Plasma insulin, C-peptide, and blood glucose and the risk of gastric cancer: The Japan Public Health Center–based prospective study

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Abbreviations: BMI: body mass index; CagA: cytotoxin associated gene A; CI: confidence interval; DM: diabetes mellitus; HbA1c: hemoglobin A1c; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-β: homeostasis model assessment of β-cell function; ICD-O: International Classification of Diseases for Oncology; IGF: insulin-like growth factor; JPHC: Japan Public Health Center–based prospective study; OR: odds ratio; PHC: public health center; SD: standard deviation

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What's new?

Diabetes mellitus related mechanisms of gastric carcinogenesis have been controversial. The authors investigated the association between plasma insulin, C-peptide, blood glucose, and homeostasis model assessment (HOMA) levels and gastric cancer risk in a large-scale population-based prospective study. The results suggest the importance of hyperinsulinemia derived from insulin resistance, rather than hyperglycemia, in gastric carcinogenesis.

Abstract

To date, the association between diabetes mellitus (DM) and gastric cancer has been controversial, including the underlying mechanism. We investigated the association between plasma diabetic biomarkers (insulin, C-peptide, and blood glucose) and gastric cancer risk. In addition, homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) were calculated. A total of 36,745 subjects aged 40–69 years in the Japan Public Health Center–based prospective study (JPHC) who returned the baseline questionnaire and provided blood samples were followed from 1990 to 2004. In the present analysis, 477 cases and 477 matched controls were used. The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) for developing gastric cancer were calculated using conditional logistic regression models. Plasma insulin was positively associated with increased risk of gastric cancer; compared to tertile 1, ORs were 1.69 (95% CI = 1.11–2.59) and 2.01 (1.19–3.38) for tertiles 2 and 3, respectively (*p* for trend = 0.009). In men, C-peptide was also positively associated with a significant risk; corresponding ORs were 1.42 (0.85–2.38) and 1.91 (1.03–3.54), respectively (*p* for trend = 0.04). These findings were confirmed for blood samples from the fasting group (≥8 h after a meal). Higher HOMA-IR was also associated with increased risk, whereas no association was observed for blood glucose. Our findings suggest that Japanese population with higher insulin and C-peptide levels derived from insulin resistance have an elevated risk of gastric cancer.

Introduction

Gastric cancer is the second leading cause of death and the fourth most common cancer in the world [1]. Although *Helicobacter pylori* (*H. pylori*) infection is well known as a major risk factor for gastric cancer, only some of the people infected with *H. pylori* will develop gastric cancer. Therefore, other risk factors might affect the association between *H. pylori* and gastric cancer occurrence.

Diabetes mellitus (DM) is associated with many types of cancer, including colorectal, liver, breast, and pancreatic cancer [2]. However, the association between DM and gastric cancer remains to be clarified. Some prospective studies reported that DM determined by questionnaire or medical records is positively associated with gastric cancer [3-6], but others found a null association [7-12]. However, DM can be easily misclassified when based on self-report of disease in questionnaire survey or medical records. To overcome this problem, several studies were directly based on diabetic biomarkers, such as hemoglobin A1c (HbA1c) and blood glucose, but the associations were also inconsistent in these prospective studies [13-16].

Another possible candidate biomarker is insulin, which may be involved in the biological mechanisms of carcinogenesis that underlie the association between DM and gastric cancer. To date, several *in vivo* and *in vitro* studies have reported a positive association between insulin and carcinogenesis including gastric mucosa [17, 18]. To our knowledge, no prospective study has evaluated the association between insulin and the risk of gastric cancer.

In this study, we investigated the association between plasma insulin, C-peptide, and blood glucose and gastric cancer risk in a case-control study nested within a large-scale population-based study.

C-peptide is a metabolic product of insulin and is more stable than insulin in blood. In addition, we calculated homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) to evaluate the extent of insulin resistance and pancreatic β-cell function [19], respectively.

