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**RESEARCH ARTICLE**

# Positive association between high-sensitivity C-reactive protein and incidence of type 2 diabetes mellitus in Japanese workers: 6-year follow-up

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## Abstract

**Background** Elevated high-sensitivity C-reactive protein (hs-CRP), a marker of low-grade systemic inflammation, may be involved in the etiology of type 2 diabetes mellitus (T2DM). However, whether inflammation precedes development of T2DM independent of cigarette smoking and obesity remains to be confirmed.

**Methods** We studied 4213 civil servants in a local government in Japan aged 35–66 years at baseline in 2002, who donated blood samples and were followed 6 years. Hazard ratios (HR) of T2DM according to the hs-CRP quartiles [range Q1: 0.02–0.18 (reference), Q2: 0.18–0.33, Q3: 0.33–0.67 and Q4: 0.67-9.62 mg/L) were estimated by Cox proportional hazards model adjusted for gender, age, body mass index, alcohol intake, smoking status (current, past and never), number of cigarettes per day, physical activity, family history of diabetes (Model 1) and variables in Model 1 + glucose (Model 2).

**Results** The geometric mean [95% confidence interval (CI)] of hs-CRP was 0.36 mg/L (0.34–0.37). During the follow-up, 156 new T2DM cases were confirmed. In total sample, Model 2 HRs (95% CIs) for hs-CRP quartiles Q2–Q4 compared with Q1 were 0.69 (0.36–1.26), 1.47 (0.91–2.39) and 1.78 (1.10–2.88), respectively ($p$ for linear trend = 0.014). Stratified analysis revealed that a statistically significant association was observed only in normal weight non-current smokers with Model 2 HRs (CIs) being 0.79 (0.29–2.17), 2.63 (1.25–5.56) and 3.19 (1.49–6.86) for Q2–Q4 compared with Q1, respectively ($p$ for linear trend = 0.0006). The relationship did not change materially after further adjusting for log-homeostasis model assessment or exclusion of past smokers.

**Conclusions** These findings imply that higher hs-CRP itself or existence of chronic systemic inflammation precedes onset of T2DM independent of obesity and smoking. Copyright © 2013 John Wiley & Sons, Ltd.

**Keywords** hs-CRP; type 2 diabetes; overweight; smoking status; incidence; cohort study

## Introduction

High-sensitivity C-reactive protein (hs-CRP) is an established risk marker that predicts development of cardiovascular events, including stroke, myocardial infarction and death from cardiovascular causes [1,2], in both western [3,4] and Asian populations [5,6]. As an acute phase reactant primarily produced

in the liver, hs-CRP is the most widely measured serum marker for low-grade systemic inflammation with stable and standardized assays for its measurement [7].

Case–control [8–13] and cohort studies [14–22] have indicated a relationship between low-grade inflammation represented by hs-CRP and type 2 diabetes mellitus (T2DM) or insulin resistance. However, one prospective study involving African-American and Caucasians in the United States reported that inflammation–diabetes association existed in the non-current smokers but not in the current smokers [8]. Another population-based cohort in Japan reported that the hs-CRP–T2DM association was observed among both current and non-current smokers [17]. Smoking itself is causally associated with inflammation [23]. It is also related to insulin resistance [23–25] and higher incidence of T2DM [26,27]. Most of the previous studies on the hs-CRP–T2DM associations included subjects' smoking status in the models; of those studies that reported the inflammation–diabetes association separately according to the smoking status of subjects, results were inconsistent (Table 1) [8,10,17,28]. Thus, it was warranted to examine the association of hs-CRP with T2DM incidence stratified by smoking status. In addition, overweight has been associated with higher hs-CRP [29,30], as well as twofold to fourfold increased risk of T2DM [31]. East Asians reportedly have lower hs-CRP levels than Caucasians [32–35]. They were also reported to have higher diabetes prevalence at a given degree of obesity than Caucasians [36]. These facts raise a possibility that the hs-CRP–T2DM association may differ by race and presence of overweight.

The aims of the present study are to examine the association of serum hs-CRP with the development of T2DM in a prospective cohort and to find out whether smoking status and the presence of overweight would modify the association.

