomyopa-

thy, coronary artery disease) (2) and noncardiac conditions (e.g., aging, obesity, sleep apnea, diabetes mellitus, metabolic syndrome, heavy alcohol consumption) (3) have been vigorously investigated.

The association of episodic heavy alcohol use with the onset of AF has been recognized as "holiday heart syndrome" for a long time (4). Recently, it has been hypothesized that not only episodic but also habitual heavy alcohol consumption is associated with the risk of AF (5). However, results from epidemiological studies that aim to confirm this hypothesis have been inconsistent, although high alcohol consumption has been associated with several major disease groups such as neoplasms and cardiovascular diseases (6). It is also important to clarify the overall impact of any degree of alcohol intake on AF risk given that moderate alcohol consumption has been associated with a lower risk of cardiovascular disease (7) or all-cause mortality (8). Therefore, our aim of this meta-analysis of observational studies is to review the risk of AF in relation to alcohol consumption, focusing on determining if there is a dose-response relation-

Abbreviations and Acronyms
AF = atrial fibrillation
CI = confidence interval
OR = odds ratio
PAF = paroxysmal atrial fibrillation
RR = relative risk

ship between any degree of alcohol consumption and the risk of AF as well as AF risk in relation to heavy alcohol drinking.

Methods

Search strategy. Electronic literature searches (Medline, January 1966 to December 2009; and Embase, January 1974 to December 2009) to identify studies describing alcohol intake and AF were conducted using medical subject headings related to alcohol (alcohol drinking OR alcohol related disorders OR alcoholism OR alcoholic beverage OR ethanol) and AF (arrhythmias OR atrial fibrillation). Reference lists from the identified articles were manually examined for relevant new articles. This process was repeated until no additional articles could be identified. No language restriction was imposed.

For inclusion, a study had to fulfill the following criteria: 1) have a cohort or case-control design; 2) identify AF as an outcome variable of interest separate from other arrhythmias; and 3) provide or allow calculation of the effect measure (i.e., relative risk [RR] in a prospective study or odds ratio [OR] in a retrospective study) with its corresponding confidence interval (CI). As an exception, because of the overlap between AF and atrial flutter, studies in which AF and atrial flutter were combined as a study outcome were also included. However, when data on risk of both AF only and the combination of AF and atrial flutter were provided simultaneously in 1 study, we used data on the risk of AF only. We excluded studies wherein alcohol consumption was classified as "yes" or "no" because the degree of daily alcohol consumption could not be ascertained through such a response.

Data extraction. Two of our investigators (S.K. and H.S.) independently reviewed all relevant articles and identified eligible studies. Discrepancies were resolved by group discussion. We extracted the following data from each publication: first author's name, year of publication, geographic region, design of the observational study (i.e., cohort or case-control), selection of study population (i.e., hospital-based or population-based), participants' characteristics (i.e., age [mean or range], proportion of men, and whether participants with heart disease that influenced AF risk were excluded), characteristics of outcome (i.e., onset or recurrence, dominantly paroxysmal atrial fibrillation [PAF] or dominantly chronic [persistent] AF, and whether atrial flutter was included in the study outcome), methods of assessment of alcohol consumption (i.e., questionnaire, interview, or reviews of medical records and registries), methods for ascertainment of AF (i.e., electrocardiogram screening, registries, or participant's report), category of alcohol intake, number of participants and cases, and study-specific controlled variables.

The effect measure in each study was extracted or, if the effect measure for AF was not provided, it was calculated based on data on the number of cases and noncases in referent and exposed groups. In principle, we defined the lowest alcohol intake category or no drinking as the referent group and the other category as the exposed group. When a study classified >2 alcohol intake categories, we extracted or calculated all available effect measures for AF. If a study provided several effect measures, such as unadjusted and adjusted effect measures, the most completely adjusted effect measure was used.

The effect measures were transformed to their natural logarithm (log OR/RR). Fundamentally, the standard error (SE) was calculated from the corresponding CI. In some studies (9–11), the SE corresponding to the log OR/RR was not provided. Then we directly calculated the SE corresponding to the log OR/RR using data on the number of cases and noncases in the exposed and referent groups in each comparison as follows:

$$SE^2 = \frac{1}{C_1} + \frac{1}{N_1} + \frac{1}{C_0} + \frac{1}{N_0}$$

(in case of log OR) (9,11) or:

$$SE^2 = \frac{1}{C_1} - \frac{1}{C_1 + N_1} + \frac{1}{C_0} - \frac{1}{C_0 + N_0}$$

(in case of log RR) (10), where C_1 and N_1 indicate the number of cases and noncases in the exposed group, respectively, and C_0 and N_0 indicate the number of cases and noncases in the referent group, respectively. If necessary, the effect measure and its corresponding SE were approximated from figures in the manuscripts using an image scanner (CanoScan LiDE 500F [resolution 600 dpi], Canon, Inc., Tokyo, Japan).

To standardize alcohol intake, we used a common scale (grams per day) for ethanol consumption. When a study used the number of drinks per day as a unit of alcohol intake, the unit was transformed into grams of ethanol according to the study-specific methods for estimating the amount of ethanol per drink. If the amount of ethanol per drink was not specified, the unit was considered equivalent to 12 g ethanol (12).

For each study, data on the mean level of daily alcohol intake for each category were extracted or calculated as point estimates of ethanol consumption. When this information was not provided, we assigned the mid-point of the upper and lower boundaries in each category as the average intake. If the highest category had an open upper boundary, mean alcohol intake was estimated to be 1.2 times the lower boundary (13).

Data synthesis. To summarize the association of habitual heavy alcohol consumption with the risk of AF, the effect measures were pooled for the highest versus lowest alcohol intake category. Based on the definition of heavy alcohol

drinking by the National Institute on Alcohol Abuse and Alcoholism (12), we limited this analysis to studies in which the highest alcohol intake category was defined as consumption of 2 or more drinks per day for men, 1 or more drinks per day for women, and 1.5 or more drinks per day for the combination of men and women. Subjects described as "alcohol abusers" and "alcoholics" were also considered to be heavy drinkers. The pooled estimate was calculated by averaging the log OR/RRs weighted by the inverse of variance based on a fixed- or random-effects model. We used the results from the random-effects model if between-study heterogeneity, which was assessed by Q statistics and I-squared (14), was significant (15). Because daily alcohol consumption in heavy alcohol drinkers varied from study to study, we conducted stratified analysis according to the degree of "heavy" drinking. Analyses were also stratified by the pre-specified study characteristics. We also conducted meta-regression analyses to assess the influence of study characteristics on study results.

The possibility of publication bias was assessed primarily by visual inspection of a funnel plot in which the effect measure in the individual study was plotted against its corresponding SE. The funnel plot is expected to be symmetrical with respect to the overall estimate if publication bias is absent. We secondarily assessed the possibility of publication bias by 2 formal tests: the Begg's adjusted rank correlation test (16) and the Egger's regression asymmetry test (17). If publication bias was statistically suspected, we also followed the Duval and Tweedie "trim and fill" procedure (18) for further estimation of the possible effect of the publication bias. This method considers the possibility of hypothetical unpublished studies that would have allowed a funnel plot to be symmetrical and recalculates a pooled

estimate after imputation of the effect measures of the hypothetical studies as though they actually existed.