Material and Methods

Study population

The Japan Public Health Center–based prospective study (JPHC) was established in 1990 for cohort I (subject age range 40–59 years) and in 1993 for cohort II (40–69 years), as described previously [20]. The JPHC consisted of 11 public health centers (PHCs) in Japan and included 140,420 subjects (68,722 men and 71,698 women). The subjects from one PHC (Tokyo) in cohort I were excluded from this study because the data on cancer incidence were not available. In addition, one subgroup of cohort II (Osaka) was excluded because the selection of subjects differed from that of other cohort subjects, which left 123,576 subjects (61,009 men and 62,567 women). This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Baseline survey

In the baseline survey, a self-administered questionnaire was used in each cohort. The study subjects were asked about various lifestyle factors, such as sociodemographic characteristics, personal medical history, family history, smoking and drinking habits, dietary habits, and physical activity. A total of 99,808

subjects (47,525 men and 52,283 women) responded (response rate: 80.8%).

We asked each subject to provide a 10-ml blood sample at the time of the health checkup. After exclusion of subjects who self-reported cancer at baseline (*n* = 2136), who were non-Japanese (*n* = 18), and who did not live in the area at the baseline (*n* = 11), 97,644 subjects (46,803 men and 50,841 women) remained eligible. (One subject both self-reported cancer at baseline and was non-Japanese.) Among the eligible subjects, 36,745 subjects (13,467 men and 23,278 women) provided blood samples at baseline. Plasma levels of blood glucose were measured at each PHC area at the time of the baseline health check-up and the values were used for the present analysis. One PHC (Niigata) in cohort II and two PHCs (Akita and Iwate) in cohort I did not routinely measure glucose (*n* = 174). According to the Osaka Medical Center for Health Science and Promotion, the accuracy of plasma blood glucose measurements in all the laboratories was found to be satisfactory [21]. The plasma and buffy coat were divided into four tubes, each holding 1.0 ml (three tubes for plasma and one for the buffy coat), and then preserved at –80°C until analysis.

The blood samples were collected from 1990 to 1992 in cohort I and from 1993 to 1995 in cohort II. Following the standard protocol, we requested that subjects avoid having a meal after 21:00 on the day before the health checkup, and recorded the approximate last time of caloric intake, including a meal and/or drinking.

Follow-up

Subjects were observed from 1 January 1990 to 31 December 2004 for cohort I and from 1 January 1993

to 31 December 2004 for cohort II. Residence status, survival, and death were identified annually through residential registries in each PHC area. In Japan, residence and death registration are required by law, and the registries are believed to be complete. Among the 36,745 subjects, 1423 (3.9%) moved outside the study area, 1610 (4.4%) died, and 11 (0.03%) were lost to follow-up during the study period.

Cancer registry for the JPHC

Incidence data on gastric cancer cases were collected for the JPHC cancer registry from two sources: local major hospitals and population-based cancer registries (usually prefecture-wide). Death certificate information was also used. In our cancer registry system, information for 7.6% of gastric cancer cases was based on the case first identified via a death certificate and 2.1% were registered based on information from the death certificate alone.

Selection of cases and controls

Over the entire study period from 1990 to 2004, 1681 new gastric cancer cases with a histologically proven diagnosis were observed in the two cohorts. Among these cases, blood samples and questionnaire responses at baseline had been obtained from 512 cases. The anatomic subsite of each case was coded on the basis of the International Classification of Diseases for Oncology (ICD-O), 3rd edition [22]. Tumor located in the upper third of the stomach was referred to as proximal gastric cancer (cardia subsite) (ICD-O code C16.0 and 16.1), and that in the lower portion of the stomach was classified as distal gastric cancer (non-cardia subsite) (ICD-O code C16.2–16.7). The remaining cases were tumors that could not be

classified because of overlapping lesions (ICD-O code C16.8) or no information (ICD-O code C16.9). The subdivisions by histological type was based on the Lauren classification [23]. For each case, we selected one control subject from those who were not diagnosed with gastric cancer during the follow-up period when the case was diagnosed. We matched case and control for gender, age (±3 years), study area, fasting time at blood donation (±5 h), and blood donation date (±2 months). Among the 512 new gastric cancer cases, 1 case was excluded due to a technical error in the measurement of *H. pylori* and 34 cases were excluded due to no volume left for the present measurement. The final analysis included 477 matched sets of cases and controls.

