Effect of high-sensitivity C-reactive protein on the development of diabetes as demonstrated by pooled logistic-regression analysis of annual health-screening information from male Japanese workers

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Abstract

Aim. – The aim of this study was to determine the relationship between the development of diabetes mellitus and high-sensitivity C-reactive protein (HsCRP) adjusted for various potential confounders.

Methods. – This 5-year prospective cohort study was conducted at a Japanese steel factory and involved male workers who had received annual health screenings between 2005 and 2010. The 7392 male participants were aged 19–75 years. The study endpoint, the development of diabetes mellitus, was defined as HbA1c greater or equal to 6.5% or the use of antidiabetic medication. The association between variables was investigated using pooled logistic regression adjusted for various covariates such as age, baseline body mass index (BMI) and increase in BMI from baseline, blood biochemistry, job schedule and job-related stress.

Results. – The incidence rate of diabetes development per 1000 person-years was 13.9. Multivariate analysis showed a significant relationship between the development of diabetes and elevated levels of baseline HsCRP and increases in levels from baseline. The Odds ratios for a 2.9-fold (±1 geometric standard deviation) increase in baseline HsCRP and increase in HsCRP level from baseline were 1.18 [95% confidence interval (CI): 1.03–1.34; P = 0.018] and 1.21 (95% CI: 1.03–1.41; P = 0.018), respectively.

Conclusion. – The present study has indicated that HsCRP is an independent predictor for the development of diabetes in men, together with various confounders such as BMI, type of job schedule and job-related stress.

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Keywords: High-sensitivity C-reactive protein; Diabetes mellitus; Male; Job-related stress; Predictive value; Incidence; Longitudinal study; Japan; HbA1c; Body mass index; Cohort study

Résumé

Effet de la C-réactive protéine ultrasensible (CRPus) sur le développement du diabète mis en évidence par régression logistique groupée à partir des bilans de santé annuels de travailleurs japonais de sexe masculin.

Objectif. – Analyser la relation entre le développement du diabète sucré (DM) et la protéine C réactive ultrasensible (CRPus) en fonction de divers autres facteurs.

Méthodes. – Une étude de cohorte prospective a été menée chez des travailleurs masculins (n = 7392) d’une entreprise sidérurgique japonaise qui ont été suivis lors d’exams annuels de santé entre 2005 et 2010. Le développement du diabète sucré était défini par une HbA1c supérieure ou égale à 6,5 % ou la prise de tout médicament antidiabétique. Nous avons réalisé une régression logistique groupée en faisant varier divers paramètres tels l’âge, l’indice de masse corporelle (IMC) initial et l’augmentation de l’IMC, le type de planification du travail et le stress au travail.

Résultats. – L’incidence annuelle du diabète sucré était de 13,9 pour 1000 personnes. En utilisant la régression logistique groupée, nous avons montré que la CRPus initiale et l’augmentation des concentrations de CRPus étaient des prédicteurs significatifs du développement du diabète sucré. L’Odds ratio pour une augmentation de 2,9 fois des concentrations initiales de CRPus (écart-type géométrique de 1) et l’augmentation des concentrations de CRPus étaient respectivement de 1,18 (1,03–1,34) et de 1,21 (1,03–1,41).

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1. Introduction

In 2007, the Ministry of Health, Labour and Welfare in Japan estimated that approximately 8.9 million people were strongly suspected of having diabetes mellitus, and approximately 22.1 million people were at risk of developing diabetes [1]. Moreover, since the 1970s, the number of diabetic patients in Japan has increased by around two-fold every 10–20 years [2]. Therefore, in 2010, the Ministry set a goal to reduce the number of diabetic patients in Japan by almost 7% [3].

C-reactive protein (CRP) is a protein marker of acute-phase and systemic inflammation [4]. Several earlier epidemiological studies documented an association between CRP and arteriosclerotic diseases [5], while other investigations have suggested that elevated CRP concentrations were significantly associated with the prevalence of underlying atherosclerotic vascular disease [6], as well as the risk of first and recurrent cardiovascular, stroke and ischaemic events [5,7–9].

Previous studies have also indicated a relationship between diabetes mellitus and CRP [10–19]. In addition, several recent case-control [12,14,17] and cohort studies [10,11,13,15,16] have demonstrated that high levels of CRP were related to the development of diabetes, especially in women. In contrast, other studies showed that CRP was not related to diabetes after adjusting for body mass index (BMI) [5,7–9].