We primarily used weighted, least-squared regression models (19) to explore the dose-response relationship between alcohol intake and the risk of AF by regressing the log OR/RR of AF on the alcohol dose. To further investigate the shape of the relationship between the level of alcohol consumption and the risk of AF, we used restricted cubic splines with knots at the 25th, 50th, and 75th centiles of the distribution of alcohol consumption. These analyses were limited to data from studies with a referent category whose mean alcohol consumption was reported or estimated to be less than 1 drink per day, so that overlapping of alcohol intake of exposed and referent groups could be avoided as much as possible. Two-sided p values of ≤ 0.05 were considered statistically significant except for tests of publication bias for which the recommended level is p value ≤ 0.10 (20). Data were analyzed using STATA software version 10 (STATA Corp., College Station, Texas).

Results

Study characteristics. Figure 1 shows details of the literature search. Our electronic literature search resulted in retrieval of 1,754 citations (591 from Medline and 1,163 from Embase). Of these, 1,673 citations were excluded after the first screening. Eighty-one papers as well as 20 additional papers identified by manual search were left for full-text review. After this review, of the 101 papers, 87 were excluded for the reasons shown in Figure 1. Finally, 14 studies (9–11,21–31), which comprised 130,820 participants and 7,558 cases, were included in this meta-analysis.

1754 Citations Found in Databases Using Search Terms
591 MEDLINE
1163 EMBASE

1673 Excluded Based on Title and Abstract

81 Potentially Relevant Studies for More Detailed Evaluations

20 Articles Obtained from Manual Searches

87 Articles Excluded for the Following Reasons:
   25 Review articles
   13 Case studies
    3 Cross-sectional studies
   10 Did not assess alcohol intake
    5 Assessed alcohol intake as Yes or No (i.e., impossible to determine whether alcohol intake is high or moderate.)
    9 Did not investigate chronic but acute effect of alcohol intake
   19 Did not provide data on odds ratios or relative risks (OR/RR) of atrial fibrillation for high vs. low/non alcohol intake
    1 Standard error of OR/RR could not be estimated
    1 Data updated by more recent studies
    1 Same cohort as one of the included studies

14 Articles Included in Our Analysis

Figure 1   **Study Flow Chart of Meta-Analysis**

Table 1	Characteristics of Studies Included in the Meta-Analysis

Authors (Ref. #)		Year of Publication	Design	Selection of Population	Country	Age (yrs), Range (Mean)	% Men
Rich et al. (9)		1985	Case-control	Hospital-based	U.S.	18–70	76
Cohen et al. (10)		1988	Cohort	Population-based	U.S.	NA	NA
Krahn et al. (21)		1995	Cohort	Population-based	Canada	18–62 (31)	100
Wilhelmsen et al. (22)		2001	Cohort	Population-based	Sweden	47–55	100
Ruigomez et al. (23)		2002	Case-control	Hospital-based	Sweden	40–89	46
Djousse et al. (24)	Men	2004	Cohort	Population-based	U.S.	28–62	100
	Women						0
Frost and Vestergaard (25)	Men	2004	Cohort	Hospital-based	Denmark	50–64	100
	Women						0
Mattioli et al. (11)		2005	Case-control	Population-based	Italy	54	74
Mukamal et al. (26)	Men	2005	Cohort	Population-based	U.S.	26–75 (51)	100
	Women					26–73 (52)	0
Ruigomez et al. (27)	PAF	2005	Case-control	Hospital-based	Sweden	40–89	47
	Chronic		Cohort	Hospital-based		40–89	49
Planas et al. (28)		2006	Cohort	Hospital-based	Spain	53	64
Mukamal et al. (29)		2007	Cohort	Population-based	U.S.	≥65	42
Conen et al. (30)		2008	Cohort	Population-based	Switzerland	≥45 (53)	0
Marcus et al. (31)		2008	Case-control	Both	U.S.	53	75

Authors (Ref. #)		Were Patients With Heart Disease Excluded?	PAF Dominant or Chronic-AF Dominant	Was Method to Distinguish PAF From Chronic AF Described?	Onset or Recurrent AF	Was Atrial Flutter Among AF Events Included?	Method of Ascertaining AF	Method for Assessment of Exposure
Rich et al. (9)		Yes	PAF	Yes	Onset	No	Medical records	Medical records
Cohen et al. (10)		No	Chronic	No	Onset	No	Medical records	Questionnaires
Krahn et al. (21)		No	Chronic	No	Onset	No	ECG screening or physicians' report	Medical records
Wilhelmsen et al. (22)		No	Chronic	No	Onset	No	Registries	Questionnaires
Ruigomez et al. (23)		No	Chronic	Yes	Onset	No	Registries	Medical records
Djousse et al. (24)		No	Chronic	No	Onset	Yes	ECG screening	Questionnaires
Frost and Vestergaard (25)		Yes	Chronic	No	Onset	Yes	Registries	Questionnaires
Mattioli et al. (11)		Yes	PAF	Yes	Onset	No	Medical records	Questionnaires
Mukamal et al. (26)		Yes	Chronic	No	Onset	Yes	ECG screening, medical records, Registries	Interviews
Ruigomez et al. (27)	PAF	No	PAF	Yes	Onset	No	Registries	Medical records
	Chronic	No	Chronic		Recurrent	No	Registries	
Planas et al. (28)		Yes	PAF	Yes	Recurrent	No	ECG screening	Medical records
Mukamal et al. (29)		No	Chronic	No	Onset	Yes	ECG screening, medical records, Registries	Questionnaires
Conen et al. (30)		Yes	Chronic	No	Onset	No	Participants' reports	Questionnaires
Marcus et al. (31)		No	PAF	No	Onset	No	Medical records	Interviews

Characteristics of the 14 included studies are shown in Table 1. Nine studies used a cohort design, 4 studies used a case-control design, and 1 study reported data from both case-control and cohort designs. All studies were conducted in Western countries (7 in Europe and 7 in North America).

For assessing daily alcohol consumption, only 4 of the 14 studies (25,26,29,31) validated methods to assess alcohol intake. All effect measures were controlled for age and sex, and most of the included reports (10 studies) made adjustments for heart disease, which potentially elevates the risk of AF, or excluded participants with heart disease. Only 3 studies (10,29,31) considered racial differences among the participants.

