

80.2%) consented to take part in a comprehensive assessment. After exclusion of 82 subjects who had already had breakfast, 10 subjects who were receiving insulin therapy, and 15 subjects who complained of nausea or general fatigue during the ingestion of glucose, a total of 2,480 subjects completed a 75-g oral glucose tolerance test. Among these, 297 subjects with diabetes, 52 subjects for whom there was no measurement of waist circumference, and 2 subjects who died before the start of follow-up were excluded, and the remaining 2,129 subjects (894 men and 1,235 women) were enrolled in the baseline examination.

The baseline subjects were followed-up prospectively from December 1988 to November 2002 by repeated health examinations. Of the baseline subjects, 1,935 subjects (793 men and 1,142 women) who underwent reexaminations were finally selected for the present study (follow-up rate, 90.9%; mean follow-up period, 11.8 years; mean frequency of follow-up examinations, 6.9 times). One subject who developed overt type 1 diabetes clinically during the follow-up period was censored at the time.

#### Clinical evaluation and laboratory measurements

In the baseline and follow-up examinations, the study subjects underwent an oral glucose tolerance test between 8:00 and 10:30 A.M. after an overnight fast of at least 12 h. Blood for the glucose assay was obtained by venipuncture into tubes containing sodium fluoride at fasting and at 2-h postload. Plasma glucose concentrations were determined by the glucose oxidase method. According to the American Diabetes Association criteria in 2003 (16), diabetes was defined as FPG concentrations of  $\geq 7.0$  mmol/l and/or 2-h postload glucose concentrations of  $\geq 11.1$  mmol/l and/or the use of antidiabetes medication. Total and HDL cholesterol and triglycerides were determined enzymatically.

At the baseline examination, waist circumference was measured by a trained staff member at the umbilical level with the subject standing. Blood pressure was obtained three times using a mercury sphygmomanometer with the subject in a sitting position; the average values were used in the analyses. Each participant completed a self-administered questionnaire covering medical history, antidiabetes and antihypertension treatments, alcohol intake, smoking habits, and phys-

**Table 1—Baseline characteristics of subjects by the presence or absence of incident type 2 diabetes, 1988**

	No developed diabetes	Developed diabetes
n	1,649	286
Age (years)	57 $\pm$ 10	56 $\pm$ 9
Men (%)	39.3	50.7
FPG (mmol/l)	5.4 $\pm$ 0.5	5.9 $\pm$ 0.6
Two-hour postload glucose (mmol/l)	6.4 $\pm$ 1.5	7.5 $\pm$ 1.8
Family history of diabetes (%)	6.3	14.0
Waist circumference (cm)	80.8 $\pm$ 9.0	85.0 $\pm$ 8.7
Total cholesterol (mmol/l)	5.35 $\pm$ 1.06	5.39 $\pm$ 1.07
HDL cholesterol (mmol/l)	1.32 $\pm$ 0.30	1.26 $\pm$ 0.30
Triglycerides (mmol/l)	1.09 (0.40–2.98)	1.43 (0.45–4.49)
Systolic blood pressure (mmHg)	130 $\pm$ 19	137 $\pm$ 19
Diastolic blood pressure (mmHg)	77 $\pm$ 11	82 $\pm$ 12
Elevated blood pressure (%)	48.8	67.8
Current drinking (%)	28.6	39.2
Current smoking (%)	21.6	31.8
Regular exercise (%)	11.3	6.6

Data are means  $\pm$  SD, %, or geometric means (95% CI) for triglycerides (because of the skewed distribution). Elevated blood pressure was defined as blood pressure  $\geq 130/85$  mmHg and/or current use of antihypertension agents.

ical activity at the screening. Diabetes in first- or second-degree relatives was taken to indicate a family history of diabetes. Alcohol intake and smoking habits were classified as either current use or not. Subjects engaging in sports at least three times per week during their leisure time were defined as the regular-exercise group.

#### Definition of metabolic syndrome

The criteria set for metabolic syndrome used in this study was defined by the updated 2005 National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) (17). By this definition, metabolic syndrome includes the presence of at least three of five factors: elevated FPG ( $\geq 5.6$  mmol/l), central obesity for Asians (waist circumference  $\geq 90$  cm in men and  $\geq 80$  cm in women), elevated triglycerides ( $\geq 1.68$  mmol/l), reduced HDL cholesterol ( $< 1.03$  mmol/l for men and  $< 1.29$  mmol/l for women), and elevated blood pressure (blood pressure  $\geq 130/85$  mmHg and/or current use of antihypertension agents).

#### Statistical analysis

The SAS software package (version 8.2; SAS Institute, Cary, NC) was used to perform all statistical analyses. Serum triglycerides were transformed into logarithms to improve the skewed distribution. Multivariate-adjusted hazard ratios (HRs) and their 95% CIs were estimated with the use

of the Cox proportional hazards model.  $P < 0.05$  was considered statistically significant in all analyses.

#### Ethical considerations

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 all participants.

**RESULTS**— During the follow-up, 286 subjects (145 men and 141 women) developed type 2 diabetes. The baseline clinical characteristics of subjects by the presence or absence of incident type 2 diabetes are shown in Table 1. The mean values of fasting and 2-h postload glucose, waist circumference, triglycerides, and systolic and diastolic blood pressures and the frequencies of men, family history of diabetes, elevated blood pressure, alcohol intake, and smoking habits were higher in subjects who developed type 2 diabetes than in those who did not develop it, and subjects with incident type 2 diabetes had lower HDL cholesterol and lower frequency of regular exercise. The mean values for age and total cholesterol did not differ between the groups.

The multivariate-adjusted HRs for the development of type 2 diabetes associated with metabolic syndrome and its individual components were estimated by sex (Table 2). The multivariate analysis showed that metabolic syndrome was a

Metabolic syndrome and incident diabetes

Table 2—Multivariate-adjusted HRs for the development of type 2 diabetes associated with metabolic syndrome and its individual components

	Population at risk (n)	No. events	Multivariate-adjusted HR (95% CI)	P
<b>Men</b>				
Updated 2005 NCEP ATP III				
(-)	597	82	1 (referent)	
(+)	196	63	2.58 (1.85–3.59)	<0.001
IFG				
(-)	401	35	1 (referent)	
(+)	392	110	3.76 (2.57–5.52)	<0.001
Central obesity				
(-)	667	103	1 (referent)	
(+)	126	42	2.28 (1.58–3.29)	<0.001
Reduced HDL cholesterol				
(-)	614	108	1 (referent)	
(+)	179	37	1.32 (0.90–1.95)	0.16
Elevated triglycerides				
(-)	579	84	1 (referent)	
(+)	214	61	2.05 (1.46–2.88)	<0.001
Elevated blood pressure				
(-)	338	41	1 (referent)	
(+)	455	104	2.17 (1.49–3.17)	<0.001
<b>Women</b>				
Updated 2005 NCEP APT III				
(-)	723	52	1 (referent)	
(+)	419	89	3.69 (2.58–5.27)	<0.001
IFG				
(-)	685	47	1 (referent)	
(+)	457	94	3.50 (2.45–5.00)	<0.001
Central obesity				
(-)	496	39	1 (referent)	
(+)	646	102	1.96 (1.35–2.85)	<0.001
Reduced HDL cholesterol				
(-)	631	64	1 (referent)	
(+)	511	77	1.55 (1.10–2.18)	0.01
Elevated triglycerides				
(-)	973	105	1 (referent)	
(+)	169	36	2.28 (1.54–3.37)	<0.001
Elevated blood pressure				
(-)	598	51	1 (referent)	
(+)	544	90	2.49 (1.74–3.58)	<0.001

Data are n or HR (95% CI). IFG, FPG levels of 5.6–6.9 mmol/l; central obesity, waist circumference of  $\geq 90$  cm in men and  $\geq 80$  cm in women; reduced HDL cholesterol, HDL cholesterol levels of  $< 1.03$  mmol/l in men and  $< 1.29$  mmol/l in women; elevated triglycerides, triglyceride levels of  $\geq 1.68$  mmol/l; elevated blood pressure, blood pressure  $\geq 130/85$  mmHg and/or current use of antihypertension agents. Multivariate adjustment was made for age, family history of diabetes, total cholesterol, alcohol intake, smoking habits, and regular exercise.

significant risk factor for type 2 diabetes in men and women, even after adjustment for the following confounding factors: age, family history of diabetes, total cholesterol, alcohol intake, smoking habits, and regular exercise (men: multivariate-adjusted HR, 2.58 [95% CI 1.85–3.59],  $P < 0.001$ ; women: 3.69 [2.58–5.27],  $P < 0.001$ ). All components of metabolic syndrome in both sexes, except for reduced HDL cholesterol in men, were sig-

nificantly associated with future type 2 diabetes. Among the individual components of metabolic syndrome, IFG was the strongest predictor of incident type 2 diabetes in both sexes (men: 3.76 [2.57–5.52],  $P < 0.001$ ; women: 3.50 [2.45–5.00],  $P < 0.001$ ). Compared with that of IFG, the multivariate-adjusted HR of metabolic syndrome for developing type 2 diabetes was slightly lower in men and similar in women. Furthermore, even

when the cutoff point of waist circumference for U.S. individuals was used ( $> 102$  cm in men and  $> 88$  cm in women) in the metabolic syndrome criteria instead of the cutoff point for Asians, the HR of metabolic syndrome for incident type 2 diabetes was substantially unchanged (men: 2.48 [1.76–3.51],  $P < 0.001$ ; women: 3.22 [2.27–4.55],  $P < 0.001$ ).

Because IFG is a strong predictor of future type 2 diabetes, the associations between the number of the other metabolic syndrome components and the development of type 2 diabetes were examined among individuals with or without IFG in men and women together (Table 3). In subjects with normal FPG levels, the multivariate-adjusted HRs for type 2 diabetes rose significantly as the number of metabolic syndrome components increased (one component: multivariate-adjusted HR 1.76 [95% CI 0.88–3.50]; two components: 2.49 [1.22–5.06]; three components: 3.71 [1.72–8.02]; and four components: 5.90 [2.24–15.53];  $P_{\text{trend}} < 0.001$ ). Similar relationships were also observed in subjects with IFG (one component: 2.38 [1.30–4.35]; two components: 2.98 [1.62–5.47]; three components: 4.61 [2.48–8.56]; and four components: 4.22 [2.01–8.83];  $P_{\text{trend}} < 0.001$ ).