In these previous studies, CRP was measured just once at baseline, while the participants were divided into various groups based on their CRP level [10–17,20–23]. Moreover, the clinical history of chronic inflammatory diseases, such as collagen diseases, was not obtained from participants in all of these studies [10–17,20–23]. For this reason, it may be said that the relationship between CRP levels and diabetes has still not been conclusively established. Thus, the aim of the present longitudinal cohort study in male Japanese workers was to evaluate the relationship between high-sensitivity CRP (HsCRP) and the development of diabetes, using pooled logistic regression adjusted for the effect of fluctuations in various potential confounders.

2. Patients and methods

2.1. Subjects

The present prospective cohort study included observations made over the 5-year period from 2005 to 2010. A total of 7392 men out of a possible 9546 male workers at a Japanese steel factory were enrolled in the study. The cohort consisted of workers of whom more than 98% had attended annual health examinations during the study observation period, although new participants could be enrolled during the follow-up period. Workers excluded from the study were individuals who had neither a health examination (869 men) nor a glyated haemoglobin A1c (HbA1c) measurement (584 men) in the subsequent year, those with either a history of collagen disease (48 men) or a diagnosis of diabetes (589 men), based on criteria used in the present study, and those with missing data for the year of entry (64 men).

The study protocol was approved by the Ethics Review Board of the Graduate School of Medicine, Chiba University.

2.2. Measurements

Data from the annual health examination included HbA1c, measured using a latex agglutination kit (RAPIDIA Auto HbA1c-L, Fujirebio Inc., Tokyo, Japan) standardized by the Japan Diabetes Society (JDS) method [24,25]. The minimum detectable concentration and interassay coefficient of variation for HbA1c were 3.0% and 4.29%, respectively. The measured HbA1c (JDS) values were then converted to HbA1c values as determined by the US National Glycohemoglobin Standardization Program (NGSP), using the following equation: HbA1c (NGSP) (%) = 1.02 × HbA1c (JDS) (%) + 0.25 (%) [26]. Serum HsCRP levels were measured by the latex turbidimetric immunoassay method. The minimum detectable concentration and interassay coefficient of variation for HsCRP were 0.02 mg/dL and 2.43%, respectively.

Health examinations, including blood sampling, were carried out between 9 a.m. and 3 p.m. throughout the study period. None of the measurements were taken within 30 min of a meal or heavy physical activity. The worker’s medical history was confirmed during individual interviews conducted by occupational physicians. The study endpoint, the development of diabetes, was defined as an HbA1c greater or equal to 6.5% [25,26] or the use of any antidiabetic medication. Age, BMI, mean arterial pressure, total serum cholesterol, aspartate aminotransferase (AST), creatinine and uric acid were measured during the study, with drinking, smoking and exercise habits serving as covariates in the analyses. All potential covariates were updated at each annual examination. However, as obtaining fasting blood samples from all participants throughout the entire observational period was not feasible, plasma glucose was not measured in our study. Similarly, as waist circumference was not available for all participants during the follow-up period, this variable was also not included in the study.

However, as weight gain over time may be an important causative factor for diabetes, baseline BMI and increases in BMI from baseline were included in the model. To evaluate the effect of the increase in HsCRP from baseline, HsCRP at

Conclusion. – Notre étude montre que la CRPus est un facteur prédictif indépendant du développement du diabète sucré chez les hommes, ainsi que l’IMC, le type de planification du travail et le stress au travail.
baseline and any increases from baseline were included in the statistical model. As much as possible, covariates were selected from the available items in the yearly health examinations used in our previous studies [27,28]. Care was taken to not overlap with other measurements, such as AST and alanine aminotransferase (ALT) for liver dysfunction, to avoid co-linearity in the logistic model. The tests were conducted in comprehensive clinical-testing laboratories that met the requirements of official certification organizations. Information on job schedules, habitual exercise, who prepared meals, soft-drink and alcohol consumption, smoking and snacking between meals were recorded during the annual health examination and obtained from self-administered questionnaires. The quantity of alcohol in each type of alcoholic beverage was calculated using the unit ‘gou’, the most popular unit in Japan for measuring alcohol consumption: 1 gou (180 mL) of Japanese sake (rice wine) contains approximately 22 g of ethanol, and is equivalent to 500 mL of beer, 60 mL of whiskey, 180 mL of wine and 110 mL of shochu (white spirits). This unit was used in the questionnaire as it was easily comprehensible by the general Japanese population for determining the amount of alcohol in the beverages consumed. Weekly alcohol intake was estimated by multiplying quantity by frequency. Smoking status was classified as either smokers or non-smokers. The other variables were categorized as: type of job schedule (daytime, three-shift work, two-shift work, or other); amount of habitual exercise (none, 1–2 times/month, 1–2 times/week, 3–4 times/week or ≥ 5 times/week); who prepared the meals (oneself, family member, eating out or catering, eating in a dormitory, or other); soft-drink consumption (rare, occasionally or frequent); and snacking between meals (rare, occasionally or frequent). Job-related stress factors were also included in the multivariate model, as evaluated by the Brief Job Stress Questionnaire (BJSQ) [29]. The BJSQ includes job demands and control, interpersonal relationships and compatibility, and has been used widely for evaluating job-related stress in Japan. Mean arterial pressure was also calculated using the equation (diastolic blood pressure × 2 + systolic blood pressure)/3 [30].

