and prevalence trends in Japan through studying published reports from 1964 to 1992, consistency of our estimates with the review's estimates was confirmed. The prevalence of diabetes in the early 1990s was 7%–10% in men and 3%–6% in women and increasing.[1] The estimates in the present study were on the projected regression curve shown in the review.[1]

We also compared the prevalence in the present study with the published report[4] of the prevalence of diabetes in the national surveys performed at nearly the same period, finding that the estimates were slightly different. The prevalence of diabetes in the national survey and the present study according to age categories is shown in eTable. Although a broader definition of diabetes was adopted in the present study than in the national survey, the prevalence of diabetes was generally lower in the national survey. In addition, the prevalence in women showed a downward trend during the 5 years in the national survey, which was not observed in the present study. The discrepancy in the results should be further investigated.

Of note, the study population of the JPHC Diabetes Study consisted of participants who responded to the questionnaire. These participants might have been more health-conscious than the participants of the national survey, which might have resulted in the lower prevalence of diabetes observed in the participants of the JPHC Diabetes Study. The difference in the sampling methods between the national survey and the JPHC Diabetes Study might have also affected the discrepancy. The national survey was performed in areas selected by geographical cluster sampling across Japan, whereas the surveys of the JPHC Diabetes Study were performed in specific PHC areas. As such, there could be a possibility that low-prevalence areas might be included in the JPHC Diabetes Study. However, the large sample size and strict standardization of HbA1c strengthen the results of our survey. In the present study, increases in BMI were also observed in most age categories and both sexes, which were more prominent in males than in females. This suggests that obesity-related lifestyles could contribute to the increase in the prevalence of diabetes.

The present study identified patients with diabetes based on both a self-reported questionnaire and laboratory measurements, enabling an analysis of unrecognized diabetes and missed opportunities to access medical treatment. At the initial survey, 4.2% of the study participants were aware of their diabetes. On the other hand, almost an equivalent number of participants (3.9%) had diabetes but were unaware of it. These participants were newly diagnosed as having diabetes based on the laboratory measurements performed in the survey. Diabetes is often asymptomatic and difficult to recognize. Considering that a non-negligible number of people who are not aware of their diabetes exist in the general population, population-based screening tests for the detection of diabetes should be promoted. When the 1985 WHO criteria, which were used in the clinical setting at the time of the initial survey, were adopted to diagnose diabetes, the number of participants with diabetes solely confirmed by laboratory data (1985 WHO) was relatively small. This result suggests that the self-reported questionnaire could provide a valid estimate of the prevalence of diabetes defined according to the 1985 WHO criteria.

In the present study, high proportions of medical attendance were reported among participants with self-reported diabetes. The proportion of those who were currently receiving diabetes treatment was 75% at the initial survey and increased to 83% at the 5-year follow-up. An increase in the proportion of participants who were currently receiving diabetes treatment was also reported in a national survey,[5] which is consistent with the findings of the present study. The upward trend and the high proportions of patients receiving treatment could reflect increased public awareness of diabetes. Once people recognize their diabetes, they are likely to access healthcare and receive medical treatment.

The distributions of HbA1c values among participants who had never received, previously received, or were currently receiving treatment for diabetes showed that the mean HbA1c value was highest among those who were currently receiving diabetes treatment. This result seems reasonable, since these patients might include those who had a poor response to diabetes treatment or those with more advanced disease. The mean HbA1c values in the participants who had never or previously received diabetes treatment were unexpectedly fair. However, it should be considered that the study population consisted of participants attending health checkups, and these participants might have therefore been more health conscious than those who did not participate. The glycemic control in the noncompliant groups may be worse if the survey were applied to the whole population, including those who do not participate in health checkups.

Also of note, poor diabetic control was observed among participants who had never or previously received diabetes treatment. At the initial survey, 14.9% of the participants who were aware of the presence of diabetes but had never received diabetes treatment had an HbA1c $\geq$ 8.4%. This finding implies that a certain proportion of diabetic participants were left untreated despite their awareness of diabetes. An effort to encourage continuous medical attendance should be promoted to reduce the number of untreated diabetes.

The present study had some limitations. First, the JPHC cohort consists of health checkup participants. Thus, whether the results can be applied to the whole population is uncertain. Another limitation was the differences in the study participants between the initial survey and the 5-year follow-up survey. Of the participants in the initial survey, those who did not participate in the 5-year follow-up survey had higher HbA1c levels (data not shown) and were more likely to have diabetes at the initial survey than those who did. This follow-up bias could have affected the results. As for the type of diabetes, the present study did not distinguish between type 1 and type 2 diabetes. Since the prevalence of type 1

diabetes is very low in Japan, the vast majority of the participants with diabetes were thought to have type 2 diabetes. In fact, of the 2282 participants with diabetes at the initial survey, only 107 participants (4.7%) were on insulin treatment, which was confirmed by the self-reported questionnaire. The validity of self-reported diabetes is another concern in the present study. A self-reported questionnaire always involves misclassification. However, one past study[16] demonstrated high specificity of self-reported diabetes in a similar setting in Japan. This suggests that, although a self-reported questionnaire is not perfect, participants with self-reported diabetes were likely to have true diabetes.

In summary, the present study assessed the growing burden of diabetes and estimated prevalence of diabetes among participants across Japan in the late 1990s and early 2000s. The 5-year change in the prevalence of diabetes in the JPHC Study was increasing, and wide variations in the prevalence were observed across the different study areas. A concerted effort to reduce the number of individuals with unrecognized or untreated diabetes is required to stop the diabetes epidemic.