Finally, we examined the combined as well as separate effects of metabolic syndrome and IFG on the development of type 2 diabetes. In this analysis, metabolic syndrome was defined as the presence of at least three metabolic syndrome components, not including the component of elevated FPG. As shown in the Figure 1, the multivariate-adjusted HR for future type 2 diabetes was significantly higher in subjects with metabolic syndrome alone and in those with IFG alone than in those with neither metabolic syndrome nor IFG; the former was slightly lower than the latter, but there was no significant difference between the two (metabolic syndrome alone: multivariate-adjusted HR 2.37 [95% CI 1.45–3.88],  $P < 0.001$ ; IFG alone: 3.49 [2.57–4.74],  $P < 0.001$ ). Furthermore, the subjects who had both metabolic syndrome and IFG had a markedly higher HR for the development of type 2 diabetes (6.76 [4.75–9.61],  $P < 0.001$ ). The risk of future type 2 diabetes was also significantly higher in subjects with both metabolic syndrome and IFG than in subjects with metabolic syndrome alone (2.82 [1.74–4.57],  $P < 0.001$ ) as well as in those with IFG alone (1.94 [1.44–2.62],  $P < 0.001$ ).

Table 3—Multivariate-adjusted HRs for the development of type 2 diabetes associated with the number of metabolic syndrome components excluding IFG by the presence or absence of IFG

FPG levels	No. of metabolic syndrome components excluding IFG	Population at risk (n)	No. events	Multivariate-adjusted HR (95%CI)	<i>P</i> <sub>trend</sub>
Normal	0	285	12	1 (referent)	<0.001
	1	399	26	1.76 (0.88–3.50)	
	2	236	22	2.49 (1.22–5.06)	
	3	126	15	3.71 (1.72–8.02)	
	4	40	7	5.90 (2.24–15.53)	
IFG	0	122	13	1 (referent)	<0.001
	1	278	61	2.38 (1.30–4.35)	
	2	243	62	2.98 (1.62–5.47)	
	3	153	51	4.61 (2.48–8.56)	
	4	53	17	4.22 (2.01–8.83)	

Data are n or HR (95% CI). Multivariate adjustment was made for age, sex, family history of diabetes, total cholesterol, alcohol intake, smoking habits, and regular exercise. Normal, FPG levels of <5.6 mmol/l; IFG, FPG levels of 5.6–6.9 mmol/l.

**CONCLUSIONS**— Using data from a 14-year follow-up study of a defined general Japanese population, we demonstrated that metabolic syndrome determined by the updated 2005 NCEP ATP III criteria was an independent risk factor for the development of type 2 diabetes in both sexes even after adjustment for comprehensive risk factors. The HR of metabolic syndrome for developing type 2 diabetes was slightly lower in men and similar in women compared with that of

IFG. When subjects were stratified by the presence or absence of IFG, the risk of future type 2 diabetes rose significantly as the number of metabolic syndrome components increased in both FPG level groups. Furthermore, metabolic syndrome that did not include the IFG component was also a significant risk factor for developing type 2 diabetes, and the coexistence of metabolic syndrome and IFG greatly increased the risk of future type 2 diabetes. These findings suggest

that the diagnosis of metabolic syndrome as well as that of IFG is a valuable tool to identify individuals at increased risk of type 2 diabetes.

In Japan, there has been only one prospective study to date that found a significant association between metabolic syndrome determined by the World Health Organization definition and incident type 2 diabetes among Japanese male workers (18). To our knowledge, the present study is the first report to indicate that metabolic syndrome was associated significantly with future type 2 diabetes for individuals of both sexes in a general Japanese population. Several epidemiological studies examined the relationship between metabolic syndrome determined by the updated 2005 NCEP ATP III criteria and incident diabetes (3,4,9–11), and the risks of incident diabetes associated with metabolic syndrome have differed among these investigations. In the Framingham Offspring Study, subjects with metabolic syndrome had an 8.6-fold higher risk of future type 2 diabetes than those without metabolic syndrome (4). The San Antonio Heart Study, which consisted predominantly of Hispanics, also showed that the diabetes risk was 6.9-fold higher in subjects with metabolic syndrome than that in subjects without metabolic syndrome (3). A similar increased risk of diabetes was observed among subjects with metabolic syndrome in an Ital-

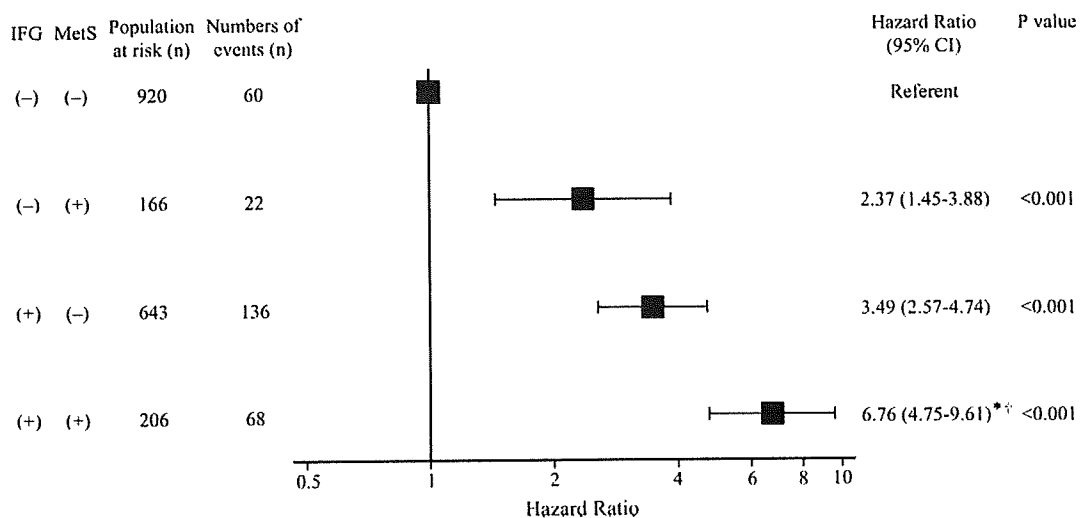


Figure 1—Multivariate-adjusted HRs for the development of type 2 diabetes according to the presence or absence of metabolic syndrome and IFG. Metabolic syndrome (MetS) was defined as the presence of at least three metabolic syndrome components other than that related to FPG. Multivariate adjustment was made for age, sex, family history of diabetes, total cholesterol, alcohol intake, smoking habits, and regular exercise. The centers of the boxes are placed at the estimates of HRs. Error bars indicate 95% CIs. IFG indicates FPG levels of 5.6–6.9 mmol/l. \**P* < 0.001 vs. IFG (–) and MetS (+). †*P* < 0.001 vs. IFG (+) and MetS (–).

ian population study (6.2-fold) (11). On the other hand, metabolic syndrome increased the risk of diabetes two- to fourfold in studies of Chinese populations (9,10). In the present analysis, the risk of developing type 2 diabetes was nearly threefold higher in men and fourfold higher in women with metabolic syndrome than in those without it, and these figures were much lower than the figures in Western populations but comparable to those of other Asian populations. Furthermore, even when the definition of waist circumference for U.S. individuals was used (>102 cm in men and >88 cm in women) in the metabolic syndrome criteria set, the risk of future type 2 diabetes among subjects with metabolic syndrome was hardly altered. Taken together, these findings suggest that metabolic syndrome is less strongly associated with increased risk of type 2 diabetes in Asian populations than in Western populations. Although the reason for this difference is unclear, the diversity of etiology for type 2 diabetes among races could explain it. That is, an epidemiological study has shown that the levels of insulin secretion and resistance differed among various ethnic groups in the U.S. (19); Asians had lower levels of insulin secretion than other ethnic groups, whereas whites, especially Hispanics, were more insulin resistant than Asians. In addition, Japanese diabetic individuals were found to have lower BMI levels than western diabetic individuals (20). Thus, we speculate that insulin resistance may play a lesser role than impaired insulin secretion in the development of type 2 diabetes among Asian populations. These findings may indicate one reason that the impact of metabolic syndrome, which has features of insulin resistance, on the development of type 2 diabetes is lower in Asian populations, including ours, than in Western populations.

There has been controversy over whether metabolic syndrome is better than IFG for detecting subjects at high risk of type 2 diabetes. The San Antonio Heart Study revealed that metabolic syndrome was a better predictor of diabetes than IFG (3), whereas other epidemiological studies including ours (2,6,7) showed that metabolic syndrome was comparable or inferior to IFG as a predictor of diabetes. This discrepancy also may result from the difference in the degree of insulin resistance among the populations, because the study subjects in the San Antonio Heart Study were more obese than those

in other studies. Thus, metabolic syndrome might be less effective in predicting incident type 2 diabetes in relatively lean ethnic groups. In our study, however, the risk of type 2 diabetes rose progressively as the number of the other metabolic syndrome components increased, not only in subjects with IFG but also in those with normal FPG levels. Moreover, our stratified analysis indicated that metabolic syndrome defined without the FPG component was also a significant risk factor for future type 2 diabetes in individuals both with and without IFG. These results imply that metabolic syndrome excluding the FPG component is also an independent risk factor for incident type 2 diabetes. On the other hand, in our study, the coexistence of metabolic syndrome and IFG appeared to increase the risk of future type 2 diabetes compared with either one alone. Other epidemiological studies have also shown that type 2 diabetes prediction was greatly enhanced by adding information on metabolic variables to that of IFG (21–23). Thus, metabolic syndrome would provide additional information beyond that provided by IFG alone in regard to the development of type 2 diabetes.

In our study, reduced HDL cholesterol was not a significant risk factor for developing type 2 diabetes in men, although lower HDL cholesterol has often been shown to be a strong predictor of diabetes in other epidemiological studies (2,6,7,9,21–23). The reasons for this discrepancy are not precisely known, but a higher prevalence of drinking habits in our men (61%) relative to our women (9%) may contribute to this phenomenon. It is known that heavy alcohol intake augments the risk of diabetes (24), whereas it increases serum HDL cholesterol levels (25). These effects of alcohol intake could weaken the association between HDL cholesterol levels and the risk of diabetes.

The strengths of our study include a longitudinal population-based design, a long duration of follow-up, a sufficient number of type 2 diabetes events, a high follow-up rate, and the use of an oral glucose tolerance test for the diagnosis of diabetes. However, two limitations of the present study should be discussed. One is that the diagnosis of metabolic syndrome was based on a single measurement of its components at baseline, as was the case in other epidemiological studies. The risk factor levels might have changed during the follow-up because of modifications in

lifestyle or medication. The other limitation is that the present study lacked information on antilipidemic drugs, such as fibrates and nicotinic acid, which could have affected the metabolism of HDL cholesterol and triglycerides, although these medications were rarely used in our country by 1988, the time of the baseline. These limitations may have led to misclassification of metabolic syndrome. These biases have the potential to underestimate the association between metabolic syndrome and incident type 2 diabetes, and thus the true impact of metabolic syndrome on the occurrence of type 2 diabetes may be stronger than that shown in our findings. For this reason, we believe that these limitations would not have substantially altered our conclusions.