# Methods

## Subjects

Baseline information was collected in 2002 from local government workers aged 35–66 years in Aichi Prefecture, an urban and a suburban areas located in central Japan. Self-administered questionnaires were returned from 7991 workers, and 5030 provided written consent to use information on their lifestyle and health check-up, of whom 4213 donated serum samples. We excluded subjects with missing hs-CRP ($n = 219$) mostly due to lack of the blood sample, hs-CRP level higher or equal to 10 mg/L ($n = 24$), missing baseline fasting blood glucose ($n = 341$), history of T2DM or prevalent diabetes mellitus (self-reported medication use or baseline glucose level higher or equal to 126 mg/dL ($n = 468$), missing information on smoking status and other variables including the following: age, gender, height, weight, alcohol intake, family history of diabetes and physical activity ($n = 121$), leaving 3040 subjects (2346 men and 694 women) for the present analysis.

Subjects were followed up until they retired. Retirement age at worksite is usually 60 years; however, some workers were reemployed after their retirement and were kept in the cohort. Those who retired and were not reemployed were contacted by mail from us. The study protocol was approved by the Ethic Review Committee of Nagoya University School of Medicine.

## Incidence of T2DM

We identified T2DM incidence by two methods. First, we used data obtained during annual mandatory health check-up through March 2007 until workers retired. We defined the incidence as the year when fasting glucose level first exceeded 126 mg/dL. We arbitrarily set the date of onset as 1 July for the analysis considering the fact that check-ups were usually carried out from October to December. Second, we utilized data from detailed self-administered questionnaire surveys on medical history conducted in 2004 and 2007. In the surveys, participants reported medical histories of several pre-specified conditions including T2DM. They provided information on the year of diabetes diagnosis as well as the name and the address of their present or past physician. They were asked to give written consent for our access to the medical records from their specified physicians. We confirmed the accuracy of self-reports (95%) by reviewing the medical records from cases with consent, and the details of the validating study have been reported elsewhere [37]. In the present analysis, we included all the self-reported T2DM.

## Anthropometric measurements and biochemical analysis

Weight and height were measured with the subjects in typical indoor clothing but without shoes, weight to the nearest 0.5 kg and the height to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kilogram) divided by the square of height (meter). Venous blood samples were drawn after the subjects fasted for 8 h or more, or overnight, and serum samples were frozen at $-80\,^{\circ}C$ until the biochemical assay. hs-CRP was measured by latex nephelometry (BNII, Dade Behring Co., Ltd.). Blood glucose was measured by solid-phase radioimmunoassay (RIABEAD II; Dinabot Co., Ltd., Chiba, Japan). Insulin resistance was evaluated with a homeostasis model assessment (HOMA-IR = fasting insulin ($\mu$UI/mL) $\times$ fasting blood glucose (mg/dL)/405 [38].

## Other variables

Smoking status was classified into current, former and never, and average number of cigarettes consumed