Risk of AF through heavy alcohol consumption. The lowest amount of alcohol consumed in the highest category in each study ranged from 1.5 to 6 drinks per day. Consequently, in all 14 studies, the criteria for heavy alcohol drinking as previously defined were met (12). Three studies reported separate results according to sex. One study analyzed 2 populations separately according to age (≤60 years or >60 years), and 1 study indicated 2 risk measures (1 for PAF and another for the progression from an AF episode to permanent AF). Finally, 19 effect measures were analyzed to

Table 1	Continued				
		Category of Alcohol Intake (Ethanol Consumption)	No. of Cases	No. of Participants	Duration (yrs)*
Rich et al. (9)		>70 ml/day or not	58	116	—
Cohen et al. (10)		6 or more drinks/day or <1 drink/day	28	3,966	—
Krahn et al. (21)		Self- and physician-reported alcoholism or not	299	3,983	44
Wilhelmsen et al. (22)		Alcohol abuse or not	754	7,495	25.2
Ruigomez et al. (23)		None, 1–5, 6–15, 16–42, >42 U/week (1 U = 10 ml)	1,035	6,035	—
Djousse et al. (24)	Men	None, 0.1–12, 12.1–24, 24.1–36, >36 g/day	544	2,921	≥24
	Women		511	2,806	
Frost and Vestergaard (25)	Men	Quintile (4.1, 12.1, 20.0, 36.1, 68.7 g/day)	374	22,528	5.7
	Women	Quintile (1.1, 4.6, 9.4, 15.6, 38.8 g/day)	182	25,421	5.8
Mattioli et al. (11)		0, 1–20, 21–50, >50 ml/day	116	232	—
Mukamal et al. (26)	Men	<1, 1–6, 7–13, 14–20, 21–27, 28–34, >34 drinks/week (1 drink = 12 g)	548	7,588	16.3
	Women	<1, 1–6, 7–13, 14–20, >20 drinks/week (1 drink = 12 g)	523	8,827	18.8
Ruigomez et al. (27)	PAF	None, 1–7, 8–21, 21 U/week (1 U = 10 ml)	525	5,525	—
	Chronic		70	418	2.7
Planas et al. (28)		>40 g/day or not (men); >20 g/day or not (women)	32	115	2.5
Mukamal et al. (29)		None, former, <1, 1–6, 7–13, >13 Drinks/week (1 drink = 13.3 g)	1,232	5,609	9.1
Conen et al. (30)		None, <1, 1–2, >2 drinks/day (1 drink = 15 g)	653	34,175	12.4
Marcus et al. (31)		>1.5 drinks/day or not	74	260	—

		Age/Sex	Smoking	Study BMI or WC	Confounders SBP or HT	Heart Disease†	Other Control Variables	Total No. of Control Variables
Rich et al. (9)		✓				✓		2
Cohen et al. (10)		✓	✓				Race	3
Krahn et al. (21)		✓						1
Wilhelmsen et al. (22)		✓						1
Ruigomez et al. (23)		✓				✓		2
Djousse et al. (24)		✓			✓	✓		3
Frost and Vestergaard (25)		✓	✓	✓	✓	✓		5
Mattioli et al. (11)		✓				✓		2
Mukamal et al. (26)		✓	✓	✓	✓	✓	Education, income, diabetes, physical activity, respiratory function	10
Ruigomez et al. (27)	PAF	✓						1
	Chronic							
Planas et al. (28)		✓			✓	✓		3
Mukamal et al. (29)		✓		✓	✓	✓	Race, income, diabetes, use of psychoactive medication	8
Conen et al. (30)		✓	✓	✓	✓	✓	Diabetes	6
Marcus et al. (31)		✓		✓	✓	✓	Race	5

*Duration is the duration between the time point of alcohol consumption and subsequent observation. †Cardiac function (e.g.. left ventricular end-systolic volume, left atrial size) was involved in heart disease.

AF = atrial fibrillation; BMI = body mass index; ECG = electrocardiogram; HT = hypertension; NA = not available; PAF = paroxysmal atrial fibrillation; SBP = systolic blood pressure; WC = waist circumference.

summarize the risk of AF in relation to heavy alcohol intake. The pooled estimate of OR/RR for the highest category of alcohol consumption compared with the lowest category in individual studies was 1.51 (95% CI: 1.31 to 1.74) (Fig. 2).

There was significant between-study heterogeneity in the effect measures (Q-squared, 33.2; I-squared, 45.8%; p = 0.02). Table 2 shows results of stratified and meta-regression analyses across a number of key study characteristics to explore causes of the study heterogeneity. On the

whole, a positive association between AF risk and heavy alcohol consumption was consistently found in all stratified analyses.