In summary, the present analysis clearly demonstrated that metabolic syndrome was a significant risk factor for developing type 2 diabetes in both sexes in a general Japanese population. Although the ability of metabolic syndrome to predict type 2 diabetes was comparable or inferior to that of IFG, the effects of metabolic syndrome on the development of type 2 diabetes were independent of IFG. These findings suggest that the diagnosis of metabolic syndrome is useful and valuable for predicting type 2 diabetes even in relatively lean Asians. Further studies are needed to verify these findings in other populations.

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## Hyperglycemia Increases Risk of Gastric Cancer Posed by *Helicobacter pylori* Infection: A Population-Based Cohort Study

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See related article, Wu C–Y et al, on page 427 in *CGH*.

**Background & Aims:** Although diabetes mellitus and hyperglycemia are considered to be possible risk factors for various types of malignancy, the epidemiologic evidence concerning gastric cancer is scarce. The aim of this study was to evaluate the impact of hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) levels on gastric cancer occurrence and their interaction with *Helicobacter pylori* infection.

**Methods:** A total of 2603 Japanese subjects aged ≥40 years were stratified into 4 groups according to baseline HbA<sub>1c</sub> levels (≤4.9%, 5.0%–5.9%, 6.0%–6.9%, and ≥7.0%) and followed up prospectively for 14 years.

**Results:** During the follow-up, 97 subjects developed gastric cancer. The age- and sex-adjusted incidence of gastric cancer significantly increased in the 6.0%–6.9% (5.1 per 1000 person-years;  $P < .05$ ) and ≥7.0% groups (5.5 per 1000 person-years;  $P < .05$ ) compared with the 5.0%–5.9% group (2.5 per 1000 person-years), whereas it was slightly but not significantly high in the ≤4.9% group (3.6 per 1000 person-years). This association remained substantially unchanged even after adjusting for the confounding factors including *Helicobacter pylori* seropositivity, (multivariate-adjusted hazard ratio [HR], 2.13; 95% confidence interval [CI]: 1.30–3.47 for the 6.0%–6.9% group and HR, 2.69; 95% CI: 1.24–5.85 for the ≥7.0% group). Among subjects who had both high HbA<sub>1c</sub> levels (≥6.0%) and *Helicobacter pylori* infection, the risk of gastric cancer was dramatically elevated (interaction term,  $P = .004$ ). **Conclusions:** Our findings suggest that casual hyperglycemia is a risk factor for gastric cancer and is a possible cofactor increasing the risk posed by *Helicobacter pylori* infection.

Despite the fact that the incidence of and mortality from gastric cancer have declined markedly worldwide over the past decades, gastric cancer is still the second most common cause of cancer-related death in the world.<sup>1</sup> Gastric cancer is considered a multifactorial disease, and various factors are involved in its develop-

ment. Among the risk factors, *Helicobacter pylori* infection is a well-known and strong risk factor for gastric cancer, and its prevalence in Japan is higher than in other Western countries, especially in middle-aged and elderly individuals.<sup>2</sup> However, only a small percentage of people with *H pylori* infection develop gastric cancer, indicating that *H pylori* infection cannot be the only etiologic factor of gastric cancer.<sup>3</sup>

Although a possible association between diabetes mellitus and an increased risk of malignant neoplasms has been discussed for many years,<sup>4</sup> very few studies have evaluated the relation of diabetes mellitus or hyperglycemia to the development of gastric cancer specifically. We have previously revealed a significant association between fasting plasma glucose levels and subsequent occurrence of gastric cancer<sup>5</sup>; however, 2 other cohort studies failed to show a similar association.<sup>6,7</sup>

A hyperglycemic state has various indicators: of them, HbA<sub>1c</sub> reflects long-term glycemic control and is a more stable measurement than fasting plasma glucose.<sup>8</sup> Although the associations between HbA<sub>1c</sub> levels and incidence or mortality for malignancies have been shown in 2 cohort studies,<sup>9,10</sup> no studies have evaluated the impact of HbA<sub>1c</sub> levels on the development of gastric cancer. In the present study, we conducted a prospective investigation of the relationship between HbA<sub>1c</sub> levels and gastric cancer occurrence in a general Japanese population, taking *H pylori* infection as well as other risk factors into consideration.

### Subjects and Methods

#### Study Population

A population-based prospective study has been underway since 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area of Kyushu Island, Japan. The age and occupational distributions for Hisayama have been almost identical to those of Japan as a whole from 1961 to the present based on data from the national census. A screening survey for the present study

Abbreviations used in this paper: HbA<sub>1c</sub>, hemoglobin A<sub>1c</sub>.

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was performed in 1988. A detailed description of this survey has been published previously.<sup>11,12</sup> Briefly, of the 3390 Hisayama residents in 1988 who were 40 years or over according to the town registry, 2742 (80.9%) consented to take part in the comprehensive assessment, including an interview covering medical history. After excluding 132 individuals with a prior history of gastrectomy or gastric cancer, 2 individuals for whom HbA<sub>1c</sub> levels were not measured, and 5 individuals who died during the screening period, a total of 2603 subjects (1070 men and 1533 women; mean age, 59 years) was enrolled in the study. This study protocol was approved by the Human Ethics Review Committee of Kyushu University Graduate School of Medical Sciences, and written informed consent for medical research was obtained from the study subjects.

#### Follow-up Survey

The subjects were followed prospectively for 14 years from December 1988 to November 2002 by repeated health examinations or 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. To identify new occurrences of gastric cancer in this cohort, 3 monitoring methods were performed. First, we interviewed subjects concerning their medical history of gastric cancer at every checkup and checked all records of the annual mass screenings for gastric cancer (performed by barium x-ray examination). Second, we also monitored all radiographic and endoscopic study records and endoscopic biopsy records for the stomach at local clinics or general hospitals in and around Hisayama. Third, to find any concealed gastric cancer, autopsies were performed on 442 (76.1%) of a total of 581 subjects who died during the follow-up period. The diagnosis of all cases of gastric cancer was confirmed by histologic examination of tissue obtained by surgery, including gastrectomy and endoscopic mucosal resection, or autopsy. Pathologic diagnosis and classification of identified gastric cancers were made according to the guidelines proposed by the Japanese Gastric Cancer Association<sup>13</sup> and the histologic classification of Laurén.<sup>14</sup> During the follow-up period, only 1 subject dropped out, and first-ever gastric cancer developed in 97 subjects (68 men and 29 women), including 3 (3.1%) concealed cases first diagnosed at autopsy. Among these positive cases, 8 subjects (8.2%) had double cancers with a total of 105 lesions.

#### Laboratory Testing and Risk Factor Measurement

At the baseline examination, HbA<sub>1c</sub> was measured by high-performance liquid chromatography (HLC-723Hb; TOSOH Inc., Tokyo, Japan). Plasma glucose levels were

determined by a glucose-oxidase method. Diabetes was determined by a 75-g oral glucose tolerance test (1998 World Health Organization criteria), fasting plasma glucose levels ( $\geq 7.0$  mmol/L), or medical history of diabetes.

To assess the independent effect of HbA<sub>1c</sub> on gastric cancer occurrence, the following baseline factors in addition to age and sex were used for analysis as confounding factors: (1) serum IgG antibodies to *H pylori* were assayed by means of a quantitative enzyme immunoassay, and the assay values were interpreted as either positive or negative based on the manufacturer's instructions; (2) information about history of peptic ulcer disease, alcohol intake, and smoking habits was obtained by means of a questionnaire administered to each subject, and the latter 2 items were categorized as in current use or not; (3) height and weight were measured with the subject in light clothes without shoes, and the body mass index was calculated (weight in kilograms/height in square meters); (4) serum cholesterol levels were determined by an enzymatic autoanalyzer; (5) data on dietary factors were obtained by the semiquantitative food frequency method, validated in a prior study<sup>15</sup>; and the daily nutrient intakes, including total energy, total fat, salt, vitamin A, vitamin B-1, vitamin B-2, vitamin C, and dietary fibers, were calculated using the 4th revision of the Standard Tables of Food Composition in Japan,<sup>16</sup> and the nutritional elements were adjusted for energy intake using the method of Willet and Stampfer.<sup>17</sup>

#### Statistical Analysis

According to the American Diabetes Association's guideline,<sup>18</sup> an HbA<sub>1c</sub> level of 4.0%–6.0% is considered normal; an HbA<sub>1c</sub> less than 7.0% is the recommended glycemic control level for adults with diabetes, and a more stringent glycemic goal is less than 6.0%. Therefore, dividing subjects with normal HbA<sub>1c</sub> by 1.0% intervals, we classified all of the subjects into 4 groups according to baseline HbA<sub>1c</sub> level:  $\leq 4.9\%$ , 5.0%–5.9%, 6.0%–6.9%, and  $\geq 7.0\%$  and considered the 5.0%–5.9% group as a reference based on the finding of the analysis with the spline model. Age- and sex-adjusted mean values of the possible risk factors were calculated by the analysis of covariance method, and their trends across HbA<sub>1c</sub> levels were tested by multiple regression analysis. The frequencies of risk factors were adjusted for age by the direct method and were examined for trends by the Cochran-Mantel-Haenszel test. Age- and sex-adjusted incidence rates were calculated by the person-year method and the direct method and compared by the Cox proportional hazards model.<sup>19</sup> For age adjustment in direct method, all study subjects were used as the standard population. The age- and sex- or multivariate-adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs) were also estimated using the Cox proportional hazards model. In the multivariate analysis, the possible risk factors for gastric cancer available in our cohort, namely, age, sex, *H pylori* seropositiv-

**Table 1.** Age- and Sex-Adjusted Mean Values or Frequencies of Risk Factors According to Hemoglobin A<sub>1c</sub> Levels at Baseline

