Table 3. Continued

Frequency, %			Haplotype in Block C					Frequency, %		
Case	Control	*P* Value	rs35387	rs40512	rs26954	rs26950	rs26948	Case	Control	*P* Value
41.5	40.9	0.63	G	C	C	A	G	34.4	31.7	0.03
14.5	16.0	0.11	C	T	T	G	G	26.0	27.3	0.26
8.1	7.2	0.17	G	C	C	A	A	23.1	24.6	0.16
4.7	5.7	0.10	G	T	C	A	A	7.5	7.0	0.51
4.1	4.7	0.33	G	C	T	G	G	2.7	2.5	0.56
4.1	3.6	0.41								
3.1	3.1	0.87								
3.5	2.7	0.10								
2.9	2.2	0.08								
1.9	2.6	0.07								
2.1	2.1	0.82								

missed true associations of modest effect. Assuming our sample size, the allele frequencies of the SNPs in our control subjects, and the relative risks of the SNPs in the initial study, the power to detect associations at a significance level of 0.05 would be greater than 99% for SNP83 and SNP56, 98.3% for SNP87, and 69.7% for SNP89 in the case–control samples. In contrast, the statistical power of the prospective cohort was <30% for the 6 SNPs. However, a meta-analysis of these samples should increase the statistical power to detect the association. Therefore, if a true association exists, our study could detect the association between SNPs or haplotypes in *PDE4D* and ischemic stroke with high probability. A recent meta-analysis of 5216 cases and 6615 control subjects also showed that allele 0 of AC008818 and haplotype G0 carriers were associated with increased risk of ischemic stroke, but these associations become nonsignificant after exclusion of the initial study.[28] These results indicate that the effect size of *PDE4D* variants on ischemic stroke, if it exists, may be small.

Because the initial study could not determine a causative SNP or haplotype in *PDE4D*, many replication studies have reported positive associations between different SNPs in *PDE4D* and various ischemic stroke subtypes.[19] This indicates the possibility that hidden causative SNPs for ischemic stroke might exist in *PDE4D*. We analyzed a total of 198 tag-SNPs that covered the 2.2-Mb region, including *PDE4D*, but none of the SNPs were significant after adjustment for multiple testing. Because we selected tag-SNPs according to strict criteria, this analysis was able to capture the most common SNPs in *PDE4D*. Therefore, the previous positive findings of different SNPs may be attributable to chance.

One possible reason for the lack of association between *PDE4D* and ischemic stroke in our study was the difference in the ethnic background. Indeed, SNP45 and SNP41, which showed the most significant association with the combined ATI and CEI phenotypes in an Icelandic population, were monomorphic and all of the Japanese populations studied were homozygotes of the risk alleles in both SNPs. If SNP45 or SNP41 or absolutely linked variations are causative, we cannot estimate the effects of these variations on ischemic stroke, because all causative variations are homozygotes of risk alleles in both cases and control subjects.

Several limitations of this study should be discussed. First, we did not genotype the microsatellite marker, AC008818-1, in this study. However, we genotyped 16 tag-SNPs selected

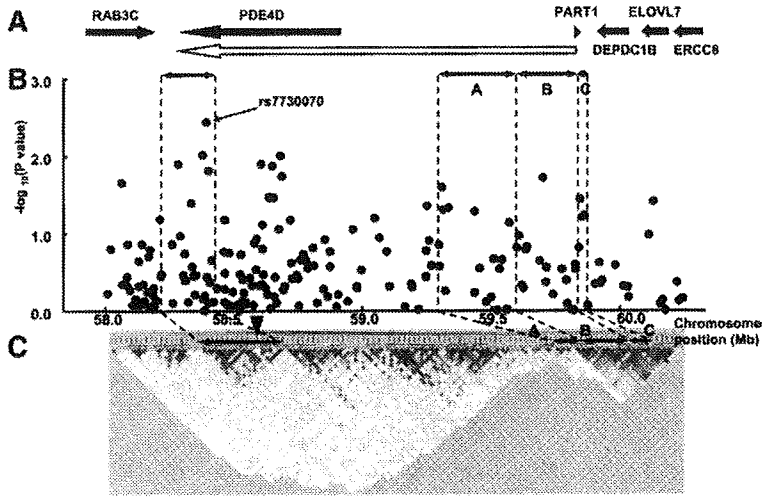

Figure. Genomic structure, case–control results, and linkage disequilibrium map of the 2.2-Mb region, including *PDE4D*. A, Genomic structure around *PDE4D*. The white arrow indicates *PDE4D* reported by the initial study. B, Case–control association results for ischemic stroke among Japanese. The log 10-transformed probability values calculated by the Cochran-Armitage trend test are plotted on the y axis. "A" indicates block A; "B," block B; "C," block C in the initial study. C, Pairwise linkage disequilibrium map between SNPs. The strength of the linkage disequilibrium increases from white to black. A black inverse triangle indicates the location of rs7730070 in the map.

from the regions of blocks B and C according to strict criteria. Therefore, we believe that the effect of AC008818-1 could be sufficiently covered by haplotype analysis using tag-SNPs. Second, we could use only 1656 of 2634 subjects in the prospective cohort. Subjects who developed ischemic stroke would have a higher mortality rate than subjects who did not, and this may have resulted in the lower participation rate in this study. There is a possibility that the results of the prospective cohort might have been distorted by a survivorship bias. Third, the criteria used for classifying ischemic stroke were different between the initial study and ours. For classification of ischemic stroke, the initial study used the Trial of Org 10172 in Acute Stroke Treatment research criteria[29] and we used NINDS-III.[23] However, these 2 classifications are similar to each other, and we diagnosed the subtypes of ischemic stroke by adequate laboratory examinations. We believe that there is no large difference in the phenotype definition.

