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Serum 1,5-anhydro-D-glucitol levels predict first-ever cardiovascular disease: An 11-year population-based Cohort study in Japan, the Suita study

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ABSTRACT

Objective: Serum 1,5-anhydro-D-glucitol (1,5-AG) is well-known to be a useful clinical marker of both short-term glycemic status and postprandial hyperglycemia. In addition, previous epidemiological studies have shown that an increased postload glucose level in an oral glucose tolerance test is a risk factor for cardiovascular diseases (CVD). However, no previous prospective study has reported the association between serum 1,5-AG levels and the risk of CVD. In this study, we examined whether serum 1,5-AG levels can predict the incidence of first-ever CVD.

Methods: Our study was a population-based cohort study in an urban area of Japan. Study subjects comprised 2095 initially healthy Japanese (991 men and 1104 women, mean age: 58.5 years) with no history of coronary heart disease (CHD) or stroke. They were followed up for an average of 11.1 years, and 147 CVD events (64 CHD and 83 strokes) were observed.

Results: The adjusted hazard ratios (HRs) of all CVD in men increased linearly ($p = 0.004$). The HR in the category with serum 1,5-AG levels of 14.0 μg/mL or less was 2.22 (95% confidence interval; 1.24–3.98) compared to the reference category (24.5 μg/mL or greater). Similar results were also shown with a sensitivity analysis in non-diabetic men. Conversely, no significant relationship between serum 1,5-AG levels and CVD risks was observed in women.

Conclusions: Our results suggest that measurement of serum 1,5-AG levels is useful to detect individuals, especially men, at higher risk for CVD, regardless of the presence or absence of diabetes.

1. Introduction

Serum 1,5-anhydro-D-glucitol (1,5-AG) levels are well-known to rapidly decrease concomitantly with the excretion of glucose in urine, and serum 1,5-AG is a useful clinical marker for short-term glycemic status and postprandial hyperglycemia [1–3].

Previous epidemiological studies have shown that an increased postload glucose level in an oral glucose tolerance test (OGTT) is a risk factor for cardiovascular diseases (CVD) [4,5]. A randomized controlled trial of individuals with impaired glucose tolerance also reported that acarbose, an α-glycosidase inhibitor that suppresses the elevation of postprandial glucose levels, reduced the incidence of CVD as well as type 2 diabetes [6]. These findings suggest that detection and improvement of postprandial hyperglycemia is important for CVD prevention.

An OGTT is useful for the detection of postprandial hyperglycemia, however, it requires overnight fasting, long time, additional costs, and is not always feasible in routine clinical settings or during health check-ups. In contrast, measurement of serum 1,5-AG levels can be performed using a single non-fasting blood sample, relatively costs less, and may be an alternative to OGTT. However, to our knowledge, no previous prospective study has shown the association between serum 1,5-AG levels and the risk of CVD in initially healthy individuals. We examined whether serum 1,5-AG levels can predict the incidence of first-ever CVD in a population-based cohort study of an urban area in Japan.

2. Methods

2.1. Study design and samples

The details of the Suita study have been described elsewhere [7–9]. Briefly, the Suita study is a prospective population-based cohort study of an urban area in Japan. In 1989, 6485 Suita city residents (age, 30–79 years) were randomly sampled and enrolled as study participants. They underwent medical examinations every 2 years. Among these participants, 2406 participants underwent medical examinations between April 1994 and February 1995, and their serum samples were collected and stored at −80 °C. In this

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study, we measured serum 1,5-AG levels in these stored samples. Of these 2406 participants, 289 were excluded from the present analysis for the following reasons: history of coronary heart disease (CHD) or stroke ($n=78$), lost to follow-up ($n=132$), serum creatinine level of 176.8 mmol/L (2.0 mg/dL) or more ($n=4$), and data missing ($n=97$). Finally, the remaining 2095 participants (991 men and 1104 women) with serum 1,5-AG measurements were included as subjects in the baseline study and were followed up until December 31, 2007. Informed consent was obtained from all subjects, and the institutional review board at the National Cerebral and Cardiovascular Center approved this study.

2.2. Baseline data collection

The baseline survey included questionnaires, anthropometric measurements, and blood sample tests. Height and weight were measured in light clothing, and body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Blood pressure was measured 3 times in more than 1-min intervals by well-trained physicians in a sitting position after at least 5 min of rest, using a standard mercury sphygmomanometer [7], and the third measurement of blood pressure was adopted for the present analyses. The levels of total serum cholesterol, high-density-lipoprotein (HDL)-cholesterol and creatinine were determined using an automatic analyzer in the laboratory of the National Cerebral and Cardiovascular Center. Estimated glomerular filtration rates (eGFR) were estimated with a following equation for the Japanese: eGFR (mL/min/1.73 m^2) = 194 × serum creatinine$^{-1.094}$ × Age$^{-0.287}$ (×0.739: if women) [10].

2.3. Measurement of 1,5-AG

In 2009, stored frozen serum samples were shipped to the clinical laboratory company for measurement of 1,5-AG (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). 1,5-AG was measured using the enzymatic method with the "Determiner L 1,5-AG" measurement kit manufactured by the Kyowa Medex Co., Ltd. (Tokyo, Japan) and an H7700 Clinical auto-analyzer, manufactured by the Hitachi High-Technologies Corporation (Tokyo, Japan). The coefficient of variation was less than 5%.

2.4. Ascertainment of outcomes

Outcome ascertainment has been previously described elsewhere [7–9]. The main outcome is the incidence of first-ever CVD events (stroke and CHD). Physicians or nurses checked the health status of each subject at biennial clinical visits to the National Cerebral and Cardiovascular Center, and all participants also completed yearly questionnaires by either mail or telephone. The patients suspected of developing stroke or CHD were confirmed by a review of medical records performed by either the registered hospital physicians or the cohort study research physicians. In addition, to complete the surveillance, we also conducted a systematic search of death certificates for fatal stroke and MI. In Japan, all death certificates are forwarded to the Ministry of Health, Welfare, and Labor and coded for the National Vital Statistics.

A stroke was defined according to criteria from the US National Survey of Stroke [11]. Classification of stroke subtypes (ischemic stroke, intracerebral hemorrhage, subarachnoid hemorrhage) was based on the examination of computed tomographic scans, magnetic resonance images, or autopsies (subarachnoid hemorrhages were excluded from the present analyses). With regard to myocardial infarction (MI), definite and probable MI were defined according to the criteria of the MONICA project [12]. The criteria for CHD were first-ever MI, coronary angioplasty, coronary artery bypass grafting and sudden cardiac death.

2.5. Statistical analysis

A previous report from Japan proposed a serum 1,5-AG level of 14.0 μg/mL, irrespective of sex, as the cut-off for the diagnosis of diabetes [13]. The distribution of serum 1,5-AG levels differed between sexes. Accordingly, we adopted a serum 1,5-AG level of 14.0 μg/mL as the lower cut-off in common, and set the median of those who had serum 1,5-AG of more than 14.0 μg/mL as the upper cut-off (overall and according to sex), overall: 23.1 μg/mL, men: 24.5 μg/mL, women: 21.3 μg/mL. These cut-offs were used to compare baseline characteristics, crude incidence rates, and hazard ratios (HRs). To calculate p values for continuous variables, one-way analysis of variance was used, and for categorical variables, Chi-square test was used. To compare in women the prevalence of medication for diabetes and current alcohol drinking status, Fisher's exact test was used. The p values to test for a linear trend in HRs were calculated.

A Cox proportional hazard model was used to estimate age- and multivariate-adjusted HRs with 95% confidence intervals (CIs). The HRs were adjusted for the following baseline covariates as follows for model 1, age; for model 2, model 1 plus BMI, hypertension (systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or the use of antihypertensive medication), hypercholesterolemia (total cholesterol ≥5.7 mmol/L (220 mg/dL) or the use of antihypercholesterolemic medication) [14], HDL-cholesterol, eGFR, current cigarette smoking (non-current and current) and current alcohol drinking (men; non-current/light to moderate/heavy, women: non-current/current);, for model 3, model 2 plus diabetes (fasting plasma glucose (FPG) ≥7.0 mmol/L (126 mg/dL), postprandial plasma glucose (PPG) ≥11.1 mmol/L (200 mg/dL), or use of anti-diabetic medication). Fasting was defined as fasting time of 8 h or more ($n=1401$, 67%), and postprandial was defined as that of less than 8 h ($n=694$, 33%). We defined current alcohol drinking as non-current drinking, light to moderate drinking (alcohol consumption of less than 46 g/day), or heavy drinking (that of 46 g/day or more). However, because women with heavy alcohol drinking were few ($n=8$, 0.7%) and had no CVD incidence, we treated current alcohol drinking as non-current/current drinking in the multivariate analyses of women. Menopause was added to model 2 and model 3 in women. Combined analyses of women and men adjusting for sex were conducted only in CHD and ischemic strokes because significant interactions between sex and serum 1,5-AG levels were observed in all CVD ($p=0.03$) and all strokes ($p=0.01$).

In addition, three sensitivity analyses were conducted: First, similar analyses were performed in non-diabetic men with FPG or PPG less than 6.1 mmol/L (110 mg/dL). Second, the definition of postprandial in the diagnostic criteria for diabetes was changed to a fasting time of 2 h or less (postprandial: $n=28$, 1%), and similar analyses were conducted to confirm the influence of diabetes diagnostic criteria by PPG. Third, adjustment for waist circumferences in model 2, instead of BMI, was conducted to estimate the influence of insulin resistance. We did not enter both BMI and waist circumferences into the models to avoid the colinearity problem because waist circumferences highly correlated with BMI (correlation coefficient: 0.84). In addition, triglycerides levels were categorized by tertile and added to the model 2 in the combined analysis of women and men with fasting time of 8 h or more ($n=1401$), and similar analyses for CHD and ischemic strokes were conducted.