Variables	Hemoglobin A <sub>1c</sub> level (%)				P value for trend
	≤4.9 (n = 390)	5.0–5.9 (n = 1685)	6.0–6.9 (n = 427)	≥7.0 (n = 101)	
Age (y)	55.3 ± 12.0	59.2 ± 11.9	62.2 ± 12.1	61.4 ± 10.1	<.001
Men (%)	39.1	38.9	53.3	52.8	<.001
Hemoglobin A <sub>1c</sub> (%)	4.7 ± 0.3	5.4 ± 0.3	6.3 ± 0.3	8.3 ± 0.3	<.001
Diabetes (%)	5.3	6.7	28.1	94.2	<.001
<i>Helicobacter pylori</i> infection (%)	68.3	73.0	70.2	74.9	.97
History of peptic ulcer disease (%)	12.0	14.5	16.0	11.6	.40
Body mass index (kg/m <sup>2</sup> )	22.5 ± 3.1	22.9 ± 3.1	23.4 ± 3.1	23.8 ± 3.1	<.001
Total cholesterol (mmol/L)	5.08 ± 1.06	5.34 ± 1.05	5.60 ± 1.06	5.71 ± 1.06	<.001
Alcohol intake (%)	28.3	30.0	34.1	36.6	.010
Smoking habits (%)	21.1	24.2	28.2	25.5	.019
Total energy intake (kJ/day)	7055 ± 1585	7169 ± 1569	7046 ± 1571	7371 ± 1572	.16
Total fat intake (g/day)	48.0 ± 10.2	48.2 ± 10.1	48.5 ± 10.2	50.4 ± 10.1	.20
Salt intake (g/day)	13.1 ± 4.7	13.1 ± 4.7	13.1 ± 4.7	12.0 ± 4.7	.15
Vitamin A intake (IU/day)	2737 ± 1097	2935 ± 1086	2836 ± 1094	3087 ± 1088	.003
Vitamin B-1 intake (mg/day)	0.77 ± 0.40	0.82 ± 0.39	0.78 ± 0.39	0.78 ± 0.39	.06
Vitamin B-2 intake (mg/day)	1.11 ± 0.31	1.15 ± 0.30	1.17 ± 0.31	1.29 ± 0.31	<.001
Vitamin C intake (mg/day)	75.6 ± 32.7	78.0 ± 32.4	73.9 ± 32.7	84.6 ± 32.5	.012
Dietary fiber intake (g/day)	10.7 ± 3.3	10.7 ± 3.3	10.9 ± 3.3	12.2 ± 3.3	<.001

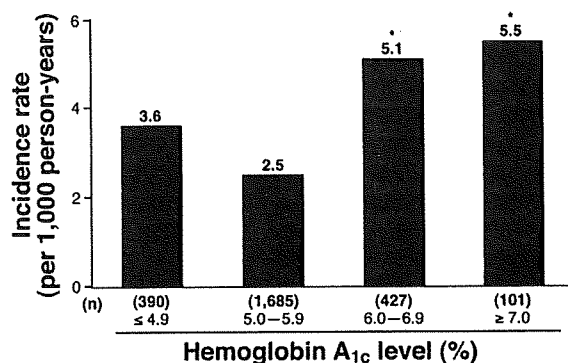
NOTE. Age is sex-adjusted; sex is age-adjusted; mean ± SD or percentage.

ity, history of peptic ulcer disease, body mass index, serum cholesterol, alcohol intake, smoking habits, and dietary factors, were included in the model all at once. These variables are known or suspected to modify the risk of gastric cancer.<sup>3,11,20–23</sup> Additionally, we examined the possibility of a nonlinear relation between HbA<sub>1c</sub> levels and the occurrence of gastric cancer using a spline model with the model selection method proposed by Kawaguchi et al.<sup>24</sup> The effect of the interaction between HbA<sub>1c</sub> and *H pylori* infection on the risk of gastric cancer was examined by the  $\chi^2$  test. All tests were 2-sided, and a *P* value of <.05 was considered statistically significant. Because there was no interaction between sex and HbA<sub>1c</sub> levels (data not shown), we included men and women together in all analyses. Statistical analyses were conducted using Statistical Analysis Software (SAS) version 8 (SAS Institute, Cary, NC).

## Results

Table 1 compares the age- and sex-adjusted mean values or frequencies of possible risk factors for gastric cancer among the HbA<sub>1c</sub> groups. The mean age and frequency of male sex tended to increase with an increase in HbA<sub>1c</sub> levels. The frequencies of diabetes, alcohol intake, and smoking habits and mean values of body mass index and total cholesterol also increased significantly with higher HbA<sub>1c</sub> levels, but such tendencies were not observed for the frequencies of *H pylori* infection and history of peptic ulcer disease. Among relevant dietary factors, the mean values of vitamin A, vitamin B-2, vitamin C, and dietary fiber intakes showed positive relationships with HbA<sub>1c</sub> levels.

The incidence of gastric cancer was 3.1 per 1000 person-years in our total subjects. Figure 1 shows the age- and sex-adjusted incidence of gastric cancer according to HbA<sub>1c</sub> levels. The incidence of cancer was significantly higher in the 6.0%–6.9% and ≥7.0% groups compared with the 5.0%–5.9% group (both, *P* < .05), and it was slightly but not significantly higher in the ≤4.9% group. The age- and sex-adjusted HR of gastric cancer was 1.97 (95% CI: 1.23–3.16; *P* = .005) in the 6.0%–6.9% group and 2.51 (95% CI: 1.18–5.32, *P* = .016) in the ≥7.0% group (Table 2). This association remained substantially unchanged even after adjustment for age; sex; *Helicobacter pylori* seropositivity; history of peptic ulcer disease; body mass index; serum cholesterol; alcohol intake; smoking habits; and dietary factors, including intake of total en-



**Figure 1.** Age- and sex-adjusted incidence rate of gastric cancer according to hemoglobin A<sub>1c</sub> levels. \**P* < .05 vs hemoglobin A<sub>1c</sub> 5.0%–5.9%.



**Table 2.** Age- and Sex-Adjusted and Multivariate-Adjusted Hazard Ratios and 95% Confidence Intervals of Gastric Cancer by Hemoglobin A<sub>1c</sub> Levels

Hemoglobin A <sub>1c</sub> level (%)	No. of population at risk	No. of events	Age- and sex-adjusted		Multivariate-adjusted <sup>a</sup>	
			HR (95% CI)	P value	HR (95% CI)	P value
≤4.9	390	14	1.49 (0.82–2.72)	.19	1.59 (0.85–3.00)	.15
5.0–5.9	1,685	47	1.00		1.00	
6.0–6.9	427	28	1.97 (1.23–3.16)	.005	2.13 (1.30–3.47)	.003
≥7.0	101	8	2.51 (1.18–5.32)	.016	2.69 (1.24–5.85)	.013
P value for trend			.025		.021	

HR, hazard ratio; CI, confidence interval.

<sup>a</sup>Adjusted for age, sex, *Helicobacter pylori* seropositivity, history of peptic ulcer disease, body mass index, serum total cholesterol, alcohol intake, smoking habits, and dietary factors (intake of total energy, total fat, salt, vitamin A, vitamin B-1, vitamin B-2, vitamin C and dietary fibers).

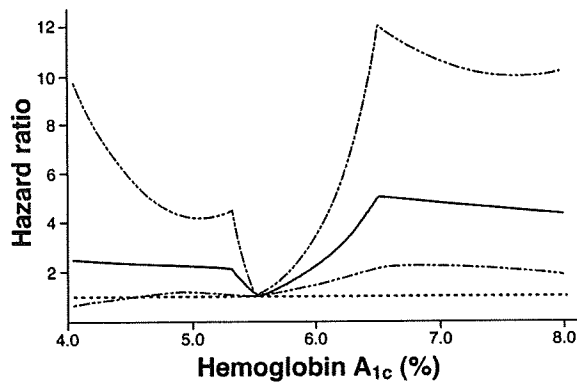
ergy, total fat, salt, vitamin A, vitamin B-1, vitamin B-2, vitamin C, and dietary fibers.

Furthermore, we examined the nonlinear relationship between HbA<sub>1c</sub> levels and the risk of gastric cancer using the spline model after adjusting for other potential risk factors (Figure 2). Compared with reference HbA<sub>1c</sub> level of 5.5%, the adjusted HRs of gastric cancer significantly increased at HbA<sub>1c</sub> levels above this reference, whereas it plateaued at HbA<sub>1c</sub> levels of >6.5%. The risk of the cancer was also higher at HbA<sub>1c</sub> levels of <5.5%, but the increase was not significant.

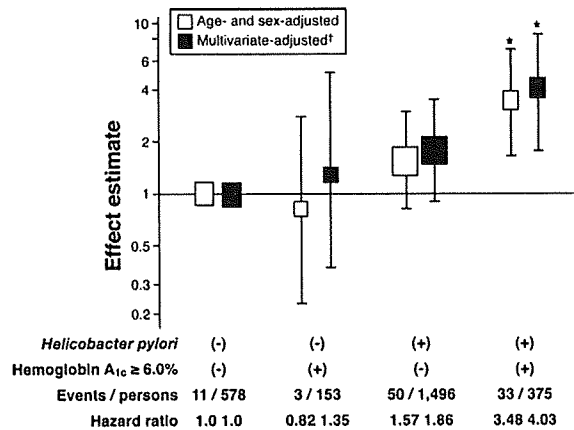
The seroprevalence of *H pylori* was 71.9% for all subjects. The age- and sex-adjusted incidence of gastric cancer was 1.6 per 1000 person-years in *H pylori*-negative subjects and 3.8 in *H pylori*-positive subjects: the difference was statistically significant (age- and sex-adjusted HR, 2.09; 95% CI: 1.18–3.68; P = .01).

To clarify the combined effects of elevated HbA<sub>1c</sub> levels and *H pylori* infection on gastric cancer occurrence, we

estimated the age- and sex-adjusted or multivariate-adjusted HRs of gastric cancer among 4 groups divided into high (≥6.0%) or low (≤5.9%) HbA<sub>1c</sub> levels, with or without *H pylori* infection (Figure 3). Compared with the reference group having neither high HbA<sub>1c</sub> levels nor *H pylori* infection, the age- and sex-adjusted HRs of gastric cancer for the groups with high HbA<sub>1c</sub> levels alone and *H pylori* infection alone were not significant, but the HR for the group having both high HbA<sub>1c</sub> levels and *H pylori* infection was significantly high (HR, 3.48; 95% CI: 1.75–6.93; P < .001). This association remained significant even after adjustment for the above-mentioned risk factors (HR, 4.03; 95% CI: 1.89–8.58; P < .001); moreover, there was a significant interaction between high HbA<sub>1c</sub> levels and *H pylori* infection (P = .004).



**Figure 2.** Multivariate adjusted splines for hemoglobin A<sub>1c</sub> (%) and gastric cancer risk, relative to hemoglobin A<sub>1c</sub> = 5.5%. The double-dashed lines are the 95% confidence intervals for the splines. The risk-changing points (shown by knots) are the hemoglobin A<sub>1c</sub> values of 5.3%, 5.5%, and 6.5%. The risks are adjusted for age, sex, history of peptic ulcer disease, body mass index, serum total cholesterol, alcohol intake, smoking habits, and total dietary factors (intake of total energy, total fat, salt, vitamin A, vitamin B-1, vitamin B-2, vitamin C, and dietary fibers).



