Original Article

High-Sensitivity CRP Reflects Insulin Resistance in Smokers

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Aim: The elevation of high-sensitivity C-reactive protein (hs-CRP) is a strong risk factor for cardiovascular disease (CVD) and is associated with insulin resistance. The hs-CRP concentration also increases in smokers known to be at a high risk for CVD. We examined whether hs-CRP concentration reflects insulin resistance in smokers.

Methods: The hs-CRP levels were measured in 121 male subjects (54 nonsmokers and 67 smokers) with a normal glucose tolerance. The hs-CRP concentration was compared to the homeostasis model assessment of insulin resistance (HOMA-IR) and other clinical variables related to insulin resistance.

Results: Smokers had a 64.5% higher hs-CRP concentration than nonsmokers ($p < 0.0001$). In both nonsmokers and smokers, hs-CRP positively correlated with HOMA-IR ($r = 0.301$, $p < 0.05$ and $r = 0.312$, $p < 0.01$) and fasting insulin ($r = 0.281$, $p < 0.05$ and $r = 0.356$, $p < 0.01$). The correlation between hs-CRP and HOMA-IR or fasting insulin was stronger in smokers than in nonsmokers. In smokers, hs-CRP significantly correlated with BMI and HDL-cholesterol ($r = 0.386$, $p < 0.01$ and $r = -0.307$, $p < 0.05$). Stepwise regression analysis revealed that BMI and HOMA-IR were significant predictors of hs-CRP in smokers ($r = 0.423$, $p < 0.01$).

Conclusions: The hs-CRP concentration reflects insulin resistance in smokers. It would be preferable to consider insulin resistance in evaluating hs-CRP concentrations, even in smokers.

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Key words; High-sensitivity C-reactive protein (hs-CRP), Insulin resistance, Homeostasis model assessment of insulin resistance (HOMA-IR), Smoker

Introduction

C-reactive protein (CRP) is an inflammatory marker whose expression is markedly up regulated during inflammation¹. It is primarily synthesized in the liver and is regulated to a large extent by the pro-inflammatory cytokine IL-6 and tumor necrosis factor- α (TNF- α)^{2, 3}. Recent prospective studies have demonstrated that subjects with low-grade inflam-

mation have a higher risk of cardiovascular disease (CVD)⁴⁻⁷. Several lines of evidence suggest that elevation of CRP may reflect not only local inflammation at atherosclerotic lesions but also systemic abnormalities related to insulin resistance, such as increases in fasting insulin, body mass index (BMI), systolic blood pressure, and triglyceride (TG) as well as a decrease in high-density lipoprotein cholesterol (HDL-C)⁸⁻¹³. All these abnormalities are responsible for atherosclerosis.

Regardless of race, sex, or geographical area, epidemiological studies have consistently supported that smokers are at a higher risk for CVD. Although smokers usually have a high level of CRP, the precise mechanism is still unclear. If CRP is further increased in smokers with insulin resistance, their absolute levels for CVD risk assessment could be as important in smokers as in nonsmokers. To clarify whether CRP

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reflects insulin resistance in smokers, we measured high-sensitivity CRP (hs-CRP) levels in smokers with a normal glucose tolerance and compared these levels to the homeostasis model assessment of insulin resistance (HOMA-IR)^{14, 15}, which is an established index of insulin resistance, and other clinical variables related to insulin resistance.

Materials and Methods

Study Subjects

Healthy men who had annual medical examinations at our institute were randomly invited to participate in this study. Because diabetic patients tend to have latent as well as overt infections, we excluded subjects whose fasting plasma glucose (FPG) or hemoglobin A_{1c} (HbA_{1c}) levels were beyond the upper limits of individual reference ranges (110 mg/dL and 5.8%, respectively). Subjects with acute or chronic infections based on clinical history, urinalysis, white blood cell count in peripheral blood, and chest roentgenogram were also excluded. The other exclusion criteria were malnutrition, liver dysfunction (transaminases greater than twice the upper limit of normal), and malignancies. Finally, 121 subjects were selected for this study and were composed of 67 smokers and 54 nonsmokers (aged 20–68 years). The Brinkmann index (BI) was used as an indicator of smoking grade and was calculated as follows: (average number of daily cigarette consumption) × (years of smoking)¹⁶. The average BI of the present smokers was $2,216 \pm 715$ (mean ± SD). All subjects gave informed consent before participation. This study was conducted in accordance with the Declaration of Helsinki.

Blood Sampling

After an overnight fast, blood samples were drawn from a cubital vein while the subject was in a sitting position. Plasma was separated after low-speed centrifugation. All variables except the hs-CRP concentration were measured on the day the blood was sampled. Aliquots of samples were frozen at -80°C . The hs-CRP concentration was measured in one analytical run with the thawed samples as described in the hs-CRP concentration section.