All p values were two-tailed, and $p<0.05$ was considered statistically significant. All analyses were conducted using SAS version 8.2 (SAS Institute, Cary, Carolina, USA).

3. Results

The mean (standard deviation) of serum 1,5-AG was 23.0 μg/mL (9.2) in men and 20.0 μg/mL (7.0) in women. The overall dis-

Table 1
Baseline characteristics by sex and serum 1,5-anhydro-D-glucitol levels, the Suita study, Japan, 1994–2007.

	Men			
	1,5-Anhydro-D-glucitol (μg/mL)			p
	≥24.5	14.1–24.4	≤14.0	
Number of subjects	423	416	152	
Age (years)	58 (12)	61 (12)	63 (11)	<0.001
Body mass index (kg/m^2)	22.7 (2.7)	22.8 (2.9)	23.1 (2.9)	0.24
HDL cholesterol (mmol/L)	1.4 (0.3)	1.4 (0.4)	1.4 (0.4)	0.48
1,5-Anhydro-D-glucitol (μg/mL)	31.3 (5.6)	19.7 (3.0)	8.8 (3.6)	<0.001
Estimated GFR (mL/min/1.73 m^2)	80.2 (15.6)	78.1 (16.0)	79.0 (18.1)	0.19
Hypertension (%)[a]	32	37	45	0.01
Hypercholesterolemia (%)[b]	23	23	21	0.85
Diabetes (%)[c]	0	3	30	<0.001
Current cigarette smoking (%)	44	39	41	0.36
Alcohol drinking (non/light to moderate/heavy) (%)	29/53/18	29/55/16	35/47/18	0.55
Hypertension medication (%)	13	15	20	0.09
Hypercholesterolemia medication (%)	4	4	5	0.81
Diabetes medication (%)	0	0	20	<0.001
	Women			
	1,5-Anhydro-D-glucitol (μg/mL)			p
	≥21.3	14.1–21.2	≤14.0	
Number of subjects	442	438	224	
Age (years)	59 (12)	55 (12)	58 (12)	<0.001
Body mass index (kg/m^2)	22.2 (3.2)	21.9 (2.7)	22.3 (3.2)	0.12
HDL cholesterol (mmol/L)	1.6 (0.4)	1.6 (0.3)	1.6 (0.3)	0.001
1,5-Anhydro-D-glucitol (μg/mL)	26.7 (4.1)	18.0 (2.0)	10.5 (3.2)	<0.001
Estimated GFR (mL/min/1.73 m^2)	80.2 (19.7)	81.2 (16.8)	81.1 (15.2)	0.71
Hypertension (%)[a]	33	26	31	0.06
Hypercholesterolemia (%)[b]	39	37	38	0.80
Diabetes (%)[c]	1	1	12	<0.001
Current cigarette smoking (%)	11	8	8	0.42
Current alcohol drinking (non/light to moderate/heavy) (%)	75/25/0	72/27/1	72/28/0	0.31
Menopause (%)	76	63	71	<0.001
Hypertension medication (%)	14	12	17	0.17
Hypercholesterolemia medication (%)	7	7	5	0.46
Diabetes medication (%)	0	0	4	<0.001

Mean (standard deviations), or percentage is shown. GFR means glomerular filtration rate.
[a] Hypertension is defined by systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or the use of antihypertensive medication.
[b] Hypercholesterolemia is defined by total cholesterol ≥5.7 mmol/L (220 mg/dL) or the use of antihypercholesterolemic medication.
[c] Diabetes is defined by fasting plasma glucose ≥7.0 mmol/L (126 mg/dL) in those with fasting time of 8 h or more, postprandial plasma glucose ≥11.1 mmol/L (200 mg/dL) in those with fasting time of less than 8 h, or the use of antidiabetic medication.

tribution (minimum, 25th percentile, median, 75th percentile, maximum) of serum 1,5-AG by sex was 1.2, 17.0, 23.1, 28.9, and 55.3 μg/mL, respectively in men, and 1.7, 15.2, 19.8, 24.8, and 41.5 μg/mL, respectively in women (data not shown). The prevalence of diabetes and medication for diabetes at baseline was highest in the category with the lowest serum 1,5-AG (≤14.0 μg/mL) in both sexes, and was much higher in men (Table 1). Age and prevalence of hypertension increased as serum 1,5-AG decreased in men only.

During the follow-up period (11.1 years average), 147 CVD events (64 CHD and 83 strokes) were observed. The CHD included 14 percutaneous coronary angioplasty, 5 coronary artery bypass grafting, 1 sudden death, 41 myocardial infarctions and 3 unclassified CHD. The strokes included 53 ischemic strokes, 14 hemorrhagic strokes and 16 unclassified strokes. The incidence rates of all CVD and each CVD subtype increased as 1,5-AG levels decreased in men, and the incidence rate of all CVD was 15.1 per 1000 person-years in the lowest 1,5-AG category (Table 2). In model 2, there was a statistically significant linear increase in the adjusted HRs of all CVD in men ($p = 0.004$), and the adjusted HR was 2.22 (95% CI 1.24–3.98) in the lowest 1,5-AG category. In model 3, the adjusted HR of all CVD in the lowest 1,5-AG category was less than model 2. However, the adjusted HR of the middle category (14.1–24.4 μg/mL) was not very different and the elevation of risk was still significant, 1.74 (95% CI 1.07–2.84). In men, similar results were observed for each CVD subtype, although the HRs of CHD were much lower than of all strokes and were not statistically significant. In women, similar results were not observed, although, for CHD, similar trends were observed (Table 3). In the combined analysis of women and men for CHD, the HRs in model 2 increased linearly with decrease in serum 1,5-AG levels ($p = 0.03$), and the adjusted HR in the lowest 1,5-AG category was 2.10 (95% CI 1.10–4.02) (Table 4).

A sensitivity analysis for non-diabetic men with FPG or PPG less than 6.1 mmol/L (110 mg/dL) showed that the adjusted HRs for all CVD in model 2 increased as 1,5-AG levels decreased ($p = 0.03$), and the adjusted HR was 2.00 (95% CI 0.88–4.55) in the lowest 1,5-AG category (Table 5). Similar results were observed with all strokes and ischemic strokes, but such a relationship was not clear in CHD.

In the sensitivity analyses, altering the definition of postprandial, entering waist circumferences or adding triglycerides levels to the models hardly alter the results. In addition, waist circumferences or triglycerides levels were not related with the risk for CVD or each CVD subtype.

Table 2
Incidence rates and adjusted hazard ratios for cardiovascular diseases by serum 1,5-anhydro-D-glucitol levels in men, the Suita study, Japan, 1994–2007.

	1,5-Anhydro-D-glucitol (μg/mL)			*p* for trend
	≥24.5	14.1–24.4	≤14.0	
Person-years	4727	4322	1455	
All cardiovascular diseases				
Cases, *n*	26	49	22	
Incidence rates/1000 person-years	5.5	11.3	15.1	
Model 1[a]	1	1.76 (1.09–2.86)	2.29 (1.29–4.07)	0.003
Model 2[a]	1	1.79 (1.10–2.91)	2.22 (1.24–3.98)	0.004
Model 3[a]	1	1.74 (1.07–2.84)	1.72 (0.89–3.34)	0.049
Coronary heart disease				
Cases, *n*	16	19	10	
Incidence rates/1000 person-years	3.4	4.4	6.9	
Model 1[a]	1	1.21 (0.61–2.38)	1.81 (0.81–4.05)	0.17
Model 2[a]	1	1.14 (0.57–2.25)	1.59 (0.70–3.59)	0.29
Model 3[a]	1	1.13 (0.57–2.24)	1.47 (0.59–3.68)	0.44
All strokes				
Cases, *n*	10	30	12	
Incidence rates/1000 person-years	2.1	6.9	8.2	
Model 1[a]	1	2.56 (1.25–5.25)	3.02 (1.31–7.01)	0.006
Model 2[a]	1	2.64 (1.28–5.45)	3.32 (1.41–7.79)	0.003
Model 3[a]	1	2.53 (1.23–5.23)	2.29 (0.87–6.01)	0.04
Ischemic strokes				
Cases, *n*	8	20	9	
Incidence rates/1000 person-years	1.7	4.6	6.2	
Model 1[a]	1	2.16 (0.95–4.92)	2.84 (1.09–7.37)	0.02
Model 2[a]	1	2.15 (0.94–4.93)	2.86 (1.09–7.49)	0.03
Model 3[a]	1	2.10 (0.92–4.82)	2.28 (0.78–6.67)	0.09

Parentheses indicate 95% confidence intervals.

[a] Model 1: adjusted for age, model 2: adjusted for model 1 plus body mass index, hypertension, hypercholesterolemia, HDL cholesterol, estimated glomerular filtration rate, current cigarette smoking, current alcohol drinking, model 3: adjusted for model 2 plus diabetes.

4. Discussion

This is the first report of a prospective cohort study showing that serum 1,5-AG levels predict CVD incidence in men, similar to HbA_{1c} [15–17] or postload glucose levels in OGTT [4,5]. More subjects with overt diabetes were included in the category with serum 1,5-AG levels of 14.0 μg/mL or less, which would lead to the greatest risk. Those with serum 1,5-AG levels of 14.1 to 24.4 μg/mL, whose preva-

Table 3
Incidence rates and adjusted hazard ratios for cardiovascular diseases by serum 1,5-anhydro-D-glucitol levels in women, the Suita study, Japan, 1994–2007.