Laboratory assays for insulin and C-peptide

Plasma levels of insulin and C-peptide were measured at GeneticLab, Hokkaido, Japan. All laboratory personnel were blinded about case and control status. Plasma diabetic biomarkers were simultaneously assayed using a Human Endocrine Milliplex Kit (#HEND-65K; Millipore Company, 6 Research Park Drive, St. Charles, Missouri 63304, USA). The kit used polystyrene bead–based assays to measure the markers in 25-μl samples across panels. Based on the measurement of eight median fluorescent intensities, a standard curve of the biomarker was used to convert optical density values into concentrations, with limits of assay detection of 5.8 pg/ml (1 pmol/L) for insulin and 3.6 pg/ml (1 pmol/L) for C-peptide. Using the curve-fit measurements for each standard, technicians also estimated coefficients of variation, which were calculated as the ratio of the observed and expected concentrations. The average coefficients of variation for plasma levels of insulin and C-peptide were 7.2% and 4.2%, respectively. Some plasma

samples could not be measured because of insufficient volume: 27 for insulin and 2 for C-peptide.

Statistical analysis

Tertiles of plasma diabetic biomarkers and HOMA-β were based on levels in control subjects. The chi-square test and Student's *t*-test were used to compare background characteristics between cases and controls. Matched odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated using conditional logistic regression models. OR1 was matched for age (±3 years), gender, PHC area, blood donation date (±2 months), and fasting time at blood donation (±5 hours). OR2 was calculated by multivariate conditional logistic regression analysis adjusting for potential confounding factors such as smoking status, alcohol consumption, total calorie intake, salt intake, body mass index (BMI), family history of gastric cancer, *H. pylori* infection status, and atrophy. OR3 was further adjusted for past history of DM and drug treatment for DM.

Smoking status was divided into four groups: never smoker, past smoker, current smoker with ≤20 cigarettes per day, and current smoker with ≥21 cigarettes per day. Alcohol consumption was divided into four groups: never drinker, occasional drinker, current drinker who intakes <300 g of ethanol per week, and current drinker who intakes ≥300 g of ethanol per week. Total calorie and salt intakes were treated as continuous variables. BMI was divided into three classes: BMI <22 kg/m^2, 22≤ BMI <25, and 25≤ BMI. Subjects who were missing value for BMI (n = 6), total calorie (n = 1), and salt intakes (n = 1) were excluded when adjusting for these confounding factors. Family history of gastric cancer was considered positive if at least one parent or sibling had gastric cancer. The *H. pylori* infection status was

regarded as positive if subjects had either *H. pylori* antibody ≥10 U/ml or cytotoxin associated gene A (CagA) antibody >10. Atrophy was regarded as positive if pepsinogen I was ≤70 ng/ml and the pepsinogen I/pepsinogen II ratio was ≤3 [24]. Because we do not have any data from upper gastrointestinal endoscopies and biopsies, the pepsinogen data were used. Urita *et al* reported that the pepsinogen I/pepsinogen II ratio ≤3 identified gastric atrophy with a sensitively of 71.7% and a specificity of 66.7% [25]. We believe that the pepsinogen data could explain the level of atrophy, to some extent, if added to the model. Past history of DM and drug treatment for DM were considered positive if subjects were diagnosed with DM before and used a diabetic drug at the time of the baseline survey, respectively. Stratified analysis based on fasting status (≥8 hours or <8 hours after a meal) was also conducted for each plasma diabetic biomarker. Furthermore, for the subjects who were in the fasting group (≥8 hours after a meal) at blood donation and not under drug treatment for DM, we calculated HOMA-IR [fasting plasma insulin level (μU/ml) × fasting plasma glucose level (mg/dl)/405] and HOMA-β [360 × fasting plasma insulin level (μU/ml)/fasting plasma glucose level (mg/dl) – 63] [19]. HOMA-IR ≥1.73 was defined as the presence of insulin resistance [26]. According to the manufacturer of the insulin measuring kit (Millipore), conversion of insulin units was based on the human insulin international reference preparation of WHO (1 μIU/ml = 35 pg/ml).

Reported *p* values are two-sided, and $p < 0.05$ was defined as statistically significant. All statistical analyses were performed with SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics of cases and controls are shown in Table 1. Family history of gastric cancer, past history of DM, *H. pylori* positivity, and atrophy were significantly more frequent among cases compared to controls. The distributions of other factors were similar in cases and controls. At baseline, 9.2% of cases and 4.4% of controls had past history of DM, and 3.1% of cases and 1.7% of controls had received drug treatment for DM.