ONLINE ONLY MATERIALS

eTable. Comparison of the prevalence of diabetes between the national surveys and the present study.
Abstract in Japanese.

ACKNOWLEDGMENT

This study was supported by Health Sciences Research Grants (Research on Health Services H10-074, Medical Frontier Strategy Research H13-008, Clinical Research for Evidence-based Medicine H14-008 and H15-006, and Comprehensive Research on Life-Style Related Diseases including Cardiovascular Diseases and Diabetes Mellitus H16-019, H17-019, H18-028, H19-016, and H25-016) and a grant-in-aid for Cancer Research and a grant-in-aid for the Third Term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan.

Conflicts of interest: None declared.

REFERENCES

1. Islam MM, Horibe H, Kobayashi F. Current trend in prevalence of diabetes mellitus in Japan, 1964–1992. J Epidemiol. 1999; 9:155–62.
2. Neville SE, Boye KS, Montgomery WS, Iwamoto K, Okamura M, Hayes RP. Diabetes in Japan: a review of disease burden and approaches to treatment. Diabetes Metab Res Rev. 2009; 25:705–16.
3. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87:4–14.
4. Report on the National Diabetes Survey in 2002 [homepage on the Internet]; c2002. Available from: http://www.mhlw.go.jp/shingi/2004/03/s0318-15.html#tyosa.
5. Report on the Results of the National Health and Nutrition Survey in Japan [homepage on the Internet]; c2007 [updated 10th Aug 2012]. Available from: http://www.mhlw.go.jp/bunya/kenkou/eiyou09/01.html.
6. Igari Y, Nakano H, Oba K. Epidemiology of elderly diabetes mellitus in Japan. Nihon Rinsho. 2006;64:12–8 (in Japanese).
7. 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(6 suppl):S24–9.
8. Noda M, Kato M, Takahashi Y, Matsushita Y, Mizoue T, Inoue M, et al. Fasting plasma glucose and 5-year incidence of diabetes in the JPHC diabetes study—suggestion for the threshold for impaired fasting glucose among Japanese. Endocr J. 2010;57: 629–37.
9. Takahashi Y, Noda M, Tsugane S, Kuzuya T, Ito C, Kadowaki T. Prevalence of diabetes estimated by plasma glucose criteria combined with standardized measurement of HbA1c among health checkup participants on Miyako Island, Japan. Diabetes Care. 2000;23:1092–6.
10. Kashiwagi A, Kasuga M, Araki E, Oka Y, Hanafusa T, Ito H, et al. International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. J Diabetes Investig. 2012;3:39–40.
11. Yamamoto S, Watanabe S. Geographic characteristics and mortality profiles in the JPHC study. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol. 2001;11(6 suppl):S8–23.
12. The 1985 Japanese model population [homepage on the Internet]. Available from: http://www.e-stat.go.jp/SG1/estat/GL08020103.do?_toGL08020103_&listID=000001049584&requestSender=estat.
13. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika. 1986;73:13–22.
14. Wolf PA, Benjamin EJ, Belanger AJ, Kannel WB, Levy D, D'Agostino RB. Secular trends in the prevalence of atrial fibrillation: The Framingham Study. Am Heart J. 1996;131: 790–5.
15. Sekikawa A, Eguchi H, Tominaga M, Igarashi K, Abe T, Manaka H, et al. Prevalence of type 2 diabetes mellitus and impaired glucose tolerance in a rural area of Japan. The Funagata diabetes study. J Diabetes Complications. 2000;14:78–83.
16. Goto A, Morita A, Goto M, Sasaki S, Miyachi M, Aiba N, et al. Validity of diabetes self-reports in the Saku diabetes study. J Epidemiol. 2013;23:295–300.

発表論文

4) Hidaka A, Sasazuki S, Goto A, Sawada N, Shimazu T, Yamaji T, Iwasaki M, Inoue M, Noda M, Tajiri H, Tsugane S, for the JPHC Study Group: Plasma insulin, C-peptide, and blood glucose and the risk of gastric cancer: The Japan Public Health Center–based prospective study. *Int J Cancer* 136: 1402-1410, 2015.

IJC
International Journal of Cancer

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

Akihisa Hidaka[1,2], Shizuka Sasazuki[1], Atsushi Goto[3], Norie Sawada[1], Taichi Shimazu[1], Taiki Yamaji[1], Motoki Iwasaki[1], Manami Inoue[1,4], Mitsuhiko Noda[3], Hisao Tajiri[2] and Shoichiro Tsugane[1] for the JPHC Study Group

[1] Epidemiology and Prevention Group, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan
[2] Division of Gastroenterology and Hepatology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan
[3] Department of Diabetes Research, Diabetes Research Center, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan
[4] Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

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

Grant sponsor: JSPS KAKENHI (Grant-in-Aid for Scientific Research); **Grant number:** 25460742; **Grant sponsor:** National Cancer Center Research and Development Fund (23-A31[toku]) (since 2011); a Grant-in-Aid for Cancer Research (1989 to 2010); Grant-in-Aid for the Third-Term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan; Health Sciences Research Grants (Comprehensive Research on Life-Style Related Diseases Including Cardiovascular Diseases and Diabetes Mellitus, H22-019 and H25-016) from the Ministry of Health, Labor and Welfare of Japan

DOI: 10.1002/ijc.29098

History: Received 8 Apr 2014; Accepted 16 July 2014; Online 28 July 2014

Correspondence to: Shizuka Sasazuki, Epidemiology and Prevention Group, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan, Tel: +81-3-3542-2511, Fax: +81-3-3547-8578, E-mail: ssasazuk@ncc.go.jp

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.

