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Fat redistribution preferentially reflects the anti-inflammatory benefits of pioglitazone treatment

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Abstract

Thiazoledinedione is known to have an anti-inflammatory effect besides a hypoglycemic effect. We investigated changes in high-sensitivity C-reactive protein (hsCRP), a proinflammatory marker, after pioglitazone treatment in association with the resulting changes in various metabolic and anthropometric parameters. A total of 93 type 2 diabetes mellitus patients (47 men and 46 women; mean age, 50.0 ± 10.8 years) who were being treated with a stable dose of sulfonylurea or metformin were enrolled in the study. Pioglitazone (15 mg/d) was added to their treatment regimen for 12 weeks, and metabolic and anthropometric measurements were taken before and after pioglitazone treatment. Pioglitazone treatment for 12 weeks decreased serum hsCRP levels (0.83 [1.14] to 0.52 [0.82] mg/L, $P < .001$) and improved glycemic control (fasting glucose, $P < .001$; glycosylated hemoglobin, $P < .001$) and lipid profiles (triglyceride, $P = .016$; high-density lipoprotein cholesterol, $P < .001$). Between responders and nonresponders to the hsCRP-lowering effect of pioglitazone, there were significant differences in baseline hsCRP levels and changes in the postprandial glucose and the ratio of visceral fat thickness (VFT) to subcutaneous fat thickness (SFT) ($P = .004$, .011, and .001, respectively). The percentage change in hsCRP levels after treatment was inversely correlated with baseline hsCRP levels ($r = -0.497$, $P < .001$) and directly correlated with the change in postprandial glucose ($r = 0.251$, $P = .021$), VFT ($r = 0.246$, $P = .030$), and VFT/SFT ratio ($r = 0.276$, $P = .015$). Logistic regression analysis revealed that the hsCRP-lowering effect of pioglitazone was affected by baseline hsCRP levels (odds ratio [OR] = 7.929, $P = .007$) as well as changes in postprandial 2-hour glucose (OR = 0.716, $P = .025$) and VFT/SFT ratio (OR = 0.055, $P = .009$). In conclusion, treatment with pioglitazone produced an anti-inflammatory effect, decreasing serum hsCRP levels; and a decrease in the VFT/SFT ratio was independently and most strongly associated with the hsCRP-decreasing effect. These results suggest that abdominal fat redistribution preferentially reflects the anti-inflammatory benefits of pioglitazone treatment.

1. Introduction

Pioglitazone, a peroxisome proliferator-activated receptor–γ (PPARγ) agonist, is an oral hypoglycemic agent that improves insulin sensitivity. In addition, pioglitazone has been reported to have antioxidant, anti-inflammatory, antiproliferative, and antiprocoagulant effects, among others [1]. Furthermore, several studies have reported that pioglitazone also improves diabetic dyslipidemia [2]. The anti-inflammatory effect of pioglitazone is marked by the

The study protocol was approved by the institutional review boards and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

This study was performed at the Diabetes Center of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

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reduction of proinflammatory markers including C-reactive protein (CRP) [3-13]. C-reactive protein is an acute-phase reactant that is known as a proinflammatory marker and a predictor of cardiovascular events [14-16]. Moreover, CRP directly facilitates the development of cardiovascular disease by hindering nitric oxide synthesis and inducing adhesion molecule expression in human endothelial cells [17,18]. Recently, CRP was reported to be independently associated with short-term mortality risk in type 2 diabetes mellitus patients [19]. In addition, Williams et al [20] reported that the levels of systolic blood pressure, CRP, and apolipoprotein B are higher in diabetic women than in diabetic men; and such conditions result in a loss of cardiovascular protection. They suggested that the sex differences of CRP and apolipoprotein B in diabetic patients were associated with differences in abdominal adiposity. Indeed, the relationship between CRP and abdominal adiposity has been reported in several studies [21-26]. A recent study from the Framingham Offspring Study used volumetric multidetector computed tomography (CT) to show that both subcutaneous adipose tissue and visceral adipose tissue are associated with plasma inflammatory markers including CRP and that the association for visceral adipose tissue by CRP is stronger than that for subcutaneous adipose tissue [23].

Peroxisome proliferator-activated receptor–γ agonists are known to increase body weight [27-30] and total fat mass [31-33] based on studies with both animals and diabetic humans. It has been suggested that PPARγ agonists promote differentiation of preadipocytes in subcutaneous fat tissue but not in visceral fat tissue. This regional variability in the response to PPARγ agonists induces increases in body weight and subcutaneous fat mass [34]. Furthermore, fat redistribution by PPARγ agonists may affect the hypoglycemic effect mediated by these agents. Interestingly, the hypoglycemic efficacy of rosiglitazone is reported to be associated with an increase in abdominal subcutaneous fat mass [35].