**Figure 3.** Age- and sex-adjusted and multivariate-adjusted hazard ratios and 95% confidence intervals of gastric cancer according to hemoglobin A<sub>1c</sub> level and *Helicobacter pylori* status. <sup>†</sup>Adjusted for age, sex, history of peptic ulcer disease, body mass index, serum total cholesterol, alcohol intake, smoking habits and total dietary factors (intake of total energy, total fat, salt, vitamin A, vitamin B-1, vitamin B-2, vitamin C, and dietary fibers). \*P < .001 vs hemoglobin A<sub>1c</sub> ≤ 5.9% and *Helicobacter pylori* negative.

CLINICAL-ALIMENTARY TRACT

## Discussion

Our findings indicated that elevated HbA<sub>1c</sub> level was a significant risk factor for the development of gastric cancer. This association remained significant even after adjusting for other risk factors, namely, age, sex, *H pylori* seropositivity, history of peptic ulcer disease, body mass index, serum total cholesterol, alcohol intake, smoking habits, and dietary factors. Moreover, the coexistence of a high HbA<sub>1c</sub> level and *H pylori* infection dramatically increased the risk of future gastric cancer. To our knowledge, this is the first epidemiologic study to reveal the association between HbA<sub>1c</sub> levels and gastric cancer incidence, while taking into account the interaction between HbA<sub>1c</sub> levels and *H pylori* status.

Diabetes and impaired glucose tolerance are thought to be possible risk factors, not only for cardiovascular events,<sup>25</sup> but also for malignancies.<sup>4-7,26</sup> Among the studies on this subject, few have examined the association between hyperglycemia and gastric cancer.<sup>5-7</sup> Our previous study showed that elevated fasting plasma glucose levels ( $\geq 5.3$  mmol/L) were clearly associated with gastric cancer incidence.<sup>5</sup> Another prospective study revealed that only men who had a fasting plasma glucose level of 6.1–6.9 mmol/L or known diabetes had a significantly elevated risk of death from gastric cancer.<sup>6</sup> However, another prospective study did not show these relationships.<sup>7</sup> On the other hand, HbA<sub>1c</sub> is a good time-integrated indicator of blood glucose concentrations over the preceding 1 to 3 months.<sup>27</sup> It is also a particularly convenient screening or monitoring tool for diabetes because it does not require subjects to fast. However, there have been no studies to date examining the association between HbA<sub>1c</sub> levels and the risk of gastric cancer.

In the present study, we demonstrated that the incidence of gastric cancer significantly increased in the HbA<sub>1c</sub> groups of  $\geq 6.0\%$  compared with the 5.0%–5.9% group. These findings suggest that a modest increase in casual blood glucose is a risk factor for the cancer. However, the risk of gastric cancer appeared to plateau at HbA<sub>1c</sub> levels over 6.5% using the spline model. It is possible that the risk did not change at the higher HbA<sub>1c</sub> levels or that this finding was a result of the competing effect of other diseases such as cardiovascular events. In fact, elevated HbA<sub>1c</sub> levels were significantly associated with the increased cardiovascular mortality in our cohort (data not shown). Meanwhile, the spline model showed that the risk of gastric cancer was lowest at an HbA<sub>1c</sub> level of around 5.5%. These observations indicate that an optimum HbA<sub>1c</sub> level for prevention of gastric cancer may exist. Further studies are needed to confirm these findings.

The precise pathogenetic role of hyperglycemia in gastric carcinogenesis remains obscure so far. One possible explanation is that hyperglycemia and its related conditions may act directly as a carcinogenic factor. A clinical study with diabetic subjects and healthy volunteers has

demonstrated that diabetes is associated with increased production of reactive oxygen species and greater oxidative damage to DNA.<sup>28</sup> In an experimental study, high glucose itself was also shown to induce DNA damage.<sup>29</sup> Thus, it is possible that increased production of reactive oxygen species or high glucose itself contributes to DNA damage, which may lead to mutational changes in oncogenes and tumor suppressor genes, and thereby to the development of gastric cancer. Another possible explanation is that hyperinsulinemia is related to gastric carcinogenesis. It is well-known that individuals with hyperglycemia are prone to insulin resistance, the effect of which is increased blood insulin levels. An experimental study has shown that insulin enhances the stimulatory effects of epidermal growth factor on the proliferation of cultured gastric epithelial cells obtained from guinea pigs.<sup>30</sup> It is speculated that an increase in cell proliferation predisposes gastric mucosa to genetic or epigenetic alterations and, therefore, to carcinogenesis. Another experimental study has also demonstrated that insulin-like growth factors, which increase in diabetic patients, may play an important role in the initiation, progression, and metastasis of gastric cancer.<sup>31</sup>

Numerous epidemiologic and experimental studies have shown a clear association between *H pylori* infection and the risk of gastric cancer.<sup>11</sup> Although the mechanisms for the increased risk of gastric cancer in the presence of *H pylori* are not clearly understood, it has been clarified that this infection contributes to modifications in epithelial cell proliferation,<sup>32</sup> which is considered to be the initial step in a cascade culminating in the development of gastric cancer. However, despite the increased risk of gastric cancer from *H pylori* infection, the majority of *H pylori*-infected subjects do not develop gastric cancer. This fact suggests that *H pylori* infection is not an absolute oncogenic factor for gastric cancer and that there must be other critical cofactors contributing to the risk posed by *H pylori* infection. Our stratified analysis showed a dramatically increased risk of gastric cancer occurrence only in subjects with both high HbA<sub>1c</sub> levels and *H pylori* infection after adjustment for other comprehensive risk factors. On the other hand, the multivariate-adjusted risk of gastric cancer for the groups with high HbA<sub>1c</sub> levels alone and *H pylori* infection alone were slightly but not significantly higher than that of the reference group. This might be due to the limited number of gastric cancer cases. However, the interaction term between high HbA<sub>1c</sub> level and *H pylori* infection was significant, indicating their synergic effect on gastric carcinogenesis. This finding suggests that hyperglycemia is one of the possible cofactors increasing the carcinogenic effects of *H pylori* infection.

There is suggestive clinical evidence to explain the synergistic effects of hyperglycemia and *H pylori* infection on gastric carcinogenesis. A population study demonstrated that hyperglycemia was associated with gastric

mucosal atrophy,<sup>33</sup> which is considered to be a precursor of gastric cancer. It is possible that increased reactive oxygen-related damage to DNA and genetic or epigenetic alterations in gastric mucosa induced by hyperglycemia or associated hyperinsulinemia stimulate a modifying effect of *H pylori* on epithelial cell proliferation, which is the initial step in a cascade of gastric carcinogenesis. On the other hand, *H pylori* infection might affect insulin release from the pancreas through gut hormones. A clinical study showed that *H pylori*-related gastritis increases glucose- and meal-stimulated insulin release by increasing gastrin secretion.<sup>34</sup> *H pylori* infection can also increase pancreatic insulin secretion by decreasing the serum concentration of somatostatin,<sup>35</sup> which has an inhibiting effect on insulin release.<sup>36</sup> Moreover, a clinical study demonstrated an increased state of insulin resistance in subjects with *H pylori* infection.<sup>37</sup> Thus, the enhanced effect of hyperglycemia and *H pylori* infection on gastric cancer might be explained partially by hyperinsulinemia or insulin resistance. Another possible explanation is that hyperglycemia affects *H pylori* and that its infection status or stimulates its carcinogenic effects. However, the association between diabetes and *H pylori* infection is controversial. A higher prevalence of *H pylori* infection in diabetic than in control subjects has been reported in some studies,<sup>38,39</sup> whereas other studies have found no significant correlation between fasting plasma glucose levels and *H pylori* status.<sup>40,41</sup> In our subjects, no significant correlation was observed between HbA<sub>1c</sub> levels and *H pylori* prevalence at baseline. Given the limited findings, these hypotheses require further consideration.

The incidence rate of gastric cancer in our cohort was higher than that of other reported data.<sup>1,2,42</sup> This discrepancy seemed to have occurred because of differences in the study design as well as in the age structure or regions examined. Most of the previous studies were registration studies, whereas ours was a prospective cohort study. In addition, even the representative cancer registry research in Japan covered only 20%–30% of the total population,<sup>43</sup> and few studies included concealed cancers.<sup>44</sup> On the other hand, we performed almost perfect follow-up of subjects, with only 1 subject dropping out of follow-up and 76% of subjects who died during the follow-up underwent autopsy. Furthermore, the diagnosis of all our cases of gastric cancer was confirmed by histologic examination. Therefore, it is considered that very few cases who developed gastric cancer were missed, resulting in the high incidence rate in our cohort.

There are some limitations in this study that merit discussion. First, changes in HbA<sub>1c</sub> and other potentially confounding factors were not reassessed over time in our subjects, although this limitation is typical of most prospective studies. It is therefore possible that, as a result of treatment for diabetes, greater modification of HbA<sub>1c</sub> and other risk factors occurred in diabetic than in nondiabetic subjects. In our subjects, however, the risk of

gastric cancer increased even at relatively low elevations of HbA<sub>1c</sub>, which do not require medical treatment. Therefore, biases of this kind should not have distorted the significant association between HbA<sub>1c</sub> levels and the risk of gastric cancer; nonetheless, the estimates of effect that we have reported here are most likely conservative. Second, because we did not perform a screening survey of the stomach in each subject at baseline examination, it is undeniable that there were presymptomatic gastric cancer patients at the baseline of this study, although this limitation is also a major problem for other registration studies of gastric cancer. However, the prevalence of gastric cancer in healthy subjects was reported to be low (0.12%) by nationwide mass screening in Japan.<sup>45</sup> We performed the same analysis with all subjects, except for those who developed gastric cancer in the first 2 years of the follow-up period, and the risk of gastric cancer was substantially unchanged (data not shown). Thus, we believe that the influence of concealed cancer at the time of baseline examination is small enough that it can be neglected.

In conclusion, this is the first population-based cohort study to investigate the association between HbA<sub>1c</sub> levels and gastric cancer incidence. Our findings suggest that an elevated HbA<sub>1c</sub> level, namely, casual hyperglycemia, is an independent risk factor for gastric cancer occurrence and is a possible cofactor increasing the risk of cancer posed by *H pylori* infection. Although the mechanism by which hyperglycemia per se is involved in gastric carcinogenesis remains obscure, early identification of hyperglycemia and appropriate behavioral and therapeutic intervention may be beneficial for the prevention of gastric cancer, especially in countries such as Japan where the number of diabetic individuals is increasing rapidly, and the risk of gastric cancer is considerable. Further cohort studies and intervention trials with strict control of blood glucose-enrolling diabetic patients and/or hyperglycemic subjects, especially those with *H pylori* infection, are needed to clarify the role of hyperglycemia in the development of gastric cancer.

