A possible mechanism for the carcinogenicity of *Helicobacter pylori* (*H. pylori*), an established cause of gastric cancer [23], is that it elicits an intense inflammatory response, which leads to DNA damage from production of reactive oxygen species and nitric oxide [24]. Vegetables and fruits contain many antioxidants, such as carotenoids, vitamin C, vitamin E compounds, and phenolics [25]. It has been suggested that these substances scavenge potentially mutagenic free radicals [26] and induce production of detoxification enzymes [25]. Therefore, vegetable and fruit intake might counteract DNA damage caused by *H. pylori*. We found a significant inverse association between total vegetable and green–yellow vegetable intakes and distal gastric cancer in men. This observation does not contradict the above putative mechanism, although a recent meta-analysis identified an association between *H. pylori* infection and gastric cancer for both non-cardia and cardia cancers in high-risk settings [27].

We did not find a clear association of total vegetable and green–yellow vegetable intakes with distal gastric cancer in women. A possible explanation for this sex difference is the relatively higher intake of vegetables among women [29, 30]. For example, the percentage of men (25%) who ate carrots and pumpkins one to two times a day or less per week was twice as high as that among women (12%) in JACC [28]. If the protective effect of vegetables and fruit on gastric cancer has a threshold, an inverse association would not be observed among a population in which most individuals had vegetable and fruit intakes above a certain level. This hypothesis is supported by the results of a nested case–control study in the JPHC, which reported that the plasma level of β-carotene was lower among men than among women and that it was inversely associated with gastric cancer risk only among men [30].

Among women, we found an inverse association of total fruit intake with differentiated gastric cancer. Only two previous prospective studies reported gastric cancer results by histologic type [5, 11]. Chyou et al. studied a cohort of Japanese American men in Hawaii and found that total intakes of vegetables and fruit were associated with significant and nonsignificant reductions in the risk of intestinal gastric cancer (differentiated cancer in the current study), respectively. These results were consistent with those of a meta-analysis of case–control studies [4]. In contrast, the EPIC-EUROGAST study showed that total fresh fruit intake was inversely associated with diffuse gastric cancer [5]. Clearly, further study of associations with gastric cancer histology is needed.

The limitations of our study warrant mention. First, we had no information on *H. pylori* infection. A large majority of the population in Japan may have been infected by *H. pylori* at the time of the baseline surveys of the cohorts included in the current analysis. In fact, seropositivity for *H. pylori* immunoglobulin G (IgG) or CagA IgG antibody was 98.8% among cases and 90.0% among controls in a nested case–control study using baseline data and blood samples from the JPHC [31]. If the proportions were similar for the cohort members included in the current pooled analysis, it is unlikely that adjustment for *H. pylori* infection status would be informative. Secondly, we used only baseline information on vegetable and fruit intake and thus could not investigate associations of lifetime intake or changes in intake during follow-up with gastric cancer risk. Thirdly, although we added factors that might be related to gastric cancer risk to the multivariate model used to investigate the independent association between vegetable/fruit intake and gastric cancer risk, we cannot completely exclude the possibility of residual confounding by unmeasured factors. Furthermore, although we used single dietary components (i.e. vegetable/fruit) as exposures in the current analyses, it is also important to consider dietary patterns in gastric cancer prevention, because actual diets comprise a variety of foods rather than a single food [32]. Finally, estimated absolute values of the cutoffs used for quintiles of vegetable/fruit intake varied among studies (supplementary Table S4, available at *Annals of Oncology* online). Differences in FFQs might be responsible for variability in estimated intakes from FFQs. If the estimated absolute value of intake reflected a difference in true intake, the pooled HR could be biased. However, on the basis of findings for mean intakes of vegetable/fruit by district in Japan, we assumed that distributions of vegetable/fruit would not differ greatly [33]. Therefore, we believe that quintiles of vegetable/fruit intake are comparable among studies.

Our study had several strengths. First, we analyzed data from large-scale population-based cohort studies that used validated questionnaires to collect data on vegetable and fruit intake. Secondly, each study controlled for a common set of available variables that are considered to be potential confounders of the association between vegetable/fruit intake and gastric cancer risk. Thirdly, by pooling data, we could conduct analyses by sex and cancer subsite and histology.

In conclusion, this pooled analysis of data from large prospective studies in Japan suggests that vegetable intake reduces the risk of gastric cancer, especially the risk of distal gastric cancer among men.

