C-Reactive Protein and Insulin Resistance in Non-obese Japanese Type 2 Diabetic Patients

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The aim of the present study was to investigate the relationship between C-reactive protein (CRP) and insulin resistance in non-obese Japanese type 2 diabetic patients. A total of 135 non-obese Japanese type 2 diabetic patients (96 men and 39 women, aged 36 to 83 years, with a body mass index [BMI] of 16.2 to 26.8 kg/m2) were studied. BMI, glycosylated hemoglobin (HbA_{1c}), fasting concentrations of plasma glucose, serum lipids (triglycerides, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol, and total cholesterol), CRP, and fibrinogen were measured. LDL cholesterol was calculated using the Friedewald formula. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment (HOMA-IR). Univariate regression analysis showed that CRP value was positively correlated to age ($r = 0.218$, $P = .012$), BMI ($r = 0.239$, $P = .006$), HOMA-IR ($r = 0.397$, $P < .0001$), triglycerides ($r = 0.310$, $P < .005$), LDL cholesterol ($r = 0.179$, $P = .038$), and fibrinogen ($r = 0.371$, $P < .0001$) levels and inversely correlated to HDL cholesterol ($r = 0.174$, $P = .044$) level in our diabetic patients. Multiple regression analysis showed that CRP was independently predicted by HOMA-IR ($P < .0001$, $F = 11.6$) and fibrinogen ($P < .0001$, $F = 34.2$), which explained 23.5% of the variability of CRP in our non-obese Japanese type 2 diabetic patients. These results indicate that insulin resistance and fibrinogen level are independent predictors of CRP in non-obese Japanese type 2 diabetic patients.

CORONARY HEART DISEASE (CHD) is the most important cause of mortality and morbidity in type 2 diabetic patients. Although the significance of smoking, hypertension, and high serum cholesterol as risk factors for CHD is established, Bierman[1] previously estimated that these 3 risk factors can account for no more than 25% to 30% of excess cardiovascular risk factor in diabetic patients. This suggests that other factors might play a key role in the progression of CHD in type 2 diabetes.

Inflammation is hypothesized to play a role in the development of CHD in humans. Fibrinogen has been shown in a wide variety of studies to consistently predict future CHD events in an independent manner.[2] Some recent investigations have demonstrated that increased circulating concentrations of C-reactive protein (CRP) are an independent risk factor for CHD in man.[3-6] Insulin resistance has also been suggested to be underlying defect leading to be the development of CHD in type 2 diabetic patients.[7] Type 2 diabetic patients not only have higher concentrations of CRP but also are insulin-resistant as compared to nondiabetic subjects.[5] It is still unknown whether elevated CRP levels are associated with insulin resistance in type 2 diabetic patients. A major problem is that the degree of being overweight or of hyperglycemia per se affects CRP level and insulin resistance in man.[5,8,9] CRP levels are known to be associated with serum lipids that are characterized by insulin resistance.[8,9] Patients with type 2 diabetes have a high prevalence of atherosclerosis. To overcome this difficulty, we recruited non-obese well-controlled Japanese type 2 diabetic patients who had no evidence of atherosclerosis and investigated the relationship between CRP and other variables including insulin resistance and serum lipids using univariate and multiple regression analyses.

METHODS

One hundred thirty-five non-obese Japanese type 2 diabetic patients who visited Kansai-Denryoku Hospital were enrolled for the present study. Type 2 diabetes mellitus was diagnosed based on World Health Organization criteri.[10] They had no evidence of current acute illness, including clinically significant infectious disease. The duration of diabetes was 9.1 ± 0.7 years (mean ± SEM). Seventy-four of 135 diabetic patients were taking sulfonylureas (gliclazide) and the rest were treated with diet alone. No patient received insulin therapy. All subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. None of the subjects had significant renal, hepatic, or cardiovascular disease. Patients did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Blood was drawn at the morning after a 12-hour fast. Plasma glucose was measured with glucose oxidase method. The triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, and fibrinogen were also measured. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.[11] CRP was measured by ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem) with an interassay coefficient of variation (CV) of 8.9 %.[12] Samples for CRP were prepared, frozen, and stored at −70°C until the assay.

The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the formula: fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5.[13]

Statistical Analysis

Data were presented as means ± SEM. Statistical analyses were conducted using the StatView 5 system (Statview, Berkeley, CA). The

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means of 2 groups (insulin-resistant *v* insulin-sensitive) were compared using Student's *t* test. Simple (Spearman's rank) correlation coefficient and stepwise multiple regression analyses were used to examine the relationships between CRP and insulin resistance, body mass index (BMI), fibrinogen, or the measures of variables including triglycerides. *P* values less than .05 were considered significant. In multivariate analyses an , an *F* value ≥ 4 was considered significant.