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

As an index of insulin resistance, we determined HOMA-IR simultaneously with FPG and fasting immunoreactive insulin (IRI) concentrations. The FPG concentration was measured by a hexokinase method with a Hitachi 7170 auto-analyzer (Hitachi,

Tokyo, Japan). The serum IRI concentration was measured by a microparticle enzyme immunoassay with an AxSYM (Abbott, Abbott Park, IL). HOMA-IR was determined by the product of FPG and fasting IRI: $\text{FPG (mg/dL)} \times \text{fasting IRI (mU/L)} / 405$ ^{14, 15}.

hs-CRP Concentration

The hs-CRP concentration was assayed by BN II nephelometry (Dade Behring, Tokyo, Japan) using polystyrene particles coated with anti-CRP monoclonal antibodies (N High Sensitivity CRP assay; Dade Behring). The standard curve was linear between 0.0461 and 0.328 mg/L for hs-CRP. The coefficients of variation (CVs) were <0.5% in the same assay and <1.5% between assays for hs-CRP.

Other Measured Variables

Height, body weight, and blood pressure were measured in all subjects. Body mass index (BMI) was calculated as the body weight (kg)/[height (m)]².

HbA_{1c}, excluding unstable fractions, was measured with high-pressure liquid chromatography using an HLC-723GHbV (Tosoh, Yamaguchi, Japan) (normal range 4.3–5.8%). Total cholesterol (TC) and TG concentrations were measured enzymatically on a Hitachi 7450 auto-analyzer. Low-density lipoprotein cholesterol (LDL-C) and HDL-C were determined using homogenous assays (LDL-EX, Denka Seiken, Tokyo, Japan; Determiner-L-HDL-C, Kyowa Medex, Tokyo, Japan, respectively).

Statistical Analysis

Data are expressed as the means ± SD. When data showed a skewed distribution (hs-CRP, HOMA-IR, IRI, TG, and HDL-C), we logarithmically transformed the measured values for statistical analysis. For comparisons between smokers and nonsmokers, Student's unpaired *t*-test was used. For correlation analysis, Pearson's correlation coefficients were determined. Using selected variables, we also performed stepwise regression analysis. All calculations were made by StatView (version 5.0; Abacus Concepts, Berkeley, CA). A *p*-value less than 0.05 was considered significant.

Results

Subject Characteristics

Although the age and anthropometric parameters were almost identical between groups, smokers had more risk factors than nonsmokers. Smokers had higher FPG and TG concentrations and lower HDL-C concentration than nonsmokers. As we expected, smokers had a 64.5% higher hs-CRP concentration

Table 1. Clinical characteristics

	Nonsmokers (<i>n</i> = 54)	Smokers (<i>n</i> = 67)	<i>p</i> -value
age (years)	49.6 ± 9.0	47.4 ± 8.8	N.S.
BMI (kg/m ²)	21.9 ± 2.6	22.8 ± 2.6	N.S.
systolic BP (mmHg)	118 ± 13	117 ± 14	N.S.
diastolic BP (mmHg)	74 ± 9	76 ± 11	N.S.
mean BP (mmHg)	89 ± 10	90 ± 11	N.S.
hs-CRP (mg/L)	0.411 ± 0.491	0.676 ± 0.516	<0.0001
HbA _{1c} (%)	4.77 ± 0.29	4.70 ± 0.28	N.S.
FPG (mg/dL)	89 ± 7	93 ± 8	<0.01
fasting IRI (mU/L)	5.9 ± 2.6	6.2 ± 2.9	N.S.
HOMA-IR	1.30 ± 0.60	1.45 ± 0.73	N.S.
TC (mg/dL)	220 ± 39	204 ± 27	<0.01
LDL-C (mg/dL)	129 ± 33	122 ± 27	N.S.
HDL-C (mg/dL)	71 ± 19	55 ± 14	<0.0001
TG (mg/dL)	81 ± 36	134 ± 71	<0.0001

Data are presented as the means ± SD. BMI, body mass index; BP, blood pressure; hs-CRP, high-sensitivity C-reactive protein; FPG, fasting plasma glucose; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

than nonsmokers (Table 1). There were no significant differences in age, BMI, blood pressure, HbA_{1c}, fasting IRI, HOMA-IR or LDL-C between smokers and nonsmokers.

Relationship between hs-CRP and HOMA-IR or Fasting IRI

In both smokers and nonsmokers, the hs-CRP concentration was significantly related to both HOMA-IR and fasting IRI (Fig. 1, 2). The correlation between the hs-CRP concentration and HOMA-IR or fasting IRI was stronger in smokers than in nonsmokers.