	1,5-Anhydro-D-glucitol (μg/mL)			*p* for trend
	≥21.3	14.1–21.2	≤14.0	
Person-years	5077	5293	2424	
All cardiovascular diseases				
Cases, *n*	22	15	13	
Incidence rates/1000 person-years	4.3	2.8	5.4	
Model 1[a]	1	0.83 (0.43–1.60)	1.23 (0.62–2.44)	0.68
Model 2[a]	1	0.92 (0.47–1.79)	1.30 (0.65–2.60)	0.54
Model 3[a]	1	0.91 (0.47–1.77)	1.04 (0.48–2.22)	0.99
Coronary heart disease				
Cases, *n*	7	5	7	
Incidence rates/1000 person-years	1.4	0.9	2.9	
Model 1[a]	1	0.82 (0.26–2.60)	2.09 (0.73–5.96)	0.21
Model 2[a]	1	0.89 (0.28–2.83)	2.33 (0.81–6.71)	0.15
Model 3[a]	1	0.87 (0.27–2.76)	1.74 (0.54–5.56)	0.42
All strokes				
Cases, *n*	15	10	6	
Incidence rates/1000 person-years	3.0	1.9	2.5	
Model 1[a]	1	0.83 (0.37–1.86)	0.83 (0.32–2.14)	0.65
Model 2[a]	1	0.93 (0.41–2.09)	0.88 (0.34–2.27)	0.77
Model 3[a]	1	0.92 (0.41–2.08)	0.75 (0.26–2.12)	0.59
Ischemic strokes				
Cases, *n*	6	7	3	
Incidence rates/1000 person-years	1.2	1.3	1.2	
Model 1[a]	1	1.48 (0.50–4.41)	1.03 (0.26–4.12)	0.84
Model 2[a]	1	2.01 (0.66–6.11)	1.20 (0.29–4.89)	0.60
Model 3[a]	1	1.99 (0.66–6.06)	1.01 (0.22–4.71)	0.71

Parentheses indicate 95% confidence intervals.

[a] Model 1: adjusted for age, model 2: adjusted for model 1 plus body mass index, hypertension, hypercholesterolemia, HDL-cholesterol, estimated glomerular filtration rate, current cigarette smoking, current alcohol drinking, menopause, model 3: adjusted for model 2 plus diabetes.

Table 4
Incidence rates and adjusted hazard ratios for coronary heart disease and ischemic strokes by serum 1,5-anhydro-D-glucitol levels in men and women, the Suita study, Japan, 1994–2007.

	1,5-Anhydro-D-glucitol (μg/mL)			*p* for trend
	≥23.1	14.1–23.0	≤14.0	
Number of subjects	854	865	376	
Person-years	9606	9814	3878	
Coronary heart diseases				
Cases, *n*	22	25	17	
Incidence rates/1000 person-years	2.3	2.5	4.4	
Model 1[a]	1	1.36 (0.76–2.44)	2.17 (1.14–4.13)	0.02
Model 2[a]	1	1.41 (0.78–2.52)	2.10 (1.10–4.02)	0.03
Model 3[a]	1	1.37 (0.76–2.46)	1.76 (0.85–3.63)	0.12
Ischemic strokes				
Cases, *n*	19	22	12	
Incidence rates/1000 person-years	2.0	2.2	3.1	
Model 1[a]	1	1.25 (0.67–2.31)	1.58 (0.76–3.27)	0.22
Model 2[a]	1	1.24 (0.67–2.31)	1.56 (0.75–3.24)	0.23
Model 3[a]	1	1.21 (0.65–2.25)	1.23 (0.54–2.82)	0.56

Parentheses indicate 95% confidence intervals.
[a] Model 1: adjusted for age, sex, model 2: adjusted for model 1 plus body mass index, hypertension, hypercholesterolemia, HDL cholesterol, estimated glomerular filtration rate, current cigarette smoking, and current alcohol drinking.

lence of diabetes or anti-diabetic medication was clearly lower than those with 14.0 μg/mL or less, also had significantly elevated risks. This suggested the possibility that many subjects without overt diabetes who had postprandial hyperglycemia with excretion of glucose in the urine were included in this middle category. Measurement of serum 1,5-AG levels can be useful to detect individuals at greater risk for CVD even among those without overt diabetes. In fact, the sensitivity analyses in non-diabetic subjects with almost normal plasma glucose levels also showed similar results, which reinforced these findings.

In men, the relationship between serum 1,5-AG levels and stroke was much clearer than that with CHD. The prevalence of hypertension increased with decrease in serum 1,5-AG levels, but the prevalence of hypercholesterolemia did not change, irrespective of serum 1,5-AG levels. Such discrepancies in the relationships between serum 1,5-AG levels and risk factors for CVD may account for the difference observed between risk of stroke and that of CHD.

In women, no significant relationship was observed between serum 1,5-AG levels and the risk for all CVD or each CVD subtype, although a similar increase in the risk for CHD was found. Previous meta-analyses have shown either that women with diabetes have a higher risk for CHD than men with diabetes [18,19], or that there was no sex difference [20]. The DECODE study also showed that the HR of death from CVD in individuals with 2-h glucose levels of 11.1 mmol/L or greater tended to be higher among women than among men [5]. The present results show an opposite sex difference, and the reason is not clear. However, the prevalence of diabetes at baseline was much lower in women than in men, and the incidence rate of all CVD and each CVD subtype was also relatively lower in women. Such discrepancies in basic characteristics

Table 5
Sensitivity analyses of incidence rates and adjusted hazard ratios for cardiovascular diseases by serum 1,5-anhydro-D-glucitol levels in non-diabetic men with fasting or postprandial plasma glucose levels of less than 6.1 mmol/L, the Suita study, Japan, 1994–2007.

	1,5-Anhydro-D-glucitol (μg/mL)			*p* for trend
	≥24.5	14.1–24.4	≤14.0	
Number of subjects	388	349	77	
Person-years	4326	3636	703	
All cardiovascular diseases				
Cases, *n*	22	40	8	
Incidence rates/1000 person-years	5.1	11.0	11.4	
Model 1[a]	1	1.75 (1.04–2.96)	1.65 (0.73–3.72)	0.07
Model 2[a]	1	1.76 (1.04–2.98)	2.00 (0.88–4.55)	0.03
Coronary heart diseases				
Cases, *n*	14	17	2	
Incidence rates/1000 person-years	3.2	4.7	2.8	
Model 1[a]	1	1.26 (0.62–2.57)	0.71 (0.16–3.15)	0.96
Model 2[a]	1	1.18 (0.57–2.43)	0. 86 (0.19–3.86)	0.89
All strokes				
Cases, *n*	8	23	6	
Incidence rates/1000 person-years	1.8	6.3	8.5	
Model 1[a]	1	2.58 (1.15–5.79)	3.11 (1.07–9.00)	0.01
Model 2[a]	1	2.51 (1.11–5.66)	3.68 (1.26–10.75)	0.01
Ischemic strokes				
Cases, *n*	7	15	5	
Incidence rates/1000 person-years	1.6	4.1	7.1	
Model 1[a]	1	1.97 (0.80–4.85)	3.05 (0.96–9.69)	0.045
Model 2[a]	1	1.92 (0.77–4.75)	3.45 (1.08–11.05)	0.03

Parentheses indicate 95% confidence intervals.
[a] Model 1: adjusted for age, model 2: adjusted for model 1 plus body mass index, hypertension, hypercholesterolemia, HDL cholesterol, estimated glomerular filtration rate, current cigarette smoking, and current alcohol drinking.

between men and women might result in the sex difference. In addition, the involvement of selection bias cannot be completely eliminated in women. Further studies with sufficient samples and CVD events in women are necessary to clarify this problem.

Measurement of serum 1,5-AG levels could detect not only those with persistent hyperglycemia but also those with transient postprandial hyperglycemia who are likely to be at higher risk for development of diabetes in the near future. Accordingly, decrease in serum 1,5-AG levels might be related with the elevated risk of CVD. The previous epidemiological studies also reported the association of postprandial hyperglycemia with risk of CVD [4–6], and the present results are not inconsistent with them. However, the mechanism remains still inconclusive, and two hypotheses could be considered. First, hyperglycemia itself is a risk for atherosclerotic diseases. Second, hyperglycemia is just a reflection of insulin resistance which is closely related to risk factors for atherosclerotic diseases. In the present study, adjustments for insulin resistance-related factors, waist circumferences or triglycerides, hardly changed the results. This indirectly suggests that serum 1,5-AG levels are independently related with a risk for CVD from insulin resistance, and we infer that hyperglycemia itself might be a risk.

OGTT cannot be conducted easily in the routine clinical setting or during health check-ups because it requires overnight fasting in blood sampling, longer time and extra costs. Conversely, measurement of serum 1,5-AG can be performed easily with a single non-fasting blood sample and is relatively low cost. Serum 1,5-AG levels do not fluctuate very much within an individual if glucose is not excreted into urine; however, it varies widely among individuals [1–3,13,21,22]. Accordingly, periodic measurement of serum 1,5-AG might be important for the early detection of a decrease from the normal level in each individual.

It is also well known that hemoglobin A_{1c} (HbA_{1c}) is useful for the diagnosis of diabetes or as a marker of glycemic control, and elevated HbA_{1c} is associated with increased risk for macro- and micro-complications [15–17,23]. HbA_{1c} can also be measured in a single non-fasting blood sample. However, red cell turnover and hemoglobinopathies influence HbA_{1c} levels, and this has been often identified as a problem [23,24]. In contrast, serum 1,5-AG levels are not affected by red cell turnover and hemoglobinopathies. In terms of screening higher risk individuals among the general population, a combination of HbA_{1c} and serum 1,5-AG measurements might be better choice.