RESULTS

The subjects studied were all Japanese type 2 diabetic patients (96 men and 39 women) with an age range of 36 to 83 years (60.8 ± 0.9) and a BMI of 16.2 to 26.8 kg/m^2 (22.8 ± 0.2). They all were non-obese.[14,15] The fasting plasma glucose was 147 ± 3 mg/dL and glycosylated hemoglobin (HbA_{1c}) was 7.1% ± 0.1 %. Fasting insulin level was 6.7 ± 0.3 U/mL. Serum triglycerides, total and HDL cholesterol levels were 116 ± 5 mg/dL and 202 ± 3 mg/dL, and 55 ± 2 mg/dL, respectively. Serum LDL cholesterol and fibrinogen levels were 147 ± 3 mg/dL and 283 ± 5 mg/dL, respectively. CRP level was 1,117 ± 129 ng/mL. There was a wide variation in insulin resistance calculated from HOMA-IR in our diabetic patients (range, 0.29 to 9.80; 2.46 ± 0.13). Forty-seven of 135 (35 %) patients had HOMA-IR greater than 2.5, indicating that they were insulin-resistant.[16]

Table 1 shows the clinical profile between insulin-resistant and insulin-sensitive type 2 diabetic patients. Compared with insulin-sensitive type 2 diabetic patients, insulin-resistant patients had significantly higher levels of BMI, HbA_{1c}, triglycerides, and CRP. No significant difference was observed in age, total, LDL and HDL cholesterol, and fibrinogen levels between the 2 groups.

Table 2 illustrates the correlation between CRP and age, BMI, or the measures of variables including HOMA-IR and serum triglycerides in our diabetic patients. CRP value was positively correlated to age ($r = 0.218$, $P = .012$), BMI ($r = 0.239$, $P = .006$), HOMA-IR ($r = 0.397$, $P < .0001$), triglycerides ($r = 0.310$, $P < .005$), LDL cholesterol ($r = 0.179$, $P = .038$), and fibrinogen ($r = 0.371$, $P < .0001$). In contrast, CRP value was negatively correlated with HDL cholesterol level ($r = 0.174$, $P = .044$). There was, however, no relationship between CRP and measures of variables including gender, duration of diabetes, systolic or diastolic blood pressure, fasting glucose, HbA_{1c}, total cholesterol, or medication status.

Table 1. Clinical Characteristics in Insulin-Resistant and Insulin-Sensitive Diabetic Patients

	Insulin-Resistant	Insulin-Sensitive	*P*
No. of subjects	49	86	
Male/female	33/16	63/23	
HOMA-IR	4.07 ± 0.24	1.60 ± 0.06	< .001
Age (yr)	61.0 ± 1.6	60.7 ± 1.1	.415
BMI (kg/m2)	24.0 ± 0.3	22.1 ± 0.2	< .001
HbA_{1c} (%)	7.4 ± 0.2	6.8 ± 0.1	.002
Fasting glucose (mg/dL)	161 ± 2	140 ± 3	< .001
Fasting insulin (μU/mL)	10.3 ± 0.5	4.7 ± 0.2	< .001
Total cholesterol (mg/dL)	207 ± 5	200 ± 3	.120
LDL cholesterol (mg/dL)	154 ± 5	144 ± 3	.054
Triglycerides (mg/dL)	135 ± 9	106 ± 6	.003
RLP cholesterol (mg/dL)	6.0 ± 0.4	5.0 ± 0.3	.037
HDL cholesterol (mg/dL)	53 ± 4	56 ± 2	.243
Fibirinogen	280 ± 8	284 ± 6	.351

Table 2. Correlation of GRP to Measures of Variables in Diabetic Patients

	Univariate		Multivariate
	r	*P*	*F*
Age	0.218	.012	1.4
BMI	0.239	.006	0.1
HOMA-IR	0.397	< .0001	11.6
Triglycerides	0.310	< .005	0.4
LDL cholesterol	0.179	.038	0.1
Fibrinogen	0.371	< .0001	34.2
HDL cholesterol	−0.174	.044	1.7
Gender	0.142	.099	—
Diabetes duration	−0.135	.099	—
Systolic blood pressure	0.109	.217	—
Diastolic blood pressure	−0.010	.906	—
Fasting glucose	0.112	.195	—
HbA_{1c}	0.004	.964	—
Total cholesterol	0.090	.295	—
Therapy for diabetes	−0.048	.581	—

Multiple regression analyses were performed using the stepwise procedure. The analysis included CRP as the dependent variable and candidate risk factors (age, BMI, HOMA-IR, triglycerides, LDL cholesterol, fibrinogen, HDL cholesterol) as independent variables. CRP was independently predicted by HOMA-IR ($P < .0001$, $F = 11.6$) and fibrinogen ($P < .0001$, $F = 34.2$), which explained 23.5% of the variability of CRP in our diabetic patients. Other variables, including lipid profile, were not associated with CRP in our non-obese Japanese type 2 diabetic patients (Table 2).