In the stratified analysis by mean alcohol intake in the highest intake group, the pooled estimates of AF for <4 drinks per day, or 48 g per day, and ≥4 drinks per day was 1.32 (95% CI: 1.15 to 1.50) and 1.74 (95% CI: 1.35 to 2.24), respectively. However, the difference was not significant (p = 0.17). When analyses were limited to the 6 studies that regarded nondrinkers as the referent group, the
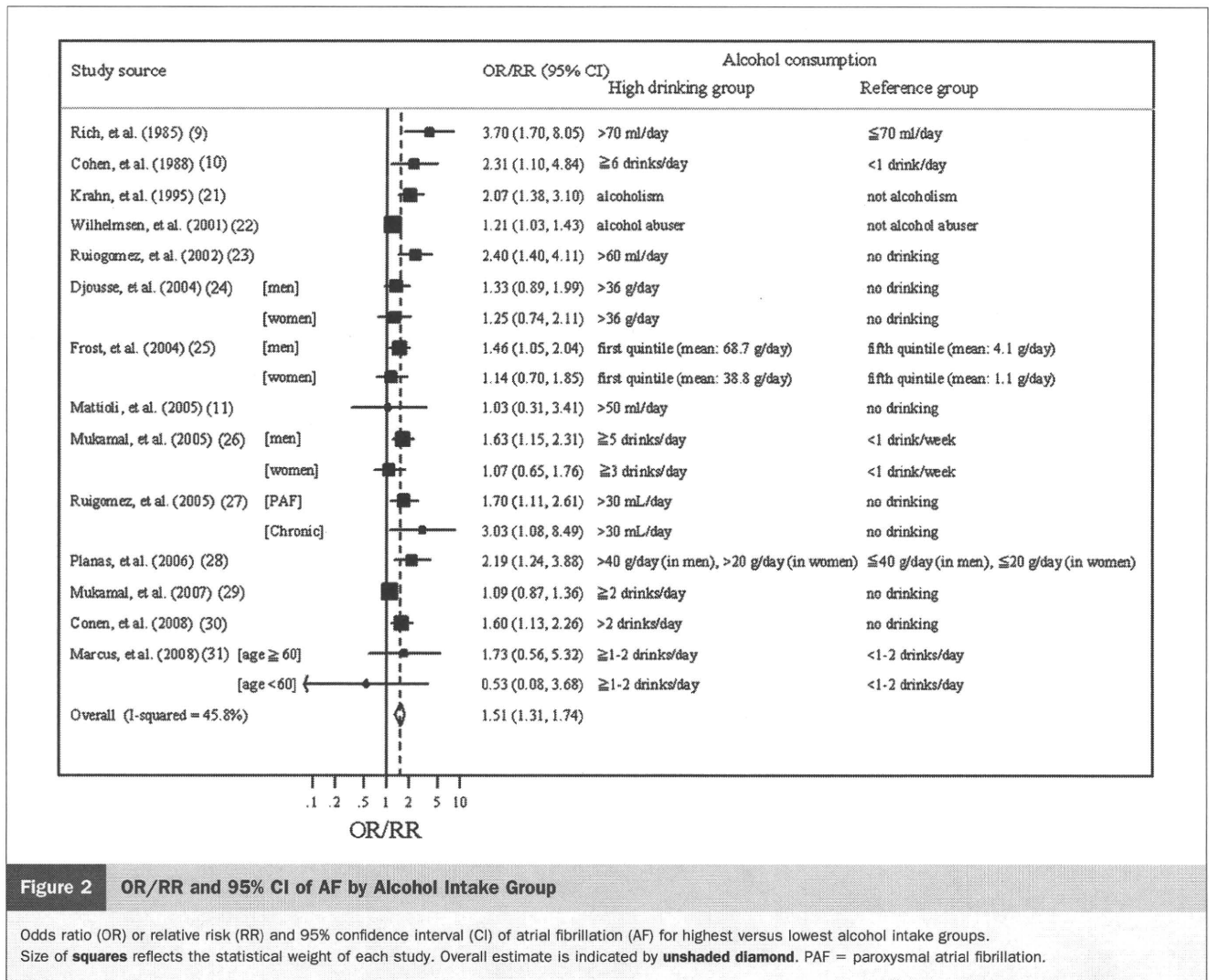
Study source		OR/RR (95% CI)	Alcohol consumption	
			High drinking group	Reference group
Rich, et al. (1985) (9)		3.70 (1.70, 8.05)	>70 ml/day	≤70 ml/day
Cohen, et al. (1988) (10)		2.31 (1.10, 4.84)	≥6 drinks/day	<1 drink/day
Krahn, et al. (1995) (21)		2.07 (1.38, 3.10)	alcoholism	not alcoholism
Wilhelmsen, et al. (2001) (22)		1.21 (1.03, 1.43)	alcohol abuser	not alcohol abuser
Ruiogomez, et al. (2002) (23)		2.40 (1.40, 4.11)	>60 ml/day	no drinking
Djousse, et al. (2004) (24)	[men]	1.33 (0.89, 1.99)	>36 g/day	no drinking
	[women]	1.25 (0.74, 2.11)	>36 g/day	no drinking
Frost, et al. (2004) (25)	[men]	1.46 (1.05, 2.04)	first quintile (mean: 68.7 g/day)	fifth quintile (mean: 4.1 g/day)
	[women]	1.14 (0.70, 1.85)	first quintile (mean: 38.8 g/day)	fifth quintile (mean: 1.1 g/day)
Mattioli, et al. (2005) (11)		1.03 (0.31, 3.41)	>50 ml/day	no drinking
Mukamal, et al. (2005) (26)	[men]	1.63 (1.15, 2.31)	≥5 drinks/day	<1 drink/week
	[women]	1.07 (0.65, 1.76)	≥3 drinks/day	<1 drink/week
Ruigomez, et al. (2005) (27)	[PAF]	1.70 (1.11, 2.61)	>30 mL/day	no drinking
	[Chronic]	3.03 (1.08, 8.49)	>30 mL/day	no drinking
Planas, et al. (2006) (28)		2.19 (1.24, 3.88)	>40 g/day (in men), >20 g/day (in women)	≤40 g/day (in men), ≤20 g/day (in women)
Mukamal, et al. (2007) (29)		1.09 (0.87, 1.36)	≥2 drinks/day	no drinking
Conen, et al. (2008) (30)		1.60 (1.13, 2.26)	>2 drinks/day	no drinking
Marcus, et al. (2008) (31)	[age ≥ 60]	1.73 (0.56, 5.32)	≥1-2 drinks/day	<1-2 drinks/day
	[age <60]	0.53 (0.08, 3.68)	≥1-2 drinks/day	<1-2 drinks/day
Overall (I-squared = 45.8%)		1.51 (1.31, 1.74)		

.1 .2 .5 1 2 5 10
OR/RR

Figure 2   **OR/RR and 95% CI of AF by Alcohol Intake Group**

Odds ratio (OR) or relative risk (RR) and 95% confidence interval (CI) of atrial fibrillation (AF) for highest versus lowest alcohol intake groups.
Size of **squares** reflects the statistical weight of each study. Overall estimate is indicated by **unshaded diamond**. PAF = paroxysmal atrial fibrillation.

pooled estimate for the highest category was 1.36 (95% CI: 1.18 to 1.57).

Stratified analysis by geographic region, participants' sex, or whether persons with heart disease were included did not show any significant difference in pooled estimates between strata. Using a case-control design seemed to produce a substantially larger AF risk (pooled estimates 1.98 [95% CI: 1.49 to 1.63]) compared with the use of other designs (pooled estimate 1.34 [95% CI: 1.22 to 1.47]). However, these differences were not borderline significant (p = 0.06). Selecting hospital-based participants produced a borderline significantly larger pooled estimate in comparison with population-based participants (pooled estimate 1.75 [95% CI: 1.45 to 2.11] vs. 1.30 [95% CI: 1.18 to 1.44]; p = 0.049).

Strong associations were observed when the type of AF end point in the study was PAF-dominant (pooled estimate 1.92 [95% CI: 1.44 to 2.56]) or AF recurrence (pooled estimate 2.37 [95% CI: 1.44 to 3.90]) whereas a significantly weaker association was observed in studies that included atrial flutter as a study end point (pooled estimate 1.25 [95% CI: 1.10 to

1.43]) than in those that did not (pooled estimate 1.83 [95% CI: 1.45 to 2.30]; p = 0.02).

The method for ascertainment of AF did not significantly affect the magnitude of the association between high alcohol consumption and AF risk. However, a significantly stronger association was observed when reviewed data on alcohol intake were based on medical records or registries (pooled estimate 2.17 [95% CI: 1.74 to 2.70]) compared with other methods of determining alcohol consumption, such as questionnaires or interviews (pooled estimate 1.28 [95% CI: 1.16 to 1.41]; p < 0.001). The influence of study adjustments for possible confounders was not significant, although AF effect measures were attenuated with adjustment for hypertension or blood pressure (pooled estimate 1.33 [95% CI: 1.17 to 1.50]).