The present analysis had several limitations. First, some aspects of medical history were unknown, including gastric resection, hyperthyroidism and renal glycosuria, which can lower 1,5-AG levels. Second, the present dataset did not include measurement of HbA_{1c} levels or OGTT; therefore, comparison of HbA_{1c} or OGTT with serum 1,5-AG was not possible. Third, a single serum 1,5-AG measurement at baseline may have lead to an underestimation of the association between serum 1,5-AG levels and CVD due to regression dilution bias [25].

In conclusion, the present analyses suggest that in men measurement of serum 1,5-AG was useful to detect individuals at increased risk for CVD, regardless of the presence or absence of diabetes. Measurement of serum 1,5-AG levels might be a useful tool for screening in the clinical setting or during health check-ups. However, this is the first report with a limited population of Japanese, and these findings should be further investigated by studies with sufficient samples and CVD events among various populations, races and geographical areas.

Conflict of interest

None to be declared.

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Association With Serum Gamma-Glutamyltransferase Levels and Alcohol Consumption on Stroke and Coronary Artery Disease

The Suita Study

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Background and Purpose—Light-to-moderate alcohol consumption is associated with reduced risk for cardiovascular disease, whereas high serum γ-glutamyltransferase (GGT) level is associated with cardiovascular disease. However, whether light-to-moderate alcohol drinking is still related to reduced risk of cardiovascular disease irrespective of GGT level is uncertain.

Methods—We performed a 12.5-year cohort study of 2336 men (excluding exdrinkers) who were free from cardiovascular disease. They were classified into 4 groups according to alcohol consumption: never, and current light, moderate, or heavy drinker. The multivariate-adjusted hazard ratios of alcohol consumption for incidence of coronary artery disease, total stroke, and ischemic stroke compared with those of never drinkers were assessed with stratification by GGT median (32 IU/L).

Results—In participants with GGT >32 IU/L, the hazard ratios of all current drinkers for total and ischemic stroke were higher than those of never drinkers. However, in all current drinkers with GGT ≤32 IU/L, the multivariate-adjusted hazard ratios for total and ischemic stroke were lower than in never drinkers.

Conclusions—In men with above GGT median, alcohol drinking even with light-to-moderate consumption could be a risk factor for ischemic stroke. **(*Stroke*. 2011;42:1764-1767.)**

Key Words: cohort ■ epidemiology ■ incidence ■ ischemic stroke

Light-to-moderate alcohol drinking is associated with reduced risk of cardiovascular disease (CVD).[1,2] On the other hand, high serum γ-glutamyltransferase (GGT) level is associated with increased risk of CVD.[3,4] However, it is unclear whether light-to-moderate alcohol drinking is still associated with lower risk of CVD irrespective of GGT level. We performed a cohort study in Japanese men to examine the relationships of alcohol drinking with stroke and coronary artery disease (CAD) events after stratification by GGT level.

Methods

The Suita Study, a cohort study of CVD, was established in 1989. The details of the methods have been described elsewhere.[5] In the present study, 2336 men aged 30 to 79 years who were free from CVD and did not include exdrinkers were followed for 12.5±5.3 years. Disease event included the first stroke or CAD incidence or mortality from stroke or CAD. This study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center.

In the baseline survey, alcohol consumption was determined by alcohol intake per typical week and calculated as ethanol intake per day. We defined 11.5 g of ethanol (half a *gou*, a traditional Japanese unit) as 1 drink; this is nearly equal to 1 "standard" drink in most countries.[6] Participants were classified into 4 groups according to alcohol consumption.

We calculated the multivariate-adjusted hazard ratios (HRs) and 95% CIs for CAD, total stroke and ischemic stroke in each drinking group compared with never drinkers by Cox proportional hazards model after adjusting for possible confounders. The same statistical models were used after the participants were classified according to GGT median (32 IU/L) at the baseline survey and when never drinkers with GGT ≤32 IU/L were used as a reference in all participants. All statistical analyses were performed by using SPSS statistical software, Version 15.0 J (SPSS, Tokyo, Japan).

Results

The baseline characteristics of the participants are shown in Table 1.

During the follow-up period, 109 CAD and 113 total stroke cases occurred. The multivariate-adjusted HRs for CAD were

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Table 1. Baseline Characteristics of Participants According to Alcohol Consumption: The Suita Study

	Alcohol Consumption				
		Current Drinkers			
	Never	Light (<2.0 Drinks/Day)	Moderate (≥2.0 and <4.0 Drinks/Day)	Heavy (≥4.0 Drinks/Day)	Trend *P*
No. of participants	521	317	796	702	
Age, y*	59±14	58±14	55±13	52±11	<0.001
GGT, IU/L†	22 (3–279)	25 (2–343)	33 (5–521)	56 (10–788)	<0.001
Body mass index, kg/m^2*	22.7±3.2	22.5±3.0	22.8±2.7	23.1±2.8	0.003
Hypertension, %‡§	29.4	32.2	32.2	35.9	0.110
Diabetes, %‡§	4.4	5.4	5.3	6.7	0.364
High-density lipoprotein cholesterol, mmol/L*	1.15±0.30	1.25±0.31	1.28±0.33	1.36±0.36	<0.001
Triglycerides, mmol/L†	1.28	1.22	1.25	1.41	<0.001
Hypercholesterolemia, %‡§	29.9	30.0	28.4	27.6	0.785
Current smoking, %‡	48.2	36.9	48.0	62.0	<0.001

Values are mean±SD unless specified otherwise. Values of GGT and triglycerides are median (range).
GGT indicates γ-glutamyltransferase.
*Analysis of variance.
†Kruskal-Wallis test.
‡Chi-square test.
§Hypertension is systolic/diastolic blood pressures of ≥140/90 mm Hg and/or current medication for hypertension. Diabetes is fasting blood glucose of ≥7 mmol/L and/or current use of insulin or oral medication for diabetes. Hypercholesterolemia is fasting serum total cholesterol of ≥5.69 mmol/L and/or current medication for dyslipidemia.

Table 2. Risk of Alcohol Consumption for Cardiovascular Disease According to GGT Level With 12.5-Year (Mean) Follow-Up: The Suita Study

	Alcohol Consumption			
		Current Drinkers		
	Never	Light (<2.0 Drinks/Day)	Moderate (≥2.0 and <4.0 Drinks/Day)	Heavy (≥4.0 Drinks/Day)
Participants with GGT median (32 IU/L) and below				
No. of participants	392	209	396	172
Person-y	4746	2564	5041	2236
Coronary artery disease				
No. of events	35	14	14	4
Multivariate-adjusted HR (95% CI)*	1.00	0.64 (0.34–1.22)	0.40 (0.21–0.76)	0.31 (0.11–0.89)
Total stroke				
No. of events	24	11	12	7
Multivariate-adjusted HR (95% CI) *	1.00	0.63 (0.30–1.31)	0.43 (0.21–0.88)	0.70 (0.29–1.69)
Ischemic stroke				
No. of events	15	9	7	4
Multivariate-adjusted HR (95% CI)*	1.00	0.80 (0.34–1.89)	0.43 (0.17–1.08)	0.63 (0.20–2.01)
Participants with above GGT median (32 IU/L)				
No. of participants	129	108	400	530
Person-y	1619	1329	4923	6831
Coronary artery disease				
No. of events	8	5	15	14
Multivariate-adjusted HR (95% CI)*	1.00	1.14 (0.37–3.54)	0.81 (0.34–1.97)	0.61 (0.24–1.56)
Total stroke				
No. of events	2	8	26	23
Multivariate-adjusted HR (95% CI)*	1.00	7.66 (1.60–36.60)	6.68 (1.56–28.66)	4.68 (1.07–20.54)
Ischemic stroke				
No. of events	2	6	21	14
Multivariate-adjusted HR (95% CI)*	1.00	5.81 (1.15–29.26)	5.27 (1.21–23.02)	2.54 (0.55–11.70)

*Multivariate-adjusted HR was calculated after adjustment for age, body mass index, high-density lipoprotein cholesterol, triglycerides (log-transformed), presence of hypertension, diabetes and hypercholesterolemia, and smoking status (current or noncurrent).
GGT indicates γ-glutamyltransferase; HR, hazard ratio.

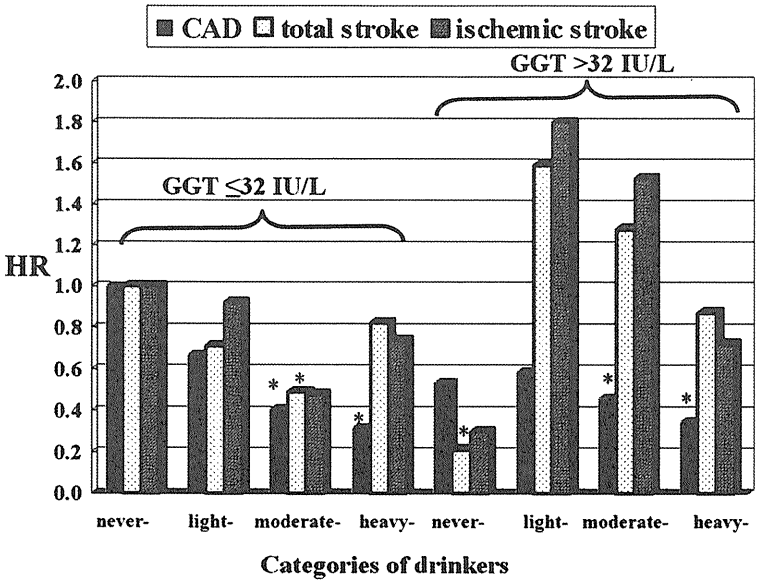

Figure. Risk of alcohol consumption for CVD according to GGT level. *$P<0.05$ compared to never-drinkers with GGT median and below. HR indicates multivariate-adjusted hazard ratio adjusted for the same confounders presented in Table 2; CVD, cardiovascular disease; GGT, γ-glutamyltransferase.

lower in drinkers than in never drinkers with statistical significance in moderate and heavy drinkers. The HRs for total and ischemic stroke were similar among all groups (data not shown).