Relationship between hs-CRP and BMI

In smokers, the hs-CRP concentration was positively correlated with BMI (Fig. 3). In nonsmokers, however, the correlation between the hs-CRP concentration and BMI was not significant. In both smokers and nonsmokers, BMI was positively correlated with HOMA-IR ($r=0.396$, $p<0.001$, and $r=0.365$, $p<0.01$, respectively). In smokers, we carried out the same analysis in those without BMI > 25. The hs-CRP concentration still showed a tendency to correlate with HOMA-IR ($r=0.226$, $p=0.1$, $n=52$).

Relationship between hs-CRP and Lipoproteins

In smokers, the hs-CRP concentration was nega-

tively correlated with HDL-C (Fig. 4). In nonsmokers, however, hs-CRP had no significant correlation with HDL-C. HDL-C was related to HOMA-IR in smokers ($r=-0.486$, $p<0.0001$), but not in nonsmokers ($r=-0.191$, N.S.).

Relationship between hs-CRP and Other Variables

In smokers, the hs-CRP concentration had no significant correlation with other variables (Table 2). In nonsmokers, the hs-CRP concentration tend to correlate to TG but not significantly ($r=0.263$, $p=0.0542$). TG was positively correlated with HOMA-IR in both smokers and nonsmokers ($r=0.504$, $p<0.0001$, and $r=0.295$, $p<0.05$, respectively).

Stepwise Regression Analysis

We performed stepwise regression analysis using the hs-CRP concentration as a dependent variable and using age, systolic blood pressure, diastolic blood pressure, HbA_{1c}, HOMA-IR, BMI, LDL-C, HDL-C and TG as independent variables (Table 3). To avoid the problem of collinearity, we excluded FPG and fasting IRI as independent variables, because HOMA-IR is calculated from FPG and fasting IRI. In smokers, BMI and HOMA-IR were selected as significant predictors of hs-CRP. This model yielded sufficient correlation ($r=0.423$, $p<0.01$). In nonsmokers, HOMA-IR was selected as a significant predictor of hs-CRP. This model also yielded significant correlation ($r=0.301$, $p<0.05$).

Discussion

This study demonstrates that hs-CRP reflects insulin resistance in smokers. We found that the hs-CRP level was positively correlated with HOMA-IR in smokers (Fig. 1). The hs-CRP concentration was also significantly correlated with other markers related to insulin resistance, such as the fasting IRI (Fig. 2), BMI (Fig. 3), and HDL-C (Fig. 4), which are also related to HOMA-IR. Interestingly, the correlation between hs-CRP and these parameters was stronger in smokers than in nonsmokers.

Previous studies have shown that the hs-CRP concentration is elevated in individuals with insulin resistance and also in those with risk factors of metabolic syndrome, such as high fasting insulin, BMI, systolic blood pressure, TG, and low HDL-C⁸⁻¹³. These relationships are explained by the following models:

The first model emphasizes the role of visceral fat obesity in increasing hs-CRP relating to insulin resistance^{8,9,17}. Adipocytes produce proinflammatory cytokines (e.g., IL-6 and TNF-alpha) that directly stimu-

Fig. 1. Relationships of hs-CRP to HOMA-IR in nonsmokers ($n=54$) and smokers ($n=67$). The hs-CRP concentration was significantly correlated with HOMA-IR in both nonsmokers and smokers (Pearson's correlation coefficient). Logarithmic axes were used for hs-CRP and HOMA-IR levels due to their skewed distribution. hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance.

Fig. 2. Relationships of hs-CRP to fasting IRI in nonsmokers ($n=54$) and smokers ($n=67$). The hs-CRP concentration was significantly correlated with fasting IRI in both nonsmokers and smokers (Pearson's correlation coefficient). Logarithmic axes were used for hs-CRP and IRI levels due to their skewed distribution. hs-CRP, high-sensitivity C-reactive protein; IRI, immunoreactive insulin.

late the synthesis of CRP by the liver^{2, 3, 18-20}. These cytokines also impair insulin signaling at adipose tissues, skeletal muscle tissues, and hepatocytes^{18, 21-23}. Furthermore, they stimulate a response from the hypothalamic-pituitary-adrenal axis²⁴. Consequently, visceral fat obesity results in insulin resistance^{12, 13, 25}, and adipose tissue can therefore cause both mild chronic inflammation and insulin resistance through the actions of cytokines. In healthy subjects in the present study, other mechanisms may be more important than visceral fat obesity in elevating the hs-CRP level

because obese subjects (BMI > 25) comprised only 11.1% of nonsmokers and 22.4% of smokers²⁶.