Publication bias was visually suggested by the asymmetrical funnel plot of the reported results (Fig. 3), which was also statistically supported by Egger's test (p = 0.03) but not Begg's test (p = 0.31). We attempted to adjust for this publication bias using the trim and fill method (18). After 4

Table 2	Stratified Analyses of Pooled Relative Risk of Atrial Fibrillation for Highest Alcohol Intake Versus Lowest Alcohol Intake					
Variable	No. of Data Units	Risk Estimates (95% CI)	Q Statistics	I-Squared	p Value of Heterogeneity	Meta-Regression*
Mean estimated alcohol intake of exposed group						
<4 drinks (48 g) per day	11	1.32 (1.15–1.50)	13.1	23.6%	0.22	Referent
≥4 drinks (48 g) per day or alcohol abuser	8	1.74 (1.35–2.24)	18.8	62.8%	0.009	0.16
Drinking status of referent group						
Nondrinkers	8	1.36 (1.18–1.57)	12.6	44.6%	0.08	Referent
Light drinkers	11	1.56 (1.27–1.91)	20.5	51.1%	0.03	0.73
Design						
Cohort	13	1.34 (1.22–1.47)	20.4	41.1%	0.06	Referent
Case-control	6	1.98 (1.49–2.63)	6.5	22.5%	0.26	0.06
Selection of study population						
Population-based	10	1.30 (1.18–1.44)	14.3	37.0%	0.11	Referent
Hospital-based	7	1.75 (1.45–2.11)	10.8	44.2%	0.10	0.06
Both population- and hospital-based	2	1.28 (0.49–3.39)	1.1	6.7%	0.30	0.88
Geographic region						
North America	10	1.50 (1.19–1.90)	19.5	53.7%	0.02	Referent
Europe	9	1.40 (1.25–1.58)	13.7	41.8%	0.09	0.76
Sex						
Men	4	1.32 (1.06–1.64)	2.3	—	0.51	Referent
Women	5	1.37 (1.21–1.55)	7.3	45.0%	0.12	0.47
Men/women	10	1.85 (1.33–2.56)	22.8	60.5%	0.007	0.32
Excluding participants with heart disease						
No	11	1.50 (1.24–1.82)	20.5	51.3%	0.03	Referent
Yes	8	1.53 (1.31–1.80)	10.6	34.1%	0.16	0.79
Was atrial flutter included as AF outcome?						
No	12	1.83 (1.45–2.30)	23.8	53.9%	0.01	Referent
Yes	7	1.25 (1.10–1.43)	5.1	—	0.52	0.02
Type of AF outcome						
Chronic AF dominant	14	1.43 (1.24–1.66)	22.0	45.4%	0.04	Referent
PAF dominant	6	1.92 (1.44–2.56)	6.0	17.2%	0.30	0.11
Was AF outcome the first episode or recurrence?						
First episode	17	1.46 (1.27–1.69)	28.5	43.8%	0.03	Referent
Recurrence	2	2.37 (1.44–3.90)	0.3	—	0.59	0.10
Methods for assessment of alcohol intake						
Questionnaires	9	1.26 (1.13–1.40)	7.4	—	0.50	Referent
Interviews	4	1.41 (1.07–1.85)	3.0	—	0.39	0.45
Historical data reviews	6	2.17 (1.74–2.70)	3.7	—	0.60	<0.001
Methods for ascertainment of AF						
ECG screening	3	1.47 (1.12–1.95)	2.5	20.0%	0.29	Referent
Medical records	5	2.14 (1.38–3.31)	5.5	27.6%	0.24	0.28
Registries	6	1.35 (1.19–1.54)	10.2	51.1%	0.07	1.00
Others†	5	1.43 (1.11–1.85)	10.8	62.9%	0.03	0.83
Study adjustment						
Smoking						
No	13	1.58 (1.29–1.95)	28.0	57.2%	0.005	Referent
Yes	6	1.47 (1.24–1.73)	4.6	—	0.46	0.68
Obesity (BMI or waist circumference)						
No	11	1.76 (1.40–2.21)	23.3	57.1%	0.01	Referent
Yes	8	1.29 (1.13–1.48)	7.9	10.9%	0.34	0.07
SBP or HT						
No	9	1.93 (1.40–2.64)	20.8	66.3%	0.004	Referent
Yes	10	1.33 (1.17–1.50)	11.0	9.4%	0.35	0.06
Heart disease						
No	5	1.74 (1.24–2.43)	11.3	64.4%	0.02	Referent
Yes	14	1.39 (1.24–1.56)	22.0	40.8%	0.06	0.44

*Represents test for significance of the study modification across strata. †Participants' report or using combination of registry with electrocardiographic screening.

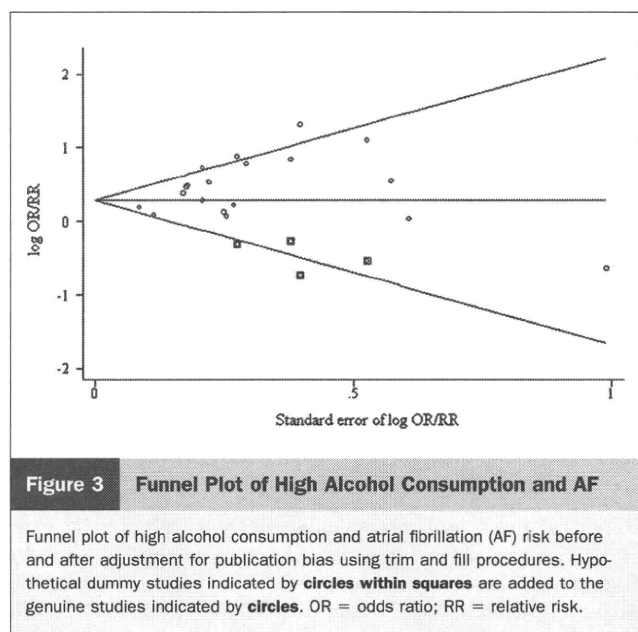CI = confidence interval; other abbreviations as in Table 1.

Figure 3 Funnel Plot of High Alcohol Consumption and AF

Funnel plot of high alcohol consumption and atrial fibrillation (AF) risk before and after adjustment for publication bias using trim and fill procedures. Hypothetical dummy studies indicated by **circles within squares** are added to the genuine studies indicated by **circles**. OR = odds ratio; RR = relative risk.

negative unpublished results were incorporated to produce a hypothetically symmetrical funnel plot, the pooled estimate of AF for heavy alcohol consumption was modestly attenuated to be 1.39 (95% CI: 1.19 to 1.62) but remained statistically significant (p < 0.001).

Dose-response relationship between alcohol intake and AF risk. Nine studies (10,11,23–27,29,30) involving 126,051 participants and 6,341 cases were eligible for analysis of the dose-response relationship between different categories of alcohol intake and AF risk. The alcohol dose in these studies ranged from 4.0 to 86.4 g per day. Figure 4 illustrates the linear and spline regression curves for AF risk related to daily alcohol intake. The linear dose-response curve showed a significant relationship between alcohol intake and AF risk (R^2 = 0.43, p < 0.001). The coefficient for the linear term was $7.4 \pm 1.3 \times 10^{-3}$, meaning that the incremental increase in relative risk of AF per 10 g alcohol consumption per day was $e^{10 \times 7.4 \pm 1.3 \times 10^{-3}}$ = 1.08 (95% CI: 1.05 to 1.10). Also, in the spline regression model, AF risk significantly increased with larger daily alcohol consumption levels (R^2 = 0.44, p < 0.001). However, the fit of this model was not significantly different from that of the linear regression model (p = 0.77).

Discussion

Our study is the first to systematically review the literature on the association between alcohol consumption and the risk of AF. In this review, high alcohol intake was shown to be associated with a significant elevation in AF risk, both by overall analysis and across a number of stratified analyses based on key characteristics of study methods, although there was substantial study heterogeneity in the magnitude of AF risk, partly due to variability in study design and methodology. Even though, in principle, observational

studies do not allow for proof of causality, there are several theoretically plausible speculations for the cause-effect relationship between high alcohol intake and the development of AF.