In conclusion, although we performed a replication study between the variations of *PDE4D* and ischemic stroke risk using 2 independent large case–control samples and a population-based prospective cohort, we failed to replicate the associations. We suggest that variations of *PDE4D* do not confer risk for ischemic stroke in the Japanese population.

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Disclosures

None.

References

1. Bak S, Gaist D, Sindrup SH, Skytthe A, Christensen K. Genetic liability in stroke: a long-term follow-up study of Danish twins. *Stroke*. 2002;33:769–774.
2. Kiely DK, Wolf PA, Cupples LA, Beiser AS, Myers RH. Familial aggregation of stroke. The Framingham Study. *Stroke*. 1993;24:1366–1371.
3. Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, Gudmundsdottir T, Bjarnadottir SM, Einarsson OB, Gudjonsdottir HM, Hawkins M, Gudmundsson G, Gudmundsdottir H, Andrason H, Gudmundsdottir AS, Sigurdardottir M, Chou TT, Nahmias J, Goss S, Sveinbjörnsdottir S, Valdimarsson EM, Jakobsson F, Agnarsson U, Gudnason V, Thorgeirsson G, Fingerle J, Gurney M, Gudbjartsson D, Frigge ML, Kong A, Stefansson K, Gulcher JR. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nat Genet*. 2003;35:131–138.
4. Lõhmussaar E, Gschwendtner A, Mueller JC, Org T, Wichmann E, Hamann G, Meitinger T, Dichgans M. ALOX5AP gene and the *PDE4D* gene in a central European population of stroke patients. *Stroke*. 2005;36:731–736.
5. Kostulas K, Gretarsdottir S, Kostulas V, Manolescu A, Helgadottir A, Thorleifsson G, Gudmundsson LJ, Thorsteinsdottir U, Gulcher JR, Stefansson K, Hillert J. PDE4D and *ALOX5AP* genetic variants and risk for ischemic cerebrovascular disease in Sweden. *J Neurol Sci*. 2007;263:113–117.
6. Meschia JF, Brott TG, Brown RD Jr, Crook R, Worrall BB, Kissela B, Brown WM, Rich SS, Case LD, Evans EW, Hague S, Singleton A, Hardy J. Phosphodiesterase 4D and 5-lipoxygenase activating protein in ischemic stroke. *Ann Neurol*. 2005;58:351–361.
7. Bevan S, Porteous L, Sitzer M, Markus HS. Phosphodiesterase 4D gene, ischemic stroke, and asymptomatic carotid atherosclerosis. *Stroke*. 2005;36:949–953.
8. Nilsson-Ardnor S, Wiklund PG, Lindgren P, Nilsson AK, Janunger T, Escher SA, Hallbeck B, Stegmayr B, Asplund K, Holmberg D. Linkage of ischemic stroke to the *PDE4D* region on 5q in a Swedish population. *Stroke*. 2005;36:1666–1671.
9. Nakayama T, Asai S, Sato N, Soma M. Genotype and haplotype association study of the *STRK1* region on 5q12 among Japanese: a case-control study. *Stroke*. 2006;37:69–76.
10. Song Q, Cole JW, O'Connell JR, Stine OC, Gallagher M, Giles WH, Mitchell BD, Wozniak MA, Stern BJ, Sorkin JD, McArdle PF, Naj AC, Xu Q, Gibbons GH, Kittner SJ. Phosphodiesterase 4D polymorphisms and the risk of cerebral infarction in a biracial population: the Stroke Prevention in Young Women Study. *Hum Mol Genet*. 2006;15:2468–2478.
11. Brophy VH, Ro SK, Rhees BK, Lui LY, Lee JM, Umblas N, Bentley LG, Li J, Cheng S, Browner WS, Erlich HA. Association of phosphodiesterase 4D polymorphisms with ischemic stroke in a US population stratified by hypertension status. *Stroke*. 2006;37:1385–1390.
12. van Rijn MJE, Slooter AJC, Schut AFC, Isaacs A, Aulchenko YS, Snijders PJLM, Kappelle LJ, van Swieten JC, Oostra BA, van Duijn CM. Familial aggregation, the *PDE4D* gene, and ischemic stroke in a genetically isolated population. *Neurology*. 2005;65:1203–1209.
13. Staton JM, Sayer MS, Hankey GJ, Attia J, Thakkinstian A, Yi Q, Cole VJ, Baker R, Eikelboom JW. Association between phosphodiesterase 4D gene and ischaemic stroke. *J Neurol Neurosurg Psychiatry*. 2006;77:1067–1069.
14. Woo D, Kaushal R, Kissela B, Sekar P, Wolujewicz M, Pal P, Alwell K, Haverbusch M, Ewing I, Miller R, Kleindorfer D, Flaherty M, Chakraborty R, Deka R, Broderick J. Association of phosphodiesterase 4D with ischemic stroke: a population-based case–control study. *Stroke*. 2006;37:371–376.
15. Fidani L, Clarimon J, Goulas A, Hatzitolios AI, Evans W, Tsirogianni E, Hardy J, Kotsis A. Association of phosphodiesterase 4D gene G0 haplotype and ischaemic stroke in a Greek population. *Eur J Neurol*. 2007;14:745–749.
16. Kuhlenbäumer G, Berger K, Huge A, Lange E, Kessler C, John U, Funke H, Nabavi DG, Stögbauer F, Ringelstein EB, Stoll M. Evaluation of single nucleotide polymorphisms in the phosphodiesterase 4D gene (*PDE4D*) and their association with ischaemic stroke in a large German cohort. *J Neurol Neurosurg Psychiatry*. 2006;77:521–524.
17. Zee RYL, Brophy VH, Cheng S, Hegener HH, Erlich HA, Ridker PM. Polymorphisms of the phosphodiesterase 4D, cAMP-specific (*PDE4D*) gene and risk of ischemic stroke: a prospective, nested case–control evaluation. *Stroke*. 2006;37:2012–2017.
18. Saleheen D, Bukhari S, Haider SR, Nazir A, Khanum S, Shafqat S, Anis MK, Frossard P. Association of phosphodiesterase 4D gene with ischemic stroke in a Pakistani population. *Stroke*. 2005;36:2275–2277.
19. Rosand J, Bayley N, Rost N, de Bakker PI. Many hypotheses but no replication for the association between *PDE4D* and stroke. *Nat Genet*. 2006;38:1091–1092.
20. Gulcher JR, Kong A, Gretarsdottir S, Thorleifsson G, Stefansson K. Reply to 'Many hypotheses but no replication for the association between *PDE4D* and stroke.' *Nat Genet*. 2006;38:1092–1093.