Acknowledgements

The authors are indebted to the Aomori, Iwate, Ibaraki, Niigata, Osaka, Kochi, Nagasaki, and Okinawa Cancer Registries for providing their incidence data. A.H. is an awardee of a Research Resident Fellowship from the Foundation for Promotion of Cancer Research (Japan) for the Third-Term Comprehensive Ten-Year Strategy for Cancer Control.

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

Members of the Japan Public Health Center–Based Prospective Study Group are: S. Tsugane (principal investigator), S. Sasazuki, M. Iwasaki, N. Sawada, T. Shimazu, T. Yamaji, and T. Hanaoka, National Cancer Center, Tokyo; J. Ogata, S. Baba, T. Mannami, A. Okayama, and Y. Kokubo, National Cerebral and Cardiovascular Center, Osaka; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, I. Hashimoto, T. Ikuta, Y. Tanaba, H. Sato, and Y. Roppongi, Iwate Prefectural Ninohe Public Health Center, Iwate; Y. Miyajima, N. Suzuki, S. Nagasawa, Y. Furusugi, N. Nagai, Y. Ito, and S. Komatsu, Akita Prefectural Yokote Public Health Center, Akita; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, Y. Miyagawa, Y. Kobayashi, M. Machida, K. Kobayashi, and M. Tsukada, Nagano Prefectural Saku Public Health Center, Nagano; Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei, and H. Sakiyama, Okinawa Prefectural Chubu Public Health Center, Okinawa; K. Imoto, H. Yazawa, T. Seo, A. Seiko, F. Ito, F. Shoji, and R. Saito, Katsushika Public Health Center, Tokyo; A. Murata, K. Minato, K. Motegi, T. Fujieda, and S. Yamato, Ibaraki Prefectural Mito Public Health Center, Ibaraki; K. Matsui, T. Abe, M. Katagiri, M. Suzuki, and K. Matsui, Niigata Prefectural Kashiwazaki and Nagaoka Public Health Center, Niigata; M. Doi, A. Terao, Y. Ishikawa, and T. Tagami, Kochi Prefectural Chuo-higashi Public Health Center, Kochi; H. Sueta, H. Doi, M. Urata, N. Okamoto, F. Ide, and H. Goto, Nagasaki Prefectural Kamigoto Public Health Center, Nagasaki; H. Sakiyama, N. Onga, H. Takaesu, M. Uehara, and T. Nakasone, Okinawa Prefectural Miyako Public Health Center, Okinawa; F. Horii, I. Asano, H. Yamaguchi, K. Aoki, S. Maruyama, M. Ichii, and M. Takano, Osaka Prefectural Suita Public Health Center, Osaka; Y. Tsubono, Tohoku University, Miyagi; K. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; Y. Honda, K. Yamagishi, S. Sakurai, and N. Tsuchiya, University of Tsukuba, Ibaraki; M. Kabuto, National Institute for Environmental Studies, Ibaraki; M. Yamaguchi, Y. Matsumura, S. Sasaki, and S. Watanabe, National Institute of Health and Nutrition, Tokyo; M. Akabane, Tokyo University of Agriculture, Tokyo; T. Kadowaki and M. Inoue, The University of Tokyo, Tokyo; M. Noda and T. Mizoue, National Center for Global Health and Medicine, Tokyo; Y. Kawaguchi, Tokyo Medical and Dental University, Tokyo; Y. Takashima and Y. Yoshida, Kyorin University, Tokyo; K. Nakamura, Niigata University, Niigata; S. Matsushima and S. Natsukawa, Saku General Hospital, Nagano; H. Shimizu, Sakihae Institute, Gifu; H. Sugimura, Hamamatsu University School of Medicine, Shizuoka; S. Tominaga, Aichi Cancer Center, Aichi; N. Hamajima, Nagoya University, Aichi; H. Iso and T. Sobue, Osaka University, Osaka; M. Iida, W. Ajiki, and A. Ioka, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka; S. Sato, Chiba Prefectural Institute of Public Health, Chiba; E. Maruyama, Kobe University, Hyogo; M. Konishi, K. Okada, and I. Saito, Ehime University, Ehime; N. Yasuda, Kochi University, Kochi; S. Kono, Kyushu University, Fukuoka; S. Akiba, Kagoshima University, Kagoshima.