One speculation is based on biological findings that suggest a harmful effect of high alcohol intake on maintenance of normal heart rhythm, including the achievement of a hyperadrenergic state (32), impairment of vagal tone (33), direct effect on myocardial structure (34), and various electrophysiological changes in atrial cells (e.g., increase in intra-atrial conduction time represented by a length of the P-wave, reduction in the refractory period, negative inotropic effect through calcium-channel inhibition in ventricular cells) (31,35,36).

Another speculation is based on reports suggesting that the development of chronic heart failure accompanied by long-term excessive alcohol consumption may result in elevated AF risk (2). Particularly, dilated cardiomyopathy is typical of alcohol abusers with chronic heart failure. The average total lifetime alcohol consumption was reported to be significantly greater in patients with dilated cardiomyopathy than in a population-based control group (37). Moreover, alcoholics were found to have progressive dilated cardiomyopathy in proportion to the duration of heavy drinking even before the clinical appearance of chronic heart failure (38).

It remains to be established whether the dose-response relationship between daily alcohol consumption and AF risk is interpreted as linear or not (e.g., J-shaped curve, threshold curve). While we identified a linear association of daily alcohol consumption with the risk of AF, a J-shaped relation or threshold value was not observed from the
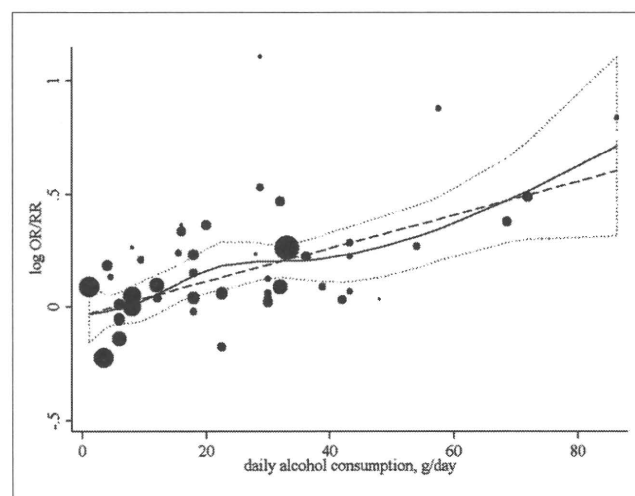

Figure 4 Regression of Natural Log OR/RR for Atrial Fibrillation on Daily Alcohol Consumption

The **solid curve** and its accompanying area indicate the log odds ratio or relative risk (OR/RR) and its corresponding 95% confidence interval based on a restricted cubic spline regression model with knots at 8, 22.5, and 33.1 g/day of alcohol consumption. This model did not significantly improve the fit compared with the linear regression model expressed by the **dotted line**. The area of each data point is proportional to its statistical weight.

current spline regression curve. These regression analyses suggest no evidence that moderate alcohol consumption is beneficial in ameliorating the risk of AF, unlike that of cardiovascular disease (7). If anything, moderate alcohol drinkers may have a greater risk of AF than nondrinkers, although the AF risk is not as large as that for heavy drinkers.

Study limitations. First, the definition of heavy drinking is heterogeneous across studies. Second, the majority of the included studies did not state whether the method used for assessment of alcohol intake was validated. Third, few studies considered racial differences among participants. Differences in ethnicity or proportion of whites and non-whites among different studies might have affected AF risk estimates. Fourth, asymptomatic PAF could have been missed in any of these studies. Given that heavy alcohol drinkers are likely to have experienced PAF, the risk of AF in relation to high alcohol intake would be underestimated in any of the examined studies. Fifth, no studies have investigated the effect of different types of alcoholic beverages on AF risk, although it has been reported that wine has a better effect on cardiovascular disease (39). Sixth, it could not be ruled out whether a particular drinking pattern, such as whether alcohol was consumed with a specific food or at meals, could have contributed to the AF risk irrespective of alcohol dosage. For example, alcohol is usually consumed during meals, as in Mediterranean countries (39), which might explain a beneficial effect.

A meta-analysis cannot completely solve problems with confounders that vary from study to study. Lack of adjustment for possible confounders could also produce a superficially strong association between high alcohol consumption and AF risk. For example, a weaker association between high alcohol consumption and AF risk was observed when studies included blood pressure values or the presence of hypertension among study confounders. In fact, hypertension was reported to be an independent risk factor for AF (2), and the risk of hypertension increases linearly with alcohol consumption (40). Possibly, the AF risk associated with high alcohol consumption is partly explained by an alcohol-related development of hypertension. In addition, other factors that could not be specified by this meta-analysis or were not specified in the individual included studies might contribute to residual confounding (e.g., objective sleep apnea [41], diabetes mellitus [42]).

Lastly, results that indicated risk of AF could be biased by study design and other methodological features. For example, larger AF risk estimates were observed in studies having a case-control design compared with studies having a cohort design or in hospital-based studies compared with population-based studies. The AF risk could have been overestimated by exaggeration of alcohol intake in patients with AF in studies with a case-control design (i.e., recall bias) or an unavoidable reduction in alcohol intake as a result of a control subject having an illness in a hospital-based setting (i.e., selection bias). However, underestima-tion of AF risk is possible if patients did not truthfully report the full extent of alcohol intake when completing questionnaires.

Conclusions

Habitual heavy alcohol drinking is associated with an increased risk of AF, although several study limitations exist and must be recognized. The relationship between daily alcohol consumption and the risk of AF was explained by a linear dose-response model, suggesting that not consuming alcohol at all is the most favorable behavior for avoiding AF rather than moderate alcohol consumption. Further investigation is needed to establish the extent to which this association is explained by a causal relationship.

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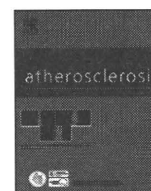
Short communication

Are serum cholesterol levels associated with silent brain infarcts? The Seiryo Clinic Study

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ABSTRACT

Objective: High levels of serum cholesterol are associated with the risk of stroke. However, the association of serum cholesterol with silent brain infarcts (SBIs) is unclear. We investigated the association between SBI and various clinical factors.

Methods: We conducted a cross-sectional study that included 324 apparently healthy Japanese men (mean age 53.8 ± 9.2 years). Combinations of three types of scan (T1-weighted, T2-weighted and FLAIR images) were used to detect and discriminate SBI.

Results: Serum cholesterol was significantly associated with SBI [total cholesterol, odds ratio (OR) 3.75 (95% confidence interval (CI) 1.45–9.68); LDL-cholesterol, OR 2.54 (95% CI 1.03–6.27), and non-HDL-cholesterol, OR 2.54 (95% CI 1.03–6.27)] after adjustment for age, smoking status, serum triglycerides, maximal-intima-media thickness, obesity, hypertension, diabetes mellitus, hyperuricemia, coronary heart disease and lipid-lowering agent use.

Conclusion: Our cross-sectional data suggest that serum cholesterol levels are associated with SBI independently of known confounders.