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disclosure

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references

1. Parkin DM, Bray F, Ferlay J et al. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74–108.
2. Lunet N, Lacerda-Vieira A, Barros H. Fruit and vegetables consumption and gastric cancer: a systematic review and meta-analysis of cohort studies. Nutr Cancer 2005; 53: 1–10.
3. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC: AICR 2007.
4. Lunet N, Valbuena C, Vieira AL et al. Fruit and vegetable consumption and gastric cancer by location and histological type: case-control and meta-analysis. Eur J Cancer Prev 2007; 16: 312–327.
5. Gonzalez CA, Lujan-Barroso L, Bueno-de-Mesquita HB et al. Fruit and vegetable intake and the risk of gastric adenocarcinoma: a reanalysis of the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study after a longer follow-up. Int J Cancer 2012; 131: 2910–2919.
6. Steevens J, Schouten LJ, Goldbohm RA et al. Vegetables and fruits consumption and risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study. Int J Cancer 2011; 129: 2681–2693.
7. Epplein M, Shu XO, Xiang YB et al. Fruit and vegetable consumption and risk of distal gastric cancer in the Shanghai Women's and Men's Health studies. Am J Epidemiol 2010; 172: 397–406.
8. Freedman ND, Subar AF, Hollenbeck AR et al. Fruit and vegetable intake and gastric cancer risk in a large United States prospective cohort study. Cancer Causes Control 2008; 19: 459–467.
9. Larsson SC, Bergkvist L, Wolk A. Fruit and vegetable consumption and incidence of gastric cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 2006; 15: 1998–2001.
10. Kobayashi M, Tsubono Y, Sasazuki S et al. Vegetables, fruit and risk of gastric cancer in Japan: a 10-year follow-up of the JPHC Study Cohort I. Int J Cancer 2002; 102: 39–44.
11. Chyou PH, Nomura AM, Hankin JH et al. A case-cohort study of diet and stomach cancer. Cancer Res 1990; 50: 7501–7504.
12. Galanis DJ, Kolonel LN, Lee J et al. Intakes of selected foods and beverages and the incidence of gastric cancer among the Japanese residents of Hawaii: a prospective study. Int J Epidemiol 1998; 27: 173–180.
13. Tsugane S, Sobue T. Baseline survey of JPHC study—design and participation rate. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol 2001; 11: S24–S29.
14. Tamakoshi A, Yoshimura T, Inaba Y et al. Profile of the JACC study. J Epidemiol 2005; 15(Suppl 1): S4–S8.
15. Tsuji I, Nishino Y, Tsubono Y et al. Follow-up and mortality profiles in the Miyagi Cohort Study. J Epidemiol 2004; 14(Suppl 1): S2–S6.
16. Tokui N, Yoshimura T, Fujino Y et al. Dietary habits and stomach cancer risk in the JACC Study. J Epidemiol 2005; 15(Suppl 2): S98–108.
17. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986; 124: 17–27.
18. World Health Organization. International Classification of Diseases for Oncology. Geneva: WHO 2000.
19. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. 10th Revision. Geneva: World Health Organization 1992.
20. Inoue M, Sasazuki S, Wakai K et al. Green tea consumption and gastric cancer in Japanese: a pooled analysis of six cohort studies. Gut 2009; 58: 1323–1332.
21. Lauren 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.
22. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7: 177–188.
23. International Agency for Research on Cancer. Schistosomes, Liver Flukes and Helicobactor Pylori. Lyon, France: IARC 1994.
24. De Luca A, Iaquinto G. Helicobacter pylori and gastric diseases: a dangerous association. Cancer Lett 2004; 213: 1–10.
25. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control 1991; 2: 427–442.
26. Drake IM, Davies MJ, Mapstone NP et al. Ascorbic acid may protect against human gastric cancer by scavenging mucosal oxygen radicals. Carcinogenesis 1996; 17: 559–562.
27. Cavaleiro-Pinto M, Peleteiro B, Lunet N et al. Helicobacter pylori infection and gastric cardia cancer: systematic review and meta-analysis. Cancer Causes Control 2011; 22: 375–387.
28. Iso H, Date C, Noda H et al. Frequency of food intake and estimated nutrient intake among men and women: the JACC Study. J Epidemiol 2005; 15(Suppl 1): S24–S42.
29. Tsugane S, Sasaki S, Kobayashi M et al. Dietary habits among the JPHC study participants at baseline survey. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol 2001; 11: S30–S43.
30. Persson C, Sasazuki S, Inoue M et al. Plasma levels of carotenoids, retinol and tocopherol and the risk of gastric cancer in Japan: a nested case-control study. Carcinogenesis 2008; 29: 1042–1048.
31. Sasazuki S, Inoue M, Iwasaki M et al. Effect of Helicobacter pylori infection combined with CagA and pepsinogen status on gastric cancer development among Japanese men and women: a nested case-control study. Cancer Epidemiol Biomarkers Prev 2006; 15: 1341–1347.
32. Bertuccio P, Rosato V, Andreano A et al. Dietary patterns and gastric cancer risk: a systematic review and meta-analysis. Ann Oncol 2013; 24: 1450–1458.
33. Ministry of Health and Welfare. The National Nutrition Survey in Japan, 1996 (in Japanese). Tokyo: Daiichi-Shuppan 1998.

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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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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 hr 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.

Epidemiology

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]

Key words: gastric cancer risk, plasma insulin, plasma C-peptide, plasma blood glucose, prospective study

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?
The idea that diabetes mellitus may play a role in some instances of gastric carcinogenesis is intriguing but controversial. Here, a positive association was identified for gastric cancer risk and plasma insulin levels, based on investigation of plasma biomarkers in a Japanese study population. The association was evident for measures of homeostasis model assessment of insulin resistance (HOMA-IR). By contrast, no association was found for blood glucose levels. The results suggest that hyperinsulinemia derived from insulin resistance, rather than hyperglycemia, is important in gastric carcinogenesis.

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, 1,423 (3.9%) moved outside the study area, 1,610 (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

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 (four cases and two controls) were deleted.
[3]Based on immunoglobulin G antibody.
[4]Atrophy: positive if pepsinogen I ≤ 70 ng ml^{-1} and pepsinogen I/pepsinogen II ratio ≤3.
Abbreviations: BMI: body mass index; CagA: cytotoxin associated gene A; DM: diabetes mellitus; SD: standard deviation.

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 were 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 hr), 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, MO). 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} (1 pmol l^{-1}) for insulin and 3.6 pg ml^{-1} (1 pmol l^{-1}) 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 hr). 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

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^{-1})	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^{-1})	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^{-1})	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 hr).
[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.

ethanol per week. Total calorie and salt intakes were treated as continuous variables. BMI was divided into three classes: BMI < 22 kg m^{-2}, $22 \leq$ BMI < 25, and $25 \leq$ 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 $\geq$10 U ml^{-1} or cytotoxin associated gene A (CagA) antibody >10. Atrophy was regarded as positive if pepsinogen I was $\leq$70 ng ml^{-1} and the pepsinogen I/pepsinogen II ratio was $\leq$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 $\leq$3 identified gastric atrophy with a sensitively of 71.7% and a specificity of 66.7%.[25] We believe that the pepsinogen data could explain the level of atrophy, to some extent, if added to the model. Past history of DM and drug treatment for DM were considered positive if subjects were diagnosed with DM before and used a diabetic drug at the time of the baseline survey, respectively. Stratified analysis based on fasting status ($\geq$8 hr or <8 hr after a meal) was also conducted for each plasma diabetic biomarker. Furthermore, for the subjects who were in the fasting group ($\geq$8 hr after a meal) at blood donation and not under drug treatment for DM, we calculated HOMA-IR [fasting plasma insulin level (μU ml^{-1}) × fasting plasma glucose level (mg dl^{-1})/405] and HOMA-β [360 × fasting plasma insulin level (μU ml^{-1})/fasting plasma glucose level (mg dl^{-1}) − 63].[19] HOMA-IR $\geq$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^{-1} = 35 pg ml^{-1}).