発表論文

5) Goto M, Goto A, Ikeda N, Noda H, Shibuya K, Noda M#: (# corresponding author)
Factors associated with untreated diabetes: Analysis of data from 20,496 participants in the Japanese National Health and Nutrition Survey.
***PLoS ONE* 10 (3): e0118749, 2015. (doi: 10.1371/journal.pone.0118749)**

PLOS | ONE

RESEARCH ARTICLE

Factors Associated with Untreated Diabetes: Analysis of Data from 20,496 Participants in the Japanese National Health and Nutrition Survey

Maki Goto[1☯], Atsushi Goto[1☯], Nayu Ikeda[2], Hiroyuki Noda[3], Kenji Shibuya[4], Mitsuhiko Noda[1]*

1 Department of Diabetes Research, Diabetes Research Center, National Center for Global Health and Medicine, Tokyo, Japan, **2** Center for International Collaboration and Partnership, National Institute of Health and Nutrition, Tokyo, Japan, **3** Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan, **4** Department of Global Health Policy, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

☯ These authors contributed equally to this work.
* mnoda@hosp.ncgm.go.jp

OPEN ACCESS

Citation: Goto M, Goto A, Ikeda N, Noda H, Shibuya K, Noda M (2015) Factors Associated with Untreated Diabetes: Analysis of Data from 20,496 Participants in the Japanese National Health and Nutrition Survey. PLoS ONE 10(3): e0118749. doi:10.1371/journal.pone.0118749

Academic Editor: Alberico Catapano, University of Milan, ITALY

Received: March 3, 2014

Accepted: January 6, 2015

Published: March 10, 2015

Funding: This study was funded by the Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (No. 24590785), and Health Sciences Research Grants (Comprehensive Research on Life-Style Related Diseases including Cardiovascular Diseases and Diabetes Mellitus H22-019 and H25-016) from the Ministry of Health, Labour and Welfare of Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have read the journal's policy and have the following conflicts: MN

Abstract

Objective

We aimed to examine factors associated with untreated diabetes in a nationally representative sample of the Japanese population.

Research Design and Methods

We pooled data from the Japanese National Health and Nutrition Survey from 2005 to 2009 (n = 20,496). Individuals aged 20 years and older were included in the analysis. We classified participants as having diabetes if they had HbA1c levels ≥6.5% (≥48 mmol/mol). People with diabetes who self-reported that they were not currently receiving diabetic treatment were considered to be untreated. We conducted a multinomial logistic regression analysis to determine factors associated with untreated diabetes relative to non-diabetic individuals.

Results

Of 20,496 participants who were included in the analysis, untreated diabetes was present in 748 (3.6%). Among participants with untreated diabetes, 48.3% were previously diagnosed with diabetes, and 46.5% had HbA1c levels ≥7.0% (≥53 mmol/mol). Participants with untreated diabetes were significantly more likely than non-diabetic participants to be male, older, and currently smoking, have lower HDL cholesterol levels and higher BMI, non-HDL cholesterol levels, and systolic blood pressure.

serves as a chairperson of the evaluation committee of the Evidence-based Practice Guideline for the Treatment of Diabetes in Japan edited by Japan Diabetes Society. He also served as a member of the editorial committee of the Treatment Guide for Diabetes in Japan edited by Japan Diabetes Society and the Health Japan 21 (the second term) plan development committee. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Conclusions

A substantial proportion of people in Japan with untreated diabetes have poor glycemic control. Targeting relevant factors for untreated diabetes in screening programs may be effective to enhance the treatment and control of diabetes.

Introduction

Effective treatment coverage of diabetes is important to prevent its complications that increase the social cost of the disease. Diabetic complications reduce patients' quality of life and increase the economic burden of diabetes [1,2]. The total cost associated with diabetes in the U.S. has increased from $174 billion in 2007 to $245 billion in 2012 [3].

In Japan, the estimated number of adults with suspected diabetes was approximately 9.5 million in 2012 [4] and diabetes accounts for 6% of the healthcare budget [5]. To promote the nation's health, the central government initiated a 10-year campaign named "Health Japan 21 (the second term)" in 2013. In the campaign, four target goals were set for diabetes: 1) increasing the number of patients with diabetes receiving medical treatment; 2) reducing the number of patients with poor glycemic control; 3) reducing the number of new diabetic nephropathy hemodialysis cases; and 4) decreasing the incidence of newly diagnosed diabetes [4]. Improving the coverage of treatment for diabetes is a key, because as many as 35% of people who are strongly suspected of having diabetes are not receiving treatment in Japan [4].

In order to improve management of diabetes, it is essential to promote detection of diabetic patients who are not on treatment in the community. Information on characteristics of individuals who have untreated diabetes would help healthcare professionals in general practice and routine physical examinations distinguish them from those who are not diabetic. However, previous studies focused on undiagnosed diabetes, investigating the development and evaluation of diabetes screening tools, identification of significant factors for appropriate glycemic control, and documentation of diabetes-related complications [6–9]. We therefore aimed to identify characteristics of individuals with untreated diabetes compared to non-diabetic population in Japan.

Materials and Methods

The National Health and Nutrition Survey (NHNS) has been conducted every November by the Ministry of Health, Labour and Welfare on a nationally representative sample of the population in Japan under the Health Promotion Law [10]. The survey started in 1947 as the National Nutrition Survey, and it was redesigned in 2003 to continue as the NHNS. After receiving permission for secondary use of survey data from the Ministry of Health, Labour and Welfare, we obtained access to anonymized individual-level data from participants who were surveyed between 2005 and 2009. This study was approved by the institutional review board (IRB) of the National Center for Global Health and Medicine. The requirement for informed consent was waived for this analysis by the IRB, because data were anonymized by the Ministry of Health, Labour and Welfare.

The survey aims at establishing measures for national health promotion and includes a cross-sectional interview and examination that obtain basic data on anthropometry, nutritional intake and diet, and lifestyle. Eligible respondents were all residents aged $\geq$1 year in a stratified random sample of 300 census tracts. Response rates of the NHNS are 60–70%, and the sample

is considered representative of the Japanese population. A blood sample was taken from all participants aged 20 years and older [11–16].