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1. Introduction

The significance of detecting silent brain infarction (SBI) and its associated factors is increasing in the primary prevention of stroke and dementia, which threaten quality of life [1]. Although increased total cholesterol (TC) levels are known to be associated with a higher risk of ischemic stroke [2], it remains unclear whether SBI is associated with serum cholesterol [1]. The aim of this study was to investigate factors associated with the development of SBI.

2. Methods

Subjects of the Seiryo Clinic Study were a cohort of apparently healthy Japanese men, aged 30–80 years who consecutively had undergone MRI of the brain as part of a health check-up between May 2000 and April 2008 in northern Japan. From the 483 persons who had MRI, measurements of maximal-intima-media thickness (maximal-IMT) and blood pressure, as well as blood tests,

we excluded women ($n = 137$) and all men who had symptomatic stroke ($n = 2$), epilepsia ($n = 1$), a space-occupying lesion shown on brain MRI due to brain tumor ($n = 2$), pituitary adenomas ($n = 2$), vascular malformations ($n = 2$), venous angioma ($n = 1$), meningioma ($n = 1$), microbleeding ($n = 1$), brain contusion ($n = 1$), brain injury ($n = 1$) or serum triglyceride (TG) ≥ 4.52 mmol/L ($n = 8$). Ultimately, 324 men were analyzed.

Blood pressure was measured in a resting state while sitting, and body mass index (BMI) was calculated by weight [kg]/(height [m])^2. The blood test was performed under fasting conditions. Serum TC and HDL-cholesterol, trygricerides, glucose and uric acid were measured by an autoanalyzer. Serum LDL-cholesterol was calculated by the Friedewald equation [3], and non-HDL-cholesterol was determined by subtracting HDL-cholesterol from TC.

Carotid arteries were evaluated independently by two trained technicians using high-resolution B-mode ultrasonography SSA-550A (Toshiba, Tokyo, Japan). The technicians were also masked to clinical information. Four segments of the common carotid artery and of the internal carotid artery at the near and far wall on both left and right sides were examined [4] and maximal-intima-media thickness (maximal-IMT) was obtained.

The following data were obtained by a questionnaire completed by the participant: smoking habit, medication and medical history.

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Table 1
Clinical characteristics of the 324 study men according to the presence of silent brain infarcts.

	All ($n=324$)	With SBI ($n=17$)	Without SBI ($n=307$)	P
Age, year	53.8 ± 9.2	63.1 ± 7.2	53.3 ± 9.0	<0.001
Body mass index, kg/m^2	24.6 ± 3.1	25.4 ± 3.4	24.5 ± 3.1	0.28
Obesity (body mass index $\geq$25)	130 (40%)	8 (47%)	122 (40%)	0.55
Systolic blood pressure, mmHg	121 ± 15	132 ± 11	120 ± 15	0.001
Diastolic blood pressure, mmHg	75.4 ± 9.9	79.6 ± 7.7	75.1 ± 10.0	0.07
Hypertension	102 (31%)	12 (71%)	90 (29%)	<0.001
Fasting blood sugar, mmol/L	5.5 ± 1.2	5.6 ± 0.6	5.5 ± 1.2	0.89
Diabetes mellitus	38 (12%)	2 (12%)	36 (12%)	1.00
Total cholesterol, mmol/L	5.19 ± 0.80	5.32 ± 0.81	5.18 ± 0.80	0.50
LDL-cholesterol, mmol/L	3.48 ± 0.77	3.57 ± 0.73	3.47 ± 0.78	0.59
Non-HDL-cholesterol, mmol/L	3.77 ± 0.83	3.84 ± 0.74	3.76 ± 0.83	0.71
HDL-cholesterol, mmol/L	1.43 ± 0.37	1.48 ± 0.37	1.42 ± 0.36	0.54
Triglycerides, mmol/L	1.45 ± 0.75	1.33 ± 0.56	1.45 ± 0.76	0.50
Use of lipid-lowering agents	26 (8%)	1 (6%)	25 (8%)	0.73
Uric acid, μmol/L	357 ± 70	378 ± 62	356 ± 71	0.20
Hyperuricemia	82 (25%)	7 (41%)	75 (24%)	0.14
Maximal-IMT, mm	1.44 ± 0.75	2.03 ± 0.63	1.41 ± 0.74	<0.001
Maximal-IMT, top tertile ($\geq$1.6 mm)	112 (35%)	14 (82%)	98 (32%)	<0.001
Current smoker	100 (31%)	6 (35%)	94 (31%)	0.69
Coronary heart disease	8 (2%)	2 (12%)	6 (2%)	0.06

Values are means $\pm$ S.D. or numbers (percentages). P values were obtained using the analysis of variance for continuous variables and chi-square test for categorical variables.

Table 2
Logistic regression model to investigate the clinical factors associated with the presence of silent brain infarcts according to each cholesterol levels in Japanese men.

Variables	Odd ratio (95% confidence interval)			
	Model 1	Model 2	Model 3	Model 4
Total cholesterol, per mmol/L	3.75 (1.45–9.68)[**]			
LDL-cholesterol, per mmol/L		2.54 (1.03–6.27)[*]		
Non-HDL-cholesterol, per mmol/L			2.54 (1.03–6.27)[*]	
HDL-cholesterol, per mmol/L				4.52 (0.97–21.0)
Triglycerides, per mmol/L	0.58 (0.19–1.73)	0.67 (0.23–1.97)	0.56 (0.18–1.76)	1.36 (0.48–3.84)
Use of lipid-lowering agent (Yes vs No)	0.54 (0.05–6.27)	0.42 (0.04–4.38)	0.42 (0.04–4.38)	0.52 (0.04–5.77)
Age, per year	1.15 (1.05–1.25)[**]	1.12 (1.04–1.21)[**]	1.12 (1.04–1.21)[**]	1.12 (1.03–1.21)[**]
Obesity (body mass index $\geq$25 kg/m^2 vs <25 kg/m^2)	0.98 (0.29–3.29)	0.81 (0.25–2.70)	0.81 (0.25–2.70)	1.12 (0.32–3.91)
Maximal-IMT ($\geq$1.6 mm vs <1.6 mm) (highest tertile vs middle or lowest tertiles)	6.48 (1.33–31.6)[*]	5.03 (1.14–22.3)[*]	5.03 (1.14–22.3)[*]	5.51 (1.31–23.1)[*]
Hypertension (Yes vs No)	7.03 (1.77–28.0)[**]	5.46 (1.51–19.8)[**]	5.46 (1.51–19.8)[**]	3.98 (1.19–13.3)[*]
Diabetes mellitus (Yes vs No)	0.46 (0.07–2.83)	0.55 (0.09–3.30)	0.55 (0.09–3.30)	0.38 (0.05–2.60)
Hyperuricemia (Yes vs No)	3.46 (0.97–12.3)	3.01 (0.87–10.4)	3.01 (0.87–10.4)	2.66 (0.80–8.88)
Coronary heart disease (Yes vs No)	3.40 (0.38–30.5)	3.33 (0.42–26.2)	3.33 (0.42–26.2)	3.46 (0.38–31.5)
Current smoker (vs never and former smoker)	4.13 (1.07–15.9)[*]	3.15 (0.87–11.4)	3.15 (0.87–11.4)	2.60 (0.76–8.91)

Odds ratios were adjusted for all of the other covariates listed in Table 2.
[*] $P<0.05$.
[**] $P<0.01$.