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**Conflict of interest**

The authors disclose no conflicts.

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# LDL Cholesterol and the Development of Stroke Subtypes and Coronary Heart Disease in a General Japanese Population

## The Hisayama Study

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**Background and Purpose**—Although the relation between serum LDL cholesterol level and coronary heart disease (CHD) is well established, its relation with stroke subtypes is less clear.

**Methods**—A total of 2351 inhabitants age  $\geq 40$  years in a Japanese community were followed up for 19 years.

**Results**—During follow-up, 271 subjects developed stroke and 144 developed CHD. Whereas the age- and sex-adjusted incidences of CHD significantly increased with increasing LDL cholesterol levels ( $P$  for trend  $< 0.001$ ), the associations between LDL cholesterol level and the incidences of ischemic or hemorrhagic stroke were not significant. The age- and sex-adjusted incidences of atherothrombotic infarctions (ATIs) and lacunar infarctions (LIs) significantly increased with increasing LDL cholesterol level ( $P$  for trend = 0.03 for ATIs and = 0.02 for LIs), but no such association was observed for cardioembolic infarction. After multivariate adjustment, the positive associations of LDL cholesterol level with the risks of ATI and CHD remained significant ( $P$  for trend = 0.02 for ATIs and = 0.03 for CHD), whereas the association with LIs was not significant. The risk of ATI significantly increased in the fourth quartile of LDL cholesterol compared with the first quartile (multivariate-adjusted hazard ratio = 2.84; 95% CI, 1.17 to 6.93). The multivariate-adjusted risks for developing nonembolic infarction (ATIs and LIs) and CHD were significantly elevated in the groups with elevated LDL cholesterol values with and without the metabolic syndrome.

**Conclusions**—Our findings suggest that an elevated LDL cholesterol level is a significant risk factor for developing ATI as well as CHD, and these associations are independent of the metabolic syndrome. (*Stroke*. 2009;40:382-388.)

**Key Words:** epidemiology ■ cholesterol ■ lipoproteins ■ risk factors

Increased blood cholesterol levels are causally related to an increased risk of coronary heart disease (CHD).<sup>1</sup> In contrast, the relation between total cholesterol levels and the risk of stroke remains unclear because of conflicting results reported in the literature.<sup>2,3</sup> The inconsistent results may be due to several reasons. First, because stroke is a heterogeneous syndrome of different etiologic origins, lipid abnormalities may be important for some subtypes of stroke but not for others. An inverse association has been observed between total cholesterol and hemorrhagic stroke,<sup>2,4</sup> and there is a positive association between total cholesterol and ischemic stroke.<sup>2,5</sup> Furthermore, the association may be different for ischemic stroke subtypes.<sup>6</sup> Second, lipoprotein subfractions are considered to exert varying influence on stroke risk.<sup>7</sup> It is possible that the protective effect of HDL cholesterol against stroke weak-

ens the positive association between total cholesterol and stroke in instances where lipoprotein subfractions are counted together. The association between cholesterol and stroke, therefore, needs to be discussed on the basis of stroke subtypes and lipoprotein subfractions.

Together with the results from prospective studies, the positive association between LDL cholesterol level and the risk of CHD has been confirmed by lipid-lowering randomized trials.<sup>8</sup> On the other hand, whereas statins significantly reduced the risk of stroke,<sup>8</sup> the risk reduction for stroke in trials in which subjects were treated with nonstatins was not significant,<sup>9</sup> suggesting that statins involve mechanisms other than cholesterol lowering for the prevention of stroke. Therefore, the true association between LDL cholesterol and the risk of stroke remains unknown. The purpose of this study was to evaluate the association between LDL cholesterol

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level and the development of stroke by its subtypes as well as of CHD in a prospective study of a general Japanese population.

## Subjects and Methods

### Study Population

Since 1961, we have been conducting a long-term, prospective cohort study of cardiovascular disease (CVD) in the town of Hisayama, a suburb of Fukuoka city in southern Japan. In 1983, a screening survey for the present study was performed in the town. A total of 2548 residents age  $\geq 40$  years (80.7% of the total population of this age group) consented to participate in the examination. Of these, 197 subjects were excluded for the following reasons: past history of stroke or myocardial infarction (MI;  $n=89$ ), blood samples not being collected or collected after a meal ( $n=86$ ), and excessively high value of triglycerides ( $\geq 4.48$  mmol/L) for which the Friedewald formula loses its validity<sup>10</sup> ( $n=22$ ). The remaining 2351 subjects (991 men, 1360 women) were included in this study.

### Follow-Up Survey

This population was followed up prospectively for 19 years, from November 1983 through October 2002, by annual health examinations. For subjects who did not undergo regular examinations or who moved out of Hisayama, health status was checked yearly by mail or telephone. We also established a daily monitoring system, which connected us with local physicians and the members of the Health and Welfare Office for the town, and through the system we gathered information on new events of CVD, including suspected cases. When stroke or CHD occurred or was suspected, physicians in the study team examined the subject and evaluated his/her detailed clinical information. The clinical diagnosis of stroke or CHD was based on the patient's history, physical and neurologic examinations, and ancillary laboratory examinations. When a subject died, an autopsy was performed at the Department of Pathology of Kyushu University. During the follow-up period, 1 subject was lost to follow-up, 707 subjects died, and 555 subjects (78.5%) underwent autopsy examination.

### Definition of Cardiovascular Events

The diagnosis and classification of stroke were determined on the basis of clinical information, including brain computed tomography and magnetic resonance imaging, cerebral angiography, echocardiography, carotid duplex imaging, or autopsy findings. In principle, stroke was defined as a sudden onset of nonconvulsive and focal neurologic deficits persisting for  $>24$  hours, and the stroke was then classified as either hemorrhagic or ischemic. Hemorrhagic stroke included cerebral hemorrhage and subarachnoid hemorrhage. Ischemic stroke was further divided into 4 clinical categories: atherothrombotic infarction (ATI), lacunar infarction (LI), cardioembolic infarction (CEI), and undetermined subtype of ischemic stroke (UND), based on the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke,<sup>11</sup> as well as on the basis of the diagnostic criteria of the Trial of Org10172 in Acute Stroke Treatment (TOAST) Study<sup>12</sup> and the Cerebral Embolism Task Force.<sup>13</sup>

Details of the diagnostic criteria for ischemic stroke subtypes have been described previously.<sup>14</sup> In brief, ATI was diagnosed when the subjects had significant stenosis ( $>50\%$ ) or occlusion of a major cerebral artery with infarct size  $\geq 1.5$  cm on brain imaging or autopsy. LI was diagnosed as the presence of a relevant brainstem, basal ganglia, or subcortical hemispheric lesion with a diameter  $<1.5$  cm demonstrated on brain imaging or autopsy and no evidence of cerebral cortical or cerebellar impairment. The diagnosis of CEI was made on the basis of primary and secondary clinical features suggestive of CEI as reported by the Cerebral Embolism Task Force.<sup>13</sup> The category of UND included all ischemic stroke cases for which the subtype could not be determined because of insufficient clinical or morphologic information. We considered morphologic findings to be significant and used clinical features as reference information. Cases with cerebrovascular diseases with distinct pa-

thology, such as collagen disease, hematologic disorder, trauma, chronic subdural hematoma, or moyamoya disease, were excluded from the evaluation.

During the follow-up period, we identified 271 first-ever stroke events. All of the stroke cases underwent morphologic evaluation that included brain imaging and autopsy; 269 subjects (99.3%) underwent brain imaging studies, and autopsies were performed on 128 subjects of 157 deceased stroke cases (81.5%), including 2 subjects who were not examined by brain imaging. When sufficient clinical and morphologic information was obtained, a diagnosis of cerebral infarction subtype was defined as "definite." When the amount of either type of information was insufficient, the diagnosis level was defined as "probable." On the basis of the aforementioned criteria, stroke cases were divided into 80 hemorrhagic strokes and 191 ischemic strokes (51 ATIs, 93 LIs, 46 CEIs, and 1 UND). Among 191 ischemic strokes, 182 were defined as definite and 9 as probable. In this study, we present the data regarding definite and probable stroke cases together, because these combined data were almost identical to those for definite cases only.

The criteria for the diagnosis of CHD included first-ever acute MI, silent MI, sudden cardiac death within 1 hour after the onset of acute illness, coronary artery angioplasty, and bypass grafting. The diagnosis of MI was based on detailed clinical information and at least 2 of the following findings: typical clinical symptoms, ECG evidence of MI, elevated cardiac enzymes, or morphologic findings including echocardiographic, scintigraphic, or angiographic abnormalities compatible with myocardial injury. Silent MI was defined as myocardial scarring without any historical indication of clinical symptoms and/or abnormal cardiac enzyme changes.<sup>15</sup> During the follow-up period, we identified 144 first-ever events of CHD.

### Risk Factors

Blood samples were drawn after an overnight fast of at least 12 hours. All measurements were done within 24 hours after venipuncture in the central study laboratory (Japan Medical Laboratory Inc, Fukuoka, Japan), which participated in the Centers for Disease Control and Prevention Lipid Standardization Program. Total cholesterol and triglyceride levels were measured enzymatically. Measurement of HDL cholesterol was performed after precipitation of VLDL and LDL with dextran sulfate and magnesium. LDL cholesterol concentration was calculated with the Friedewald formula.<sup>10</sup> Plasma glucose levels were determined by the glucose oxidase method. Sitting blood pressure (BP) was measured with a sphygmomanometer 3 times at the right upper arm after at least 5 minutes of rest, and the mean of the 3 measurements was used in the analysis. Hypertension was defined as a BP  $\geq 140/90$  mm Hg and/or current treatment with antihypertensive agents. ECG abnormalities were defined as left ventricular hypertrophy (Minnesota code 3-1), ST-segment depression (Minnesota codes 4-1,2,3), or atrial fibrillation (Minnesota code 8-3). Body height and weight were measured in light clothing without shoes, and body mass index (BMI;  $\text{kg}/\text{m}^2$ ) was calculated. Information on alcohol consumption, smoking habits, and physical activity during leisure time was obtained by the use of a questionnaire. Alcohol consumption and smoking habits were classified as either current use or not. Those subjects who engaged in sports or other forms of exertion  $\geq 3$  times per week during their leisure time were designated the regular-exercise group. We defined the presence of the metabolic syndrome according to the National Cholesterol Education Program Expert Panel criteria<sup>16</sup> with a minor modification. The presence of the metabolic syndrome was based on the existence of 3 or more of the following components: (1) BMI  $\geq 25$   $\text{kg}/\text{m}^2$  as a substitute for waist circumference<sup>17</sup>; (2) fasting triglyceride concentration  $\geq 1.68$  mmol/L; (3) HDL cholesterol concentration  $<1.03$  mmol/L in men and  $<1.29$  mmol/L in women; (4) BP  $\geq 130/85$  mm Hg or use of antihypertensive drugs; and (5) fasting plasma glucose value  $\geq 6.1$  mmol/L or current use of antidiabetic drugs.