In participants with GGT ≤32 IU/L, the HRs for CAD, total stroke, and ischemic stroke were lower among all current-drinker groups than in never drinkers (Table 2). In participants with GGT >32 IU/L, HRs for CAD were still lower in moderate and heavy drinkers than in never drinkers. However, for total and ischemic stroke, HRs were higher among current drinkers than in never drinkers. These tendencies did not change when never drinkers with GGT ≤32 IU/L were used as a reference in all participants (Figure), although the HRs were different from those presented in Table 2.

Discussion

To our knowledge, this is the first cohort study to show the consumption-specific risks of alcohol drinking for CVD stratified by GGT levels. Generally, habitual drinkers are interested to know, "What is the upper limit of alcohol intake?" and "Who should not drink?" The answer from our study is "Especially from the viewpoint of stroke prevention, serum GGT levels should be examined; people with high GGT levels should not drink alcohol," although we need to accumulate more evidence concerning this question in future studies.

Increased GGT levels are associated with CVD risk factors such as high blood pressure, high cholesterol, and high blood glucose levels[7] and with high levels of oxidative stress markers.[8] These risk factors might be associated with our current results, although we adjusted for the presence of hypertension, hypercholesterolemia, and diabetes mellitus in the baseline survey.

This study had several limitations. First, because of the small number of cases, we could not assess the risk of hemorrhagic stroke. Second, use of a single GGT measurement and a single assessment of alcohol consumption at the baseline survey might have caused misclassification or underestimation of the relationships among alcohol drinking, GGT, and events because of regression dilution bias.[9] Third, we could not accurately validate our questionnaire for alcohol consumption and investigate the effects of binge drinking, type of alcoholic beverage consumed,[6] and genetic differences.[10] Fourth, the small event number of never drinkers with GGT >32 IU/L was vulnerable to fluctuation and could affect the results.

In conclusion, for individuals with relatively high GGT levels, alcohol drinking could be a risk factor for ischemic stroke. Examination of serum GGT levels might be important for the evaluation of ischemic stroke risk in consumers of alcohol.

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Disclosures

None.

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ORIGINAL ARTICLE

Body mass index and stroke incidence in a Japanese community: the Hisayama study

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Although obesity is one of the major risk factors for coronary heart disease, its role in the development of stroke remains controversial. A total of 2421 residents, aged 40–79 years of a Japanese community were followed up prospectively for 12 years. The subjects were divided into four groups according to body mass index (BMI) levels (<21.0, 21.0–22.9, 23.0–24.9 and ⩾25.0 kg m^{-2}). During the follow-up, 107 ischemic and 51 hemorrhagic strokes occurred. The age-adjusted incidence of ischemic stroke for men significantly increased with increasing BMI levels (*P* for trend=0.005). This association remained substantially unchanged even after adjustment for other risk factors: namely, systolic blood pressure, electrocardiogram abnormalities, diabetes, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, smoking habits, alcohol intake and regular exercise (*P* for trend<0.001). Compared with that of the BMI levels of <21.0 kg m^{-2}, the multivariate-adjusted risk of ischemic stroke was significant even in the BMI levels of 23.0–24.9 kg m^{-2} (multivariate-adjusted hazard ratio (HR)=3.12; 95% confidence interval (CI), 1.24–7.87; *P*=0.02) as well as in the BMI levels of ⩾25 kg m^{-2} (multivariate-adjusted HR=5.59; 95% CI, 2.09–14.91; *P*<0.001). In stratified analyses, the risk of ischemic stroke for men synergistically increased in subjects having both obesity and diabetes or a smoking habit. We found no significant associations between BMI levels and ischemic stroke in women and between BMI levels and hemorrhagic stroke in either sex. In conclusion, our findings suggest that overweight and obesity are independent risk factors for ischemic stroke in Japanese men.
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Keywords: body mass index; incidence; obesity; prospective study; stroke

INTRODUCTION

Stroke is a leading cause of death[1] and permanent disability in middle-aged and elderly people in Japan[2–4] as well as in other developed countries.[5] In Japan, the prevalence of obesity has increased rapidly along with the westernization of lifestyle,[6] although it remains considerably lower than that in Western populations.[7] Increased body mass index (BMI) is tightly related to an increased risk of coronary heart disease,[8] but its association with stroke is less well recognized because of conflicting results reported in the literature. Some cohort studies have found a positive association between BMI and the risk of stroke,[8–14] whereas others have shown no apparent association[15–18] or have even reported an inverse or a U-shaped association.[19–22] In Japan, no prospective study has provided incidence data on this issue nor observed a positive association between BMI and the risk of stroke until now.[21,22] Based on its pathogenesis, stroke is divided into several clinical subtypes, and the effects of BMI on stroke are considered to be different among these subtypes.[8,19] In addition, obesity is an important risk factor for hypertension, diabetes mellitus and dyslipidemia, which are known as major risk factors for stroke,[23,24] and therefore, whether obesity itself independently increases the risk of stroke remains controversial.

In the present article, we investigated the association between BMI and the occurrence of stroke by its subtype based on records of a prospective study of a general Japanese population, taking other known risk factors into account.

METHODS

Study population

In 1988, a screening survey for the present study was performed in the town of Hisayama, a suburb of the Fukuoka metropolitan area in southern Japan. Of a total of 3227 residents aged 40–79 years on the town registry, 2587 consented to participate in the examination (participation rate, 80.2%) and underwent a comprehensive assessment. After excluding 82 subjects who had already had breakfast, 10 who were on insulin therapy and 15 due to complaints of nausea or general fatigue during the ingestion of glucose, a total of 2480 subjects completed a 75-g oral glucose tolerance test. From a total of 2490 subjects including 10 on insulin therapy, 68 who had a history of stroke or coronary heart disease based on questionnaires and medical records, and one who died

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before follow-up was started were excluded. The remaining 2421 (1037 men and 1384 women) were enrolled in this study.

Baseline data collection

At baseline, body height and weight were measured in light clothing without shoes, and BMI ($kg\,m^{-2}$) was calculated as an indicator of obesity. Information on antihypertensive treatment, smoking habits, alcohol intake and regular exercise were obtained with the use of a standard questionnaire. Subjects who reported smoking at least one cigarette per day were defined as current smokers, and subjects who reported consuming alcohol at least once a month were regarded as current drinkers. Subjects engaging in sports at least three times a week during their leisure time made up a regular exercise group. Sitting systolic and diastolic blood pressures were measured three times after a rest of at least 5 min by a standard mercury sphygmomanometer with a standard cuff. The average of three measurements was used for data analysis. Hypertension was defined as a systolic blood pressure $\geqslant 140$ mm Hg, a diastolic blood pressure $\geqslant 90$ mm Hg or current use of antihypertensive agents. ECG abnormalities were defined as left ventricular hypertrophy (Minnesota code 3–1), ST depression (4–1, 2 and 3) and/or atrial fibrillation (8–3). Blood samples were drawn after an overnight fast of at least 12 h. Fasting and 2-h post-load plasma glucose levels were determined by the glucose-oxidase method. Diabetes mellitus was defined as fasting plasma glucose $\geqslant 7.0\,mmol\,l^{-1}$, 2-hour post-load plasma glucose $\geqslant 11.1\,mmol\,l^{-1}$, or current use of insulin or oral medication for diabetes. Total cholesterol, high-density lipoprotein-cholesterol and triglyceride levels were all determined enzymatically.

Follow-up survey

The subjects were followed up prospectively for 12 years from December 1988 to November 2000 by repeated health examinations and by a daily monitoring system established by the study team and local physicians or members of the Health and Welfare Office of the town. Health status was checked once yearly by mail or telephone for any subjects who did not undergo a regular examination or who moved out of town. Study-team physicians performed physical and neurological examinations on all subjects who developed stroke and collected the relevant clinical information, including that on the disease course. During the follow-up period, only one subject was lost to follow-up, and 339 subjects died; among those who died, autopsy was performed on 253 (74.6%).

Stroke, defined as sudden onset of a non-convulsive and focal neurological deficit persisting for >24 h, was classified as ischemic stroke, cerebral hemorrhage, subarachnoid hemorrhage or undetermined type.[25] The clinical diagnosis of stroke and its subtypes was determined on the basis of a detailed history, neurological examination and ancillary laboratory examinations. In this paper, we focused on ischemic and hemorrhagic stroke (cerebral hemorrhage and subarachnoid hemorrhage). During the follow-up period, we identified 107 cases of first-ever ischemic stroke (47 men and 60 women) and 51 cases of first-ever hemorrhagic stroke (21 men and 30 women), consisting of 34 cases of cerebral hemorrhage and 17 cases of subarachnoid hemorrhage. All of the stroke cases were examined by computed tomography and/or magnetic resonance imaging.