No subject had a previous transient ischemic attack nor was atrial fibrillation (AF) shown by electrocardiogram.

Hypertension was defined as systolic blood pressure (SBP) $\geq$140 mmHg or diastolic blood pressure (DBP) $\geq$90 mmHg, a previous diagnosis of hypertension, or treatment with antihypertensive medications agents. Diabetes mellitus was defined as fasting blood sugar $\geq$126 mg/dL (7 mmol/L), a previous diagnosis of diabetes mellitus or the use of antidiabetic agents. Hyperuricemia was defined as uric acid $\geq$7 mg/dL (416 μmol/L), use of medication for hyperuricemia or a history of hyperuricemia. Smoking status was defined as current smoker and past or never smoker. Coronary heart disease was defined as a history or the presence of myocardial infarction or angina pectoris.

SBI and other brain lesions were assessed by a SIGNA 1.5T MRI scanner (GE Healthcare, Waukesha, WI) with T1-weighted, T2-weighted and FLAIR (fluid attenuated inversion recovery) scans with an axial AC-PC line, 5-mm slice thickness and a 2-mm inter-slice gap. We defined SBI according to the criteria of the Japanese Society for the Detection of Asymptomatic Brain Disease [5]: namely, a focal hyperintensity area $\geq$2 mm in diameter on T2-weighted images and low intensity on T1-weighted images. We also used a combination of the three types of scan to discriminate SBI from white matter lesions [6] or dilated perivascular spaces. Two board-certified radiologists who were masked to clinical data independently assessed the images. Final decisions were made by including two or more radiologists for consensus.

To examine the potential risk factors associated with SBI, logistic regression analysis was performed by entering all possible confounders determined by biological measurements and results of the questionnaire as explanatory variables. A two-sided P value of less than 0.05 was statistically significant. All analyses were performed with SPSS (Ver.15.0J for Windows, Chicago, IL) software. The institutional review board of this institution approved this study.

3. Results

Seventeen participants (5.2%) had SBI. A total of 31 infarcts were detected, with locations in the basal ganglia (77%,) subcortical area (17%), and cerebellum (6%). No infarcts were noted in the cortical area.

Table 1 shows baseline characteristics of participants with and without SBI. Participants with SBI were significantly older and had higher blood pressure and maximal-IMT values than those without SBI. Odds ratios (OR) of increased risk of SBI per unit IMT, SBP

and DBP were 2.09 (95% confidence interval (CI) 1.30–3.35, *P* value 0.002), 1.05 (95% CI 1.02–1.09, *P* value 0.002), and 1.07 (95% CI 0.99–1.09, *P* value 0.074), respectively, by univariate analysis.

Results of logistic regression analyses to examine the possible factors associated with SBI are shown in Table 2. Serum cholesterol (TC, LDL and Non-HDL) levels were significantly associated with SBI (*P* value, 0.006 for TC; 0.042 for LDL-cholesterol; 0.042 for non-HDL-cholesterol) after adjustment for age, smoking status, triglycerides, obesity, highest tertile of maximal-IMT (≥ 1.6 mm) and history of hypertension, diabetes mellitus, hyperuricemia, coronary heart disease and use of lipid-lowering agents in the multivariate analysis (Models 1, 2 and 3). There was a tendency for a positive association of HDL-cholesterol with SBI (*P* value, 0.06) (Model 4).

4. Discussion

Our results showed an independent association between serum cholesterol levels and SBI after controlling for classical risk factors and IMT. This supports results of a recent study [7]; however, most previous studies have not indicated a possible association between cholesterol values and the presence of SBI [1,8,9].

Hypertension is known to be strongly associated with SBI [1]. We also found significant association between hypertension and SBI. However, we simultaneously found significant association between serum cholesterol levels and SBI. Results of a meta-analysis [10] indicated that TC was significantly associated with ischemic stroke in subjects with normal blood pressure (SBP ≤ 125 mmHg). Consequently, this might account for our findings, because the average SBP of our subjects was 121 mmHg. In contrast, the association between SBI and IMT has been established [4,8,9].

In population studies in the United States [8] and Europe [9], SBI included not only non-cortical but also cortical lesions. In addition, those study participants were older than those in the present study and a few percent had AF. In contrast, our subjects, who had undergone health screening, had neither cortical infarcts nor AF. Most of the infarcts detected were in the basal ganglia. We speculate that the inconsistency between our results and those of others was due to differences in the brain infarction site, as well as in age and race, which could influence the association of serum cholesterol with SBI.

Uehara [11] suggested dyslipidemia as a potential associated factor for SBI in the basal ganglia, not in white matter. In subjects who use antihypertensive agents, LDL-3 levels are associated with the prevalence of silent lacunar infarcts in basal ganglia not in white matter [12]. Therefore, cholesterol is possibly that serum cholesterol is associated more with non-cortical infarcts, especially in basal ganglia.

In this study, HDL-cholesterol tended to be positively associated with SBI. However, the TC-to-HDL-cholesterol ratio (TC/HDL-C) was not significantly associated with SBI [OR 0.92 (95% CI 0.42–2.03), *P* value 0.84]. Although a recent study recommended the usefulness of TC/HDL-C for predicting stroke [2], SBI was not associated with TC/HDL-C.

Our study has one strong point: SBI was evaluated by a combination of three images involving FLAIR to ensure a higher reliability of discrimination [6]. In most previous studies, a lack of proton density or FLAIR images for diagnosis of SBI might have reduced the specificity [13].

We should address several limitations. First, the relatively small number of participants resulted in a small number of cases with SBI. Second, our results cannot be applied to women, as women were excluded because too few attended the health screening for an investigation by multivariate analysis. Third, our subjects may not have been representative of the general population because of

selection bias. These participants might have been more concerned about their health than the general male population, thus allowing more early detection of hypertension and diabetes mellitus. Fourth, although obstructive sleep apnoea is a risk factor for SBI, we did not investigate its association with SBI. Fifth, we could not elucidate risk factors because of the cross-sectional study design.

Additionally, we unfortunately did not evaluate serum lipoprotein (a) [Lp (a)], although Lp (a), which has a structure like LDL-cholesterol, has been reported to be associated with lacunar infarcts defined by MRI [14]. Therefore, we cannot predict the extent to which Lp (a) confounded the relationship between cholesterol and SBI and it is possible that the observed significant association between cholesterol and SBI could be partially explained by Lp (a).

In conclusion, our cross-sectional data suggest that serum cholesterol is a key factor in the development of SBI. From these findings, evaluating serum cholesterol levels together with brain MRI might be recommended in the primary prevention of SBI as well as prevention of symptomatic infarction. Further studies are necessary to clarify what characteristics in a population and what infarction sites would indicate that serum cholesterol is strongly associated with SBI.

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