Table 2 shows ORs and 95% CIs for the associations between plasma levels of diabetic biomarkers and gastric cancer risk using conditional logistic regression models. We found that plasma insulin was dose-dependently associated with an increased risk of gastric cancer. Compared to tertile 1, OR2 (adjusted for smoking, alcohol consumption, BMI, total calories, salt intake, family history of gastric cancer, *H. pylori* infection status, and atrophy) for tertiles 2 and 3 was 1.63 (95% CI = 1.08–2.47) and 1.91 (1.15–3.18), respectively (*p* for trend 0.01). When further adjusted for past history of DM and drug treatment for DM, corresponding values for OR3 were 1.68 (1.10–2.56) and 2.03 (1.21–3.41), respectively (*p* for trend 0.007). We found no association between the other diabetic biomarkers and risk of gastric cancer.

In Table 3, the associations between plasma levels of diabetic biomarkers and gastric cancer risk are shown for men and women separately. In men, besides insulin, plasma C-peptide was also dose-dependently associated with gastric cancer risk; OR2 was 1.39 (0.83–2.30) and 1.90 (1.04–3.48) for tertiles 2 and 3, respectively (*p* for trend 0.04). Corresponding values for OR3 were 1.43 (0.86–2.40) and 1.96 (1.06–3.64), respectively (*p* for trend 0.03). In women, plasma C-peptide was inversely associated with gastric cancer risk (OR1), but it lost statistical significance after further adjustment (OR2 and OR3).

Participants who provided blood samples more than 8 hours after a meal were defined as the fasting group. Because plasma insulin and C-peptide showed positive associations with gastric cancer (Tables 2 and 3), further stratified analysis by fasting status (≥8 hours and <8 hours after a meal) was performed for these biomarkers, as well as HOMA-IR and HOMA-β. After excluding pairs with different fasting status, conditional logistic regression analysis was conducted (Table 4). The levels of these biomarkers differed by fasting status. We found that higher levels of plasma insulin and C-peptide were marginally associated with gastric cancer risk in the fasting group (≥8 hours after a meal). For the non-fasting group (<8 hours after a meal), whose biomarker levels may be strongly influenced by the meal, a weakly increased risk was also observed, but not significantly so. Moreover, a higher HOMA-IR was associated with increased risk of gastric cancer; OR2 for HOMA-IR ≥1.73 was 1.88 (1.03–3.45) compared to HOMA-IR <1.73. Corresponding values for OR3 were 1.97 (1.07–3.65). Higher HOMA-β also showed a trend toward a positive association.

We conducted stratified analyses by alcohol consumption, smoking status, menopausal status (menopausal or not menopausal), and atrophy, and no differences according to such stratification were observed. Higher insulin and C-peptide levels were positively associated with the distal subsite and intestinal type of gastric cancer risk, but not significantly so. In addition, the cardia subsite and diffuse type of gastric cancer also showed a trend toward a positive association with insulin, but not with C-peptide, possibly due to the small number of subjects (data not shown). When we excluded the subjects with a past history of DM and drug treatment for DM, similar associations were observed between plasma insulin and C-peptide and gastric cancer risk. Higher HOMA-IR and HOMA-β values also showed

similar associations when subjects with past history of DM were excluded (data not shown). Finally, when we excluded the subjects who developed gastric cancer within 2 years of blood donation and their matched controls, similar associations were observed (data not shown).

Discussion

In this case-control study nested within a large-scale population-based study, we observed an increased risk of gastric cancer according to higher insulin levels, C-peptide levels, and HOMA-IR, independent of several confounding factors. The positive association was also observed when excluding subjects who had past history of DM and drug treatment for DM. In contrast, plasma levels of blood glucose were not associated with gastric cancer risk. No association was observed for any of the diabetic biomarkers in women.

