Initiation of Insulin Therapy Reduces Serum Concentrations of High-Sensitivity C-Reactive Protein in Patients With Type 2 Diabetes

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Atherosclerosis has highly important chronic inflammatory aspects. We investigated anti-inflammatory effects upon initiating insulin therapy by measuring serum high-sensitivity C-reactive protein (hsCRP) and plasma fibrinogen and serum monocyte chemoattractant protein (MCP)-1in patients with poorly controlled type 2 diabetes. In 18 inpatients with type 2 diabetes, we measured serum hsCRP, plasma fibrinogen, serum MCP-1, body weight (BW), girth, and fasting plasma glucose (FPG) before and 2 weeks (14.0 ± 2.5 days) after initiation of insulin therapy. Daily insulin doses (in units) were approximately 0.2 × BW (in kilograms). Various changes (ratio) were calculated as the ratio of the value during treatment to the pretreatment value. Significant decreases occurred for $\log_{10}$ hsCRP and FPG (−0.025 ± 0.557 mg/L, 215 ± 64.3 mg/dL *v* −0.213 ± 0.571 mg/L, 129.8 ± 32.1 mg/dL; *P* = .0121, and *P* = .00002, respectively). This was particularly true for $\log_{10}$ hsCRP in patients whose BW was unchanged or increased between measurement (*P* = .0050). There were no significant differences between pretreatment and treatment values for fibrinogen and MCP-1. However, MCP-1 decreased significantly in the group with high-value in the first time point (MCP-1 > 250 pg/mL, n = 9; *P* = .0224) compared with the low-value group (MCP-1 < 250 pg/mL, n = 9; *P* = .3164). No significant correlation was found between hsCRP ratio and fibrinogen ratio, MCP-1 ratio, BW ratio, waist girth ratio, or FPG ratio. In conclusion, newly initiated insulin therapy in patients with poorly controlled type 2 diabetes decreased serum hsCRP. The decrease in hsCRP may have resulted largely from anti-inflammatory effects of insulin.

ATHEROSCLEROSIS NOW is considered to result from a chronic inflammatory response in arterial walls.[1,2] Proinflammatory cytokines[3-5] including chemokines, such as monocyte chemoattractant protein (MCP)-1, adhesion molecules,[6,7] and acute-phase reactants, such as C-reactive protein (CRP) and fibrinogen,[8,9] have seen use as circulating markers for atherosclerosis as well as inflammation. High-sensitivity CRP (hsCRP) has attracted particular attention because of good assay reproducibility and demonstrated association with cardiovascular risk.[8-14] In addition to serving as a markers, CRP contributes to progression of atherosclerosis by activation of complement,[15,16] enhancement of MCP-1 production,[17] participation in low-density lipoprotein (LDL) uptake by endothelial macrophages,[18] and other mechanisms.

Several recent reports have indicated that insulin inhibits a transcription factor, nuclear factor (NF) κB, in endothelial cells and mononuclear cells while showing acute anti-inflammatory effects, such as decreases in cytokines.[19,20] CRP is produced by the liver in response to cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 induced by activation of NFκB. Thus, if insulin truly has an inhibitory effect on NFκB, CRP as well as proinflammatory cytokines might be decreased by insulin therapy. Effective insulin therapy for diabetic patients would inhibit atherosclerosis not only by suppressing glycation, but also more directly by decreasing proinflammatory cytokines and CRP.

To determine whether insulin can rapidly decrease CRP in patients with type 2 diabetes, we measured hsCRP in serum before and after induction of insulin therapy. We also measured plasma fibrinogen as an acute-phase reactant and serum MCP-1 as key chemokines for atherogenesis.

PATIENTS AND METHODS

Patients

We studied 18 Japanese patients with type 2 diabetes who had been hospitalized to initiate insulin therapy because of poor diabetic control. Patients included 7 men and 11 women.

Five patients had been managed with observation and dietary modification; the other 13 patients had been treated before admission with a sulfonylurea (glibenclamide or glimepiride). No patient had been treated with insulin. Although no patients had a systolic blood pressure (SBP) exceeding 140 mm Hg or a diastolic blood pressure (DBP) exceeding 90 mm Hg during the hospital stay, 5 of them were taking antihypertensive drugs, specifically an angiotensin-converting enzyme inhibitor (ACE-I) or a calcium channel blocker. No patient had a history of liver disease, such as viral or autoimmune hepatitis, and none showed evidence of biochemical liver dysfunction on admission. Any patient suspected of having any infectious disease including a common cold shortly before or during the admission or autoimmune disease, such as rheumatoid arthritis, was excluded from study. Five patients were smokers.