21. NCI-NHGRI Working Group on Replication in Association Studies. Replicating genotype–phenotype associations. *Nature.* 2007;447:655–670.
22. Kubo M, Hata J, Ninomiya T, Matsuda K, Yonemoto K, Nakano T, Matsushita T, Yamazaki K, Ohnishi Y, Saito S, Kitazono T, Ibayashi S, Sueishi K, Iida M, Nakamura Y, Kiyohara Y. A nonsynonymous SNP in *PRKCH* (protein kinase Cη) increases the risk of cerebral infarction. *Nat Genet.* 2007;39:212–217.
23. Special report from the National Institute of Neurological Disorders and Stroke. Classification of cerebrovascular diseases III. *Stroke.* 1990;21: 637–676.
24. Nakamura Y. The BioBank Japan Project. *Clin Adv Hematol Oncol.* 2007;5:696–697.
25. Kubo M, Kiyohara Y, Kato I, Tanizaki Y, Arima H, Tanaka K, Nakamura H, Okubo K, Iida M. Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community. The Hisayama Study. *Stroke.* 2003;34:2349–2354.
26. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. *J Hum Genet.* 2001;46:471–478.
27. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. *Science.* 2002; 296:2225–2229.
28. Bevan S, Dichgans M, Gschwendtner A, Kuhlenbäumer G, Ringelstein EB, Markus HS. Variation in the *PDE4D* gene and ischemic stroke risk. A systematic review and meta-analysis on 5200 cases and 6600 controls. *Stroke.* 2008;39:1966–1971.
29. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE III. Classification of subtype of acute ischemic stroke definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke.* 1993;24:35–41.

Impact of Metabolic Syndrome Compared With Impaired Fasting Glucose on the Development of Type 2 Diabetes in a General Japanese Population

The Hisayama study

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OBJECTIVE — We examined whether metabolic syndrome predicts incident type 2 diabetes more effectively than impaired fasting glucose (IFG) in a general Japanese population.

RESEARCH DESIGN AND METHODS — A total of 1,935 nondiabetic subjects aged 40–79 years were followed-up prospectively for a mean of 11.8 years.

RESULTS — During the follow-up, 286 subjects developed type 2 diabetes. Compared with those without metabolic syndrome, the multivariate-adjusted hazard ratio (HR) for incident type 2 diabetes was significantly higher in subjects of both sexes with metabolic syndrome, even after adjustment for confounding factors, age, family history of diabetes, total cholesterol, alcohol intake, smoking habits, and regular exercise (men: HR 2.58 [95% CI 1.85–3.59]; women: 3.69 [2.58–5.27]). The multivariate-adjusted HR of metabolic syndrome for type 2 diabetes was slightly lower in men and similar in women compared with that of IFG. The multivariate-adjusted HR for type 2 diabetes rose progressively as the number of metabolic syndrome components increased in both subjects with and without IFG. In stratified analysis, the multivariate-adjusted risk of type 2 diabetes was significantly higher in subjects with metabolic syndrome alone (2.37 [1.45–3.88]) or IFG alone (3.49 [2.57–4.74]) and markedly increased in subjects with both metabolic syndrome and IFG (6.76 [4.75–9.61]) than in subjects with neither metabolic syndrome nor IFG. Furthermore, the multivariate-adjusted risk for type 2 diabetes was also significantly higher in subjects with both metabolic syndrome and IFG than in those with either one alone (both $P < 0.001$).

CONCLUSIONS — Our findings suggest that metabolic syndrome significantly increases the risk of incident type 2 diabetes, independent of IFG, and is therefore a valuable tool to identify individuals at high risk of type 2 diabetes.

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Metabolic syndrome consists of a clustering of cardiovascular risk factors, such as central obesity, elevated blood pressure, glucose intolerance, and dyslipidemia, and individuals with this condition have an elevated risk of developing cardiovascular diseases (1–5) and type 2 diabetes in different ethnic populations (1–4,6–11). Thus, the concept of metabolic syndrome could be used to reduce the incidence of these diseases worldwide. However, a number of experts in the field of diabetes have questioned whether the idea of metabolic syndrome is useful and valuable (12–14). Because all of the criteria sets for metabolic syndrome have included the component of impaired fasting glucose (IFG), which is a powerful predictor of type 2 diabetes, detractors have questioned whether the more complex definition of metabolic syndrome is better than a simple measurement of fasting plasma glucose (FPG). However, reported findings concerning this issue are controversial: a cohort study has shown that the ability of metabolic syndrome to predict type 2 diabetes was superior to that of IFG alone (3), whereas in other studies, the value of metabolic syndrome was comparable or inferior to that of IFG alone (2,6,7). Furthermore, most of these epidemiological studies were performed in Western populations, and this subject has not been assessed sufficiently in Asian populations.