In the second model, insulin resistance initiates or exaggerates the elevation of hs-CRP due to insufficient insulin-induced suppression of the synthesis of CRP²⁷. When nondiabetic patients with either coronary artery disease or metabolic syndrome are treated with thiazolidinedione (TZD; an insulin-sensitizing antidiabetic agent), they do not lose weight, but instead significantly decrease the level of hs-CRP^{28, 29}. These findings agree well with this model.

Fig.3. Relationships of hs-CRP to BMI in nonsmokers ($n=54$) and smokers ($n=67$).

The hs-CRP level was significantly correlated with BMI not in nonsmokers but in smokers (Pearson's correlation coefficient). Logarithmic axis was used for the hs-CRP level due to its skewed distribution. hs-CRP, high-sensitivity C-reactive protein; BMI, body mass index.

Fig.4. Relationships of hs-CRP to HDL-C in nonsmokers ($n=54$) and smokers ($n=67$).

The hs-CRP level was significantly correlated with HDL-C not in nonsmokers but in smokers (Pearson's correlation coefficient). Logarithmic axes were used for hs-CRP and HDL-C levels due to their skewed distribution. hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol.

In the third model, inflammation causes insulin resistance. CRP is bound to the membranes of damaged vascular cells where it activates complement proteins or enhances the production of thrombogenic agents. These instances of vascular inflammation may contribute to the development of insulin resistance^{30, 31}. Insulin resistance and vascular inflammation may create a vicious cycle *in vivo*⁷.

The hs-CRP concentration is also elevated by smoking itself³², which can be explained by three possible mechanisms. First, smokers have chronic airway inflammation because cigarettes contain potent airway

irritants³³. Indeed, smokers have a higher prevalence of chronic bronchitis, bronchial asthma, and pulmonary emphysema than nonsmokers³⁴. Smokers also have a predisposition to non-respiratory inflammation, such as periodontitis³⁵. Second, smokers are likely to have atherosclerotic lesions, a local and chronic inflammation of vessel walls. Smokers have a tendency to have dyslipidemia, coronary vasomotor reactivity, platelet aggregation, and a prothrombotic state³⁶⁻³⁸. Accumulation of these risk factors promotes the initiation and progression of atherosclerosis. This hypothesis is supported by reports that smokers have elevated

Table 2. Correlation between hs-CRP and clinical parameters

	Nonsmokers (<i>n</i> = 54)		Smokers (<i>n</i> = 67)	
	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
age	0.107	N.S.	0.091	N.S.
systolic BP	0.022	N.S.	0.013	N.S.
diastolic BP	-0.024	N.S.	0.049	N.S.
mean BP	-0.005	N.S.	0.037	N.S.
HbA _{1c}	0.053	N.S.	0.194	0.117
FPG	0.219	0.112	-0.108	N.S.
LDL-C	0.168	N.S.	0.184	0.137
TG	0.263	0.054	0.201	0.104

For correlation analysis, Pearson's correlation coefficients were determined. hs-CRP, high-sensitivity C-reactive protein; BP, blood pressure; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.

concentrations of soluble intercellular adhesion molecule type 1 (sICAM-1), E-selectin, interleukin-5, and P-selectin³⁹⁾. Third, smoking deteriorates insulin resistance, resulting in an increase in hs-CRP⁴⁰⁾. Insulin resistance in smokers could be caused by an increase in counter-regulatory hormones, such as GH, cortisol, glucagon, and catecholamines, all of which raise blood glucose levels⁴¹⁾. In this study, smokers had higher levels of hs-CRP, FPG and TG but lower levels of HDL-C than nonsmokers. These data suggest that smoking may induce a vascular inflammatory response, eventually leading to atherothrombosis^{42, 43)}.

Although the precise mechanism is still unclear, insulin resistance and smoking are likely to increase the hs-CRP concentration in a synergistic manner; therefore, hs-CRP is a good indicator of cardiovascular risk in both smokers and nonsmokers. In conclusion, hs-CRP reflects insulin resistance in smokers. Our study suggests that hs-CRP can be used to predict cardiovascular risk even under chronic inflammatory conditions. Further investigation is needed to confirm this observation.

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Table 3. Stepwise regression analysis of selected variables on hs-CRP

Independent variables	standardized regression coefficient	<i>F</i> -value
Nonsmokers (<i>n</i> = 54)		
HOMA-IR	0.301	5.162
Smokers (<i>n</i> = 67)		
HOMA-IR	0.189	2.338
BMI	0.312	6.385

Stepwise multiple regression analysis was performed using the hs-CRP concentration as a dependent variable. The *F*-value to enter was set at 2.0 at each step. hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; BMI, body mass index.

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