On the basis of these studies, we hypothesized that changes in abdominal adiposity is one of the factors that affects the anti-inflammatory activity of pioglitazone in decreasing hsCRP levels. Although PPARγ agonists are widely prescribed in clinical practice, the anti-inflammatory effect of these agents is not yet fully understood. In the present study, we investigated the change in hsCRP after pioglitazone treatment and analyzed the interrelationship between the hsCRP-lowering effect of pioglitazone and various metabolic and anthropometric parameters. Mechanisms of the anti-inflammatory actions of pioglitazone may also be suggested through this study.

2. Materials and methods

2.1. Subjects

A total of 93 patients (47 men and 46 women; mean age, 50.0 ± 10.8 years) were enrolled in our study. Type 2 diabetes mellitus patients who were being treated at the Diabetes Center of Severance Hospital, Yonsei University Health System (Seoul, Korea), with a stable dose of sulfonylurea or metformin and who had not experienced a change in body weight for at least 3 months before enrollment were included. Patients were excluded if they were receiving lipid-lowering agents (eg, statins or fibrates) or if they had a history of ketoacidosis; unstable or rapidly progressive diabetic retinopathy, nephropathy, or neuropathy; *impaired hepatic function* (defined as plasma aspartate transaminase and/or alanine transaminase levels 2-fold higher than the upper normal limit); *impaired renal function* (defined as serum creatinine concentrations higher than 177 μmol/L); and/or severe anemia. Patients with severe cardiovascular disease or who had experienced a cerebrovascular condition within 6 months of study enrollment were also excluded, as were women who were pregnant or breast-feeding. The Institutional Review Board of Yonsei University College of Medicine approved this study, and informed consent was obtained from each patient before participation.

2.2. Treatment

All patients received dietary supervision and were instructed to maintain the same level of energy intake and physical activity throughout the study. Patients received 1 tablet (15 mg) of pioglitazone (Actos; Dakeda Pharmaceutical, Osaka, Japan) after breakfast every day in addition to their other medications. There were no other changes in medication during the study period. Anthropometric and metabolic measurements were taken before and after 12 weeks of pioglitazone treatment.

2.3. Anthropometric data

We measured height and weight of patients in light clothing and without shoes to the nearest 0.1 cm and 0.1 kg, respectively. Waist circumference was measured at the midpoint between the lateral iliac crest and the lowest rib. Hip circumference was measured at the maximal protrusion of the greater trochanter. Body composition assessments were performed using the bioelectric impedance assay method with InBody 2.0 (Biospace, Seoul, Korea). Abdominal ultrasonography was performed using a high-resolution ultrasonographic system (SA 9900; Medison, Seoul, Korea) as described by Suzuki et al [36] and Armellini et al [37]. Transverse scanning was performed 1 cm above the umbilicus using a 7.5-MHz probe and a 3.5-MHz probe to measure the subcutaneous fat thickness (SFT) and visceral fat thickness (VFT), respectively. SFT was defined as the thickness between the skin-fat interface and the linea alba, whereas *VFT* was defined as the distance between the anterior wall of the aorta and the internal face of the rectoabdominal muscle perpendicular to the aorta. All subjects were scanned in the supine position, and all indices

were measured directly from frozen images using an electronic caliper.

2.4. Laboratory data

Fasting plasma glucose (FPG), glycosylated hemoglobin (HbA_{1c}), serum insulin, C-peptide, uric acid, blood urea nitrogen, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), free fatty acid (FFA), total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol levels were measured in the mornings after patients fasted overnight. Plasma glucose was measured using the glucose oxidase method, and HbA_{1c} was measured by high-performance liquid chromatography. Plasma lipids, blood urea nitrogen, creatinine, AST, ALT, and uric acid were assayed by routine automated laboratory methods. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. Serum insulin (RIABEAD II; Abbott, Abbott Park, IL) and C-peptide (DiaSorin, Stillwater, MN; coefficient of variation = 2.7%) levels were measured with a commercially available enzyme immunoassay. Serum hsCRP levels were measured with a BN II Nephelometer (Dade Behring, Newark, DE) using a latex-enhanced immunonephelometric assay method with the detection limit of 0.17 mg/L. Insulin resistance indices were assessed using the homeostasis model assessment (HOMA-IR; fasting insulin [in microunits per milliliter] × FPG [in millimoles per liter]/22.5).