The purpose of the present study was to investigate the association between metabolic syndrome and the development of type 2 diabetes in a prospective study of a defined Japanese population, taking into account comprehensive risk factors. In addition, we compared which of the two measures, metabolic syndrome or IFG, better predicted incident type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study population and follow-up survey

A population-based prospective study of cardiovascular disease and its risk factors has been underway since 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area on Japan's Kyushu Island. In 1988, a screening survey for the present study was performed in the town. A detailed description of this survey was published previously (15). In brief, of the total of 3,227 residents aged 40–79 years based on the town registry, 2,587 residents (participation rate,

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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.

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 ± 10	56 ± 9
Men (%)	39.3	50.7
FPG (mmol/l)	5.4 ± 0.5	5.9 ± 0.6
Two-hour postload glucose (mmol/l)	6.4 ± 1.5	7.5 ± 1.8
Family history of diabetes (%)	6.3	14.0
Waist circumference (cm)	80.8 ± 9.0	85.0 ± 8.7
Total cholesterol (mmol/l)	5.35 ± 1.06	5.39 ± 1.07
HDL cholesterol (mmol/l)	1.32 ± 0.30	1.26 ± 0.30
Triglycerides (mmol/l)	1.09 (0.40–2.98)	1.43 (0.45–4.49)
Systolic blood pressure (mmHg)	130 ± 19	137 ± 19
Diastolic blood pressure (mmHg)	77 ± 11	82 ± 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 ± SD, %, or geometric means (95% CI) for triglycerides (because of the skewed distribution). Elevated blood pressure was defined as blood pressure ≥130/85 mmHg and/or current use of antihypertension agents.

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 ≥7.0 mmol/l and/or 2-h postload glucose concentrations of ≥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 physical 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 (≥5.6 mmol/l), central obesity for Asians (waist circumference ≥90 cm in men and ≥80 cm in women), elevated triglycerides (≥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 ≥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

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*
Men				
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
Women				
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 ≥90 cm in men and ≥80cm 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 ≥1.68 mmol/l; elevated blood pressure, blood pressure ≥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 significantly 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]; $P_{trend} < 0.001$). 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]; $P_{trend} < 0.001$).

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)	P_{trend}
Normal	0	285	12	1 (referent)	
	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)	<0.001
IFG	0	122	13	1 (referent)	
	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)	<0.001

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-

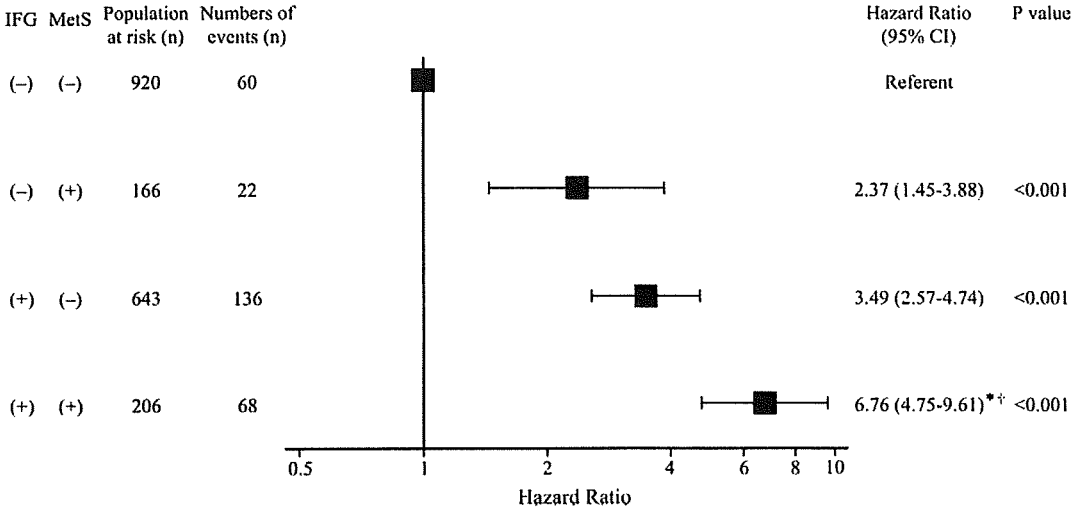

Figure 1—*Multivariate-adjusted HRs for the development of type 2 diabetes according to the presence or absence of metabolic syndrome and IFG. Metabolic syndrome (MetS) was defined as the presence of at least three metabolic syndrome components other than that related to FPG. Multivariate adjustment 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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No potential conflicts of interest relevant to this article were reported.

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References

1. Wannamethee SG, Shaper AG, Lennon L, Morris RW. Metabolic syndrome vs Framingham Risk Score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. Arch Intern Med 2005; 165:2644–2650
2. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and