**Table 1. Studies of inflammatory markers in relations to diabetes incidence by subgroups**

| Study name | *n*/*N*[a] | Stratified by | Covariates included in subgroup analysis | Summary of the finding |
|---|---|---|---|---|
| MESA [16] | 410/5571 | Ethnicity (White, Chinese, Black and Hispanic) | Age, gender, BMI, race, education, site, alcohol drinking, smoking status, exercise, systolic blood pressure, antihypertensive medication use and HOMA-IR | hs-CRP quartile was significantly associated with incident diabetes in all ethnic groups except for Chinese and was significantly attenuated by additional adjustment for BMI. In a given hs-CRP quartile, incidence rate tended to be higher in non-White ethnic groups than White group. |
| Finnish [18] | 321/12 861 | Age group (35–54/55–74 years); gender (men/women); smoking status (current/ non-current); BMI ($<30/\geq 30$ kg/m$^2$); alcohol drinking (yes/no); family history of diabetes (yes/no) | Age, education, physical activity, smoking status, alcohol drinking, coffee consumption, family history of diabetes, antihypertensive medication use, cholesterol-lowering medication use, hormone replacement therapy, systolic blood pressure, HDL cholesterol and TG | The hs-CRP–diabetes association was stronger in women than in men and remained significant in all the other subgroup analysis. |
| MONICA/KORA Augsburg [28] | 527/2225 | Gender (men/women); smoking status (current/ non-current); BMI ($<30/\geq 30$ kg/m$^2$) | Age, different times of survey, smoking status, alcohol drinking, physical activity, BMI, systolic blood pressure, total-to-HDL cholesterol ratio, parental history of diabetes and IL-6 | The hs-CRP–diabetes association was stronger in women than men in total sample, in men with BMI $<30$ kg/m$^2$ than obese men and in non-current smoking women than current smoking women. The hs-CRP–diabetes association was not modified by smoking status in men. |
| Hisayama [17] | 131/1759 | BMI ($\leq$21.5, 21.6–24.2 and $\geq$24.3 kg/m$^2$); TG ($\leq$0.88, 0.89–1.34 and $\geq$1.35 mmol/L); HDL ($\leq$1.14, 1.15–1.40 and $\geq$1.41 mmol/L); hypertension (yes/no); smoking status (current/ non-current); alcohol drinking (yes/no) | Age and gender | The positive hs-CRP–diabetes association was seen in subjects with BMI $\leq$21.5 kg/m$^2$, without hypertension and drinking habit, with lower TG, with higher HDL cholesterol and in both current and non-current smokers. |
| KIHD [10] | 78/762 | Smoking status (current/ non-current); BMI ($<27/\geq 27$ kg/m$^2$) | Age, socioeconomic status, physical activity, presence of cardiovascular disease, alcohol consumption, smoking, family history of diabetes, BMI, waist-to-hip ratio, insulin, fasting glucose, TG, systolic blood pressure and antihypertensive medication use | The positive hs-CRP–diabetes associations existed after exclusion of smokers and were similar in both BMI subgroups. |
| ARIC[b] [8] | Case: 581; control: 572 | Smoking status (current/ non-current); ethnicity (White/Black) | Age, gender, education level, parental history of diabetes, hypertension, BMI, waist-to-hip ratio, fasting glucose and insulin | The positive inflammation–diabetes association existed only among Whites and non-current smokers. |
| Nakanishi, *et al*.[c] [42] | 154/2953 | Smoking status (never/past/current) | Age, BMI, family history of diabetes, alcohol consumption, physical activity, systolic blood pressure, fasting glucose, total cholesterol, HDL cholesterol, TG, uric acid and hematocrit | The positive inflammation–diabetes association existed only in past and never smokers. |

MESA, Multi-Ethnic Study of Atherosclerosis; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; ARIC, the Atherosclerosis Risk in Communities Study; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; HDL cholesterol, high density lipoprotein cholesterol; TG, triglyceride; IL-6, interleukin-6; hs-CRP; elevated high-sensitivity C-reactive protein.

[a]*n*, number of cases; *N*, number of subjects.

[b]This analysis of the ARIC study used a nested case–control study design, and calculated an inflammatory score from hs-CRP, IL-6, orosomucoid and sialic acid.

[c]White blood cell count was used as the representation of inflammation.


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per day for current and former smokers was assessed by self-reports. Alcohol consumption was calculated by multiplying weekly frequency and the amount drunk per one occasion and then converted into grams of ethanol consumed per day. Physically active individuals were defined as those engaged in moderate or vigorous leisure-time physical activity for 12 days or more and at least 360 min in total per month. Family history of T2DM was assessed by self-report and defined as the one in their first-degree relatives.

## Statistical analysis

Fasting blood glucose, HOMA-IR and hs-CRP values were natural-logarithmically transformed to approximately normalize their distribution prior to the analyses, and geometric means and the 95% confidence intervals (CIs) were presented. To investigate the association of hs-CRP with T2DM incidence with no assumption for linearity, the level of hs-CRP was divided into quartiles (cutoff values: 0.18, 0.33 and 0.67 mg/L). Descriptive statistics were utilized to compare baseline characteristics of participants across the hs-CRP quartile groups. Age-adjusted and gender-adjusted incidence rates in each quartile were estimated using Poisson regression analysis. Cox proportional hazard model was used to estimate hazard ratios (HRs) across quartiles taking the lowest quartile (Q1) as the reference. Model 1 adjusted for age, gender, BMI, alcohol intake, physical activity, smoking status, average number of cigarettes consumed per day and family history of diabetes. Model 2 included Model 1 variables + log-glucose. Model 3 included Model 1 variables + log-HOMA-IR. The linear trend of T2DM incidence risk was assessed by using the median value of each hs-CRP quartile group and treating it as a continuous variable in the corresponding models.

Subsequently, we performed stratified analyses by current smoking and BMI-defined overweight (25 kg/m$^2$). Then, we further stratified non-current smokers by overweight. Even in these stratified analyses by BMI, the Cox models included continuous values of BMI.