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, Cary, NC).

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

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^{-1})	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^{-1})	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^{-1})	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^{-1})	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^{-1})	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^{-1})	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 hr).
[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.

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 hr 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 hr and <8 hr 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 hr after a meal). For the non-fasting group (<8 hr 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

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^{-1})	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^{-1})	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^{-1})	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^{-1})	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 hr after a meal; Non-fasting group: <8 hr 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.

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 hr 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 hr 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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References

1. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108.
2. Onitilo AA, Engel JM, Glurich I, et al. Diabetes and cancer I: risk, survival, and implications for screening. *Cancer Causes Control* 2012;23:967–81.
3. 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;89:1360–5.
4. Inoue M, Iwasaki M, Otani T, et al. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006;166:1871–7.
5. Lin SW, Freedman ND, Hollenbeck AR, et al. Prospective study of self-reported diabetes and risk of upper gastrointestinal cancers. *Cancer Epidemiol Biomarkers Prev* 2011;20:954–61.
6. Carstensen B, Witte DR, Friis S. Cancer occurrence in Danish diabetic patients: duration and insulin effects. *Diabetologia* 2012;55:948–58.
7. Wotton CJ, Yeates DG, Goldacre MJ. Cancer in patients admitted to hospital with diabetes mellitus aged 30 years and over: record linkage studies. *Diabetologia* 2011;54:527–34.
8. Ogunleye AA, Ogston SA, Morris AD, et al. A cohort study of the risk of cancer associated with type 2 diabetes. *Br J Cancer* 2009;101:1199–201.
9. Chodick G, Heymann AD, Rosenmann L, et al. Diabetes and risk of incident cancer: a large population-based cohort study in Israel. *Cancer Causes Control* 2010;21:879–87.
10. Khan M, Mori M, Fujino Y, et al. Site-specific cancer risk due to diabetes mellitus history: evidence from the Japan Collaborative Cohort (JACC) Study. *Asian Pac J Cancer Prev* 2006;7: 253–9.
11. Adami HO, McLaughlin J, Ekbom A, et al. Cancer risk in patients with diabetes mellitus. *Cancer Causes Control* 1991;2:307–14.
12. Atchison EA, Gridley G, Carreon JD, et al. Risk of cancer in a large cohort of US veterans with diabetes. *Int J Cancer* 2011;128:635–43.
13. Ikeda F, Doi Y, Yonemoto K, et al. Hyperglycemia increases risk of gastric cancer posed by *Helicobacter pylori* infection: a population-based cohort study. *Gastroenterology* 2009;136:1234–41.
14. 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.
15. 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: 945–52.
16. Jun JK, Gwack J, Park SK, et al. Fasting serum glucose level and gastric cancer risk in a nested case-control study. *J Prev Med Public Health* 2006;39:493–8 (In Korean with English abstract).
17. 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;37:2257–63.
18. Kasuga M, Ueki K, Tajima N, et al. Report of the Japan diabetes society/japanese cancer association joint committee on diabetes and cancer. *Cancer Sci* 2013;104:965–76.
19. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–9.
20. Watanabe S, Tsugane S, Sobue T, et al. Study design and organization of the JPHC study. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. *J Epidemiol* 2001;11:S3–S7.
21. Iida M, Sato S, Nakamura M. Standardization of laboratory test in the JPHC study. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. *J Epidemiol* 2001;11: S81–S6.
22. WHO. International classification of diseases for oncology. Geneva, Switzerland: World Health Organization, 2000.
23. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. *Acta Pathol Microbiol Scand* 1965;64:31–49.
24. Miki K, Morita M, Sasajima M, et al. Usefulness of gastric cancer screening using the serum pepsinogen test method. *Am J Gastroenterol* 2003;98: 735–9.
25. Urita Y, Hike K, Torii N, et al. Serum pepsinogens as a predicator of the topography of intestinal metaplasia in patients with atrophic gastritis. *Dig Dis Sci* 2004;49:795–801.
26. Ohnishi H, Saitoh S, Takagi S, et al. Incidence of insulin resistance in obese subjects in a rural Japanese population: the Tanno and Sobetsu study. *Diabetes Obes Metab* 2005;7:83–7.
27. Giovannucci E, Harlan DM, Archer MC, et al. Diabetes and cancer: a consensus report. *Diabetes Care* 2010;33:1674–85.
28. Stocks T, Rapp K, Bjørge T, et al. Blood glucose and risk of incident and fatal cancer in the metabolic syndrome and cancer project (me-can): analysis of six prospective cohorts. *PLoS Med* 2009;6:e1000201.
29. Pisani P. Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies. *Arch Physiol Biochem* 2008;114:63–70.
30. Wolpin BM, Bao Y, Qian ZR, et al. Hyperglycemia, insulin resistance, impaired pancreatic β-cell function, and risk of pancreatic cancer. *J Natl Cancer Inst* 2013;105:1027–35.
31. Ollberding NJ, Cheng I, Wilkens LR, et al. Genetic variants, prediagnostic circulating levels of insulin-like growth factors, insulin, and glucose and the risk of colorectal cancer: the Multiethnic Cohort study. *Cancer Epidemiol Biomarkers Prev* 2012;21:810–20.
32. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. *J Nutr* 2001;131:3109S–20S.
33. Camargo MC, Goto Y, Zabaleta J, et al. Sex hormones, hormonal interventions, and gastric cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2012;21:20–38.
34. Key TJ, Allen NE, Verkasalo PK, et al. Energy balance and cancer: the role of sex hormones. *Proc Nutr Soc* 2001;60:81–9.
35. Lindtner C, Scherer T, Zielinski E, et al. Binge drinking induces whole-body insulin resistance by impairing hypothalamic insulin action. *Sci Transl Med* 2013;5:170ra14.
36. Facchini FS, Hollenbeck CB, Jeppesen J, et al. Insulin resistance and cigarette smoking. *Lancet* 1992;339:1128–30.
37. Butler AE, Janson J, Bonner-Weir S, et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003;52: 102–10.
38. Polyzos SA, Kountouras J, Zavos C, et al. The association between *Helicobacter pylori* infection and insulin resistance: a systematic review. *Helicobacter* 2011;16:79–88.
39. Polyzos SA, Kountouras J, Zavos C, et al. Comment on: Jeon et al. *Helicobacter pylori* infection is associated with an increased rate of diabetes. *Diabetes Care* 2012;35:520–525; e53; author reply e54.
40. Iwasaki M, Otani T, Yamamoto S, et al. Background characteristics of basic health examination participants: the JPHC Study Baseline Survey. *J Epidemiol* 2003;13:216–25.