2.5. Definition of response

The hsCRP-lowering response to pioglitazone treatment was defined by a greater than 20% decrease in hsCRP level after 12 weeks of pioglitazone treatment. *Nonresponders* were defined as patients who did not meet this criterion. The 20% decrease in hsCRP level was a median value of percentage changes in plasma hsCRP levels.

2.6. Statistical analysis

All statistical analyses were performed with SPSS software (version 15.0; SPSS, Chicago, IL). Values with normal distribution were expressed as means ± SD, and values with nonnormal distribution were expressed as medians (interquartile range). Statistical comparison between responders and nonresponders was performed using independent *t* test or Mann-Whitney *U* test. Paired *t* test or Wilcoxon signed rank test was used for the comparison between baseline and posttreatment parameters. The Spearman correlation coefficient was used to estimate the relationships between the percentage change in hsCRP and the continuous variables. Binary logistic regression analysis was used to estimate the multiple correlations between the hsCRP-lowering effect of pioglitazone treatment and the metabolic and anthropometric data (baseline and their changes). Data with a P value < .05 were considered significant.

3. Results

3.1. Changes in metabolic and anthropometric data after pioglitazone treatment

Table 1 shows the baseline metabolic and anthropometric data of all patients. A total of 93 patients (47 men and 46 women; mean age, 50.0 ± 10.8 years) completed the 12-week study. The FPG, HbA_{1c}, and hsCRP were significantly decreased after pioglitazone treatment; however, postprandial 2-hour glucose levels did not change significantly. Triglyceride levels decreased, and HDL cholesterol levels increased. However, no changes in total cholesterol and LDL cholesterol were observed during the treatment period. The FFA, uric acid, and ALT also decreased after the treatment course was completed. The concentrations of fasting and postprandial 2-hour C-peptide and fasting insulin and the value of HOMA-IR fell significantly after pioglitazone treatment.

Whereas pioglitazone treatment improved glycemic control and insulin resistance, it led to a significant increase in body weight, body mass index (BMI), and fat mass, and a decrease in waist to hip ratio. Lean body mass exhibited no change during treatment. Abdominal

Table 1
Metabolic and anthropometric data before and after 12-week pioglitazone treatment

	Before	After	*P* value
Fasting glucose (mmol/L)	8.42 ± 2.24	7.18 ± 2.12	<.001[a]
Postprandial 2-h glucose (mmol/L)	11.93 ± 3.86	11.45 ± 4.40	NS[a]
HbA_{1c} (%)	7.90 ± 1.47	7.20 ± 1.10	<.001[a]
hsCRP (mg/L)	0.83 (1.14)	0.52 (0.82)	<.001[b]
Total cholesterol (mmol/L)	4.94 ± 1.04	4.95 ± 0.88	NS[a]
LDL cholesterol (mmol/L)	2.68 ± 0.98	2.74 ± 0.78	NS[a]
Triglyceride (mmol/L)	2.40 ± 2.01	1.93 ± 1.27	.016[a]
HDL cholesterol (mmol/L)	1.17 ± 0.24	1.32 ± 0.29	<.001[a]
FFA (mmol/L)	21.73 ± 8.39	19.37 ± 7.28	.022[a]
Uric acid (mmol/L)	0.29 ± 0.08	0.27 ± 0.07	.021[a]
AST (IU/L)	24.0 ± 13.0	22.8 ± 11.7	NS[a]
ALT (IU/L)	31.0 ± 23.9	24.8 ± 18.6	.001[a]
Fasting insulin (pmol/L)	55.5 (35.7)	44.2 (30.4)	<.001[b]
Postprandial 2-h insulin (pmol/L)	189.7 (227.2)	195.9 (147.2)	NS[b]
Fasting C-peptide (nmol/L)	0.58 (0.25)	0.41 (0.17)	<.001[b]
Postprandial 2-h C-peptide (nmol/L)	1.22 (0.64)	1.12 (0.54)	.018[b]
HOMA-IR	3.19 ± 1.75	2.15 ± 1.27	<.001[a]
Weight (kg)	68.5 ± 12.1	69.6 ± 11.9	<.001[a]
BMI (kg/m^2)	25.8 ± 3.3	26.3 ± 3.3	<.001[a]
Waist circumference (cm)	88.2 ± 8.6	89.6 ± 9.2	.006[a]
Hip circumference (cm)	97.6 ± 7.1	100.6 ± 7.4	<.001[a]
Waist to hip ratio	0.90 ± 0.05	0.89 ± 0.05	.009[a]
Fat mass (kg)	20.7 ± 7.1	22.1 ± 8.1	<.001[a]
Lean body mass (kg)	48.3 ± 9.0	48.0 ± 8.7	NS[a]
SFT (mm)	24.8 ± 8.6	26.2 ± 9.2	<.001[a]
VFT (mm)	56.0 ± 21.2	53.8 ± 22.5	NS[a]
VFT/SFT ratio	2.68 ± 1.90	2.41 ± 1.71	.002[a]