Statistical analysis

All statistical analyses were performed with the SAS program package Ver 9.2 (SAS Institute Inc, Cary, NC, USA). All tests were two-sided, and values of $P<0.05$ were considered statistically significant in all analyses. The subjects were divided into four groups according to BMI levels (<21.0, 21.0–22.9, 23.0–24.9 and $\geqslant 25.0\,kg\,m^{-2}$). Because of the skewed distribution of serum triglycerides, this value was log-transformed for statistical analysis. The age-adjusted mean values of risk factors were calculated by the analysis of covariance method, and their trends across BMI levels were tested by multiple regression analysis. Frequencies of risk factors were adjusted for age by the direct method and were examined for trends by the Cochran–Mantel–Haenszel test. The incidence of stroke was calculated by the person-year method and was adjusted for the age distribution of the study population by the direct method. Differences in the incidence of stroke among BMI levels were tested by the Cox proportional hazards model. The age- and multivariate-adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs) were also calculated using the Cox proportional hazards model. The multivariate adjustment was made for age, systolic blood pressure, ECG abnormalities, diabetes, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, smoking habits, drinking status and regular exercise. To assess whether synergistic effect was observed between obesity and each of other risk factors, we added a multiplicative interaction term to the relevant Cox model.

Ethical considerations

The study protocol was approved by the Human Ethics Review Committee of Kyushu University Graduate School of Medical Sciences, and a written informed consent was obtained from the study participants.

RESULTS

Characteristics of the subjects

The age-adjusted mean values or frequencies of risk factors by BMI levels at baseline are shown by sex (Table 1). Mean age significantly decreased with rising BMI levels for men, but such an association was not observed for women. In both sexes, the mean values of systolic and diastolic blood pressures, total cholesterol and triglycerides, and the frequencies of hypertension, antihypertensive drug use and diabetes increased significantly, whereas the mean high-density lipoprotein-cholesterol levels decreased significantly with increasing BMI levels. The frequency of smoking habits for men and that of ECG abnormalities for women decreased significantly with increasing BMI levels. No dose-response relationships were observed between BMI levels and the frequencies of alcohol intake or regular exercise for both sexes.

Impact of BMI on stroke

As shown in Figure 1, the age-adjusted incidence of ischemic stroke for men increased with increasing BMI levels: the difference was significant between the BMI level of $<21.0\,kg\,m^{-2}$ and that of $\geqslant 25.0\,kg\,m^{-2}$ (age-adjusted HR=3.32; 95% CI, 1.43–7.72; $P=0.005$; Table 2). This association remained substantially unchanged even after adjustment for other risk factors (Table 2). The multivariate-adjusted risk of ischemic stroke was significant even in the subjects with BMI levels of 23.0–24.9 $kg\,m^{-2}$ (multivariate-adjusted HR=3.12; 95% CI, 1.24–7.87; $P=0.02$) as well as in those with BMI levels of $\geqslant 25\,kg\,m^{-2}$ (multivariate-adjusted HR=5.59; 95% CI, 2.09–14.91; $P<0.001$). We found no significant associations between BMI levels and the incidence of ischemic stroke in women and between BMI levels and the incidence of hemorrhagic stroke in either sex (Figure1 and Table 2).

Combined effects of obesity and other risk factors

Because hypertension, diabetes and smoking habits are major risk factors for ischemic stroke and are concurrently associated with obesity, we examined the combined effects of obesity and these risk factors on the development of ischemic stroke for men after adjustment for the above-mentioned confounding factors, except for the factor which was used for the grouping. As shown in Table 3, multivariate-adjusted HRs of ischemic stroke were significantly higher in the group of obese subjects irrespective of the presence or absence of hypertension. On the other hand, the risk of ischemic stroke synergistically increased in obese subjects with diabetes compared with non-obese subjects without diabetes (multivariate-adjusted HR=7.91; 95% CI, 3.08–20.28; $P<0.001$), whereas such an increased risk was not observed in non-obese subjects with diabetes or in obese subjects without diabetes. A similar synergistic pattern was observed for the coexistence of obesity and smoking habits (multivariate-adjusted HR=3.62; 95% CI, 1.39–9.43; $P=0.008$). A significant interaction between obesity and diabetes was revealed in the risk of ischemic

Table 1 Age-adjusted baseline characteristics according to body mass index level by sex, the Hisayama Study, 1988

	Body mass index, kg m^{-2}				
	<21	*21–22.9*	*23–24.9*	*≥25*	P *for trend*
Men					
No at risk	283	255	247	252	—
Age (years)	60.5 (0.6)	56.8 (0.6)	56.2 (0.7)	54.4 (0.6)	<0.001
SBP (mm Hg)	127.1 (1.1)	132.2 (1.2)	135.5 (1.2)	141.2 (1.2)	<0.001
DBP (mm Hg)	75.5 (0.6)	79.3 (0.7)	82.0 (0.7)	86.3 (0.7)	<0.001
Hypertension (%)	32.6	37.4	46.9	58.7	<0.001
Antihypertensive drug (%)	9.0	10.8	15.1	23.6	<0.001
ECG abnormalities (%)[a]	20.6	20.9	19.3	18.7	0.28
Diabetes (%)	10.1	16.9	13.6	20.9	0.005
Total cholesterol (mmol l^{-1})	4.95 (0.06)	5.05 (0.07)	5.13 (0.07)	5.31 (0.07)	<0.001
HDL cholesterol (mmol l^{-1})	1.37 (0.02)	1.30 (0.02)	1.22 (0.02)	1.14 (0.02)	<0.001
Triglycerides (mmol l^{-1})	1.01 (0.94–1.07)	1.28 (1.20–1.37)	1.46 (1.36–1.56)	1.77 (1.65–1.90)	<0.001
Smoking (%)	68.7	47.0	44.5	36.6	<0.001
Drinking (%)	59.7	65.9	64.7	58.6	0.63
Regular exercise (%)[b]	12.8	11.1	11.0	10.9	0.34
Women					
No at risk	380	347	318	339	
Age (years)	59.1 (0.5)	57.0 (0.6)	57.0 (0.6)	57.6 (0.6)	0.052
SBP (mm Hg)	125.2 (1.0)	130.2 (1.0)	131.1 (1.1)	136.9 (1.0)	<0.001
DBP (mm Hg)	71.8 (0.5)	74.4 (0.6)	77.0 (0.6)	80.0 (0.6)	<0.001
Hypertension (%)	24.2	30.9	34.5	50.3	<0.001
Antihypertensive drug (%)	7.5	14.1	14.2	21.5	<0.001
ECG abnormalities (%)[a]	15.2	14.3	9.4	11.6	0.03
Diabetes (%)	7.5	6.8	8.8	16.6	<0.001
Total cholesterol (mmol l^{-1})	5.31 (0.05)	5.54 (0.06)	5.74 (0.06)	5.66 (0.06)	<0.001
HDL cholesterol (mmol l^{-1})	1.44 (0.01)	1.35 (0.02)	1.30 (0.02)	1.26 (0.02)	<0.001
Triglycerides (mmol l^{-1})	0.88 (0.84–0.92)	1.04 (0.99–1.09)	1.15 (1.10–1.21)	1.24 (1.18–1.30)	<0.001
Smoking (%)	8.1	3.5	6.6	8.1	0.72
Drinking (%)	9.5	10.3	5.1	10.7	0.79
Regular exercise (%)[b]	9.4	10.5	8.9	6.3	0.11

Abbreviations: DBP, diastolic blood pressure; HDL, high-density lipoprotein; SBP, systolic blood pressure.
Data are shown as the means (standard error) or a percentage. Geometric mean values and 95% confidence intervals of serum triglycerides are shown attributable to the skewed distribution. Mean age was not age-adjusted.
[a]Minnesota codes: 3–1, 4–1, 2, 3 or 8–3
[b]Engaging in sports or other forms of exertion regularly ≥three times a week during leisure time.

stroke (P=0.01), whereas the interactions between obesity and hypertension and between obesity and smoking habits were not significant.

DISCUSSION

In this prospective study of a community-dwelling Japanese population, we demonstrated that higher BMI was a significant risk factor for the development of ischemic stroke in men. This association remained unchanged even after adjustment for other risk factors. In addition, the combinations of obesity plus diabetes or obesity plus a smoking habit synergistically increased the risk of ischemic stroke. However, there was no significant association between BMI levels and the risk of hemorrhagic stroke in either sex.

Some cohort studies have shown an increased risk of total stroke or ischemic stroke with elevating BMI,[8–14] which is in accord with the findings of the risk of ischemic stroke in our male subjects. On the other hand, other studies have found no association,[15–18] an inverse or a U-shaped association.[19–22] One possible explanation for this difference in findings may be that stroke was not evaluated by its subtype in all these studies, as the effect of obesity is different among stroke subtypes. Another explanation may be that most of these studies used mortality data as an endpoint. Our previous study showed that lower BMI was a significant risk factor for death after total stroke and ischemic stroke.[26] Epidemiological studies of body weight and mortality are affected by methodological problems, such as failure to control the harmful biological effects of smoking and subclinical diseases resulting in weight loss. Thus, the association of BMI with stroke mortality should be interpreted with caution.

In the literature, the associations between BMI levels and the risk of hemorrhagic stroke have been inconsistent, with some studies showing a positive association,[8,11,14] and others showing no, a negative or a U-shaped, association.[9,12,13,16,19,21,22] In the present study, we did not find a clear association between BMI levels and hemorrhagic stroke in men or women. The lack of a clear consensus on this association may be partly due to the low number of cases of hemorrhagic stroke in most of the studies, including our present work, or differences in ethnicities, study populations or study methods. Future studies will be needed to resolve this issue.