type 2 diabetes mellitus. Circulation 2005; 112:3066–3072
3. Lorenzo C, Williams K, Hunt KJ, Haffner SM. The National Cholesterol Education Program-Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions of the metabolic syndrome as predictors of incident cardiovascular disease and diabetes. Diabetes Care 2007;30:8–13
4. Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB Sr, Wilson PW. Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. Diabetes Care 2007;30:1219–1225
5. Ninomiya T, Kubo M, Doi Y, Yonemoto K, Tanizaki Y, Rahman M, Arima H, Tsuruya K, Iida M, Kiyohara Y. Impact of metabolic syndrome on the development of cardiovascular disease in a general Japanese population: the Hisayama Study. Stroke 2007;38:2063–2069
6. Cameron AJ, Magliano DJ, Zimmet PZ, Welborn TA, Colagiuri S, Tonkin AM, Shaw JE. The metabolic syndrome as a tool for predicting future diabetes: the AusDiab Study. J Intern Med 2008;264: 177–186
7. Sattar N, McConnachie A, Shaper AG, Blauw GJ, Buckley BM, de Craen AJ, Ford I, Forouhi NG, Freeman DJ, Jukema JW, Lennon L, Macfarlane PW, Murphy MB, Packard CJ, Stott DJ, Westendorp RG, Whincup PH, Shepherd J, Wannamethee SG. Can metabolic syndrome usefully predict cardiovascular disease and diabetes? Outcome data from two prospective studies. Lancet 2008;371:1927–1935
8. Laaksonen DE, Lakka HM, Niskanen LK, Kaplan GA, Salonen JT, Lakka TA. Metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. Am J Epidemiol 2002;156: 1070–1077
9. Cheung BM, Wat NM, Man YB, Tam S, Thomas GN, Leung GM, Cheng CH, Woo J, Janus ED, Lau CP, Lam TH, Lam KS. Development of diabetes in Chinese with the metabolic syndrome: a 6-year prospective study. Diabetes Care 2007;30: 1430–1436
10. Wang JJ, Li HB, Kinnunen L, Hu G, Jarvinen TM, Miettinen ME, Yuan S, Tuomilehto J. How well does the metabolic syndrome defined by five definitions predict incident diabetes and incident coronary heart disease in a Chinese population? Atherosclerosis 2007;192:161–168
11. Mannucci E, Monami M, Cresci B, Pala L, Bardini G, Petracca MG, Dicembrini I, Pasqua A, Buiatti E, Rotella CM. National Cholesterol Education Program and International Diabetes Federation definitions of metabolic syndrome in the prediction of diabetes: results from the FIrenze-Bagno A Ripoli Study. Diabetes Obes Metab 2008;10:430–435
12. Kahn R, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal. Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2005;28:2289–2304
13. Ford ES, Li C, Sattar N. The metabolic syndrome and incident diabetes: current state of the evidence. Diabetes Care 2008; 31:1898–1904
14. Kahn R. Metabolic syndrome: what is the clinical usefulness? Lancet 2008;371: 1892–1893
15. Ohmura T, Ueda K, Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Nomiyama K, Ohmori S, Yoshitake T, Shinkawa A, Hasuo Y, Fujishima M. Prevalence of type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in the Japanese general population: the Hisayama study. Diabetologia 1993;36:1198–1203
16. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26: 3160–3167
17. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735–2752
18. Nakanishi N, Takatorige T, Fukuda H, Shirai K, Li W, Okamoto M, Yoshida H, Matsuo Y, Suzuki K, Tatara K. Components of the metabolic syndrome as predictors of cardiovascular disease and type 2 diabetes in middle-aged Japanese men. Diabetes Res Clin Pract 2004;64:59–70
19. Jensen CC, Cnop M, Hull RL, Fujimoto WY, Kahn SE. β-Cell function is a major contributor to oral glucose tolerance in high-risk relatives of four ethnic groups in the U.S. Diabetes 2002;51:2170–2178
20. Sone H, Ito H, Ohashi Y, Akanuma Y, Yamada N. Obesity and type 2 diabetes in Japanese patients. Lancet 2003;361:85
21. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med 2007;167:1068–1074
22. Nichols GA, Hillier TA, Brown JB. Progression from newly acquired impaired fasting glucose to type 2 diabetes. Diabetes Care 2007;30:228–233
23. Nichols GA, Hillier TA, Brown JB. Normal fasting plasma glucose and risk of type 2 diabetes diagnosis. Am J Med 2008;121: 519–524
24. Howard AA, Arnsten JH, Gourevitch MN. Effect of alcohol consumption on diabetes mellitus: a systematic review. Ann Intern Med 2004;140:211–219
25. Kato I, Kiyohara Y, Kubo M, Tanizaki Y, Arima H, Iwamoto H, Shinohara N, Nakayama K, Fujishima M. Insulin-mediated effects of alcohol intake on serum lipid levels in a general population: the Hisayama study. J Clin Epidemiol 2003; 56:196–204

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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_{1c} (HbA_{1c}) 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_{1c} 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_{1c} 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.[1] Gastric cancer is considered a multifactorial disease, and various factors are involved in its development. 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.[2] 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.[3]

Although a possible association between diabetes mellitus and an increased risk of malignant neoplasms has been discussed for many years,[4] 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[5]; however, 2 other cohort studies failed to show a similar association.[6,7]

A hyperglycemic state has various indicators: of them, HbA_{1c} reflects long-term glycemic control and is a more stable measurement than fasting plasma glucose.[8] Although the associations between HbA_{1c} levels and incidence or mortality for malignancies have been shown in 2 cohort studies,[9,10] no studies have evaluated the impact of HbA_{1c} levels on the development of gastric cancer. In the present study, we conducted a prospective investigation of the relationship between HbA_{1c} 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_{1c}, hemoglobin A_{1c}.

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was performed in 1988. A detailed description of this survey has been published previously.[11,12] 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_{1c} 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[13] and the histologic classification of Laurén.[14] 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_{1c} 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 (≥7.0 mmol/L), or medical history of diabetes.