Additional analyses were conducted using the same models by excluding past smokers from the non-current smokers group ($n = 660$) or by including the subjects with hs-CRP 10 mg/L or higher ($n = 24$).

The likelihood ratio test was used to test the interaction of current smoking and of the presence of overweight with hs-CRP quartile. Statistical analyses were conducted with R version 2.15.0 (30-03-2012) [39], package of epicalc [40] and survival [41]. A $p$ value of less than 0.05 was considered statistically significant.

# Results

Mean age and BMI (standard deviation) of the subjects were 47.7 (7.2) years and 22.8 (2.7) kg/m$^2$ at baseline. The geometric mean (95% CI) of hs-CRP was 0.36 mg/L (0.34–0.37). Age, BMI, HOMA-IR, the proportion of men and the prevalence of current smoker were positively associated with the serum hs-CRP levels (Table 2). By contrast, the proportion of those with family history of diabetes was lowest in Q2. During 6 years of follow-up (15 782 person-years), 156 new T2DM incidences were ascertained (men: 127; women: 29). The annual incidence rate was 9.8 per 1000 person-years (men: 10.4; women: 8.2). The age-adjusted and gender-adjusted incidence rate was highest in Q4 (14.5 per 1000 person-years) and lowest in Q2 (4.9 per 1000 person-years) (Table 3). Model 1 HRs (95% CI) of Q3 and Q4 compared with Q1 were 1.39 (0.86–2.25) and 1.48 (0.91–2.41), respectively ($p$ for linear trend = 0.016). The linear associations were similar after further adjustment for log-transformed fasting blood glucose (Model 2) or HOMA-IR (Model 3).

Stratified analyses revealed that Model 2 HRs of Q3 and Q4 against Q1 were statistically significant only in non-smokers (1.96 and 2.40, respectively, both $p < 0.05$) and in normal weight subjects (1.87 and 1.83, respectively, both $p < 0.05$). And, the results did not change

**Table 2. Means (SD) or percentages of all participants at baseline according to hs-CRP quartiles, Aichi, 2002**

| | | Q1 | Q2 | Q3 | Q4 | $p$[b] |
|---|---|---|---|---|---|---|
| | | 0.01–0.18 | 0.19–0.33 | 0.34–0.67 | 0.68–9.62 | value |
| hs-CRP[a] | mg/L | 0.104 (0.100–0.107) | 0.250 (0.247–0.253) | 0.462 (0.456–0.468) | 1.49 (1.42–1.57) | <0.001 |
| Number of subjects | | 794 | 731 | 766 | 749 | |
| Age | years | 46.4 (7.0) | 47.1 (7.1) | 48.6 (6.8) | 48.6 (7.4) | <0.001 |
| Men | % | 67.3 | 76.6 | 81.2 | 84.1 | <0.001 |
| Postmenopausal women | % | 7.7 | 5.7 | 7.3 | 5.5 | <0.001 |
| Current smoker | % | 20.4 | 26.5 | 29.6 | 38.6 | <0.001 |
| Presence of physical activity | % | 53.7 | 55.7 | 56.7 | 53.4 | 0.51 |
| Body mass index | kg/m$^2$ | 21.7 (2.3) | 22.6 (2.5) | 23.2 (2.6) | 23.9 (2.8) | <0.001 |
| Alcohol intake | g/day | 12.5 (18.3) | 14.1 (20.1) | 14.8 (21.3) | 14.9 (21.6) | 0.06 |
| Presence of diabetes family history | % | 15.1 | 13.5 | 15.0 | 16.3 | 0.53 |
| Fasting blood glucose[a] | mg/dL | 89.2 (88.5–89.9) | 89.0 (88.3–89.8) | 89.5 (88.7–90.3) | 88.7 (87.9–89.4) | 0.58 |
| HOMA-IR[a] | | 1.17 (1.12–1.23) | 1.34 (1.28–1.41) | 1.49 (1.42–1.57) | 1.57 (1.49–1.65) | <0.001 |

SD, standard deviation; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance.
[a]Geometric mean and 95% confidence interval.
[b]$p$ value was calculated by using analysis of variance to compare the difference between hs-CRP quartile groups.