2.3. Statistical analysis

In the longitudinal multivariate analysis, a pooled logistic-regression analysis was used to evaluate the relationship between HsCRP and the development of diabetes. The baseline HsCRP and increase in levels from baseline, total serum cholesterol, creatinine, AST and uric acid were log-transformed, using a base of ±1 geometric standard deviation (GSD). To ensure the inclusion of all available annual measurements taken during the follow-up period, a method that involved pooling the repeated observations was used [31]. Thus, each examination interval of 1 year was treated as a mini follow-up study. The parameters obtained at the beginning of each 1-year follow-up were linked to the development of diabetes at the end of the follow-up. These 1-year follow-ups were then pooled and the relationship of the parameters obtained to the development of diabetes analyzed using a logistic-regression model [31]. The method therefore included the concept of person-years. The parameters obtained were adjusted for the effects of other time-variable covariates.

Table 1

<table>
<thead>
<tr>
<th>Characteristics of participants at the time (year) of study entry.</th>
<th>Mean (SD)</th>
<th>GM (GSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.4 (12.1)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.7 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>129.4 (12.9)</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78.7 (8.8)</td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>95.6 (9.6)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption (gou/week)</td>
<td>5.3 (6.0)</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>0.05 (2.9)</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Total serum cholesterol (mg/dL)</td>
<td>196.3 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase (IU/L)</td>
<td>21.8 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.80 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.8 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Type of job schedule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td>60.9%</td>
<td></td>
</tr>
<tr>
<td>Three-shift work</td>
<td>32.4%</td>
<td></td>
</tr>
<tr>
<td>Two-shift work</td>
<td>5.2%</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1.5%</td>
<td></td>
</tr>
<tr>
<td>Smoking habits</td>
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<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>46.4%</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>53.6%</td>
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<tr>
<td>Habitual exercise</td>
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<td></td>
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<tr>
<td>None</td>
<td>38.1%</td>
<td></td>
</tr>
<tr>
<td>1–2 times/month</td>
<td>19.6%</td>
<td></td>
</tr>
<tr>
<td>1–2 times/week</td>
<td>26.7%</td>
<td></td>
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<tr>
<td>3–4 times/week</td>
<td>9.9%</td>
<td></td>
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<tr>
<td>≥ 5 times/week</td>
<td>5.7%</td>
<td></td>
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<tr>
<td>Meals</td>
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<tr>
<td>Cooked by oneself</td>
<td>5.9%</td>
<td></td>
</tr>
<tr>
<td>Cooked by family member</td>
<td>80.9%</td>
<td></td>
</tr>
<tr>
<td>Eating out or catering</td>
<td>6.1%</td>
<td></td>
</tr>
<tr>
<td>Eating in a dormitory</td>
<td>6.5%</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.6%</td>
<td></td>
</tr>
<tr>
<td>Soft-drink consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rare</td>
<td>26.7%</td>
<td></td>
</tr>
<tr>
<td>Occasionally</td>
<td>46.7%</td>
<td></td>
</tr>
<tr>
<td>Frequent</td>
<td>26.6%</td>
<td></td>
</tr>
<tr>
<td>Snacking between meals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rare</td>
<td>40.9%</td>
<td></td>
</tr>
<tr>
<td>Occasionally</td>
<td>50.7%</td>
<td></td>
</tr>
<tr>
<td>Frequent</td>
<td>8.4%</td>
<td></td>
</tr>
<tr>
<td>Brief Job Stress Questionnaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Job demands (high)</td>
<td>43.9%</td>
<td></td>
</tr>
<tr>
<td>Job control (low)</td>
<td>38.7%</td>
<td></td>
</tr>
<tr>
<td>Interpersonal relationships (low)</td>
<td>17.4%</td>
<td></td>
</tr>
<tr>
<td>Compatibility (low)</td>
<td>19.8%</td>
<td></td>
</tr>
</tbody>
</table>

GM: geometric mean; GSD: geometric standard deviation.