Data are means ± SD or median (interquartile range). NS indicates not significant.
[a] Derived from a independent *t* test.
[b] Derived from a Mann-Whitney *U* test.

ultrasonography before and after pioglitazone treatment revealed a significant increase in SFT and a decrease in the VFT/SFT ratio. No change in VFT was observed during the treatment.

3.2. Comparison of metabolic and anthropometric data according to the hsCRP-lowering response to pioglitazone treatment

Between responders and nonresponders to the hsCRP-lowering effect of pioglitazone, there was no significant difference in age, sex, duration of diabetes, and baseline hypoglycemic agents (Table 2). In baseline metabolic data, the responders group had significantly higher hsCRP levels before pioglitazone treatment. Other baseline metabolic and anthropometric data exhibited no difference between responders and nonresponders (Table 2).

A comparison of the changes in metabolic and anthropometric data between responders and nonresponders is shown in Table 3. Although the changes in fasting glucose and HbA_{1c} after pioglitazone treatment showed no significant differences between responders and nonresponders, the decrease in postprandial 2-hour glucose was prominent in the responders group (Fig. 1). In addition, the changes in lipid profiles were not significantly different between the 2 groups. Visceral fat thickness and the VFT/SFT ratio decreased only in the responders, whereas changes in body weight, BMI, waist to hip ratio, and fat mass exhibited no significant difference between responders and nonresponders. Fig. 2 shows the changes in the VFT/SFT ratio after pioglitazone treatment in responders and nonresponders.

Table 2
Basal characteristics according to the hsCRP-lowering response to pioglitazone

	Responder	Nonresponder	*P* value
n (%)	52 (55.9%)	41 (44.1%)	
Sex (male/female)	24/28	22/19	NS[a]
Age (y)	49.8 ± 10.9	50.5 ± 10.9	NS[b]
DM duration (y)	5.5 ± 4.4	5.4 ± 5.4	NS[b]
Treatment (n)			NS[a]
Sulfonylurea	13	8	
Metformin	17	13	
Sulfonylurea + metformin	22	20	
Fasting glucose (mmol/L)	8.52 ± 2.21	8.41 ± 2.44	NS[b]
Postprandial 2-h glucose (mmol/L)	12.44 ± 4.09	11.10 ± 3.76	NS[b]
HbA_{1c} (%)	8.02 ± 1.34	7.79 ± 1.66	NS[b]
Fasting insulin (pmol/L)	55.5 (36.5)	56.7 (40.3)	NS[c]
Postprandial 2-h insulin (pmol/L)	226.5 (235.7)	187.8 (157.2)	NS[c]
Fasting C-peptide (nmol/L)	0.57 (0.25)	0.56 (0.32)	NS[c]
Postprandial 2-h C-peptide (nmol/L)	1.22 (0.60)	1.16 (0.88)	NS[c]
HOMA-IR	3.02 ± 1.63	3.08 ± 1.69	NS[b]
hsCRP (mg/L)	1.12 (1.67)	0.62 (0.89)	0.004[c]
Total cholesterol (mmol/L)	4.84 ± 1.05	4.97 ± 1.03	NS[b]
LDL cholesterol (mmol/L)	2.55 ± 0.89	2.74 ± 1.02	NS[b]
Triglyceride (mmol/L)	2.46 ± 2.18	2.30 ± 1.93	NS[b]
HDL cholesterol (mmol/L)	1.16 ± 0.23	1.17 ± 0.27	NS[b]
FFA (mmol/L)	21.90 ± 7.03	21.23 ± 10.10	NS[b]
Weight (kg)	69.1 ± 12.6	66.8 ± 10.4	NS[b]
BMI (kg/m^2)	26.1 ± 3.6	25.2 ± 2.5	NS[b]
Waist to hip ratio	0.90 ± 0.05	0.90 ± 0.06	NS[b]
Fat mass (kg)	21.4 ± 7.7	19.2 ± 6.0	NS[b]
Lean body mass (kg)	47.7 ± 9.0	47.7 ± 8.6	NS[b]
SFT (mm)	25.5 ± 8.4	24.5 ± 8.6	NS[b]
VFT (mm)	56.8 ± 22.8	52.0 ± 20.6	NS[b]
VFT/SFT ratio	2.61 ± 1.80	2.46 ± 1.58	NS[b]

Data are means ± SD or median (interquartile range). DM indicates diabetes mellitus.
[a] Derived from a χ^2 test.
[b] Derived from a independent *t* test.
[c] Derived from a Mann-Whitney *U* test.