To assess the independent effect of HbA_{1c} 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[15]; 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,[16] and the nutritional elements were adjusted for energy intake using the method of Willet and Stampfer.[17]

Statistical Analysis

According to the American Diabetes Association's guideline,[18] an HbA_{1c} level of 4.0%–6.0% is considered normal; an HbA_{1c} 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_{1c} by 1.0% intervals, we classified all of the subjects into 4 groups according to baseline HbA_{1c} level: ≤4.9%, 5.0%–5.9%, 6.0%–6.9%, and ≥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_{1c} 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.[19] 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_{1c} Levels at Baseline

Variables	Hemoglobin A_{1c} level (%) ≤4.9 (n = 390)	5.0–5.9 (n = 1685)	6.0–6.9 (n = 427)	≥7.0 (n = 101)	*P* value for trend
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_{1c} (%)	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
Helicobacter pylori 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^2)	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.[3,11,20–23] Additionally, we examined the possibility of a nonlinear relation between HbA_{1c} levels and the occurrence of gastric cancer using a spline model with the model selection method proposed by Kawaguchi et al.[24] The effect of the interaction between HbA_{1c} and *H pylori* infection on the risk of gastric cancer was examined by the χ^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_{1c} 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_{1c} groups. The mean age and frequency of male sex tended to increase with an increase in HbA_{1c} 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_{1c} 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_{1c} 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_{1c} 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-

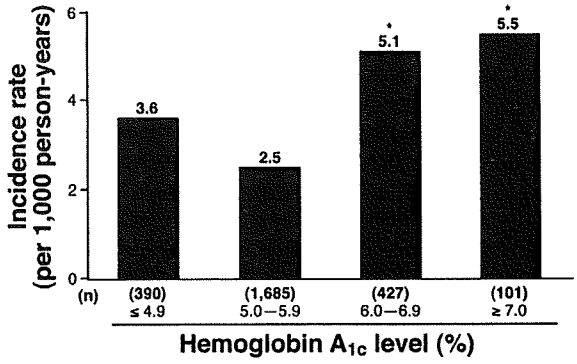

Figure 1. Age- and sex-adjusted incidence rate of gastric cancer according to hemoglobin A_{1c} levels. *$P < .05$ vs hemoglobin A_{1c} 5.0%–5.9%.

Table 2. Age- and Sex-Adjusted and Multivariate-Adjusted Hazard Ratios and 95% Confidence Intervals of Gastric Cancer by Hemoglobin A_{1c} Levels

Hemoglobin A_{1c} level (%)	No. of population at risk	No. of events	Age- and sex-adjusted		Multivariate-adjusted[a]	
			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.
[a]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_{1c} levels and the risk of gastric cancer using the spline model after adjusting for other potential risk factors (Figure 2). Compared with reference HbA_{1c} level of 5.5%, the adjusted HRs of gastric cancer significantly increased at HbA_{1c} levels above this reference, whereas it plateaued at HbA_{1c} levels of >6.5%. The risk of the cancer was also higher at HbA_{1c} 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_{1c} 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_{1c} levels, with or without *H pylori* infection (Figure 3). Compared with the reference group having neither high HbA_{1c} levels nor *H pylori* infection, the age- and sex-adjusted HRs of gastric cancer for the groups with high HbA_{1c} levels alone and *H pylori* infection alone were not significant, but the HR for the group having both high HbA_{1c} 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_{1c} levels and *H pylori* infection ($P = .004$).

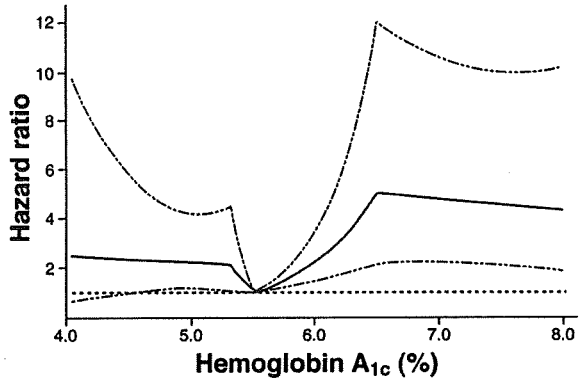

Figure 2. Multivariate adjusted splines for hemoglobin A_{1c} (%) and gastric cancer risk, relative to hemoglobin A_{1c} = 5.5%. The *double-dashed* lines are the 95% confidence intervals for the splines. The risk-changing points (shown by knots) are the hemoglobin A_{1c} values of 5.3%, 5.5%, and 6.5%. 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).

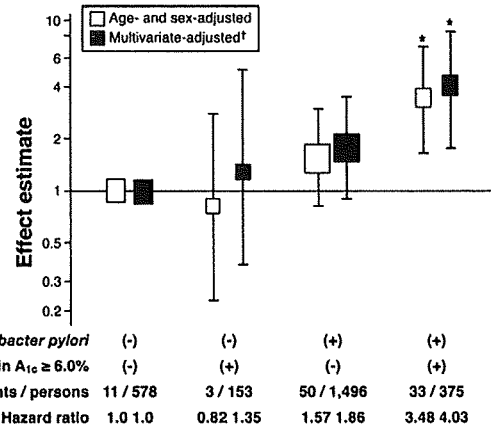

Figure 3. Age- and sex-adjusted and multivariate-adjusted hazard ratios and 95% confidence intervals of gastric cancer according to hemoglobin A_{1c} level and *Helicobacter pylori* status. †Adjusted for age, sex, history of peptic ulcer disease, body mass index, serum total cholesterol, alcohol intake, 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_{1c} ≤5.9% and *Helicobacter pylori* negative.

Discussion

Our findings indicated that elevated HbA_{1c} 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_{1c} 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_{1c} levels and gastric cancer incidence, while taking into account the interaction between HbA_{1c} levels and *H pylori* status.