The analyses were performed using IBM SPSS 19J statistical software (IBM Business Analytics, Tokyo, Japan). P values < 0.05 were considered statistically significant.

3. Results

Table 1 summarizes the characteristics of the study participants at the time (year) of study entry. Mean age was 44.4 years, and the geometric mean of HsCRP was 0.05 mg/dL.

The number of person-years and incidence rate are shown in Table 2. The per-person mean observed years were 3.08. A total
of 316 cases developed diabetes during the follow-up period. Three cases were diagnosed based solely on the self-declared use of antidiabetic drugs, confirmed by interviews carried out by occupational physicians. The remaining 313 cases had an HbA1c greater or equal to 6.5% at diagnosis. The incidence rate per 1000 person-years was 13.9. Kaplan–Meier diagrams for quartile groups of baseline HsCRP were generally consistent during the 4-year follow-up period (Fig. S1; supplementary material associated with this article online).

Table 3 shows the odds ratio (OR) and 95% confidence interval (CI) for the development of diabetes. For these log-transformed continuous variables, the OR and 95% CI were estimated for a 1-GSD-fold increase in variables. For other continuous variables, the OR and 95% CI were estimated for a ±1 standard deviation (SD) increase. This showed that both the baseline HsCRP and its increase from baseline were significantly associated with the development of diabetes mellitus in men. The OR for a 2.9 (1-GSD)-fold increase in baseline HsCRP was 1.18 (95% CI: 1.03–1.34; P = 0.018), and the OR for a 2.9 (1-GSD)-fold increase in HsCRP from baseline was 1.21 (95% CI: 1.03–1.41; P = 0.018).

Age, baseline BMI, increase in BMI from baseline, total cholesterol, AST and smoking habits all showed a significant positive association with the development of diabetes. In contrast, alcohol consumption, creatinine level and two-shift work showed significant negative associations with the development of diabetes.

4. Discussion

The present large, 5-year, prospective cohort study examined whether HsCRP was associated with the development of diabetes mellitus. The main finding of the study was that a high concentration of HsCRP had a significant effect on the development of diabetes in men, even after adjusting for a comprehensive number of risk factors. Brunner et al. [32] examined the nature of the association between the CRP phenotype and risk of diabetes using the haplotype of the CRP gene as an instrumental variable. They hypothesized that such an association was likely to be causal if the genetic variants that affect CRP level were associated with diabetes according to the Mendelian randomization method. In their study, the haplotype of the CRP gene, which showed different median CRP levels, was not significantly related to the risk of diabetes. It was therefore suggested that variants of the CRP gene do not play a major role in the relationship between serum CRP and the risk of diabetes. Thus, they also concluded that inflammation may have a causal role in diabetes via upstream effectors rather than the downstream marker CRP [32]. Our present findings support the hypothesis that low-grade chronic inflammation is an independent factor for the development of diabetes.

A notable feature of the present study was its longitudinal cohort design involving more than 7000 participants. Previous case-control and nested case-control studies on the relationship between CRP concentration and the development of diabetes mellitus may have been influenced by biases, such as the effect of healthy workers [11,12,14,17,20,23]. The longitudinal cohort design of the present study reduced such an effect. Moreover,
the number of participants in the earlier cohort studies 
[10,13,15,16,21,22] was 6000 at most [15].

Another noteworthy feature of the present study was the statistical method used in the analyses. The strength of the method was that the OR obtained was adjusted for covariates in the multivariate model, which was updated after each yearly examination [31]. Thus, the results obtained with this method represented annual fluctuations in HsCRP and other covariates. Previous cohort studies were not able to adjust for such annual fluctuations [10,13,15,16,21,22]. In addition, the yearly measurements in the present study provided accurate and immediate detection of the development of diabetes. Several cohort studies [16,22] were not able to achieve such immediate detection, as diagnosis was carried out only at baseline and at the end of the follow-up period.

Furthermore, the present study included various potential covariates such as blood chemistry, job-related stress and type of shift work, factors that were not taken into account in earlier cohort studies of CRP and diabetes mellitus [10,13,15,16,21,22].