Table 3
Changes in metabolic and anthropometric data according to the hsCRP-lowering response to pioglitazone

	Responder	Nonresponder	*P* value
ΔhsCRP (mg/L)	−0.59 (1.24)	−0.13 (0.51)	<.001[a]
ΔFasting glucose (mmol/L)	−1.33 ± 2.40	−1.09 ± 1.87	NS[b]
ΔPostprandial 2-h glucose (mmol/L)	−1.41 ± 4.29	1.09 ± 4.42	.011[b]
ΔHbA_{1c} (%)	−0.84 ± 1.18	−0.59 ± 1.04	NS[b]
ΔFasting insulin (pmol/L)	−6.30 (28.53)	−13.51 (32.31)	NS[a]
ΔPostprandial 2-h insulin (pmol/L)	−18.76 (131.60)	−0.77 (225.40)	NS[a]
ΔFasting C-peptide (nmol/L)	−0.11 (0.20)	−0.13 (0.23)	NS[a]
ΔPostprandial 2-h C-peptide (nmol/L)	−0.10 (0.32)	−0.03 (0.46)	NS[a]
ΔHOMA-IR	−0.95 ± 1.48	−1.03 ± 1.45	NS[b]
ΔTotal cholesterol (mmol/L)	0.11 ± 0.78	−0.06 ± 0.89	NS[b]
ΔLDL cholesterol (mmol/L)	0.23 ± 0.95	−0.07 ± 0.97	NS[b]
ΔTriglyceride (mmol/L)	−0.66 ± 2.08	−0.18 ± 1.53	NS[b]
ΔHDL cholesterol (mmol/L)	0.18 ± 0.26	0.10 ± 0.21	NS[b]
ΔFFA (mmol/L)	−3.33 ± 8.11	−0.72 ± 9.89	NS[b]
ΔWeight (kg)	1.34 ± 1.67	0.96 ± 1.76	NS[b]
ΔBMI (kg/m^2)	0.52 ± 0.64	0.39 ± 0.71	NS[b]
ΔWaist to hip ratio	−0.02 ± 0.05	−0.01 ± 0.04	NS[b]
ΔFat mass (kg)	1.67 ± 2.87	0.90 ± 1.68	NS[b]
ΔSFT (mm)	1.81 ± 3.10	0.81 ± 2.71	NS[b]
ΔVFT (mm)	−5.01 ± 13.01	1.98 ± 8.44	.012[b]
ΔVFT/SFT ratio	−0.47 ± 0.80	0.12 ± 0.60	.001[b]

Data are mean ± SD or median (interquartile range).
[a] Derived from a Mann-Whitney *U* test.
[b] Derived from a independent *t* test.

3.3. Correlation between changes in hsCRP after pioglitazone treatment and metabolic and anthropometric data

The percentage change in hsCRP after treatment was inversely correlated with baseline hsCRP levels ($r = -0.497$, $P < .001$) and directly correlated with changes in postprandial glucose ($r = 0.251$, $P = .021$), VFT ($r = 0.246$, $P = .030$), and VFT/SFT ratio ($r = 0.276$, $P = .015$). Other metabolic and anthropometric data (baseline and respective changes) had no significant relationship with the percentage change of hsCRP.