Several postulated DM-related mechanisms of carcinogenesis, including hyperglycemia itself and/or decreased bioactivity of insulin such as hyperinsulinemia or insulin resistance, have been controversial [27, 28]. A meta-analysis of several prospective studies reported that not only higher levels of insulin and C-peptide but also higher levels of blood glucose significantly increased the risk of pancreatic and colorectal cancers [29]. But this meta-analysis had a critical limitation, in that few studies took fasting status into account. In more recent reports of large population-based nested case-control studies of pancreatic and colorectal cancer, fasting group (≥8 hours after a meal) was considered. For the risk of pancreatic cancer, when HbA1c and insulin were adjusted, only a higher level of plasma proinsulin was found to increase the risk, whereas the proinsulin/insulin ratio, a marker of pancreatic β-cell function, was

not [30]. For the risk of colorectal cancer, higher insulin level and HOMA-IR were associated with an increased risk, whereas no association was observed for blood glucose [31]. Therefore, the authors concluded that their results did not support the hypothesis that hyperglycemia is causally associated with increased risk of pancreatic and colorectal cancers. We observed that higher levels of insulin and C-peptide significantly increase the risk of gastric cancer, not blood glucose levels. This may suggest the importance of hyperinsulinemia, rather than hyperglycemia, in gastric carcinogenesis as well as other cancer sites, such as pancreatic and colorectal cancer.

Insulin is a well-known key regulator of carcinogenesis, including gastric cancer [17, 18, 32]. Insulin can enhance insulin-like growth factor (IGF)-1 bioavailability by inhibiting the production of IGF-binding proteins [18, 32]. Insulin and bioavailable IGF-1 signal transduction occurs through insulin, IGF-1, and hybrid receptors in the cell membrane [18]. Inhibition of apoptosis and stimulation of cellular proliferation and carcinogenesis occurs because of the several downstream pathways activated by these receptors. The binding of insulin or bioavailable IGF-1 to the receptors activates phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) and Ras/MAPK (mitogen-activated protein kinase) pathways [18].

In our study, the positive associations between plasma insulin and C-peptide levels and gastric cancer occurrence were clearly observed in men, but not in women. One possible explanation is hormonal differences. A recent meta-analysis showed that women with longer exposure to estrogen by either ovarian (fertility) or exogenous origin (hormone replacement therapy) may be protected from gastric cancer [33], and that the body mass of postmenopausal women correlates with blood estrogen levels [34]. The possible protective effect of estrogen might mask the risk of developing gastric cancer in women,

although the analysis stratified by menopausal status (menopausal or not menopausal) did not show a clear difference between the two. Another explanation is that alcohol consumption [35] and smoking [36] may determine insulin resistance and hyperinsulinemia thereby resulting in gastric carcinogenesis. In our study, most alcohol drinkers and smokers were male. However, additional analysis did not show any clear interaction between smoking status or alcohol consumption and diabetic biomarkers.

In the fasting group (≥8 hours after a meal), we analyzed not only plasma insulin and C-peptide levels, but also HOMA-IR and HOMA-β. By calculating HOMA, we can estimate the background of hyperinsulinemia at fasting group such as insulin resistance (HOMA-IR) and/or greater functioning of pancreatic β-cell function (HOMA-β). We found that higher HOMA-IR was positively associated with gastric cancer risk. Therefore, our findings suggest that insulin resistance is the main mechanism underlying the positive association between hyperinsulinemia and gastric cancer risk. HOMA-β also showed a marginal association. One previous study showed an increasing pancreatic β-cell volume to compensate for insulin resistance [37], which may result in increased β-cell function. A possible explanation for insulin resistance leading to hyperinsulinemia may be that it is a consequence of *H. pylori* infection. According to a recent systematic review, a positive trend toward an association between *H. pylori* infection and insulin resistance was found [38]. Several mechanisms underlying the relationship between *H. pylori* infection and insulin resistance suggest that reactive oxygen species, proatherogenic substances, and inflammatory substances are released by *H. pylori* infection. *H. pylori* infection also promotes the activation/aggregation of platelets and apoptosis [39].

This is the first population-based prospective study to indicate a positive association between

higher levels of insulin and C-peptide and gastric cancer risk. Based on the study design, the blood samples were collected before subjects were diagnosed with gastric cancer, which enabled us to investigate the factors associated with a subsequent risk of gastric cancer incidence. In addition, we have robust data on other factors including fasting status, history of DM, drug treatment for DM, lifestyle factors, atrophy, CagA, and *H. pylori* infection.