In addition, the present study excluded participants with chronic inflammatory diseases because of their known effects on HsCRP levels [4]. Indeed, our study was the first to exclude chronic inflammatory diseases when examining the relationship between diabetes and HsCRP levels. Moreover, CRP levels were used as a continuous variable whereas, in previous studies, CRP was divided into either tertiles, quartiles or quintiles [10,13,15,16,21,22], thereby resulting in loss of information.

On the basis of the above features, the epidemiological accuracy and detection power of the present study were improved considerably compared with those of previous cohort studies [10,13,15,16,21,22].

HbA1c is a measure of average glycaemic levels over a time scale of weeks, whereas plasma glucose varies greatly within any given day and from one day to the next [33]. For the HbA1c test, the subjects require no fasting or other preparation, and blood samples can be drawn at any time of day [33]. Thus, the HbA1c level is widely accepted as a useful index of mean blood glucose in the treatment of patients with diabetes, with a systematic review showing that HbA1c and fasting plasma glucose (FPG) are equally effective as screening tools for detection of diabetes in epidemiological studies [34]. The JDS also acknowledges the usefulness of HbA1c as a screening test, and has declared that an HbA1c greater or equal to 6.5% can be used to estimate the prevalence of diabetes in epidemiological studies [25,26], as measurements of fasting blood sugar may be difficult to evaluate, given the need for participants to record the duration of fasting. Recently, an International Expert Committee, appointed by several major scientific societies of diabetes mellitus, reported that the HbA1c correlates well with the risk of long-term diabetes complications and may be a better means of diagnosing diabetes than the measurement of glucose levels [35]. Our present study reflects those guidelines, and our diagnostic method was more accurate than using only either a questionnaire [11,21] or medical interview [14], or measuring fasting glucose [10].

Earlier studies led to controversial results that CRP, after adjusting for BMI, was an independent risk factor for the development of diabetes mellitus. Several studies also demonstrated that CRP was consistently associated with the incidence of type 2 diabetes [10–17], whereas others showed no such association after adjusting for BMI [20–23]. As the prevalence of type 2 diabetes is closely associated with obesity, it is important to determine the effect of BMI on the relationship between CRP levels and onset of the disorder. In fact, our present study has demonstrated that the CRP level was related to the development of diabetes even after adjusting for BMI.

In addition, in the present study, two-shift work was associated negatively with the development of diabetes. On the other hand, alternating three-shift work was a significant risk factor for the development of diabetes and increased HbA1c levels [28,36]. In fact, there were differences between two-shift and three-shift work that may have yielded fewer mismatches of circadian rhythms, and less psychosocial, behavioural and physiological stress in two-shift workers [37]: for example, there was no evening shift in one of the two-shift work schedules; and overtime work was less frequent with two-shift work. AST and creatinine levels, and alcohol consumption, were all shown to be other significant factors for diabetes mellitus in the present study. Previous studies had shown that ALT predicted new-onset type 2 diabetes, possibly as a consequence of the presence of fatty liver and visceral adiposity indicated by high levels of this liver enzyme [38]. On the other hand, creatinine levels have been shown to have a significant negative association with the development of diabetes in men, while a recent longitudinal study also reported a negative relationship between serum creatinine levels and the development of diabetes [39]. These results have led to speculation that lower serum creatinine may reflect a smaller volume of skeletal muscle [39] that, in turn, could mean fewer target sites for insulin [39], thereby explaining, in part, the pathogenesis of type 2 diabetes [39]. Although exercise was not significant factor in our present study, alcohol consumption was negatively related to the development of diabetes mellitus in men. Several other studies have also shown a relationship between alcohol consumption and the development of diabetes [40,41], with the risk of the disorder decreasing with moderate alcohol consumption. This finding suggests a J-curve relationship. However, to clarify the effect of such lifestyle factors, further longitudinal evaluation of various categories of alcohol consumption should be performed for longer periods of observation.

5. Conclusion

Using a distinctive statistical method, our present study has shown that elevated HsCRP levels are an independent predictor of the development of diabetes mellitus in male Japanese workers, after adjusting for fluctuations in a variety of potential covariates. This could lead to the use of HsCRP as a major index of the disorder in health assessments.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.


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Appendix A. Supplementary data

Supplementary material (Fig. S1) associated with this article can be found at http://www.sciencedirect.com, at doi:10.1016/j.diabet.2012.03.004.

References