3.4. Factors associated with the hsCRP-lowering effect of pioglitazone

Binary logistic regression analysis revealed that baseline hsCRP levels and the decrease in postprandial 2-hour

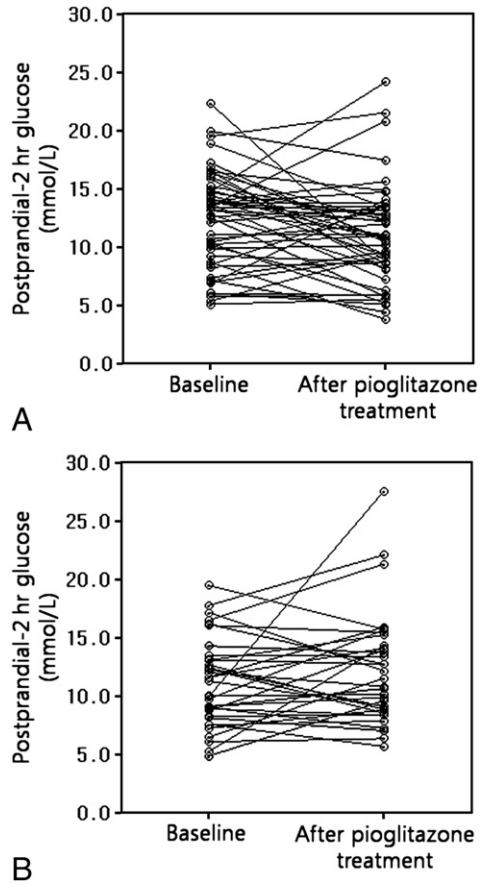

Fig. 1. Change in postprandial 2-hour glucose in responders and nonresponders to hsCRP-lowering effect of pioglitazone. (A) Responder group (n = 52). (B) Nonresponder group (n = 41).

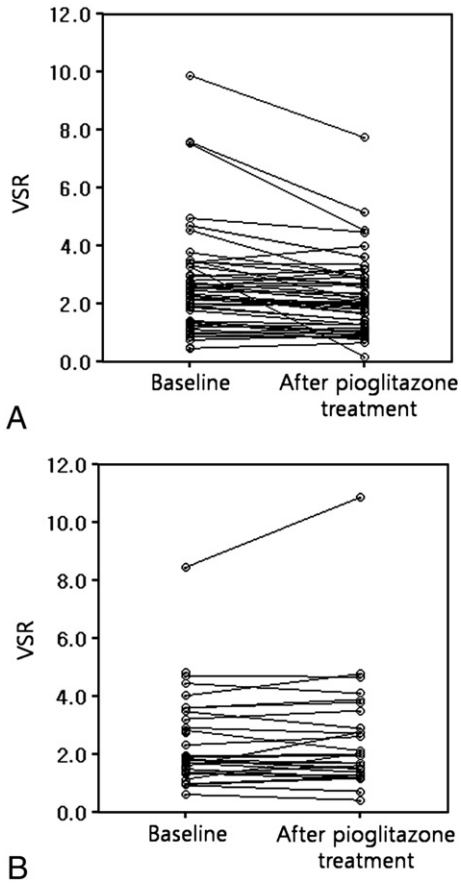

Fig. 2. Change in VFT/SFT ratio in responders and nonresponders to hsCRP-lowering effect of pioglitazone. (A) Responder group (n = 52). (B) Nonresponder group (n = 41). VSR indicates ratio of VFT to SFT.

glucose and VFT/SFT ratio were independently correlated with the hsCRP-lowering effect of pioglitazone (Table 4). However, baseline data of postprandial glucose, FFA, BMI, fat mass, and the VFT/SFT ratio, as well as changes in FFA, BMI, and fat mass, did not independently affect the hsCRP-lowering response to pioglitazone.

4. Discussion

Here, we have demonstrated that 12 weeks of pioglitazone treatment improved glycemic control, insulin resistance, and lipid profiles in type 2 diabetes mellitus patients. Although PPARγ agonists reduce both fasting and postprandial glucose [38,39], these agents may be less effective in postprandial glucose reduction compared with other hypoglycemic agents [40]. In our data, whereas fasting glucose level decreased, postprandial glucose level did not decline significantly. Pioglitazone also increased body weight and resulted in altered abdominal adiposity. Specifically, body weight in patients treated with pioglitazone

Table 4
Logistic regression analysis of the metabolic and anthropometric indices with the hsCRP-lowering response to pioglitazone

	Odds ratio	95% Confidence interval for odds ratio		*P* value
		Lower	Upper	
hsCRP	7.929	1.748	35.971	.007
Postprandial 2-h glucose	0.989	0.976	1.002	NS
FFA	0.946	0.839	1.067	NS
BMI	1.245	0.743	2.086	NS
Fat mass	0.915	0.724	1.158	NS
VFT/SFT ratio	0.635	0.318	1.265	NS
ΔPostprandial 2-h glucose	0.716	0.535	0.959	.025
ΔFFA	0.943	0.848	1.050	NS
ΔBMI	3.443	0.855	13.862	NS
ΔFat mass	0.894	0.618	1.293	NS
ΔVFT/SFT ratio	0.055	0.006	0.489	.009