Diabetes and impaired glucose tolerance are thought to be possible risk factors, not only for cardiovascular events,[25] but also for malignancies.[4–7,26] Among the studies on this subject, few have examined the association between hyperglycemia and gastric cancer.[5–7] Our previous study showed that elevated fasting plasma glucose levels (≥5.3 mmol/L) were clearly associated with gastric cancer incidence.[5] 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.[6] However, another prospective study did not show these relationships.[7] On the other hand, HbA_{1c} is a good time-integrated indicator of blood glucose concentrations over the preceding 1 to 3 months.[27] 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_{1c} levels and the risk of gastric cancer.

In the present study, we demonstrated that the incidence of gastric cancer significantly increased in the HbA_{1c} groups of ≥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_{1c} levels over 6.5% using the spline model. It is possible that the risk did not change at the higher HbA_{1c} levels or that this finding was a result of the competing effect of other diseases such as cardiovascular events. In fact, elevated HbA_{1c} 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_{1c} level of around 5.5%. These observations indicate that an optimum HbA_{1c} 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.[28] In an experimental study, high glucose itself was also shown to induce DNA damage.[29] 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.[30] 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.[31]

Numerous epidemiologic and experimental studies have shown a clear association between *H pylori* infection and the risk of gastric cancer.[11] 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,[32] 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_{1c} 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_{1c} 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_{1c} 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,[33] 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.[34] *H pylori* infection can also increase pancreatic insulin secretion by decreasing the serum concentration of somatostatin,[35] which has an inhibiting effect on insulin release.[36] Moreover, a clinical study demonstrated an increased state of insulin resistance in subjects with *H pylori* infection.[37] 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,[38,39] whereas other studies have found no significant correlation between fasting plasma glucose levels and *H pylori* status.[40,41] In our subjects, no significant correlation was observed between HbA_{1c} 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.[1,2,42] 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,[43] and few studies included concealed cancers.[44] 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_{1c} 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_{1c} 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_{1c}, which do not require medical treatment. Therefore, biases of this kind should not have distorted the significant association between HbA_{1c} 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.[45] 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_{1c} levels and gastric cancer incidence. Our findings suggest that an elevated HbA_{1c} 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.

References

1. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2. The EUROGAST Study Group. An international association between *Helicobacter pylori* infection and gastric cancer. Lancet 1993;341:1359–1362.
3. Yamaguchi N, Kakizoe T. Synergistic interaction between *Helicobacter pylori* gastritis and diet in gastric cancer. Lancet Oncol 2001;2:88–94.
4. Wideroff L, Gridley G, Mellemkjaer L, et al. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst 1997;89:1360–1365.
5. Yamagata H, Kiyohara Y, Nakamura S, et al. Impact of fasting plasma glucose levels on gastric cancer incidence in a general Japanese population: the Hisayama Study. Diabetes Care 2005; 28:789–794.
6. Jee SH, Ohrr H, Sull JW, et al. Fasting serum glucose level and cancer risk in Korean men and women. JAMA 2005;293:194–202.