### Statistical Analysis

To analyze LDL cholesterol level as a categorical variable, we classified the subjects into 4 groups according to quartiles of LDL

**Table 1. Age- and Sex-Adjusted Mean Values or Frequencies of Risk Factors for CVD According to LDL Cholesterol Quartiles at Baseline**

Risk Factor	Quartile of LDL Cholesterol Levels, mmol/L				P Value for Trend
	≤2.65 (n=586)	2.66 to 3.24 (n=591)	3.25 to 3.88 (n=585)	≥3.89 (n=589)	
Men, %	57.4	44.1	39.2	31.5	<0.001
Age, y	56±11	57±11	57±11	59±11	<0.001
Total cholesterol, mmol/L	4.03±0.57	4.81±0.41	5.40±0.43	6.45±0.68	<0.001
HDL cholesterol, mmol/L	1.36±0.42	1.35±0.36	1.34±0.37	1.31±0.33	<0.001
Triglycerides, mmol/L	1.15±0.75	1.07±0.51	1.12±0.53	1.32±0.58	<0.001
Fasting blood glucose, mmol/L	4.66±0.92	4.75±0.96	4.76±0.93	4.96±1.14	<0.001
Systolic BP, mm Hg	132±22	132±21	135±22	138±21	<0.001
Diastolic BP, mm Hg	81±12	81±12	82±11	83±10	<0.001
Hypertension, %	39.7	41.4	43.8	48.5	0.01
ECG abnormalities,* %	20.6	19.4	21.0	18.4	0.12
BMI, kg/m <sup>2</sup>	21.9±3.0	22.2±3.1	23.0±3.1	23.5±3.1	<0.001
Current drinking, %	42.2	33.3	31.8	27.9	<0.001
Current smoking, %	30.7	28.5	28.3	26.5	<0.001
Regular exercise,† %	9.0	7.9	9.5	5.7	0.03

Data are mean±SD or percent. Percentage of men was age adjusted. Mean age was sex adjusted.

\*Minnesota codes 3-1; 4-1, -2, -3; or 8-3.

†Engaging in sports or other forms of exertion regularly ≥3 times per week during leisure time.

cholesterol level: ≤2.65, 2.66 to 3.24, 3.25 to 3.88, and ≥3.89 mmol/L. Serum triglyceride levels were logarithmically transformed to improve the skewed distribution. Age- and sex-adjusted mean values of the possible risk factors were calculated by the ANCOVA method, and their trends across LDL cholesterol levels were tested by multiple-regression analysis. Frequencies of risk factors were adjusted for age and sex by the direct method and were examined for trends by the Cochran-Mantel-Haenszel test. The incidences of CVD were calculated by the person-year method and were adjusted for age and sex by the direct method according to 10-year age groups. Differences in age- and sex-adjusted incidences between LDL cholesterol quartiles were tested by Cox proportional-hazards regression analysis. The age- and sex-adjusted or multivariate-adjusted hazard ratios (HRs) and 95% CIs were also calculated by the Cox proportional-hazards model. All statistical analyses were performed with the SAS program package.  $P < 0.05$  was considered statistically significant in all analyses.

## Results

The age- and sex-adjusted mean values or frequencies of risk factors for CVD are listed by quartiles of LDL cholesterol levels at baseline in Table 1. The frequencies of male sex, current drinking, current smoking, and regular exercise and the mean values of HDL cholesterol declined with increasing LDL cholesterol level, whereas mean values of age, total cholesterol, triglycerides, fasting blood glucose, systolic and diastolic BPs, BMI, and frequency of hypertension significantly increased with rising LDL cholesterol level. The frequency of ECG abnormalities was not different among serum LDL cholesterol levels.

Table 2 shows the age- and sex-adjusted incidences of CVD according to quartiles of LDL cholesterol levels. No significant associations were observed between LDL cholesterol levels and the incidences of stroke, whether ischemic or hemorrhagic. In regard to subtypes of ischemic stroke, the incidences of ATI and LI significantly increased with increasing LDL cholesterol level ( $P$  for trend=0.03 for ATI

and=0.02 for LI), and there were significant differences between the first and fourth quartiles of LDL cholesterol for both subtypes (age- and sex-adjusted HR=2.31; 95% CI, 1.03 to 5.16;  $P=0.04$  for ATI; age- and sex-adjusted HR=2.00; 95% CI, 1.05 to 3.80;  $P=0.03$  for LI; Table 3). No such association was observed for CEI. The incidence of CHD also significantly increased with increasing LDL cholesterol level ( $P$  for trend <0.001), and compared with the first quartile, the incidence was significantly higher in the third (age- and sex-adjusted HR=1.77; 95% CI, 1.07 to 2.91;  $P=0.03$ ; Table 3) and fourth (age- and sex-adjusted HR=2.00; 95% CI, 1.22 to 3.28;  $P=0.006$ ) quartiles.

As shown in Table 3, the positive associations between LDL cholesterol level and risk of ATI and CHD remained significant even after adjustment for age, sex, HDL cholesterol, triglycerides, systolic BP, ECG abnormalities, fasting blood glucose, BMI, current drinking, current smoking, and regular exercise ( $P$  for trend=0.02 for ATI and=0.03 for CHD). Compared with the first quartile, the risk of ATI was significantly high in the fourth quartile after adjustment for the aforementioned confounding factors (multivariate-adjusted HR=2.84; 95% CI, 1.17 to 6.93;  $P=0.02$ ). On the other hand, the negative association between LDL cholesterol and the risk of CEI appeared to be significant after multivariate adjustment ( $P$  for trend=0.03), and the risk of CEI was significantly lower in the fourth quartile than in the first quartile (multivariate-adjusted HR=0.34; 95% CI, 0.12 to 0.96;  $P=0.04$ ). A similar association was observed when LDL cholesterol was examined on a continuous scale.

Because not only LDL cholesterol but also other metabolic factors may be strong risk factors for CVD, we examined the combined as well as the separate effects of elevated LDL cholesterol level and the metabolic syndrome on the development of selected CVDs. As shown in the Figure, we



**Table 2. Age- and Sex-Adjusted Incidences (per 1000 Person-Years) of CVD According to LDL Cholesterol Quartiles**

	Quartile of LDL Cholesterol Levels, mmol/L				P Value for Trend
	≤2.65 (n=586)	2.66 to 3.24 (n=591)	3.25 to 3.88 (n=585)	≥3.89 (n=589)	
<b>Stroke</b>					
No. of events	56	62	74	79	
Age- and sex-adjusted incidence	7.4	8.1	10.1	10.2	0.13
<b>Ischemic stroke</b>					
No. of events	37	47	47	60	
Age- and sex-adjusted incidence	4.9	6.3	6.8	7.9	0.07
<b>Atherothrombotic</b>					
No. of events	9	12	9	21	
Age- and sex-adjusted incidence	1.2	1.6	1.2	3.3*	0.03
<b>Lacunar</b>					
No. of events	14	21	25	33	
Age- and sex-adjusted incidence	2.0	2.6	2.9	3.8*	0.02
<b>Cardioembolic</b>					
No. of events	14	14	12	6	
Age- and sex-adjusted incidence	1.7	2.1	2.4	0.8	0.07
<b>Hemorrhagic stroke</b>					
No. of events	19	15	27	19	
Age- and sex-adjusted incidence	2.6	1.8	3.3	2.3	0.95
<b>CHD</b>					
No. of events	25	28	43	48	
Age- and sex-adjusted incidence	3.4	3.4	5.5*	6.6†	<0.001

\*P<0.05, †P<0.01 vs lowest quartile.

estimated the HRs for the occurrence of nonembolic infarction, including ATI and LI, as well as of CHD, by dividing the subjects into 4 groups according to the presence or absence of high LDL cholesterol levels (the fourth quartile, ≥3.89 mmol/L for nonembolic infarction; the third and fourth quartiles, ≥3.25 mmol/L for CHD) and the metabolic syndrome after adjustments for age, sex, ECG abnormalities, current drinking, current smoking, and regular exercise. Compared with a reference group with neither high LDL cholesterol levels nor the metabolic syndrome, the risk of developing nonembolic infarction was significantly high in the group with high LDL cholesterol levels alone and in the group with both high LDL cholesterol levels and the metabolic syndrome, whereas it was marginally significant for the group with the metabolic syndrome alone. Similarly, the risk for the development of CHD was elevated in both the group with high LDL cholesterol without the metabolic syndrome and the group with high LDL cholesterol and the metabolic syndrome. The risk of CHD was also significant for the group with the metabolic syndrome alone.

**Discussion**

In a long-term, prospective study of a general Japanese population, we demonstrated positive and significant associations between serum LDL cholesterol level and risk for the development of ATI and CHD. These associations remained unchanged even after adjustment for other lipid fractions as well as other confounding factors, namely, age, sex, systolic

BP, ECG abnormalities, fasting blood glucose, BMI, current drinking, current smoking, and regular exercise. In addition, the impact of high LDL cholesterol on CVD appeared to be similar to that of the metabolic syndrome. On the other hand, the association between LDL cholesterol level and the risk of CEI was negative and significant after adjusting for the aforementioned risk factors. To our knowledge, this is the first prospective cohort study to investigate the association between LDL cholesterol and the development of subtypes of ischemic stroke.

Several prospective studies have investigated the association between LDL cholesterol and ischemic stroke, but the results were not unanimous. The Cardiovascular Health Study<sup>18</sup> reported a positive association between LDL cholesterol and the risk of ischemic stroke, whereas the Atherosclerosis Risk in Communities Study<sup>19</sup> and the Framingham Study<sup>20</sup> found no clear associations. In the present analysis, LDL cholesterol level was not clearly associated with the risks of stroke and ischemic stroke, but these associations were heterogeneous across ischemic stroke subtypes. Because LI and CEI seem to have a less potent relation with elevated LDL cholesterol,<sup>21,22</sup> inclusion of those subtypes may mask the positive association between LDL cholesterol and ATI. This heterogeneity in the associations of LDL cholesterol level and ischemic stroke subtypes may be a reason for the controversial results obtained from previous studies that investigated the outcome of “total” ischemic stroke.