A number of studies have reported that the association between BMI and total or ischemic stroke was attenuated or eliminated after adjustment for potential mediators, such as hypertension, diabetes

and dyslipidemia.[9,10,12–14,19,22] In our study, however, the association between BMI and ischemic stroke was not attenuated even after adjusting for these risk factors. This finding indicates an independent effect of overweight and obesity on the development of ischemic stroke. A similar independent association has been observed in other studies of stroke.[10,12,14] These findings, together with our present results, suggest a link between overweight/obesity and ischemic stroke independent of established risk factors. Some investigators have proposed that the increase in prothrombotic factors[27–29] and inflammatory markers,[30–33] and the enhancement of insulin resistance and metabolic syndrome[34] observed among overweight and obese individuals may have a role in their increased risk of ischemic stroke.

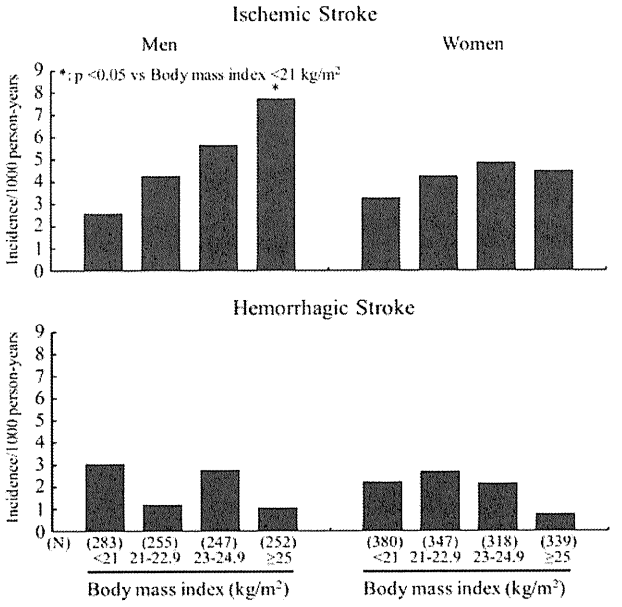

Figure 1 Age-adjusted incidence of stroke by body mass index levels during 12-year follow-up, the Hisayama Study, 1988–2000.

Our stratified analysis showed an extremely increased risk of ischemic stroke in men who have both obesity and diabetes or smoking habits. Although the mechanisms underlying this phenomenon are not clearly understood, a possible explanation can be proposed. Because diabetes and smoking are strong risk factors for the progression of systemic arteriosclerosis, it is reasonable to consider that subjects with these risk factors already have vascular injuries to some extent. Obesity-related disorders, such as inflammation, insulin resistance and metabolic syndrome, may accelerate the progression of preexisting vascular injuries, resulting in an increased risk of ischemic stroke. However, in the present study we did not find that obesity enhanced the effect of hypertension on stroke risk. Although the precise reason for this is not known, the popularization of antihypertensive treatment in our study population might have weakened the synergistic effects of these factors.

In our female subjects, we did not observe a significant association between BMI and the risk of ischemic stroke. Several cohort studies have also examined the effects of BMI on the risk of ischemic stroke in women,[9,13–15,21,22] but the findings were inconsistent, with some studies showing a positive association,[9,13,14] and others showing no association[15,21] like our study. Further studies will be needed to clarify the true association between BMI and stroke in women.

Table 2 Adjusted hazard ratio for stroke incidence according to body mass index level by sex, the Hisayama Study, 1988–2000

Body mass index, kg m^{-2}	*Person year*	*No. of events*	*Age-adjusted HR*	*95% CI*	*Multivariate-adjusted HR*[a]	*95% CI*
Men						
Ischemic stroke						
<21.0	2907	9	1.00	Referent	1.00	Referent
21.0–22.9	2736	10	1.70	0.69–4.20	2.34	0.91–6.00
23.0–24.9	2692	12	2.09	0.88–5.00	3.12	1.24–7.87
25.0≥	2790	16	3.32	1.43–7.73	5.59	2.09–14.91
P for trend			0.005		<0.001	
Hemorrhagic stroke						
<21.0	2907	9	1.00	Referent	1.00	Referent
21.0–22.9	2736	3	0.44	0.12–1.63	0.38	0.10–1.50
23.0–24.9	2692	6	0.89	0.31–2.55	0.90	0.28–2.87
25.0≥	2790	3	0.47	0.12–1.80	0.36	0.08–1.57
P for trend			0.41		0.31	
Women						
Ischemic stroke						
<21.0	4214	15	1.00	Referent	1.00	Referent
21.0–22.9	3935	15	1.41	0.69–2.90	1.37	0.65–2.88
23.0–24.9	3652	15	1.51	0.73–3.10	1.56	0.71–3.43
25.0≥	3794	15	1.41	0.69–2.91	1.27	0.58–2.80
P for trend			0.32		0.55	
Hemorrhagic stroke						
<21.0	4214	10	1.00	Referent	1.00	Referent
21.0–22.9	3935	10	1.26	0.52–3.04	1.32	0.52–3.35
23.0–24.9	3652	7	0.94	0.36–2.49	1.13	0.39–3.25
25.0≥	3794	3	0.38	0.10–1.39	0.35	0.09–1.35
P for trend			0.16		0.16	

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.
[a]Multivariate adjustment was made for age, systolic blood pressure, ECG abnormalities, diabetes, total and high-density lipoprotein-cholesterols, triglycerides, smoking, drinking and regular exercise.

Table 3 Multivariate-adjusted[a] hazard ratios for the development of ischemic stroke according to the presence or absence of obesity and each established risk factor in men, the Hisayama Study, 1988–2000

		Population at risk	*No. of events*	*HR*	*95% CI*	P *value*
Obesity[b]	*Hypertension*					
No	No	477	14	1.00	Referent	
No	Yes	308	17	1.59	0.76–3.34	0.22
Yes	No	111	7	3.79	1.44–10.00	0.007
Yes	Yes	141	9	2.95	1.19–7.30	0.02
Obesity[b]	*Diabetes*					
No	No	678	25	1.00	Referent	
No	Yes	107	6	1.60	0.65–3.97	0.31
Yes	No	200	8	1.83	0.77–4.38	0.17
Yes	Yes	52	8	7.91	3.08–20.28	<0.001
Obesity[b]	*Smoking*					
No	No	369	17	1.00	Referent	
No	Yes	416	14	1.18	0.56–2.48	0.67
Yes	No	148	8	2.13	0.83–5.46	0.11
Yes	Yes	104	8	3.62	1.39–9.43	0.008

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.
[a]Multivariate adjustment was made for age, systolic blood pressure, ECG abnormalities, diabetes, total and high-density lipoprotein-cholesterols, triglycerides, smoking, drinking and regular exercise, but the factor which was used for each grouping was excluded from the confounding factors.
[b]Obesity is defined as a body mass index $\geqslant 25\,kg\,m^{-2}$.

The strengths of our study include its longitudinal population-based design, the direct collection of height, weight and biological markers from all participants, long duration of follow-up, perfect follow-up of subjects and accuracy of diagnosis of stroke. One limitation of our study is that our findings are based on a one-time measurement of BMI, as was the case in most other epidemiological studies. During the follow-up, BMI and other risk factor levels were changed due to modifications in lifestyle or medication, and misclassification of BMI categories was possible. This could have weakened the association found in this study, biasing the results toward the null hypothesis. Therefore, the true association may be stronger than that shown here.

In conclusion, our data suggest that overweight and obesity are significant risk factors for the development of ischemic stroke in contemporary Japanese men. In Japan, BMI levels have increased steadily over the last several decades. For prevention of stroke, it is important to correct obesity while controlling other risk factors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Combined Effects of Smoking and Hypercholesterolemia on the Risk of Stroke and Coronary Heart Disease in Japanese: The Hisayama Study

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Key Words
Smoking · Hypercholesterolemia · Stroke · Coronary heart disease · Cohort study

Abstract
Background: Cigarette smoking is an established risk factor for stroke and coronary heart disease (CHD) in Western countries. However, it is uncertain whether or not smoking raises the risk of stroke in Japanese. We examined the influence of smoking on the development of stroke and CHD and the effects of interactions between smoking and hypercholesterolemia on these outcomes in a general Japanese population. ***Methods:*** A total of 2,421 community-dwelling Japanese individuals, aged 40–79 years, with no history of cardiovascular disease, were followed up for 14 years. ***Results:*** During the follow-up, 194 total stroke and 112 CHD events occurred. Compared with never smokers, the multivariate-adjusted hazard ratios for the occurrence of total stroke were 1.53 (95% confidence interval = 0.90–2.61) in former smokers, 1.90 (1.18–3.06) in current light smokers (<20 cigarettes/day) and 2.01 (1.11–3.65) in current heavy smokers (≥20 cigarettes/day). The multivariate-adjusted hazard ratios for the development of CHD were 1.10 (0.56–2.15), 1.88 (1.02–3.47) and 2.31 (1.17–4.57), respectively. In regard to stroke subtypes, current smoking was an independently significant risk factor for ischemic stroke and subarachnoid hemorrhage. Furthermore, the combination of smoking and hypercholesterolemia synergistically increased the risks of total stroke and CHD (all p for interaction <0.05). ***Conclusion:*** Our findings suggest that smoking raises the risks of ischemic stroke, subarachnoid hemorrhage and CHD occurrence in the Japanese population, and that this effect is strengthened by hypercholesterolemia.