The dependent variable was the hsCRP-decreasing response to pioglitazone treatment, and the independent variables were baseline values and the changes in the various metabolic and anthropometric parameters.

increased by 1.1 ± 1.8 kg; and BMI increased by 0.5 ± 0.7 kg/m^2. The gain in body weight can be explained by an increase in fat mass, especially for the subcutaneous fat mass, considering that it increased by 1.4 ± 2.6 kg (vs body weight, +1.1 ± 1.8 kg) and also that SFT, but not VFT, rose. These results are consistent with a prior randomized controlled study using pioglitazone [30].

In this study, we focused on the change in hsCRP after pioglitazone treatment. C-reactive protein is an acute-phase reactant known to be a proinflammatory marker and a predictor of cardiovascular events [14-16]. Moreover, CRP directly facilitates the development of cardiovascular disease by decreasing nitric oxide synthesis and inducing adhesion molecule expression in human endothelial cells [17,18]. Recently, CRP was reported to be independently associated with short-term mortality risk in type 2 diabetes mellitus patients [19]; and several studies have shown that pioglitazone reduces plasma CRP levels [3-13]. Moreover, 16 weeks of pioglitazone treatment decreased CRP levels by 42% in type 2 diabetes mellitus patients [12]; and pioglitazone further reduced CRP in patients with hypertension and diabetes who were receiving angiotensin II receptor blockers [5]. In a 6-month randomized double blind study, pioglitazone in combination with insulin treatment was shown to decrease hsCRP levels more than did insulin monotherapy alone [9]. In our study, hsCRP decreased by 32.7 (74.6)% after 12 weeks of pioglitazone treatment.

To identify the factors associated with the hsCRP-reducing effect of pioglitazone, we compared metabolic and anthropometric parameters (baseline and their changes) between the responder and nonresponder groups. Only baseline hsCRP levels were significantly higher in the responder group than in the nonresponder group and correlated with the percentage change in hsCRP after pioglitazone treatment. Other baseline parameters including age, sex, and prior use of hypoglycemic agents showed no significant differences between the 2 groups. The correlation of baseline hsCRP with percentage change in hsCRP after pioglitazone treatment suggests that the anti-inflammatory effect of pioglitazone might be prominent in patients with somewhat progressed inflammatory status or diabetes-related complications. During the enrollment period of this study, we excluded the patients with unstable or progressive complications. Nevertheless, we cannot ensure the homogeneity of the status of diabetes-related complications in our patient population. Considering the relation of inflammation and diabetes-related complications, responders who showed higher plasma hsCRP levels might have more progressed complications. Although we found no differences in baseline hsCRP level or the percentage change in hsCRP between men and women (data not shown), a study with 1423 subjects (including 258 diabetic women and 220 diabetic men) found that CRP levels were 2 times higher in diabetic women than in diabetic men [20]. In addition, a study with 382 diabetic patients from the Carotid intimal-medial tHICkness in Atherosclerosis using pioGlitazOne (CHICAGO) trial cohort showed similar results [26]. Together, these results may suggest that the CRP-lowering effect of pioglitazone is greater in diabetic women than in diabetic men in sufficiently large populations.

Interestingly, when we compared the changes in metabolic and anthropometric parameters after pioglitazone treatment between responders and nonresponders, postprandial 2-hour glucose levels decreased only in the responder group; and the changes in fasting glucose and HbA_{1c} were not different between the 2 groups. Postprandial hyperglycemia has attracted considerable attention as a strong predictor of cardiovascular disease; the association for postprandial hyperglycemia is stronger than that for fasting glucose or HbA_{1c} [41]. Several epidemiologic studies have demonstrated the importance of postprandial glucose in association with cardiovascular disease and mortality in diabetic and nondiabetic patients [42-45]. Postprandial hyperglycemia results in endothelial dysfunction by increasing a number of proinflammatory mediators, oxidative stress, and oxidized LDL [41]. As noted above, CRP is one of the proinflammatory markers indicative of subclinical inflammation and associated with cardiovascular events. A study with 1043 nondiabetic subjects from the Insulin Resistance Atherosclerosis Study revealed that elevated circulating CRP levels are more strongly associated with postchallenge glycemia than with fasting glucose levels [46]. In our data, baseline hsCRP levels were correlated with both fasting and postprandial glucose levels (data not shown); however, the percentage change in hsCRP after pioglitazone treatment was correlated only with altered postprandial glucose and not with fasting glucose. Considering the induction of proinflammatory mediators by postprandial hyperglycemia, this result may suggest that the hsCRP-reducing anti-inflammatory effect of pioglitazone occurs partially through the control of postprandial hyperglycemia.