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**Table 3. Hazard ratios (95% confidence interval) of T2DM incidence according to hs-CRP quartiles stratified by current smoking status and the presence of overweight Aichi, 2002–2007**

| | Q1<br>0.01–0.18 | Q2<br>0.19–0.33 | Q3<br>0.34–0.67 | Q4<br>0.68–9.62 | $p^a$<br>for trend |
|---|---|---|---|---|---|
| All participants | | | | | |
| $n/N^b$ | 27/794 | 19/731 | 51/766 | 59/749 | |
| Incidence rate[c] | 6.7 | 4.9 | 12.0 | 14.7 | |
| Model 1 | 1 | 0.64 (0.35–1.16) | 1.39 (0.86–2.25) | 1.48 (0.91–2.41) | 0.016 |
| Model 2 | 1 | 0.69 (0.38–1.26) | 1.47 (0.91–2.39) | 1.78 (1.10–2.88) | 0.014 |
| Model 3 | 1 | 0.62 (0.34–1.12) | 1.31 (0.80–2.12) | 1.41 (0.80–2.29) | 0.024 |
| Non-current smokers | | | | | |
| $n/N^b$ | 16/632 | 10/537 | 34/539 | 34/460 | |
| Incidence rate[c] | 4.7 | 3.5 | 11.1 | 13.5 | |
| Model 1 | 1 | 0.64 (0.29–1.43) | 1.79 (0.97–3.32) | 1.94 (1.03–3.64) | 0.086 |
| Model 2 | 1 | 0.76 (0.34–1.69) | 1.96 (1.05–3.64) | 2.40 (1.28–4.50) | 0.012 |
| Model 3 | 1 | 0.76 (0.34–1.69) | 1.96 (0.80–3.64) | 2.40 (1.28–4.51) | 0.012 |
| Current smokers | | | | | |
| $n/N^b$ | 11/162 | 9/194 | 17/227 | 25/289 | |
| Incidence rate[c] | 12.5 | 7.4 | 12.5 | 14.9 | |
| Model 1 | 1 | 0.52 (0.21–1.28) | 0.77 (0.35–1.69) | 0.81 (0.38–1.72) | 0.76 |
| Model 2 | 1 | 0.54 (0.22–1.33) | 0.81 (0.37–1.78) | 0.93 (0.44–1.98) | 0.48 |
| Model 3 | 1 | 0.54 (0.22–1.34) | 0.83 (0.38–1.82) | 0.94 (0.44–2.01) | 0.75 |
| Body mass index $< 25$ kg/m$^2$ | | | | | |
| $n/N^b$ | 21/727 | 12/613 | 33/596 | 29/505 | |
| Incidence rate[c] | 5.4 | 3.6 | 9.4 | 9.7 | |
| Model 1 | 1 | 0.65 (0.32–1.33) | 1.71 (0.97–3.00) | 1.63 (0.90–2.94) | 0.039 |
| Model 2 | 1 | 0.70 (0.34–1.43) | 1.87 (1.07–3.28) | 1.83 (1.01–3.30) | 0.016 |
| Model 3 | 1 | 0.70 (0.34–1.43) | 1.31 (1.07–3.32) | 1.84 (1.02–3.31) | 0.015 |
| Body mass index $\geq 25$ kg/m$^2$ | | | | | |
| $n/N^b$ | 6/67 | 7/118 | 18/173 | 30/244 | |
| Incidence rate[c] | 17.1 | 11.1 | 19.9 | 25.1 | |
| Model 1 | 1 | 0.60 (0.20–1.81) | 1.02 (0.40–2.61) | 1.25 (0.51–3.08) | 0.17 |
| Model 2 | 1 | 0.62 (0.21–1.88) | 0.92 (0.35–2.41) | 1.66 (0.67–4.13) | 0.015 |
| Model 3 | 1 | 0.47 (0.15–1.44) | 0.82 (0.32–2.13) | 1.03 (0.41–2.56) | 0.17 |

T2DM, type 2 diabetes mellitus; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance. Model 1 adjusted for age, gender, body mass index, alcohol intake, physical activity and family history of diabetes (smoking status and average number of cigarettes consumed per day were adjusted among current smokers); Model 2 adjusted for Model 1 + fasting blood glucose; Model 3 adjusted for Model 1 + HOMA-IR.
Values of fasting blood glucose and HOMA-IR were natural-logarithmically transformed in the models.
In current smokers, models also included the number of cigarettes smoked per day reported by the subjects.
[a]*p* for linear trend was calculated by using the median values of each hs-CRP quartile group and treating it as a continuous variable in the corresponding models.
[b]*n*, number of cases; *N*, number of subjects.
[c]Age-adjusted and gender-adjusted incidence rate was calculated using Poisson regression model and expressed as rate per 1000 person-years.

materially after adjusting for log-transformed HOMA-IR (Model 3). The interactions of current smoking and of overweight with hs-CRP quartile were statistically significant ($p = 0.011$ and 0.039, respectively).