Our study did have some limitations. First, among the 97,644 eligible subjects who responded to a self-administered questionnaire in this study, only 36,745 (37.6%) subjects provided a blood sample. Those subjects who participated in the health checkup survey had a more favorable lifestyle, such as less smoking and alcohol consumption, as compared to those who did not participate. Therefore, generalizing the findings of this study to a large population needs to be performed carefully, as described previously [40]. Second, these diabetic biomarkers were measured only once at the baseline. We do not have information regarding the onset of DM in those with high-level diabetic biomarkers, so we cannot speculate regarding the length of suffering attributable to DM. Moreover, given that the follow-up of the subjects lasted for many years, it is possible that these levels might have changed over the course of the years. However, this is not different between cases and controls and likely would have led to underestimation of the results. Third, it is difficult to completely exclude undiagnosed gastric cancer at the baseline survey because past history of gastric cancer was based on self-administered questionnaire. However, when we excluded those subjects who developed gastric cancer within 2 years of blood donation based on the cancer registry, similar associations were obtained. Fourth, with regard to asking past history of DM, we did not distinguish between type 1 and type 2 DM in the questionnaire. However, because type 1 DM is far less

frequent then type 2 DM, especially in the adult population, it would be reasonable to suppose that most of the subjects had type 2 DM. Fifth, we did not have data regarding HbA1c or adequate samples to measure HbA1c. HbA1c levels reflect mean blood glucose over the preceding 3 months. Thus, it is possible that we might have missed subjects who were pre-diabetic or subjects with optimal blood glucose control. Sixth, the proportion of the subjects in the non-fasting group was much higher than that in the fasting group, which may have an effect on the validity of our observations. Therefore, caution should be used when interpreting the results. Finally, the number of subjects may not have been sufficient to identify the association in some anatomic sites or histological types. Therefore, additional large prospective studies are needed to confirm the association in cardia subsite and diffuse type gastric cancer.

In conclusion, our findings suggest that Japanese population with higher insulin and C-peptide levels derived from insulin resistance have an elevated risk of gastric cancer.

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Appendix

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Table 1. Baseline characteristics of cases and controls

Characteristics	Cases	Controls	*p* value[1]
n	477	477	
Age, mean (SD)	57.2 (7.19)	57.2 (7.21)	Matching value
Men (%)	319 (66.9)	319 (66.9)	Matching value
Smoking status			
Never smoker (%)	218 (45.7)	237 (49.7)	
Past smoker (%)	88 (18.5)	93 (19.5)	
Current ≤20 cigarettes/day (%)	132 (27.7)	106 (22.2)	
Current ≥21 cigarettes/day (%)	39 (8.1)	41 (8.6)	0.28
Alcohol consumption			
Never or occasional (%)	229 (48.0)	236 (49.5)	
≥1 day, <300 g/week (%)	185 (38.8)	194 (40.7)	
≥1 day, ≥300 g/week (%)	63 (13.2)	47 (9.8)	0.27
BMI (kg/m^2)[2]			
BMI<22 (%)	169 (35.7)	158 (33.3)	
22≤BMI<25 (%)	207 (43.8)	198 (41.7)	
25≤BMI (%)	97 (20.5)	119 (25.0)	0.25
Family history of gastric cancer (%)	58 (12.2)	39 (8.2)	0.04
Past history of DM (%)	44 (9.2)	21 (4.4)	0.003
Drug treatment for DM (%)	15 (3.1)	8 (1.7)	0.14
Helicobacter pylori positive (%)[3]	449 (94.1)	357 (74.8)	<0.001
CagA positive (%)	359 (75.3)	335 (70.2)	0.08
Atrophy (%)[4]	390 (81.8)	278 (58.3)	<0.001

[1] Based on chi-square test or Student's *t*-test.

[2] Subjects for whom we were unable to calculate body mass index due to missing height or weight data (4 cases and 2 controls) were deleted.

[3] Based on immunoglobulin G antibody.

[4] Atrophy: positive if pepsinogen I ≤70 ng/ml and pepsinogen I/pepsinogen II ratio ≤3.

Abbreviations: BMI: body mass index; CagA: cytotoxin associated gene A; DM: diabetes mellitus; SD: standard deviation.