HbA1c levels were measured using latex agglutination-turbidimetric immunoassay by SRL Inc., a commercial laboratory in Tokyo, Japan, which analyzed all of the NHNS blood samples [11]. HbA1c values were initially determined using Japan Diabetes Society (JDS) values, and we converted them to the National Glycohemoglobin Standardization Program (NGSP) values using the following conversion formula: HbA1c (NGSP) = 1.02 × HbA1c (JDS) + 0.25% [17]. We classified participants as having diabetes if they self-reported that they were currently receiving diabetes treatment or had HbA1c levels ≥6.5% (≥48 mmol/mol) [18]. We made no distinction between type 1 diabetes and type 2 diabetes. We defined untreated diabetes as participants who had diabetes and self-reported that they were not currently receiving diabetes treatment.

The subjects of the present study were adults aged ≥20 years. Participants were excluded from the analysis if they were pregnant, had missing HbA1c measurement values, or missing information for covariates or exposure variables.

In order to determine and analyze the characteristics of individuals with diabetes who were not receiving treatment (untreated diabetes), we compared this group and the group of respondents having diabetes who were on treatment (treated diabetes) with those who did not have diabetes (no diabetes). We used *t*-tests and Chi-squared tests to compare continuous and categorical baseline characteristics, respectively. We further conducted a multinomial logistic regression on the status of diabetes (untreated diabetes, treated diabetes, or non-diabetic) both for sexes combined and separately by sex. We treated the non-diabetic group as the base outcome of the dependent variable in the model. Independent variables of the regression model included sex (men/women), age (years), BMI (kg/m^2), exercise habits (have exercise habits or have no exercise habit), smoking status (never, past, or current smoker), HDL cholesterol (mg/dL), non-HDL cholesterol (mg/dL), and systolic blood pressure (mmHg). We assessed factors for untreated diabetes against treated diabetes by using the estimated covariance matrix of regression coefficients to test the hypothesis that there was no difference in coefficients between untreated and treated diabetes.

All statistical tests were 2-sided and a *P*-value <0.05 was regarded as statistically significant. All statistical analyses were conducted using SAS (version 9.3; SAS Institute, Inc., Cary, NC, USA) and Stata software (version 12; Stata Corp, College Station, TX, USA).

Results

Out of 20,496 participants included in this study, 748 (3.6%) had untreated diabetes and 1,213 (5.9%) had treated diabetes (Fig. 1). Compared to people without diabetes, people with untreated diabetes had a significantly larger proportion of men, had significantly higher age, HbA1c levels, BMI, total and non-HDL cholesterol levels, triglyceride levels, and blood pressure, had significantly lower HDL cholesterol levels, and reported significantly higher rates of exercise habits and past and current smoking (Table 1). Among the 748 people with untreated diabetes, 361 (48.3%) were previously diagnosed with diabetes, and 348 (46.5%) had HbA1c levels ≥7.0% [≥53 mmol/mol] (Fig. 2).

In the multinomial logistic regression for sexes combined, relative to the non-diabetic group, respondents with untreated diabetes were significantly more likely to be male (*P*-value < 0.001), older (*P*-value < 0.001), current smokers (*P*-value = 0.006), have a higher BMI (*P*-value < 0.001), non-HDL cholesterol level (*P*-value < 0.001), and systolic blood pressure (*P*-value < 0.001), and a lower HDL cholesterol (*P*-value = 0.005) (Table 2). For treated diabetes, similar associations were observed, although the association with current smoking was not