DISCUSSION

Our main observation in the present study is that CRP is independently associated with insulin resistance in non-obese Japanese type 2 diabetic patients. The patients studied were unique in that they were non-obese and were well controlled in terms of HbA_{1c} (mean HbA_{1c}, 7.1 %) and blood pressure (mean, 126/72 mm Hg). They had no evidence for CHD, ischemic stroke, and chronic renal failure.

In the present study, we estimated insulin resistance using HOMA-IR in diet-treated and sulfonylurea-treated diabetic patients. One might argue that the use of sulfonylureas in patients with diabetes might significantly affect the estimate of insulin resistance by HOMA, as these drugs are known to decrease fasting plasma glucose without substantially changing fasting plasma insulin.[17] It seems, however, unlikely, since Bonora et al[18] and Emoto et al[19] confirmed that in the validation studies of HOMA, the correlation of insulin sensitivity estimated by such method and that measured by the glucose clamp were not substantially different in diet-treated and sulfonylurea-treated type 2 diabetes.

Non-obese Japanese type 2 diabetic patients are unique in that they are divided into 2 variants: one with insulin resistance and the other with normal insulin sensitivity.[20,21] The former group is characterized by higher serum triglycerides, higher remnant-like particle (RLP) cholesterol and lower HDL cho-

lesterol as compared to the latter group.[16] All of these lipid abnormalities have been identified as risk factors for CHD in type 2 diabetic patients. Thus, insulin resistance seems to play a role in increasing the risk of CHD in non-obese Japanese type 2 diabetic patients.

Elevated levels of CRP, although still for the most part in the healthy reference range, have been associated with increased risk of future CHD events.[3-6] With the use of highly sensitive CRP assays, CRP was identified as an independent, prospective CHD risk factor in the higher-risk middle-aged men of the MRFIT study,[3] the healthy middle-aged men of the PHS[4] and the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA)-Augsburg cohort,[5] and the healthy elderly men and women of the Cardiovascular Health Study (CHS) and the Rural Health Promotion Project.[6] One might argue that smoking habit per se affects CRP levels and CHD events in diabetic patients, but it seems unlikely since CRP predicted future CHD events just as well as in nonsmokers as in smokers in the PHS.[4]

With univariate analysis, we found that the levels of CRP were positively correlated with age, BMI, triglycerides, LDL cholesterol, and fibrinogen levels and inversely correlated with HDL cholesterol level. These variables are associated with insulin resistance in man. Thus, we next analyzed the relationship between CRP and these variables associated with insulin resistance using multiple stepwise regression analysis. With multivariate analysis, we first found a strong and independent association of CRP with insulin resistance in our non-obese well-controlled unique type 2 diabetic patients without major clinical cardiovascular disease. Festa et al[22] recently reported that CRP was independently associated with insulin resistance in nondiabetic subjects without clinical coronary artery disease. Therefore, the associations of elevated CRP with HOMA-IR found in the present study may potentially explain the association of CRP with CHD in non-obese Japanese type 2 diabetic patients.

The mechanisms by which CRP is independently associated with insulin resistance in our non-obese Japanese type 2 diabetic patients are currently unknown.

In the present study, we demonstrated that CRP was also independently associated with fibrinogen. CRP and fibrinogen are considered to be inflammatory markers. Thus, chronic subclinical inflammation might emerge as part of insulin resistance in our non-obese Japanese type 2 diabetic patients. If so, pharmacologic treatment aiming at improving insulin resistance might decrease chronic inflammation and CHD events. Lipid-lowering agent such as bezafibrate is known to lower not only glucose level and insulin resistance, but also fibrinogen level in type 2 diabetic patients.[23-25] Bezafibrate is also reported to reduce the incidence of CHD events.[26] Alternatively, chronic inflammation may represent a triggering factor in the origin of insulin resistance in our non-obese Japanese type 2 diabetic patients. If it is so, anti-inflammatory agent may be beneficial in the treatment of insulin resistance and/or CHD. Whereas aspirin is known to be effective in reducing CHD events, it has been suggested that the effect of aspirin may be mediated through its anti-inflammatory rather than antiplatelet properties.[4] Some cross-sectional and case-control studies have reported elevated antibody titers directed against *Chlamydia pneumoniae*, *Helicobacter pylori*, and cytomegalovirus among those with prevalent heart disease.[27] Irrespective of the mechanisms, the results of the present study might have additional benefits in the treatment of insulin resistance and/or CHD events in non-obese Japanese type 2 diabetic patients.

In summary, although our present study is performed among the limited number of patients (n = 135), it can be concluded that CRP is independently associated with insulin resistance in non-obese Japanese type 2 diabetic patients. Further study should be undertaken to clarify the mechanisms underlying the relationship between CRP and insulin resistance in non-obese Japanese type 2 diabetic patients.

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