Changes in abdominal adiposity were different between responders and nonresponders. Specifically, VFT as measured by ultrasonography and the VFT/SFT ratio decreased only in the responder group. The relationship between CRP and abdominal adiposity has been reported in several studies [21-26]. A recent study with 1250 Framingham Offspring Study participants (10% diabetic patients) using volumetric multidetector CT showed that both subcutaneous adipose tissue and visceral adipose tissue are associated with plasma inflammatory markers including CRP and that the association for visceral adipose tissue is stronger [23]. A study of 382 type 2 diabetes mellitus subjects in the CHICAGO trial cohort who underwent abdominal CT to determine subcutaneous and visceral adipose tissue distribution revealed that, before adjustment for BMI, visceral adipose tissue is positively related to CRP [26]. The relation of CRP to abdominal adiposity has been reported to be due to the production of cytokines in adipose tissue, tumor necrosis factor (TNF)–α ,and interleukin (IL)-6. Indeed, the serum concentrations of TNF-α [47-50] and soluble serum TNF-receptor-2 [49] are higher in obesity; and TNF-α induces

hepatic CRP production [51]. Likewise, IL-6 also induces hepatic production of CRP [52]; and serum IL-6 concentrations are higher in obesity [50]. Our data revealed that changes in VFT and VFT/SFT ratio were correlated with the percentage change in hsCRP after pioglitazone treatment. These results suggest that the change in abdominal adiposity is one of the possible mechanisms through which pioglitazone reduces hsCRP.

We performed binary logistic regression analysis to investigate factors that were independently associated with the hsCRP-reducing effect of pioglitazone. The dependent variable was the hsCRP-decreasing response to pioglitazone treatment, whereas the independent variables were the baseline values and the changes in the various metabolic and anthropometric parameters. There were no differences in sex, age, duration of diabetes, and underlying hypoglycemic agents between responders and nonresponders; and through logistical regression, we adjusted for BMI and fat mass. In our data, we demonstrated that baseline hsCRP, the decrease in postprandial glucose, and the decrease in VFT/SFT ratio were independently associated with the hsCRP-reducing effect of pioglitazone. Although there was correlation between the change in postprandial glucose and the change in VFT/SFT ratio (data not shown), these 2 factors showed independent association with hsCRP-reducing effect in binary logistic regression analysis. This result reaffirmed the suggestion that the hsCRP-reducing effect of pioglitazone occurs through the control of postprandial hyperglycemia and changes in abdominal adiposity. Considering the odds ratio, the redistribution of abdominal adipose tissue after pioglitazone treatment was associated with the anti-inflammatory effect of pioglitazone to the greatest extent.

In the present study, ultrasonography was performed to assess body fat distribution. Although several studies have used CT or magnetic resonance imaging as a standard method, ultrasonography has been reported to be a more reliable and convenient method to measure the amount of visceral fat [53]. Indeed, Kim et al [35] reported that SFT and VFT, the same parameters used in the present study, are well correlated with subcutaneous and visceral fat areas as measured by CT ($r = 0.806$ and 0.799, $P = .001$, respectively).

One of the limitations of the present study was the relatively small study population. In addition, the inflammatory status of type 2 diabetes mellitus was based on the sole parameter of plasma hsCRP levels because we did not measure other proinflammatory cytokines, like TNF-α or IL-6. Investigation of other proinflammatory markers may have presented additional clues regarding the mechanism by which CRP is reduced by pioglitazone. Nevertheless, the results of the present study clearly demonstrated both metabolic and anthropometric changes after pioglitazone treatment and investigated various factors affecting the effect of pioglitazone on reducing hsCRP, and suggested the mechanism of anti-inflammatory action of pioglitazone using clinical evidence.

In conclusion, 12 weeks of pioglitazone treatment reduced hsCRP levels in type 2 diabetes mellitus patients; and this effect of pioglitazone was associated with baseline hsCRP levels, as well as the decrease in postprandial glucose levels and VFT/SFT ratio. Binary logistic regression analysis revealed that a decrease in the VFT/SFT ratio was independently and most strongly associated with the hsCRP reduction by pioglitazone. Together, the results of the present study suggest that abdominal fat redistribution preferentially reflects the anti-inflammatory benefits of pioglitazone treatment.

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