Table 2. ORs and 95% CIs for the association between plasma levels of diabetic biomarkers and gastric cancer risk

		Cases (*n*)/Controls (*n*)	OR1 (95%CI)[1]	OR2 (95% CI)[2]	OR3 (95% CI)[3]
Insulin (pg/ml)	Tertile 1 (10.7–228.7)	137/152	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (233.1–468.7)	163/153	1.25 (0.87–1.80)	1.63 (1.08–2.47)	1.68 (1.10–2.56)
	Tertile 3 (471.0–7933.3)	157/152	1.36 (0.88–2.11)	1.91 (1.15–3.18)	2.03 (1.21–3.41)
	p for trend		0.17	0.01	0.007
C-peptide (pg/ml)	Tertile 1 (130.5–653.6)	160/158	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (659.7–1292.8)	160/159	0.99 (0.70–1.40)	1.15 (0.77–1.71)	1.15 (0.77–1.72)
	Tertile 3 (1303.0–8739.4)	155/158	1.02 (0.68–1.55)	1.31 (0.82–2.11)	1.30 (0.81–2.10)
	p for trend		0.92	0.26	0.28
Blood glucose (mg/dl)	Tertile 1 (72.0–92.0)	138/124	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (93.0–106.0)	114/124	0.81 (0.55–1.18)	1.01 (0.66–1.55)	0.98 (0.63–1.50)
	Tertile 3 (107.0–406.0)	121/125	0.85 (0.57–1.29)	0.96 (0.61–1.53)	0.84 (0.52–1.36)
	p for trend		0.41	0.88	0.50

[1] Matched for age (±3 years), gender, public health center area, blood donation date (±2 months), and fasting time at blood donation (±5 hours).

[2] Adjusted for smoking, alcohol consumption, body mass index, total calories, salt intake, family history of gastric cancer, *Helicobacter pylori* infection status, and atrophy.

[3] Further adjusted for past history of diabetes mellitus and drug treatment for diabetes mellitus.

Abbreviations: CI: confidence interval; OR: odds ratio.

Table 3. ORs and 95% CIs for the association between plasma levels of diabetic biomarkers and gastric cancer risk in men and women

		Cases (*n*)/Controls (*n*)	OR1 (95% CI)[1]	OR2 (95% CI)[2]	OR3 (95% CI)[3]
Men					
Insulin (pg/ml)	Tertile 1 (10.7–224.3)	92/102	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (226.4–491.0)	108/103	1.29 (0.82–2.03)	1.76 (1.00–3.09)	1.75 (0.99–3.10)
	Tertile 3 (495.9–7933.3)	107/102	1.50 (0.87–2.60)	2.43 (1.23–4.78)	2.49 (1.25–4.96)
	p for trend		0.15	0.01	0.01
C-peptide (pg/ml)	Tertile 1 (130.5–643.1)	95/106	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (644.2–1380.9)	111/106	1.25 (0.82–1.90)	1.39 (0.83–2.30)	1.43 (0.86–2.40)
	Tertile 3 (1388.3–8739.4)	112/106	1.42 (0.85–2.38)	1.90 (1.04–3.48)	1.96 (1.06–3.64)
	p for trend		0.18	0.04	0.03
Blood glucose (mg/dl)	Tertile 1 (73.0–94.0)	91/87	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (95.0–108.0)	70/81	0.81 (0.51–1.29)	0.91 (0.53–1.57)	0.92 (0.54–1.59)
	Tertile 3 (109.0–406.0)	89/82	1.07 (0.66–1.74)	1.18 (0.67–2.08)	1.02 (0.57–1.83)
	p for trend		0.85	0.59	0.98
Women					
Insulin (pg/ml)	Tertile 1 (41.1–238.4)	49/50	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (239.8–429.1)	54/50	1.05 (0.57–1.93)	1.44 (0.71–2.94)	1.61 (0.77–3.37)
	Tertile 3 (430.1–5237.4)	47/50	0.91 (0.45–1.84)	1.08 (0.48–2.46)	1.27 (0.54–3.00)
	p for trend		0.79	0.81	0.56
C-peptide (pg/ml)	Tertile 1 (158.2–679.1)	69/52	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (685.7–1181.6)	43/53	0.44 (0.22–0.88)	0.58 (0.27–1.26)	0.54 (0.25–1.20)
	Tertile 3 (1183.2–3496.9)	45/52	0.46 (0.22–0.97)	0.59 (0.25–1.39)	0.58 (0.25–1.38)
	p for trend		0.04	0.23	0.23
Blood glucose (mg/dl)	Tertile 1 (72.0–90.0)	50/41	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (91.0–103.0)	37/42	0.69 (0.36–1.35)	0.89 (0.41–1.97)	0.88 (0.39–1.98)
	Tertile 3 (104.0–235.0)	36/40	0.69 (0.32–1.51)	0.59 (0.22–1.57)	0.48 (0.17–1.33)
	p for trend		0.29	0.32	0.19

[1] Matched for age (±3 years), public health center area, blood donation date (±2 months), and fasting time at blood donation (±5 hours).