With these findings, we further divided non-current smokers ($n = 2168$) by the presence of overweight, and the results are shown in Table 4. The lowest incidence rate was observed in Q2 in normal weight non-current smokers (2.3 per 1000 person-years). Overweight non-current smokers had higher incidence rate in every quartile group than the corresponding quartile group of normal weight non-current smokers. The HR for developing T2DM was increased more than 200% in Q4 compared with Q1 in normal weight non-smokers (Model 2). These results did not change materially after adjusting for log-transformed HOMA-IR (Model 3).

Additional analysis by excluding past smokers from non-current smokers yielded essentially similar results: the HRs (95% CIs) for Q2–Q4 compared with Q1 in Model 2 in normal weight never smokers were 1.11 (0.31–3.98), 3.06 (1.11–8.47) and 5.58 (2.06–15.11), respectively. Another analysis including subjects with hs-CRP higher or equal to 10 mg/L ($n = 24$) into the Q4 group also produced similar results: the HRs (95% CIs) for Q2–Q4 compared with Q1 in Model 2 in normal weight non-current smokers were 0.80 (0.29–2.17), 2.63 (1.25–5.56) and 3.02 (1.41–6.49), respectively.

## Discussion

We found that higher baseline hs-CRP was positively associated with higher incidence of T2DM in middle-aged Japanese workers. The main findings of the present study were twofolds: first, a higher concentration of hs-CRP was significantly positively associated with the development of T2DM independent of smoking, BMI and other confounding variables in the total sample; second, smoking status and overweight significantly modified the relationship between hs-CRP and T2DM incidence. It would be possible that statistically significant trend found



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**Table 4. Hazard ratios (95% confidence interval) of T2DM incidence according to hs-CRP quartiles stratified by the presence of overweight in non-current smokers Aichi, 2002–2007**

| | Q1<br>0.01–0.18 | Q2<br>0.19–0.33 | Q3<br>0.34–0.67 | Q4<br>0.68–9.62 | $p^a$<br>for trend |
|---|---|---|---|---|---|
| Body mass index $< 25$ kg/m$^2$ | | | | | |
| $n/N^b$ | 11/585 | 6/455 | 21/417 | 20/308 | |
| Incidence rate[c] | 3.2 | 2.3 | 7.9 | 10.2 | |
| Model 1 | 1 | 0.71 (0.26–1.87) | 2.32 (1.10–4.91) | 2.89 (1.35–6.20) | 0.0012 |
| Model 2 | 1 | 0.79 (0.29–2.17) | 2.63 (1.25–5.56) | 3.19 (1.49–6.86) | 0.0006 |
| Model 3 | 1 | 0.70 (0.26–1.91) | 2.29 (1.08–4.83) | 2.74 (1.28–5.90) | 0.002 |
| Body mass index $\geq 25$ kg/m$^2$ | | | | | |
| $n/N^b$ | 5/47 | 4/82 | 13/122 | 14/152 | |
| Incidence rate[c] | 21.8 | 9.5 | 21.4 | 19.2 | |
| Model 1 | 1 | 0.44 (0.12–1.67) | 0.90 (0.31–2.58) | 0.80 (0.28–2.30) | 0.82 |
| Model 2 | 1 | 0.56 (0.15–2.18) | 0.79 (0.27–2.31) | 1.23 (0.41–3.69) | 0.24 |
| Model 3 | 1 | 0.35 (0.09–1.38) | 0.69 (0.23–2.03) | 0.59 (0.20–1.77) | 0.94 |

T2DM, type 2 diabetes mellitus; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance. Model 1 adjusted for age, gender, body mass index, alcohol intake, physical activity, and family history of diabetes (smoking status and average number of cigarettes consumed per day were adjusted among current smokers); Model 2 adjusted for Model 1 + fasting blood glucose; Model 3 adjusted for Model 1 + HOMA-IR.
Values of fasting blood glucose and HOMA-IR were natural-logarithmically transformed in the models.
In current smokers, models also included the number of cigarettes smoked per day reported by the subjects.
[a]*p* for linear trend was calculated by using the median values of each hs-CRP quartile group and treating it as a continuous variable in the corresponding models.
[b]*n*, number of cases; *N*, number of subjects.
[c]Age-adjusted and gender-adjusted incidence rate was calculated using Poisson regression model and expressed as rate per 1000 person-years.

in all samples could simply be due the strong association in non-current smokers, although we had adjusted for smoking status.