Introduction

In Western countries, cigarette smoking is an established major risk factor for cardiovascular diseases (CVD) such as stroke [1] and coronary heart disease (CHD) [2]. Therefore, smoking cessation is currently recognized as a key target of prevention strategies for CVD [3]. While most cohort studies in Japan confirmed the harmful effect of smoking on the risk of CHD [4, 5], there is no con-

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sensus on whether or not smoking increases the risk of stroke in Japanese [4–8]. Furthermore, some studies have evaluated the interaction between smoking and hypercholesterolemia, which is also an important risk factor for CVD, but their conclusions have not been consistent [4, 9–13]. The purposes of the present study were to assess the effect of smoking on the development of stroke and CHD, and to clarify the interactions between smoking and hypercholesterolemia as well as other risk factors in a population-based cohort study in Japan.

Methods

Study Subjects

In 1988, a screening examination for the present study was performed in the town of Hisayama, a suburban community in the Fukuoka metropolitan area on Kyushu Island, Japan. A detailed description of this examination was published previously [14, 15]. Briefly, a total of 2,587 residents aged 40–79 years (80.2% of the total population in this age range) participated in the examination. After the exclusion of 88 subjects with a history of stroke or CHD, 77 who did not complete a 75-gram oral glucose tolerance test, and 1 who died before the initiation of follow-up, the remaining 2,421 (1,037 men and 1,384 women) were enrolled in the present study. This study was conducted with the approval of the ethics committee of the Faculty of Medicine, Kyushu University, and written informed consent was obtained from the subjects.

Risk Factors

At the baseline examination, each subject completed a self-administered questionnaire covering medical history, treatment for hypertension and diabetes, smoking status, alcohol intake and leisure time activity. The smoking status was classified into 4 categories: never smokers, former smokers, current light smokers (<20 cigarettes per day) and current heavy smokers (≥20 cigarettes per day). Alcohol intake was defined as customary drinking of an alcoholic beverage at least once a month. Subjects engaging in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group.

The sitting blood pressure was measured 3 times using a standard mercury sphygmomanometer after rest for at least 5 min. The mean of the 3 measurements was used for the analysis. Hypertension was defined as blood pressure ≥140/90 mm Hg or current use of antihypertensive agents. Obesity was defined as body mass index ≥25. Electrocardiogram abnormalities were defined as left ventricular hypertrophy (Minnesota Code, 3-1), ST depression (4-1, 2 or 3) or atrial fibrillation (8-3).

We performed the 75-gram glucose tolerance test after at least a 12-hour overnight fast. The plasma glucose levels were determined by the glucose-oxidase method. Diabetes mellitus was defined as any of the following: fasting plasma glucose ≥7.0 mmol/l, 2-hour postload glucose ≥11.1 mmol/l, or current use of oral hypoglycemic agents or insulin. The total cholesterol levels were determined enzymatically. Hypercholesterolemia was defined as total cholesterol ≥5.69 mmol/l.

Follow-Up Survey

The subjects were followed up prospectively for 14 years from December 1988 to November 2002 by repeated health examinations. Their health status was checked yearly by mail or telephone for any subjects who did not undergo a regular examination or who had moved out of town. We also established a daily monitoring system among the study team and local physicians or members of the town's health and welfare office. Using this system, we gathered information on new events of stroke and CHD, including suspected cases. When a new CVD event occurred or was suspected, physicians in the study team examined the subject and evaluated his or her detailed clinical information, including medical history and physical, neurological, laboratory and radiological examinations, to determine whether or not this event met the definition of an outcome. In addition, when a subject died, an autopsy was usually performed at the Department of Pathology of Kyushu University. During the follow-up period, 1 subject was lost to follow-up and 418 died, of whom 312 (74.6%) underwent autopsy.

Study Outcomes

Study outcomes were the development of CVD consisting of stroke and CHD. Stroke was defined in principle as a sudden onset of nonconvulsive and focal neurological deficit persisting for ≥24 h and was classified into 3 subtypes: ischemic stroke, intracerebral hemorrhage and subarachnoid hemorrhage. All stroke events were morphologically examined by computed tomography, magnetic resonance imaging or autopsy findings. CHD included acute and silent myocardial infarction, sudden cardiac death within 1 h after the onset of acute illness, and coronary artery disease treated by coronary artery bypass surgery or angioplasty. Acute myocardial infarction was diagnosed when a subject met at least 2 of 4 criteria: (1) typical symptoms including prolonged severe anterior chest pain; (2) evolving diagnostic electrocardiographic changes; (3) cardiac enzyme levels more than twice the upper limit of the normal range; (4) morphological changes (local asynergy of cardiac wall motion on echocardiography, persistent perfusion defect on cardiac scintigraphy, or myocardial necrosis or scars ≥1 cm long accompanied by coronary atherosclerosis at autopsy). Silent myocardial infarction was defined as a morphological change of the myocardium without any historical indication of clinical symptoms or abnormal cardiac enzyme changes. During the follow-up period, 281 subjects developed CVD for the first time. These included 194 cases of all forms of stroke (132 ischemic stroke, 43 intracerebral hemorrhage and 19 subarachnoid hemorrhage) and 112 cases of CHD.

Statistical Analysis

SAS software version 9.2 was used to perform all statistical analyses. The frequency of each risk factor at baseline across the smoking status was adjusted for age and sex by a direct method and compared by logistic regression analysis. The age- and sex-adjusted mean of each risk factor at baseline was estimated and compared by the analysis of covariance. The age- and sex-adjusted and multivariate-adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs) were estimated using the Cox proportional hazards model. The interaction between smoking and each of the other risk factors was tested by adding an interaction term to the relevant Cox model. $p < 0.05$ was considered statistically significant.

Table 1. Age- and sex-adjusted mean values or frequencies of cardiovascular risk factors by smoking status at baseline

	Never smoker (n = 1,477)	Former smoker (n = 332)	Current smoker	
			<20 cigarettes/day (n = 348)	≥20 cigarettes/day (n = 264)
Age, years (sex-adjusted)	57 ± 12	61 ± 12*	59 ± 11	55 ± 12*
Men, % (age-adjusted)	14.3	92.6*	77.0*	94.8*
Systolic blood pressure, mm Hg	133 ± 23	133 ± 22	130 ± 21	129 ± 22
Diastolic blood pressure, mm Hg	79 ± 13	79 ± 12	75 ± 12*	75 ± 12*
Hypertension, %	38.6	43.0	35.2	21.1*
Fasting plasma glucose, mmol/l	5.8 ± 1.5	6.0 ± 1.5	5.8 ± 1.4	5.7 ± 1.4
Two-hour postload glucose, mmol/l	7.5 ± 4.3	7.9 ± 4.1	7.5 ± 3.8	7.4 ± 4.0
Diabetes, %	10.3	12.1	12.5	11.0
Total cholesterol, mmol/l	5.37 ± 1.27	5.44 ± 1.21	5.20 ± 1.14	5.40 ± 1.19
Hypercholesterolemia, %	35.3	38.0	37.1	22.5
Body mass index	23.1 ± 3.7	23.3 ± 3.5	22.3 ± 3.3*	22.5 ± 3.4*
Obesity, %	25.1	27.1	19.8*	23.9
Electrocardiogram abnormalities, %	17.4	16.1	16.3	13.4
Current alcohol intake, %	26.6	56.0*	46.3*	51.9*
Regular exercise, %	10.0	10.9	9.9	3.5

Values presented are means ± SD or percentages. * $p < 0.05$ compared with never smokers.

Results

The baseline characteristics of the study subjects are summarized in table 1. Compared with never smokers, the mean age was higher in former smokers but lower in current heavy smokers. The proportions of men and alcohol drinkers were higher in former and current smokers. Current heavy smokers had a lower prevalence of hypertension. Current light and heavy smokers had a lower body mass index.

In men, the risk for the development of CVD was significantly higher in current smokers than in never smokers (age-adjusted HR = 1.65; 95% CI = 1.04–2.63), and the risk of CVD was almost the same in women as in men (age-adjusted HR = 1.68; 95% CI = 0.94–2.98). Because there was no evidence of interaction between sex and current smoking (p for interaction = 0.97), we analyzed both sexes together in the following evaluations.

Table 2 shows the effects of smoking on the development of CVD, total stroke and CHD. The age- and sex-adjusted HRs for CVD and total stroke were significantly higher in current light and heavy smokers, and that for CHD was significantly higher in current heavy smokers than in never smokers. Former smoking was not a significant risk factor for each outcome. After adjusting for risk factors (age, sex, systolic blood pressure, diabetes, total cholesterol, body mass index, electrocardiogram abnormalities, alcohol intake and regular exercise), both current light and heavy smokers had a significantly higher risk of each outcome than never smokers. Table 3 shows the effects of smoking on the risks of stroke subtypes. Current light and heavy smokers were combined here because of the limited number of events. Current smoking was an independently significant risk factor for ischemic stroke and subarachnoid hemorrhage, but not for intracerebral hemorrhage, after adjustment for confounding factors.

As shown in table 4, we assessed the combined and separate effects of smoking and each of the other established risk factors on the development of CVD. Compared with nonsmokers (never or former smokers) without hypercholesterolemia, current smokers with hypercholesterolemia had significantly higher multivariate-adjusted HRs for CVD. However, no significant elevations in HRs were observed in nonsmokers with hypercholesterolemia or in current smokers without hypercholesterolemia. A significant interaction between smoking and hypercholesterolemia was revealed in the risk of CVD, while we failed to detect any significant interaction between current smoking and hypertension, diabetes, obesity, alcohol intake or regular exercise. Table 5 shows the interaction analyses between current smoking and hypercholesterolemia on the development of stroke and CHD. The combination of current smoking and hyper-