[2] Adjusted for smoking, alcohol consumption, body mass index, total calories, salt intake, family history of gastric cancer, *Helicobacter pylori* infection status, and atrophy.

[3] Further adjusted for past history of diabetes mellitus and drug treatment for diabetes mellitus.

Abbreviations: CI: confidence interval; OR: odds ratio.

Table 4. ORs and 95% CIs by fasting status for the association between insulin, C-peptide, HOMA-IR, and HOMA-β and gastric cancer risk

		Cases (*n*)/Controls (*n*)	OR1 (95%CI)[1]	OR2 (95%CI)[2]	OR3 (95%CI)[3]
Non-fasting group[4]					
Insulin (pg/ml)	Tertile 1 (92.3–366.5)	92/86	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (367.4–621.1)	81/87	0.84 (0.51–1.36)	1.07 (0.58–1.98)	1.03 (0.56–1.91)
	Tertile 3 (628.1–7933.3)	86/86	0.94 (0.56–1.59)	1.26 (0.66–2.42)	1.21 (0.63–2.32)
	p for trend		0.84	0.47	0.56
C-peptide (pg/ml)	Tertile 1 (140.4–1012.2)	93/89	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (1022.3–1755.5)	87/89	0.94 (0.57–1.54)	1.29 (0.72–2.30)	1.26 (0.70–2.27)
	Tertile 3 (1762.0–8739.4)	87/89	0.96 (0.56–1.64)	1.52 (0.79–2.93)	1.54 (0.79–2.98)
	p for trend		0.89	0.21	0.20
Fasting group[4]					
Insulin (pg/ml)	Tertile 1 (10.7–179.5)	51/62	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (180.3–283.3)	72/63	1.42 (0.84–2.41)	1.62 (0.89–2.93)	1.58 (0.87–2.88)
	Tertile 3 (286.0–4457.3)	65/63	1.35 (0.76–2.40)	1.84 (0.93–3.63)	1.89 (0.95–3.77)
	p for trend		0.31	0.08	0.07
C-peptide (pg/ml)	Tertile 1 (130.5–493.6)	54/65	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (497.5–755.4)	78/66	1.39 (0.86–2.26)	1.68 (0.95–2.97)	1.80 (1.00–3.24)
	Tertile 3 (776.0–2717.4)	65/66	1.23 (0.72–2.08)	1.80 (0.92–3.53)	1.76 (0.89–3.47)
	p for trend		0.46	0.09	0.10
HOMA-IR[5]	<1.73	96/104	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	≥1.73	60/52	1.29 (0.79–2.11)	1.88 (1.03–3.45)	1.97 (1.07–3.65)
HOMA-β (%)[5]	Tertile 1 (17.6–52.7)	41/52	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (53.3–89.0)	58/52	1.49 (0.82–2.69)	1.34 (0.67–2.67)	1.45 (0.71–2.93)
	Tertile 3 (89.3–1580.9)	57/52	1.47 (0.81–2.66)	1.60 (0.81–3.14)	1.94 (0.94–4.03)
	p for trend		0.23	0.17	0.08

[1] Matched for age (±3 years), gender, public health center area, and blood donation date (±2 months).

[2] Adjusted for smoking, alcohol consumption, body mass index, total calories, salt intake, family history of gastric cancer, *Helicobacter pylori* infection status, and atrophy.

[3] Further adjusted for past history of diabetes mellitus and drug treatment for diabetes mellitus.

[4] Fasting group: ≥8 hours after a meal; Non-fasting group: <8 hours after a meal.

[5] Subjects under drug treatment for diabetes mellitus were excluded, and OR3 was further adjusted for past history of diabetes mellitus only.

Abbreviations: HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-β: homeostasis model assessment of β-cell function; CI: confidence interval; OR: odds ratio.