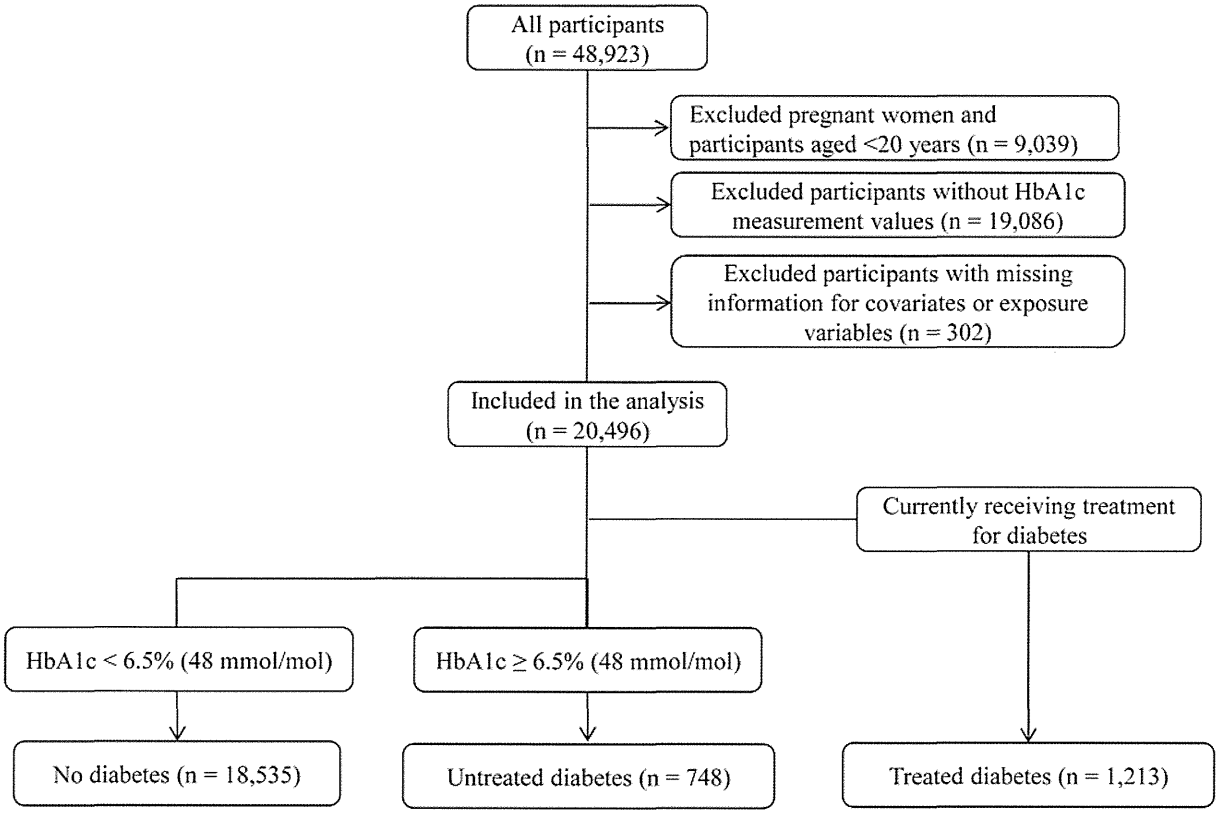

Fig 1. Flowchart of participant selection from the Japanese National Health and Nutrition Survey (NHNS). Abbreviations: HbA1c, hemoglobin A1c.

doi:10.1371/journal.pone.0118749.g001

significant (*P*-value = 0.063) and that with having exercise habits was significant (*P*-value < 0.001) (Table 2). Although the differences in the coefficients between untreated diabetes and treated diabetes were statistically significant for age (*P*-value < 0.001), exercise habits (*P*-value < 0.001), non-HDL cholesterol (*P*-value < 0.001), and systolic blood pressure (*P*-value = 0.002), most factors exhibited directional consistency in the sign of the coefficients, with the exception of non-HDL cholesterol. Although the results were similar in the analyses stratified by sex, the positive association of current smoking with untreated diabetes was significant only in men, and the inverse associations of HDL cholesterol with treated and untreated diabetes were significant only in women (Table 2).

Discussion

This study explored factors that are associated with untreated diabetes in the Japanese population. The likelihood of having untreated diabetes increased in the presence of male sex, older age, higher BMI, current smoking status, decreased HDL cholesterol levels, and increased non-HDL cholesterol and systolic blood pressure levels. Using a multinomial logistic regression analysis, we compared the findings for untreated and treated diabetes and observed directional consistency for all factors, with the exception that non-HDL cholesterol was positively associated with untreated diabetes and inversely associated with treated diabetes. The frequent use of anti-hyperlipidemic agents among participants who were being treated for diabetes may have improved their serum lipid control, which may explain the difference in direction of the association.

Table 1. Summary statistics of characteristics of participants included in the study by the status of diabetes.

Characteristics	No diabetes	Untreated diabetes	Treated diabetes	*P* value** (untreated vs. no diabetes)	*P* value**(treated vs. no diabetes)
	n = 18,535	n = 748	n = 1,213		
Men*	39.0	55.5	56.4	<0.001	<0.001
Age (years)	56.2 ± 16.6	64.9 ± 11.5	67.6 ± 9.8	<0.001	<0.001
HbA1c (%)	5.5 ± 0.4	7.6 ± 1.5	7.3 ± 1.3	<0.001	<0.001
	(37 ± 4 mmol/mol)	(60 ± 16 mmol/mol)	(56 ± 14 mmol/mol)		
BMI (kg/m^2)	22.9 ± 3.4	25.1 ± 4.3	24.6 ± 3.7	<0.001	<0.001
Total cholesterol (mg/dL)	203.1 ± 34.8	211.3 ±38.2	198.9 ± 34.5	<0.001	<0.001
Triglycerides (mg/dL)	130.1 ± 88.4	188.1 ± 127.2	156.3 ± 102.5	<0.001	<0.001
HDL cholesterol (mg/dL)	62.7 ± 16.2	55.6 ± 15.1	56.5 ± 16.7	<0.001	<0.001
non-HDL cholesterol (mg/dL)	140.4 ± 35.5	155.8 ± 39.1	142.3 ± 35.0	<0.001	<0.001
Systolic blood pressure (mmHg)	131.0 ± 20.0	143.8 ± 19.9	141.4 ± 17.8	<0.001	<0.001
Diastolic blood pressure (mmHg)	79.3 ± 11.6	83.2 ± 12.3	79.1 ± 10.8	<0.001	<0.001
Have exercise habits*	28.8	33.3	42.9	0.01	<0.001
Past smoker*	19.5	25.3	26.4	<0.001	<0.001
Current smoker*	20.1	23.3	19.4	0.03	0.55

Results are presented as mean ± SD otherwise indicated. Abbreviations: HbA1c, hemoglobin A1c; BMI, body mass index; SD, standard deviation.

*Results are presented as percentages.