Appendix

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Public Health Report

Diabetes Mellitus and Liver Cancer Risk: An Evaluation Based on a Systematic Review of Epidemiologic Evidence among the Japanese Population

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Objective: The potential associations of diabetes mellitus with malignant neoplasms including liver cancer have become a great concern from both clinical and preventive perspectives. Although sufficient evidence for a positive association between diabetes and liver cancer already exists, it would be informative to summarize up-to-date epidemiologic data in Japan.
Methods: We systematically reviewed epidemiologic studies on diabetes and liver cancer among Japanese populations. Original data were obtained by searching the MEDLINE (PubMed) and *Ichushi* databases, complemented with manual searches. The evaluation was performed in terms of the magnitude of association in each study and the strength of evidence ('convincing', 'probable', 'possible' or 'insufficient'), together with biological plausibility.
Results: We identified 19 cohort studies, one pooled-analysis of seven cohort studies, and seven case–control studies. Of 24 relative risk estimates of liver cancer for diabetes reported in those cohort studies, 17 showed a weak to strong positive association, six revealed no association and one demonstrated a weak inverse association (summary relative risk 2.10, 95% confidence interval 1.60–2.76). Ten relative risk estimates from the case–control studies showed a weak to strong positive association ($n = 9$) or no association ($n = 1$; summary relative risk 2.32, confidence interval 1.73–3.12). Overall, the summary relative risk became 2.18 (confidence interval 1.78–2.69). Heterogeneity in relative risks was significant for the difference in categories of study population ($P = 0.01$), but not in study type ($P = 0.39$) or sex ($P = 0.33$).
Conclusions: Diabetes mellitus 'probably' increases the risk of liver cancer among the Japanese population.

Key words: liver cancer – diabetes – systematic review – epidemiology – Japanese

INTRODUCTION

The prevalence of diabetes mellitus has been increasing in Japan (1), and the potential associations of diabetes with major chronic diseases including malignant neoplasms have become a great concern from both clinical and preventive points of view. For primary liver cancer, most of which (>90%) comprises hepatocellular carcinoma (2), sufficient evidence already exists for a positive association with diabetes mellitus, as illustrated by several meta-analyses showing ~2–4-fold increase of summary relative risk (RR) in diabetic vs. non-diabetic individuals (3–7). Since the publication of these meta-analyses, however, relevant epidemiologic data including those in a large pooled analysis (8) have still been accumulating, particularly in Japan, and summarizing the most recent and previous data would be informative in considering the prevention of liver cancer in this country.

We aimed to review and summarize up-to-date epidemiologic findings on diabetes mellitus and liver cancer among the Japanese, whose dominant risk factors of liver cancer represent hepatitis C and B virus infection (2,9) and alcohol consumption (10). This work was conducted as part of a project of systematic evaluation of the epidemiological evidence regarding lifestyles and cancers in Japan (11).

PATIENTS AND METHODS

The details of the evaluation method have been described elsewhere (11). In brief, original data for this review were identified by searching the MEDLINE (PubMed) and *Ichushi* (*Japana Centra Revuo Medicina*) databases, complemented by manual searches of references from relevant articles where necessary. All epidemiologic studies on the association between diabetes mellitus and liver cancer incidence/mortality among the Japanese from 1950 (or 1983 for the *Ichushi* database) to March 2014, including papers in press if available, were identified using the search terms 'diabetes', 'liver neoplasms', 'hepatocellular', 'cohort', 'follow-up', 'case–control', 'Japan' and 'Japanese' as keywords. Papers written in either English or Japanese were reviewed, and only studies on Japanese populations living in Japan were included. The individual results were summarized in the tables separately by study design as cohort or case–control studies.

The evaluation was made based on the magnitude of association and the strength of evidence. First, the former was assessed by classifying the RR in each study into the following four categories, while considering statistical significance (SS) or no statistical significance (NS): (i) 'strong' (symbol ↓↓↓ or ↑↑↑) when RR < 0.5 (SS) or RR > 2.0 (SS); (ii) 'moderate' (symbol ↓↓ or ↑↑) when RR < 0.5 (NS), 0.5 ≤ RR < 0.67 (SS), 1.5 < RR ≤ 2.0 (SS) or RR > 2.0 (NS); (iii) 'weak' (symbol ↓ or ↑) when 0.5 ≤ RR < 0.67 (NS), 0.67 ≤ RR ≤ 1.5 (SS) or 1.5 < RR ≤ 2.0 (NS) and (iv) 'no association' (symbol –) when 0.67 ≤ RR ≤ 1.5 (NS); the RR used in this paper denotes ratio measures of effect, including risk ratios, rate ratios, hazard ratios and odds ratios. The ratios of observed to expected number of deaths, which were reported in early follow-up studies of only diabetic patients with a general population as a reference group, were also used although their nature was somewhat different from that of RRs. In the case of multiple publications of analyses of the same or overlapping datasets, only data from the largest or most updated results were included. Studies that reported RRs for impaired glucose tolerance only, or did not provide RRs or data necessary for the present authors to calculate relevant RRs were excluded.