7. Rapp K, Schroeder J, Klenk J, et al. Fasting blood glucose and cancer risk in a cohort of more than 140,000 adults in Austria. Diabetologia 2006;49:945–952.
8. Rohlfing C, Little R, Wiedmeyer H, et al. Use of GHb (HbA_{1c}) in screening for undiagnosed diabetes in the US population. Diabetes Care 2000;23:187–191.
9. Nakanishi S, Yamada M, Hattori N, et al. Relationship between HbA_1c and mortality in a Japanese population. Diabetologia 2005;48:230–234.
10. Khaw KT, Wareham N, Bingham S, et al. Preliminary communication: glycated hemoglobin, diabetes, and incident colorectal cancer in men and women: a prospective analysis from the European prospective investigation into cancer-Norfolk study. Cancer Epidemiol Biomarkers Prev 2004;13:915–919.
11. Yamagata H, Kiyohara Y, Aoyagi K, et al. Impact of *Helicobacter pylori* infection on gastric cancer incidence in a general Japanese population: the Hisayama Study. Arch Intern Med 2000;160: 1962–1968.
12. Ohmura T, Ueda K, Kiyohara Y, et al. Prevalence of type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in the Japanese general population: the Hisayama Study. Diabetologia 1993;36:1198–1203.
13. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma, 2nd English ed. Gastric Cancer 1998;1:10–24.
14. Laurén P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965; 64:31–49.
15. Shirota T, Yoshizumi E. Accuracy of nutritional survey using the simple method (in Japanese). Jpn J Public Health 1990;37:100–108.
16. Resources Council of the Science and Technology Agency. Standard tables of food composition in Japan. 4th ed. Tokyo: Ministry of Finance Printing Bureau, 1982.
17. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
18. The American Diabetes association. Standards of medical care in diabetes—2008. Diabetes Care 2008;31(Suppl 1):S12–54.
19. Cox DR. Regression models and life-tables. J R Stat Soc 1972; 34:187–220.
20. Shikata K, Kiyohara Y, Kubo M, et al. A prospective study of dietary salt intake and gastric cancer incidence in a defined Japanese population: the Hisayama Study. Int J Cancer 2006; 119:196–201.
21. Forman D, Burley VJ. Gastric cancer: global pattern of the disease and an overview of environmental risk factors. Best Pract Res Clin Gastroenterol 2006;20:633–649.
22. Asano K, Kubo M, Yonemoto K, et al. Impact of serum total cholesterol on the incidence of gastric cancer in a population-based prospective study: the Hisayama Study. Int J Cancer 2008; 122:909–914.
23. Shikata K, Doi Y, Yonemoto K, et al. Population-based prospective study of the combined influence of cigarette smoking and *Helicobacter pylori* infection on gastric cancer incidence: the Hisayama Study. Am J Epidemiol 2008;168:1409–1415.
24. Kawaguchi A, Yonemoto K, Tanizaki Y, et al. Application of functional ANOVA models for hazard regression to the Hisayama data. Stat Med 2008;27:3515–3527.
25. Coutinho M, Gerstein HC, Wang Y, et al. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 1999;22: 233–240.
26. Saydah SH, Loria CM, Eberhardt MS, et al. Abnormal glucose tolerance and the risk of cancer death in the United States. Am J Epidemiol 2003;157:1092–1100.
27. Goldstein DE, Little RR, Lorenz RA, et al. Tests of glycemia in diabetes. Diabetes Care 2004;27:1761–1773.
28. Dandona P, Thusu K, Cook S, et al. Oxidative damage to DNA in diabetes mellitus. Lancet 1996;347:444–445.
29. Lorenzi M, Montisano DF, Toledo S, et al. High glucose induces DNA damage in cultured human endothelial cells. J Clin Invest 1986;77:322–325.
30. Ogihara S, Yamada M, Saito T, et al. Insulin potentiates mitogenic effect of epidermal growth factor on cultured guinea pig gastric mucous cells. Am J Physiol 1996;271:G104–112.
31. Yi HK, Hwang PH, Yang DH, et al. Expression of the insulin-like growth factors (IGFs) and the IGF-binding proteins (IGFBPs) in human gastric cancer cells. Eur J Cancer 2001;37:2257–2263.
32. Higashi H, Tsutsumi R, Muto S, et al. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 2002;295:683–686.
33. Kawamura A, Adachi K, Takashima T, et al. *Helicobacter pylori*-independent effect of hyperglycemia on gastric mucosal atrophy. Am J Gastroenterol 2002;97:2479–2480.
34. Acbay O, Celik AF, Gundogdu S. Does *Helicobacter pylori*-induced gastritis enhance food-stimulated insulin release? Dig Dis Sci 1996;41:1327–1331.
35. Kaneko H, Konagaya T, Kusugami K. *Helicobacter pylori* and gut hormones. J Gastroenterol 2002;37:77–86.
36. Colturi TJ, Unger RH, Feldman M. Role of circulating somatostatin in regulation of gastric acid secretion, gastrin release, and islet cell function. studies in healthy subjects and duodenal ulcer patients. J Clin Invest 1984;74:417–423.
37. Aydemir S, Bayraktaroglu T, Sert M, et al. The effect of *Helicobacter pylori* on insulin resistance. Dig Dis Sci 2005;50:2090–2093.
38. Oldenburg B, Diepersloot RJ, Hoekstra JB. High seroprevalence of *Helicobacter pylori* in diabetes mellitus patients. Dig Dis Sci 1996;41:458–461.
39. Marrollo M, Latella G, Melideo D, et al. Increased prevalence of *Helicobacter pylori* in patients with diabetes mellitus. Dig Liver Dis 2001;33:21–29.
40. Xia HH, Talley NJ, Kam EP, et al. *Helicobacter pylori* infection is not associated with diabetes mellitus nor with upper gastrointestinal symptoms in diabetes mellitus. Am J Gastroenterol 2001; 96:1039–1046.
41. Dore MP, Bilotta M, Malaty HM, et al. Diabetes mellitus and *Helicobacter pylori* infection. Nutrition 2000;16:407–410.
42. Truong Minh P, Fujino Y, Yoshimura T, et al. Mortality and incidence rates of stomach cancer in the JACC Study. J Epidemiol 2005;15(Suppl 2):S89–97.
43. Sobue T. Current activities and future directions of the cancer registration system in Japan. Int J Clin Oncol 2008;13:97–101.
44. Hasuo Y, Ueda K, Kiyohara Y, et al. Accuracy of diagnosis on death certificates for underlying causes of death in a long-term autopsy-based population study in Hisayama, Japan; with special reference to cardiovascular diseases. J Clin Epidemiol 1989;42: 577–584.
45. Hisamichi S. Screening for gastric cancer. World J Surg 1989; 13:31–37.

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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 ≥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).[1] In contrast, the relation between total cholesterol levels and the risk of stroke remains unclear because of conflicting results reported in the literature.[2,3] 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,[2,4] and there is a positive association between total cholesterol and ischemic stroke.[2,5] Furthermore, the association may be different for ischemic stroke subtypes.[6] Second, lipoprotein subfractions are considered to exert varying influence on stroke risk.[7] It is possible that the protective effect of HDL cholesterol against stroke weakens 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.[8] On the other hand, whereas statins significantly reduced the risk of stroke,[8] the risk reduction for stroke in trials in which subjects were treated with nonstatins was not significant,[9] 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 ≥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 (≥4.48 mmol/L) for which the Friedewald formula loses its validity[10] (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,[11] as well as on the basis of the diagnostic criteria of the Trial of Org10172 in Acute Stroke Treatment (TOAST) Study[12] and the Cerebral Embolism Task Force.[13]

Details of the diagnostic criteria for ischemic stroke subtypes have been described previously.[14] In brief, ATI was diagnosed when the subjects had significant stenosis (>50%) or occlusion of a major cerebral artery with infarct size ≥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.[13] 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 pathology, 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.[15] 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.[10] 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 ≥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; kg/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 ≥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[16] 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 ≥25 kg/m^2 as a substitute for waist circumference[17]; (2) fasting triglyceride concentration ≥1.68 mmol/L; (3) HDL cholesterol concentration <1.03 mmol/L in men and <1.29 mmol/L in women; (4) BP ≥130/85 mm Hg or use of antihypertensive drugs; and (5) fasting plasma glucose value ≥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

	Quartile of LDL Cholesterol Levels, mmol/L				
Risk Factor	≤2.65 (n=586)	2.66 to 3.24 (n=591)	3.25 to 3.88 (n=585)	≥3.89 (n=589)	*P* Value for Trend
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^2	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