**P-values for the differences were computed by *t*-tests for continuous variables and by Chi-squared tests for categorical variables.

doi:10.1371/journal.pone.0118749.t001

Previous studies have focused on the factors associated with undiagnosed diabetes [7,19,20], but not the factors for untreated diabetes. Although the factors that are associated with untreated and undiagnosed diabetes may differ, it is relevant to compare our results with previous studies that examined the factors associated with undiagnosed diabetes. In a systematic review and meta-analysis of studies that examined screening scores to detect undiagnosed diabetes,

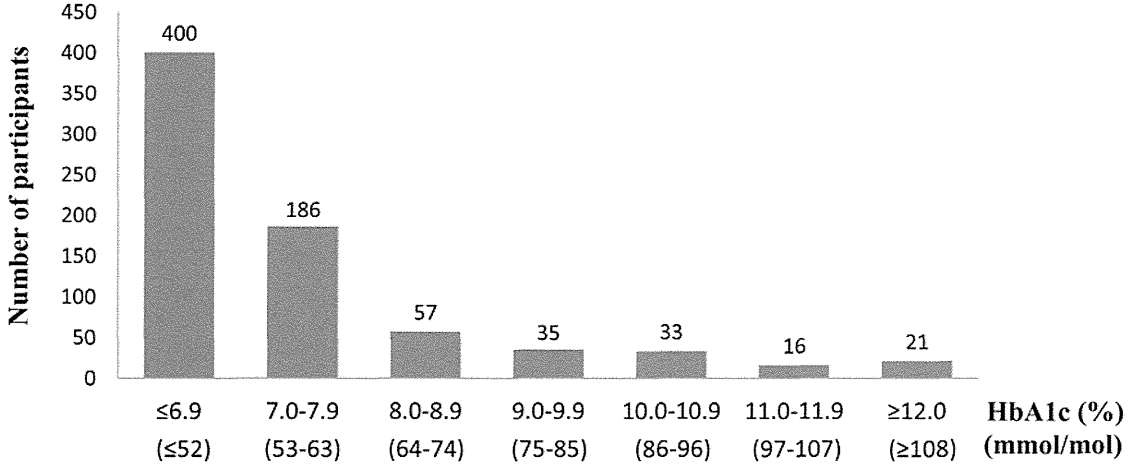

Fig 2. Distribution of HbA1c values among people with untreated diabetes (n = 748). Abbreviations: HbA1c, hemoglobin A1c.

doi:10.1371/journal.pone.0118749.g002

Table 2. Factors associated with untreated and treated diabetes vs. no diabetes.

	Total		Men		Women	
	n = 20,496		n = 8,326		n = 12,170	
Independent variables	Untreated diabetes	Treated diabetes	Untreated diabetes	Treated diabetes	Untreated diabetes	Treated diabetes
Sex (men)	1.46 (1.21, 1.78)	1.53 (1.32, 1.79)				
Age (in 10-year increments)	1.38 (1.30, 1.47)	1.60 (1.52, 1.69)	1.57 (1.44, 1.72)	1.60 (1.49, 1.71)	1.15 (1.05, 1.26)	1.57 (1.45, 1.70)
BMI (kg/m^2)	1.13 (1.11, 1.16)	1.13 (1.11, 1.15)	1.11 (1.07, 1.14)	1.10 (1.08, 1.13)	1.15 (1.12, 1.18)	1.14 (1.12, 1.70)
Have exercise habits	1.10 (0.94, 1.29)	1.58 (1.40, 1.78)	1.08 (0.87, 1.33)	1.62 (1.37, 1.91)	1.12 (0.88, 1.18)	1.55 (1.29, 1.87)
Past smoker	1.19 (0.96, 1.47)	1.07 (0.90, 1.27)	1.15 (0.89, 1.49)	1.07 (0.88, 1.30)	1.23 (0.81, 1.43)	1.04 (0.71, 1.53)
Current smoker	1.37 (1.10, 1.71)	1.19 (0.99, 1.43)	1.57 (1.20, 2.06)	1.19 (0.96, 1.48)	0.90 (0.57, 1.89)	1.18 (0.81, 1.71)
HDL cholesterol (in 10 mg/dL increments)	0.92 (0.87, 0.98)	0.91 (0.87, 0.95)	0.99 (0.92, 1.07)	0.96 (0.91, 1.02)	0.84 (0.77, 0.91)	0.84 (0.79, 0.90)
Non-HDL cholesterol (in 10 mg/dL increments)	1.07 (1.05, 1.09)	0.97 (0.95, 0.99)	1.07 (1.04, 1.10)	0.97 (0.95, 0.997)	1.07 (1.04, 1.11)	0.96 (0.94, 0.99)
Systolic blood pressure (in 10 mmHg increments)	1.17 (1.12, 1.21)	1.08 (1.04, 1.12)	1.12 (1.06, 1.18)	1.04 (0.99, 1.08)	1.23 (1.16, 1.30)	1.13 (1.07, 1.19)

Results are presented as adjusted RPR (95% CI). A multinomial logistic regression analysis was used to estimate adjusted RPRs with the independent variables in the table. Abbreviations: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; RPR, ratio of prevalence ratio.

doi:10.1371/journal.pone.0118749.t002

age and adiposity measures (e.g., BMI) were the most commonly used factors to detect undiagnosed diabetes [19]. This result is consistent with our observation that age and BMI were strongly and positively associated with untreated diabetes. Other factors that were identified by our study, such as sex, current smoking, hypertension, and exercise habits, were also commonly used to detect undiagnosed diabetes. In previous studies, family history of diabetes was another strong predictor of undiagnosed diabetes [7,19], although this information was not available for our study. Although decreased HDL cholesterol and increased non-HDL cholesterol levels were predictors of untreated diabetes in our study, lipid levels have rarely been used to detect undiagnosed diabetes [7,19], possibly because blood testing is needed to evaluate lipid levels.