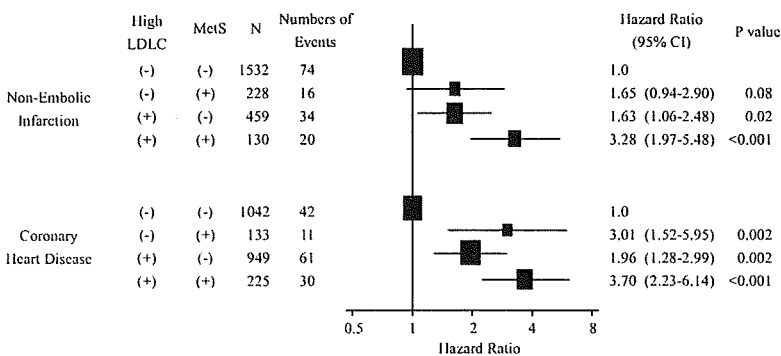
**Table 3. Age-, Sex-, and Multivariate-Adjusted HRs and 95% CIs for the Development of CVD According to LDL Cholesterol Quartiles**

	Quartile of LDL Cholesterol Levels, mmol/L				P Value for Trend	Continuous Scale
	≤2.65 (n=586)	2.66 to 3.24 (n=591)	3.25 to 3.88 (n=585)	≥3.89 (n=589)		
<b>Stroke</b>						
No. of events	56	62	74	79		
Age- and sex-adjusted HR (95% CI)	1.0	0.96 (0.66–1.37)	1.12 (0.84–1.69)	1.23 (0.86–1.75)	0.13	1.08 (0.95–1.23)
Multivariate-adjusted HR (95% CI)	1.0	0.94 (0.64–1.38)	1.15 (0.79–1.67)	1.23 (0.84–1.81)	0.16	1.07 (0.93–1.24)
<b>Ischemic stroke</b>						
No. of events	37	47	47	60		
Age- and sex-adjusted HR (95% CI)	1.0	1.08 (0.70–1.67)	1.17 (0.75–1.80)	1.45 (0.95–2.21)	0.07	1.15 (0.99–1.35)
Multivariate-adjusted HR (95% CI)	1.0	1.05 (0.66–1.66)	1.05 (0.66–1.68)	1.35 (0.85–2.14)	1.19	1.11 (0.94–1.31)
<b>Atherothrombotic</b>						
No. of events	9	12	9	21		
Age- and sex-adjusted HR (95% CI)	1.0	1.14 (0.48–2.71)	0.98 (0.39–2.50)	2.31 (1.03–5.16)*	0.03	1.51 (1.14–1.99)§
Multivariate-adjusted HR (95% CI)	1.0	1.35 (0.54–3.35)	1.19 (0.45–3.17)	2.84 (1.17–6.93)*	0.02	1.60 (1.19–2.16)§
<b>Lacunar</b>						
No. of events	14	21	25	33		
Age- and sex-adjusted HR (95% CI)	1.0	1.29 (0.65–2.54)	1.58 (0.81–3.05)	2.00 (1.05–3.80)*	0.02	1.23 (1.00–1.53)
Multivariate-adjusted HR (95% CI)	1.0	1.19 (0.57–2.50)	1.41 (0.69–2.89)	1.69 (0.83–3.43)	0.11	1.13 (0.90–1.43)
<b>Cardioembolic</b>						
No. of events	14	14	12	6		
Age- and sex-adjusted HR (95% CI)	1.0	0.83 (0.39–1.75)	0.80 (0.37–1.75)	0.39 (0.15–1.04)	0.07	0.71 (0.51–1.00)
Multivariate-adjusted HR (95% CI)	1.0	0.75 (0.34–1.63)	0.59 (0.25–1.38)	0.44 (0.12–0.96)*	0.03	0.64 (0.44–0.94)‡
<b>Hemorrhagic stroke</b>						
No. of events	19	15	27	19		
Age- and sex-adjusted HR (95% CI)	1.0	0.69 (0.35–1.36)	1.24 (0.68–2.24)	0.83 (0.43–1.59)	0.95	0.94 (0.74–1.20)
Multivariate-adjusted HR (95% CI)	1.0	0.71 (0.35–1.47)	1.41 (0.75–2.65)	1.01 (0.50–2.05)	0.53	1.02 (0.79–1.33)
<b>CHD</b>						
No. of events	25	28	43	48		
Age- and sex-adjusted HR (95% CI)	1.0	1.02 (0.60–1.76)	1.77 (1.07–2.91)*	2.00 (1.22–3.28)†	<0.001	1.29 (1.08–1.53)§
Multivariate-adjusted HR (95% CI)	1.0	1.01 (0.56–1.80)	1.68 (0.99–2.84)	1.57 (0.91–2.73)	0.03	1.15 (0.95–1.39)

Multivariate adjustment was made for age, sex, HDL cholesterol, triglycerides, systolic BP, ECG abnormalities, fasting blood glucose, BMI, current drinking, current smoking, and regular exercise. For the continuous scale, HR is given for each 1-mmol/L increase in LDL cholesterol.  
 \* $P < 0.05$ , † $P < 0.01$  vs lowest quartile; ‡ $P < 0.05$ , § $P < 0.01$ .

The atherogenesis of LDL cholesterol to large vessels, including coronary arteries and other peripheral arteries, is well known, and clinical studies have shown that an elevated LDL cholesterol level is also significantly related to the development of atherosclerotic lesions in extracranial or

intracranial large vessels.<sup>23,24</sup> Because ATI is caused by atherosclerotic lesions of those large vessels, the significant association between elevated LDL cholesterol level and the risk of ATI observed in the present analysis is compatible with the evidence of the atherogenic role of LDL cholesterol.



**Figure.** Multivariate-adjusted HRs for the development of nonembolic infarction and CHD according to the presence or absence of high LDL cholesterol and the metabolic syndrome. Multivariate adjustment was made for age, sex, ECG abnormalities, current drinking, current smoking, and regular exercise. Centers of the boxes are placed at the estimates of HRs. Horizontal lines indicate 95% CIs, and sizes of boxes are proportional to the numbers of events. LDLc indicates LDL cholesterol; MetS, metabolic syndrome.

In our cohort, the association between LDL cholesterol and the risk of LI was no longer significant after multivariate adjustment, suggesting that elevated LDL cholesterol was not an independent risk factor for the development of LI. Our previous report showed that multiple risk factors were related to the occurrence of LI,<sup>14</sup> and case-control studies on the relation between LDL cholesterol level and LI have reported varied associations.<sup>21,22,25</sup> One study reported a significant association between elevated LDL cholesterol and the risk of LI,<sup>25</sup> another study observed lower LDL cholesterol levels in LI cases,<sup>22</sup> and another study found no significant association.<sup>21</sup> Lacunar infarcts occur as a result of multiple mechanisms, such as (1) lipohyalinosis and/or fibrinoid necrosis, (2) microatheroma, (3) atherosclerosis of the basilar and middle cerebral artery stem or proximal division of large vessels, or (4) cardioembolic occlusion.<sup>26</sup> Lipohyalinosis is a vasculopathy caused by hypertension,<sup>26</sup> whereas large-vessel atherosclerosis is affected by risk factors including LDL cholesterol,<sup>23,24</sup> but cardioembolism seems less related to elevated LDL cholesterol.<sup>22,25</sup> These heterogeneous roles for LDL cholesterol in the multiple pathogenesis of LI occurrence might account for the weak association between LDL cholesterol and the risk of LI.

An inverse relation between LDL cholesterol level and the risk of CEI was observed in our earlier population in the 1960s,<sup>14</sup> and the same association was found in the present investigation after adjustment for confounding factors. Although the reason for this association is unknown, a plausible explanation is that a lowered cholesterol level might increase the risk of CEI through the increased occurrence of atrial fibrillation,<sup>27</sup> a predominant risk factor for CEI. Additional clinical and experimental evidence is needed to elucidate the mechanism underlying this association.

The results of previous prospective studies of the association between LDL cholesterol and hemorrhagic stroke have been inconsistent; a significant inverse association was reported in women in the Framingham Study,<sup>20</sup> whereas a nonsignificant association was observed in the Cardiovascular Health Study.<sup>18</sup> Lipid-lowering trials recently conducted in Japan<sup>28,29</sup> and a meta-analysis of >90 000 subjects enrolled in statin trials<sup>8</sup> found no apparent increase in the risk of hemorrhagic stroke. A nonsignificant association between LDL cholesterol and the risk of hemorrhagic stroke observed in our data was in accord with the findings of a previous prospective study<sup>18</sup> and intervention trials.<sup>8,28,29</sup>

Several prospective studies conducted in Western countries have reported positive associations between LDL cholesterol and the risk of CHD.<sup>30</sup> Among Japanese, no study has investigated the association between LDL cholesterol level and the risk of CHD, but several prospective studies have shown that total cholesterol is a strong risk factor for CHD.<sup>31,32</sup> The findings obtained from the present analysis support the results from those prospective studies and, for the first time, have demonstrated a positive association between calculated LDL cholesterol and the risk of CHD in a general Japanese population.

The metabolic syndrome has been shown to be a clear risk factor for CVD,<sup>33</sup> but LDL cholesterol level is not involved in the definition of the metabolic syndrome. In the present analysis, comparable and independent effects were observed

for elevated LDL cholesterol and the metabolic syndrome on the risks of nonembolic infarction and CHD. The highest risk was observed for the subgroup with both an elevated LDL cholesterol value and the metabolic syndrome. Similar results were found in a prospective study of a Danish cohort.<sup>34</sup> All of these results imply that management of LDL cholesterol as well as the metabolic syndrome is important for the prevention of ischemic stroke and CHD.

The strengths of our study include its longitudinal population-based study design, long duration of follow-up, almost perfect follow-up of subjects, sufficient number of cardiovascular events, and accuracy for diagnosis of CVD, including ischemic stroke subtypes. One limitation of our study is that our findings are based on a 1-time measurement of serum lipids. Subsequent use of cholesterol-lowering agents could have altered lipid levels in some participants; however, this source of variability could not account for the relation observed in the present study, because a random misclassification of such nature would tend to cause an underestimation of study findings and bias the results toward the null hypothesis. Therefore, the true association could be stronger than that observed in our study. Another limitation is that the value of LDL cholesterol was not directly assayed but was calculated by the Friedewald equation. This equation has been adopted in substantial epidemiologic and clinical studies of LDL cholesterol and CVD. It is unlikely that the bias of LDL cholesterol values that occurred through calculation, if any, would have strengthened the association between LDL cholesterol and ATI or CHD observed in the present analysis.

In conclusion, we have shown that elevated LDL cholesterol is a significant risk factor for developing ATI as well as CHD in a general Japanese population. Because LDL cholesterol level is independent of the metabolic syndrome for the development of CVD, lowering a patient's LDL cholesterol level should be considered together with treatment of other metabolic disorders for the prevention of CVD.

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### Disclosures

None.

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