After the above process, the strength of evidence was evaluated in a manner similar to that used in the WHO/FAO Expert Consultation Report, in which evidence was classified as 'convincing', 'probable', 'possible' and 'insufficient' (12). Biological plausibility was also taken into account for this evaluation. The final judgment was made based on a consensus of the research group members. When we reach a conclusion that there is 'convincing' or 'probable' evidence of an association, we conduct a meta-analysis to obtain summary estimates for the overall magnitude of association.

In meta-analyses of this paper, we estimated the summary RR of liver cancer for diabetes mellitus by using random effects models according to the method of DerSimonian and Laird because individual RRs across studies were significantly heterogeneous based on the Q statistic (13,14). We also performed random-effects meta-regression analyses with covariates of study type (two categories: cohort or case–control), sex (three categories: men, women or both) and study population (three categories: general population, diabetic patients or patients with chronic liver disease [CLD]) to explore a potential source of the above heterogeneity. The covariate for the difference in event (death or incidence) was not included in these analyses due to the limited number of RRs for liver cancer deaths. All statistical analyses were performed with the STATA statistical package (Stata Corp., College Station, TX, USA). Two-sided P values <0.05 were considered statistically significant.

RESULTS

We identified 19 cohort studies (15–33) and one pooled analysis including seven cohort studies (8) (Table 1) as well as seven case–control studies (34–40) (Table 2). For convenience, the pooled analysis (8) was treated as a single study hereafter. Of those cohort studies, three presented results by sex (8,15,33), two presented results for men only (20,23) and 15 presented results only for men and women combined (16–19,21,22,24–32). The respective numbers for the case–control studies are three (35,37,40), one (34) and three (36,38,39). In one cohort study (17), RRs were estimated separately for patients with chronic hepatitis and those with cirrhosis. As a result, 24 and 10 RR estimates in the cohort

Table 1. Cohort studies on diabetes mellitus and liver cancer among Japanese

Reference	Study period	Study population				Category	Number among cases	Relative risk (RR) (95% CI or *P*)	Confounding variables considered	Comments
		Number of subjects for analysis	Source of subjects	Event followed	Number of incident cases or deaths					
Tsukuma *et al.* (15)	1970–82	858 (484 Men and 374 women)	Diabetic patients admitted for education at Osaka Prefectural Hospital	Death	20 (19 Men and 1 woman)	O/E ratio for men	19	9.50 (5.72–14.84)	Age and observation period	The 95% CIs were not described in the original paper and were estimated by one of the authors (K.T.).
						O/E ratio for women	1	1.49 (0.038–8.32)		HBsAg and anti-HCV were not tested.
Sasaki *et al.* (16)	1960–93	1939 (1200 Men and 739 women)	Patients with NIDDM at Osaka Seijinbyo Center	Death	73	O/E ratio for liver cancer	73	3.02 (2.37–3.80)	Sex, age and observation period	The 95% CI was not described in the original paper and were estimated by one of the authors (K.T.).
										HBsAg and anti-HCV were not tested.
Kato *et al.* (17)	?–1995	542 (329 Men and 213 women)	Patients with chronic hepatitis or cirrhosis due to hepatitis B or C virus infection	Incidence	Not described	Chronic hepatitis ($n = 355$)			No adjustment	The RRs and 95% CIs were not described in the original paper and were estimated by one of the authors (K.T.).
						No diabetes ($n = 30$)		1.00		
						Diabetes ($n = 325$)		1.73 (0.42–7.15)		
						Liver cirrhosis ($n = 187$)				All patients were HBsAg-positive and/or anti-HCV positive.
						No diabetes ($n = 39$)		1.00		
						Diabetes ($n = 148$)		1.17 (0.78–1.75)		
Tazawa *et al.* (18)	1987–?	279 (190 Men and 89 women)	HCV-infected patients with chronic hepatitis without cirrhosis at Tsuchiura Kyodo General Hospital	Incidence	13 (11 Men and 2 women)	No diabetes ($n = 256$)		1.00	No adjustment	The age-adjusted RR was 9.4 ($P = 0.002$), but its CI was not shown.
						Diabetes ($n = 23$)		5.68 (1.80–18.18)		All patients were anti-HCV and HCV-RNA positive.