In our study, the association between untreated diabetes and current smoking status was observed only in men. This may be explained by the fact that the proportion of current smokers in the group of untreated diabetes was lower in women (6.6% [22/333]) than in men (36.6% [152/415]). Our study also showed that the inverse association between untreated diabetes and HDL cholesterol was observed only in women. This result is supported by findings in the follow-up of the Finnmark study, which reported that HDL cholesterol was a strong independent risk factor of diabetes in women, but not in men [21]. Although possible mechanisms responsible for the sex-difference remain to be examined, the difference may reflect effects of sex hormones on glucose and lipid metabolism [22,23].

Our findings support the notion that selective or targeted screening programs performed in a subgroup with factors such as male sex, older age, and higher BMI may be effective to reduce the proportion of untreated diabetes. Further, our results indicated that as many as half of

people with untreated diabetes had previously been diagnosed with diabetes; that is, treatment did not follow diagnosis in these participants. However, it remains uncertain which environments are most suitable to provide motivation for people to access medical services.

The major strength of this study is the use of nationwide data that represents the Japanese population. However, some limitations of this study need to be addressed. First, although the response rate was relatively high [14], the risk for selection and reporting bias may still exist. Second, additional information about a person's history of diabetes, such as family history, duration, and complications, was not available from the NHNS. Third, we excluded participants who had a missing value on HbA1c, potentially resulting in selection bias. Finally, we were unable to establish from this cross-sectional analysis a temporal relationship required for causality, and the results need to be interpreted cautiously.

In conclusion, in Japan, untreated diabetes were associated with male sex, current smoking, older age, higher BMI, higher non-HDL cholesterol levels, higher systolic blood pressure, and lower HDL cholesterol levels. A substantial proportion of people with untreated diabetes are previously diagnosed with diabetes and have poor glycemic control. Our findings support the notion that selective or targeted screening programs performed in a subgroup with factors that were associated with a lack of treatment in diabetes may be effective to reduce the proportion of untreated diabetes.

Author Contributions

Conceived and designed the experiments: MG AG NI HN KS MN. Performed the experiments: MG AG. Analyzed the data: MG AG. Contributed reagents/materials/analysis tools: MG AG NI HN KS MN. Wrote the paper: MG AG NI.

References

1. Huang ES, Brown SE, Ewigman BG, Foley EC, Meltzer DO (2007) Patient perceptions of quality of life with diabetes-related complications and treatments. Diabetes Care 30: 2478–2483. PMID: 17623824
2. Clarke P, Gray A, Legood R, Briggs A, Holman R (2003) The impact of diabetes-related complications on healthcare costs: results from the United Kingdom Prospective Diabetes Study (UKPDS Study No. 65). Diabetic Medicine 20: 442–450. PMID: 12786677
3. Herman WH (2013) The economic costs of diabetes: is it time for a new treatment paradigm? Diabetes Care 36: 775–776. doi: 10.2337/dc13-0270 PMID: 23520368
4. Ministry of Health, Labour and Welfare (2012) Reference data of Healthy Japan 21 (Second campaign) (in Japanese). Available: http://www.mhlw.go.jp/bunya/kenkou/dl/kenkounippon21_02.pdf.
5. Neville SE, Boye KS, Montgomery WS, Iwamoto K, Okamura M, Hayes RP. (2009) Diabetes in Japan: a review of disease burden and approaches to treatment. Diabetes Metab Res Rev 25: 705–716. doi: 10.1002/dmrr.1012 PMID: 19795421
6. Tekumit H, Cenal AR, Polat A, Uzun K, Tataroglu C, Akinci E. (2010) Diagnostic value of hemoglobin A1c and fasting plasma glucose levels in coronary artery bypass grafting patients with undiagnosed diabetes mellitus. Ann Thorac Surg 89: 1482–1487. doi: 10.1016/j.athoracsur.2009.11.033 PMID: 20417764
7. Heianza Y, Arase Y, Saito K, Hsieh SD, Tsuji H, Kodama S, et al. (2013) Development of a screening score for undiagnosed diabetes and its application in estimating absolute risk of future type 2 diabetes in Japan: Toranomon Hospital Health Management Center Study 10 (TOPICS 10). J Clin Endocrinol Metab 98: 1051–1060. doi: 10.1210/jc.2012-3092 PMID: 23393174
8. Lauruschkat AH, Arnrich B, Albert AA, Walter JA, Amann B, Rosendahl UP, et al. (2005) Prevalence and risks of undiagnosed diabetes mellitus in patients undergoing coronary artery bypass grafting. Circulation 112: 2397–2402. PMID: 16230496
9. Choi KM, Lee KW, Kim SG, Kim NH, Park CG, Seo HS, et al. (2005) Inflammation, insulin resistance, and glucose intolerance in acute myocardial infarction patients without a previous diagnosis of diabetes mellitus. J Clin Endocrinol Metab 90: 175–180. PMID: 15509644
10. Ministry of Health, Labour and Welfare (2011) The National Health and Nutrition Survey, 2009. Tokyo: Ministry of Health, Labour and Welfare.