Because cigarette smoking and overweight were positively associated with the risk for developing T2DM in the present study (HR for current smoking and overweight: 1.68 and 2.70, respectively), the influence of hs-CRP concentration on development of T2DM might have been obscured by their stronger influences. Our findings would indicate that hs-CRP itself could be related to the incidence of T2DM.

The present study would be consistent with previous reports [8,42]. Nakanishi *et al*. described an association of elevated inflammation, represented by white blood cell count, with incident diabetes or impaired fasting glucose among non-smokers in Japanese male workers; however, the interaction of smoking was not statistically tested in their report. In the Atherosclerosis Risk in Communities (ARIC) Study, which used inflammation score calculated from hs-CRP, orosomucoid, interleukin-6 and sialic acid, however, they did not find significant interaction of smoking status in fully adjusted models. The positive associations were observed in both smokers and non-smokers in the Hisayama study [17], which is inconsistent with the present findings. This inconsistency might be explained by a fact that the Hisayama study did not include the degree of overweight and the number of cigarettes to the covariates in smoking-stratified analysis. In addition, compared with the present study, participants in the Hisayama study were recruited from a rural area in Japan, were older (mean age: 57.4 vs. 47.7 years), more likely to be women (60.5% vs. 22.8%), with 14 years earlier baseline (1988 vs. 2002) and with different current smoker proportions (men/women: 47.6%/5.4% vs. 35.1%/6.9%).

The mechanisms explaining the association between higher level of hs-CRP and higher incidence of T2DM are still unclear. One of them may be a genetic factor. Single nucleotide polymorphisms have been identified in the human hs-CRP locus that are associated with high serum hs-CRP levels [43], low insulin sensitivity [44] and an increased risk of diabetes [20,45]. Among such single nucleotide polymorphisms, the variants rs3093059 ($-757$ A $>$ G) [45] and rs2794521 ($-717$ A G) [46] were reported to be associated with an increase risk of T2DM. Other mechanisms include increased oxidative stress and dysregulated immune system [19,47]. Increased oxidative stress and systemic inflammation have been proposed as the common pathogenic mechanisms linking insulin resistance, T2DM and cardiovascular disease. [48] Direct harmful effects of hs-CRP on vessel walls [49–52] and pancreatic β-cells [53] were also proposed, which may increase the rate of apoptotic cell death, alter endothelial permeability and eventually induce insulin resistance. Further studies are still necessary to elucidate the mechanism of hs-CRP–T2DM association.

The present study had several limitations. First, hs-CRP was assessed from single measurement of the baseline blood sample. Twenty-four subjects with 10 mg/L or higher hs-CRP were considered as having an acute inflammation and excluded in the initial analysis. However, because subjects did not have any apparent diseases and such hs-CRP elevations could also be seen as chronic inflammation [54], we carried out an additional analysis including these 24 subjects in the highest hs-CRP level group and found similar results. Second, the subjects were mostly men (77.2 %) in the present study. The issue of gender difference in hs-CRP level remains controversial [55]. In our study, men had higher hs-CRP concentration than did women (mean: 0.75 vs. 0.53 mg/L). But, it is important to note that women in the present analysis had much lower rates of current

smoking (6.9% vs. 35.1%) and overweight (9.5% vs. 22.8%) than men. Nevertheless, in the gender-stratified analysis, we found similar hs-CRP–T2DM associations in both men and women (data not shown).

In summary, we found a positive association of elevated hs-CRP concentration with higher T2DM incidence independent of BMI, baseline fasting blood glucose, insulin resistance, family history of diabetes, alcohol intake and physical activity especially in normal weight non-smoking middle-aged Japanese; elevated hs-CRP concentration in apparently healthy subjects may help to identify high-risk individuals for the development of T2DM. Our findings support the hypothesis that hs-CRP itself might be related to the development of T2DM, and existence of low-grade systemic inflammation precedes T2DM.

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## Conflict of interest

None declared.

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