Ohata *et al.* (19)	1980–2000	161 (106 Men and 55 women)	Patients with chronic hepatitis or cirrhosis due to HCV infection	Incidence	70	No diabetes		1.00	Sex, age, body mass index, drinking, ALT, HCV serotype, HCV core titer, interferon treatment, cirrhosis, histological grading and steatosis	All patients were anti-HCV-positive and HBsAg-negative.
						Diabetes		1.58 (0.62–3.99)		
Uetake *et al.* (20)	1988–2000	91 Men	Patients with HBsAg(−) anti-HCV(−) alcoholic cirrhosis at Jikei University Hospital	Incidence	13 Men	No diabetes	10	1.00	No adjustment	The RR and 95% CI were not described in the original paper and were estimated by one of the authors (K.T.).
						Diabetes	3	0.75 (0.22–2.51)		All patients were HBsAg-negative, anti-HCV-negative, and alcoholic.
Khan *et al.*(21)	1977–2002	1989 (908 Men and 1081 women)	Residents of Tanno and Sohbetsu towns of Hokkaido	Death	8 (6 Men and 2 women)	Normal	1	1.00	Sex, age, albumin and hypertension treatment	HBsAg and anti-HCV were not tested.
						IGT	5	11.36 (1.31–98.38)		
						Diabetes	2	3.38 (0.30–38.73)		
Muto *et al.* (22)	Not described	622 (294 Men and 328 women)	Patients with decompensated cirrhosis who had hypoalbuminemia	Incidence	89	No diabetes		1.00	Treatment group (BCAA supplementation and diet therapy)	Anti-HCV and, probably, HBsAg status was available but was not adjusted for.
						Diabetes		1.57 (1.00–2.45)		
Torisu *et al.* (23)	1978–2005	47 Men	Patients with alcoholic cirrhosis at Toranomon Hospital	Incidence	9 Men	No diabetes	4	1.0	Age	All patients were HBsAg-negative, anti-HCV-negative, and alcoholic.
						Diabetes	5	21.7 (2.4–193.7)		
Ohki *et al.* (24)	1994–2006	1431 (727 Men and 704 women)	Patients with positive HCV-RNA at Tokyo University Hospital	Incidence	340	No diabetes		1.00	Age, sex, alcohol, body mass index, serum albumin, bilirubin, ALT, prothrombin time, platelets and alpha-fetoprotein	All subjects were anti-HCV-positive and HBsAg-negative.
						Diabetes		1.26 (0.92–1.71)		
Tomiyama *et al.* (25)	1989–2007	95 (19 Men and 76 women)	Patients with primary biliary cirrhosis at Kawasaki Medical School Hospital	Incidence	7 (3 Men and 4 women)	No diabetes		1.00	Age, history of blood transfusion, platelet count and Scheuer's histological classification	All subjects were negative for hepatitis B and C virus markers.
						Diabetes		4.54 (0.48–42.93)		
Ikeda *et al.* (26)	1976–2004	82 (67 Men and 15 women)	Patients with non-B, non-C cirrhosis at Toranomon Hospital	Incidence	16	No diabetes		1.00	Sex, age, serum HBV-DNA and total alcohol intake	All subjects were HBsAg-negative and anti-HCV-negative.
						Diabetes		3.89 (1.22–12.47)		
Konishi *et al.* (27)	1992–?	197 (126 Men and 71 women)	Patients with HCV who had interferon therapy at Ehime University Hospital	Incidence	18	Based on 75 g OGTT			Age, hepatic fibrosis stage and γ-GTP	All subjects were anti-HCV-positive and HBsAg-negative.
						Normal/IGT		1.000		
						DM pattern		4.627 (1.677–12.766)		

Continued

Table 1. *Continued*

Reference	Study period	Study population				Category	Number among cases	Relative risk (RR) (95% CI or *P*)	Confounding variables considered	Comments
		Number of subjects for analysis	Source of subjects	Event followed	Number of incident cases or deaths					
Kurosaki *et al.* (28)	1994–?	1279 (643 Men and 636 women)	Patients with chronic hepatitis C who received interferon therapy at Musashino Red Cross Hospital	Incidence	68	No diabetes		1.00	Age, sex, stage of fibrosis, grade of steatosis, response to interferon, ethanol consumption and body mass index	All subjects were anti-HCV-positive and HBsAg-negative.
						Diabetes		0.75 (0.42–1.33)		
Kuroda *et al.* (29)	1998–?	133 (80 Men and 53 women)	Cirrhotic patients with HCV infection at Iwate Medical School	Incidence	60	No diabetes		1.00	No adjustment	All subjects were anti-HCV-positive and HBsAg-negative.
						Diabetes		0.91 (0.55–1.59)		
Takahashi *et al.* (30)	2002–?	203 (108 Men and 95 women)	HCV-positive patients who underwent liver biopsy and 75 g OGTT and who were treated with interferon	Incidence	13 (12 Men and 1 woman)	120 min post-challenge hyperglycemia (>200 mg/dl)			Sex, age, alcohol, response to interferon therapy, fibrosis stage, alpha-fetoprotein and steatosis	All subjects were anti-HCV-positive and HBsAg-negative.
						Absent	8	1.0		
						Present	5	19.5 (3.7–104.1)		
Kawamura *et al.* (31)	1997–?	6508 (5709 Men and 799 women)	Patients with non-alcoholic fatty liver disease at Toranomon Hospital	Incidence	16	No diabetes	9	1.00	Age, aspartate aminotransferase and platelet count	All subjects were anti-HCV-negative and HBsAg-negative.
						Diabetes	7	3.21 (1.09–9.50)		
Arase *et al.* (32)	1990–?	4302 (2528 Men and 1774 women)	HCV-positive patients with chronic hepatitis or cirrhosis who were treated with interferon at Toranomon Hospital	Incidence	393 (272 Men and 121 women)	No diabetes		1.00	Age, sex, total alcohol intake, presence of cirrhosis, and response to interferon therapy	All subjects were anti-HCV-positive and HBsAg-negative.
						Diabetes		1.73 (1.30–2.30)		
Nakamura *et al.* (33)	1992–2008	30 720 (14 173 Men and 16 547 women)	Residents of Takayama, Gifu prefecture	Incidence	176 (106 Men and 70 women)	For men			Age, smoking, body mass index, physical activity, education, histories of hypertension, stroke and ischemic heart disease, and intakes of total energy, fat, ethanol and coffee	HBsAg and anti-HCV were not evaluated.
						No diabetes	90	1.00		
						Diabetes	16	2.18 (1.27–3.74)		
						For women				
						No diabetes	68	1.00		
						Diabetes	2	0.65 (0.16–2.69)		

Sasazuki *et al.* (8)	1984–2009	308 739 (142 744 Men and 165 995 women)	Seven cohort studies in Japan (JPHC-I, JPHC-II, JACC, MIYAGI, Ohsaki, 3-pref Miyagi, 3-pref AICHI)	Incidence	1844 (1279 Men and 565 women)	For men			Study, age, area, history of cerebrovascular disease and coronary heart disease, smoking, alcohol, body mass index, physical activity, green leafy vegetables and coffee	HBsAg and anti-HCV were not evaluated.
						No diabetes	1078	1.00		
						Diabetes	201	2.07 (1.70–2.53)		
						For women				
						No diabetes	515	1.00		
						Diabetes	50	1.71 (1.14–2.57)		

CI, confidence interval; O/E ratio, ratio of observed to expected number; HBsAg, hepatitis B surface antigen; anti-HCV, antibody to hepatitis C virus; NIDDM, non-insulin-dependent diabetes mellitus; HCV, hepatitis C virus; HCV-RNA, hepatitis C virus RNA; ALT, alanine aminotransferase; IGT, impaired glucose tolerance; BCAA, branched-chain amino acids; HBV-DNA, hepatitis B virus DNA; OGTT, oral glucose tolerance test; DM, diabetes mellitus; γ-GTP, gamma-glutamyl transpeptidase; JPHC, Japan Public Health Center-based prospective Study; JACC, The Japan Collaborative Cohort Study; MIYAGI, The Miyagi Cohort Study; Ohsaki, The Ohsaki National Health Insurance Cohort Study; 3-pref MIYAGI, The Three Prefecture Study—Miyagi portion; 3-pref AICHI, The Three Prefecture Study—Aichi portion.

and case–control studies, respectively, were used for this evaluation.

Study populations in the cohort studies were classified broadly into three categories: apparently healthy subjects (local residents) from a general population (8,21,33) ($n = 3$), diabetic patients (15,16) ($n = 2$) and patients with CLD (17–20,22–32) ($n = 15$) (Table 1). Chronic infection with both hepatitis C virus (HCV) and hepatitis B virus (HBV) was taken into account in 13 cohort studies (18–20,23–32). In the case–control studies, a similar classification was possible based on the type of controls: apparently healthy subjects (local residents (34,35), first-visit cancer-free outpatients (37) or atomic bomb survivors (38)) ($n = 4$) and patients with CLD (36,39,40) ($n = 3$) (Table 2). Four case–control studies took into account both HCV and HBV infection (36,38–40).

A summary of the magnitude of association for the cohort studies and the case–control studies is shown in Tables 3 and 4, respectively. Of 24 RR estimates reported in 20 cohort studies, 10 (8,15,16,18,23,26,27,30,31,33) showed a strong positive association between diabetes and liver cancer, five (8,21,22,32) revealed a moderate positive association and two (17,19) demonstrated a weak positive association, while the remaining seven presented no association (15,17,20,24,28,29) or a weak inverse association (33). Of 10 RR estimates in seven case–control studies, nine (34–38,40) showed a weak to strong positive association and only one (39) presented no association.

Figure 1 illustrates a forest plot of the RRs of liver cancer for diabetes in individual studies and the corresponding summary RR. In this figure, sex-specific estimates are separately plotted. For both the cohort and case–control studies as well as all studies combined, the RRs were turned out to be significantly heterogeneous ($P < 0.001$, 0.011 and <0.001, respectively), and so the summary RRs were estimated by a random effects model. The summary RR was estimated as 2.10 (95% CI 1.60–2.76) and 2.32 (95% CI 1.73–3.12) for the cohort and case–control studies, respectively. The summary RR for all studies combined became 2.18 (95% CI 1.78–2.69).

To explore a potential source of the heterogeneity between studies, we carried out random-effects meta-regression analyses with covariates of study type, sex and study population (Table 5). Table 5 also presents the summary RR of liver cancer for diabetes in each subgroup by a random-effects model. No significant differences in RRs were evident between subgroups by study type ($\chi^2 = 0.75$ with 1 degree of freedom [DF], $P = 0.39$) or sex ($\chi^2 = 2.24$ with 2 DF, $P = 0.33$), but subgroups by study population revealed a significant difference ($\chi^2 = 8.96$ with 2 DF, $P = 0.01$). More specifically, the summary RR in the subgroup of diabetic patients was significantly higher than that in the subgroup of general population ($P = 0.004$) or CLD patients ($P = 0.01$). The residual I^2 statistic was 76% without any covariates and 63% with all covariates, and the model with all covariates showed an adjusted R^2 of 28% with an overall model P of 0.03.

Table 2. Case–control studies on diabetes mellitus and liver cancer among Japanese

Reference	Study period	Study subjects				Category	RR (95% CI or p)	Confounding variables considered	Comments
		Type and source	Definition	Number of cases	Number of controls				
Shibata *et al.* (34)	1992–95	Hospital-based (Kurume University Hospital)	Cases: confirmed as HCC by histological, angiographical and/or other findings;	115 Males	115 Male HCs and 115 male CCs	Based on CCs		Matched (1:1) for sex, age (±5 years for HCs and ±3 years for CCs), residence (for HCs), and time of hospitalization (for HCs) No adjustment.	The RR and 95% CI were not described in the original paper and were estimated by one of the authors (K.T.). Anti-HCV and HBsAg status was not available for CCs.
			Hospital controls (HCs): inpatients without chronic hepatitis or cirrhosis in two general hospitals in Kurume;			No diabetes	1.00		
			Community controls (CCs): randomly sampled citizens of Kurume			Diabetes	3.54 (1.63–7.67)		
Matsuo (35)	1995–2000	Hospital-based (Kurume University Hospital)	Cases: confirmed as HCC by histological, angiographical, and/or other findings;	222 (177 Men and 45 women)	326 HCs (177 men and 149 women) and 222 CCs (177 men and 45 women)	For males based on CCs		Matched for sex (1:4 for female HCs and 1:1 for other controls), age (±5 years for HCs and ±3 years for CCs), residence (for HCs) and time of hospitalization (for HCs) adjusted for matching factors, history of blood transfusion, smoking and drinking	Anti-HCV and HBsAg status was not available for CCs.
			HCs: inpatients without chronic hepatitis or cirrhosis in two general hospitals in Kurume;			No diabetes	1.00		
			CCs: randomly sampled citizens of Kurume			Diabetes	2.52 (1.27–5.02)		
						For females based on CCs			
						No diabetes	1.00		
						Diabetes	4.20 (0.81–21.81)		
Kabutake *et al.* (36)	1994–2006	Hospital-based (Tokyo Women's Medical University Hospital)	Cases: patients with alcoholic liver injury complicated with HCC;	96 (92 Men and 4 women)	65 (58 Men and 7 women)	No diabetes	1.00	No adjustment	The RR and 95% CI were not described in the original paper and were estimated by one of the authors (K.T.).
			Controls: patients with alcoholic cirrhosis without HCC			Diabetes	2.29 (1.20–4.37)		
Kuriki *et al.* (37)	1989–2000	Hospital-based (details not described)	Cases: patients with primary liver cancer (International Classification of Diseases, 10th revision: C22);	340 (265 Men and 75 women)	47 768 (14 199 men and 33 569 women)	For men		No matching adjusted for age, body mass index, drinking and smoking habits, physical exercise, bowel movement, family history of liver cancer, family history of diabetes, dietary restriction, raw vegetable intake, greasy food intake and snacking	Anti-HCV and HBsAg status was unknown.
			HCs: first- visit outpatients without past/present history of cancer			No diabetes	1.00		
						Diabetes	2.19 (1.56–3.07)		
						For women			
						No diabetes	1.00		
						Diabetes	2.26 (1.05–4.88)		

Ohishi *et al.* (38)	1970–2002	Nested case–control (atomic bomb survivors in Hiroshima and Nagasaki)	Cases: patients with incident HCC who had stored serum samples available; Controls: survivors without HCC who had stored serum samples available	224 (136 Men and 88 women)	644 (387 men and 257 women)	Diabetes 10 years before diagnosis No Yes	 1.00 1.98 (0.63–6.27)	Matched (1:3) for sex, age, city, time and method of serum storage and radiation exposure adjusted for matching factors, hepatitis virus infection, alcohol consumption, smoking, coffee, body mass index and radiation dose to the liver	HBsAg and anti-HCV status was adjusted for.
Taniguchi *et al.* (39)	Not described	Hospital-based (details not described)	Cases: patients with HCV-associated chronic hepatitis or cirrhosis with HCC; Controls: patients with HCV-associated chronic hepatitis or cirrhosis without HCC	230	219	No diabetes Diabetes	1.00 1.35 (0.93–1.95)	No adjustment	The RR and 95% CI were not described in the original paper and were estimated by one of the authors (K.T.). All subjects were anti-HCV-positive.
Horie *et al.* (40)	2007–08	Hospital-based (72 facilities throughout Japan)	Cases: patients with alcoholic cirrhosis with HCC; Controls: patients with alcoholic cirrhosis without HCC	243 Men and 22 women	509 men and 89 women	For men No diabetes Diabetes For women No diabetes Diabetes	 1.00 1.71 (1.26–2.33) 1.00 13.8 (4.65–40.7)	No adjustment No adjustment	The RRs and 95% CIs were not described in the original paper and were estimated by one of the authors (K.T.). All subjects were negative for HBsAg and anti-HCV.

RR, relative risk; CI, confidence interval; HCC, hepatocellular carcinoma; HCs, hospital controls; CCs, community controls; anti-HCV, antibody to hepatitis C virus; HBsAg, hepatitis B surface antigen.

Table 3. Summary of cohort studies on diabetes mellitus and liver cancer among Japanese

Reference	Study period	Study population					Magnitude of association
		Sex	Number of subjects	Age range	Event	Number of incident cases or deaths	
Tsukuma *et al.* (15)	1970–82	Men	484	Not specified	Death	19	↑↑↑
		Women	374	Not specified	Death	1	–
Sasaki *et al.* (16)	1960–93	Men and women	1939	Not specified	Death	73	↑↑↑
Kato *et al.* (17)	?–1995	Men and women	335 (Chronic hepatitis)	Not specified	Incidence	Not described	↑
			187 (Cirrhosis)	Not specified	Incidence	Not described	–
Tazawa *et al.* (18)	1987–?	Men and women	279 (HCV-associated chronic hepatitis)	23–72 years	Incidence	13	↑↑↑
Ohata *et al.* (19)	1980–2000	Men and women	161 (HCV-associated chronic hepatitis or cirrhosis)	Not specified	Incidence	70	↑
Uetake *et al.* (20)	1988–2000	Men	91 (Alcoholic cirrhosis)	34–72 years	Incidence	13	–
Khan *et al.* (21)	1977–2002	Men and women	1989	30–77 years	Death	8	↑↑
Muto *et al.* (22)	Not described	Men and women	622 (Decompensated cirrhosis)	20–75 years	Incidence	89	↑↑
Torisu *et al.* (23)	1978–2005	Men	47 (Alcoholic cirrhosis)	Not specified	Incidence	9	↑↑↑
Ohki *et al.* (24)	1994–2006	Men and women	1431 (HCV-associated chronic liver disease)	Not specified	Incidence	340	–
Tomiyama *et al.* (25)	1989–2007	Men and women	95 (Primary biliary cirrhosis)	29–84 years	Incidence	7	↑↑
Ikeda *et al.* (26)	1976–2004	Men and women	82 (Non-B, non-C cirrhosis)	34–80 years	Incidence	16	↑↑↑
Konishi *et al.* (27)	1992–?	Men and women	197 (Patients with HCV)	Not specified	Incidence	18	↑↑↑
Kurosaki *et al.* (28)	1994–?	Men and women	1279 (Patients with chronic hepatitis C)	Not specified	Incidence	68	–
Kuroda *et al.* (29)	1998–?	Men and women	133 (Cirrhotic patients with HCV infection)	51–88 years	Incidence	60	–
Takahashi *et al.* (30)	2002–?	Men and women	203 (HCV-positive patients treated with interferon)	Not specified	Incidence	13	↑↑↑
Kawamura *et al.* (31)	1997–?	Men and women	6508 (Patients with non-alcoholic fatty liver disease)	23–86 years	Incidence	16	↑↑↑
Arase *et al.* (32)	1990–?	Men and women	4302 (HCV-positive patients treated with interferon)	30–80 years	Incidence	393	↑↑
Nakamura *et al.* (33)	1992–2008	Men	14 173	>35 years	Incidence	106	↑↑↑
		Women	16 547	>35 years	Incidence	70	↓
Sasazuki et al. (8)	1984–2009	Men	142 744	40–103 years	Incidence	1279	↑↑↑
		Women	165 995	40–103 years	Incidence	565	↑↑

HCV, hepatitis C virus.