Diabetic nephropathy was assessed according to urinary albumin excretion (UAE), and patients were assigned 1 of 3 groups: UAE < 30 mg/g of creatinine (Cr), normoalbuminuria (NAU) (n = 11); 30 ≤UAE ≤ 300 mg/gCr, microalbuminuria (MIAU) (n = 4), and UAE > 300 mg/gCr, macroalbuminuria (MAAU) (n = 3). Diabetic retinopathy was classified according to Davis' criteria[21] by each patient's ophthalmologist: no diabetic retinopathy (NDR) (n = 12); simple diabetic retinopathy (SDR) (n = 3); and proliferative diabetic retinopathy (PDR) (n = 3).

Clinical data are summarized in Table 1. These data for FPG, glycosylated hemoglobin (HbA_{1C}), body mass index (BMI), body weight (BW), waist girth, SBP, and DBP were obtained before breakfast on the day after admission.

Methods

Insulin was injected subcutaneously twice a day after admission. The daily dose (in units) was approximately 0.2 × BW (in kilograms). The sulfonylureas administrated for 13 patients were suspended on the day before insulin therapy was initiated.

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Submitted May 24, 2003; accepted January 14, 2004.

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0026-0495/04/5306-0016$30.00/0
doi:10.1016/j.metabol.2004.01.003

Table 1. Clinical Characteristics of the Diabetic Subjects

Variant	No. of Patients or Mean Value	Percentile	
		25th	75th
No. (male/female)	18 (7/11)		
Age (yr)	58.2 ± 12.2	53.3	67.3
Duration of diabetes (yr)	7.3 ± 6.4	2.0	10.0
FPG (mg/dL)	215.6 ± 64.3	167.0	275.0
HbA_{1C} (%)	11.0 ± 2.3	9.3	11.7
BMI (kg/m^2)	24.3 ± 4.4	20.7	27.9
BW (kg)	58.8 ± 14.4	46.8	69.8
Waist girth (cm)	83.7 ± 13.9	73.5	94.5
SBP (mm Hg)	124.9 ± 14.3	119.0	133.8
DBP (mm Hg)	74.8 ± 10.3	64.0	82.5
Smoking (n)	5		
Therapy (n)			
Diet	5		
OHA	5		

Abbreviations: FPG, fasting plasma glucose; BMI, body mass index; BW, body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; OHA, oral hypoglycemic agents; n, number of patients.

All venous blood samples for chemical examinations were collected from patients in the morning before breakfast after at least 10 hours of overnight fasting.

Serum hsCRP, plasma fibrinogen, and serum MCP-1 were measured twice, before beginning insulin therapy and again about 14 days (14 ± 2.5) after injection of insulin. At these time points, BW and girth also were measured.

Serum hsCRP Assay

Blood was centrifuged at 1,500 rpm for 5 minutes to separate serum from the clot containing blood cells. Sera were stored at −70°C until analysis. The BN II N High Sensitivity CRP assay (Dade Behring, Marburg, Germany), which has been approved by the US Food and Drug Administration (FDA) for use in evaluating risk of cardiovascular and peripheral vascular disease, was used. The lowest detectable concentration of hsCRP with this assay was 0.05 mg/L. Only 1 patient on 1 occasion showed a value for hsCRP below 0.05 mg/L; this value was taken to be 0.05 mg/L.

Serum MCP-1 Assay

The same serums in which hsCRP was measured were also used for the measurement of MCP-1. Serum MCP-1 was assayed by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN).

Measurement of Plasma Glucose, HbA_{1C}, and Serum Lipids

Fasting plasma glucose was assessed by an automated glucose oxidase method (Glucose Auto Stat GA1160; Arkray, Kyoto, Japan). HbA_{1C} was measured by high-performance lipid chromatography (HPLC; Hi-auto A1C, HA8150, Arkray). With this method, only stable HbA_{1C} was detected. The normal range was 4.3% to 5.8%. Serum total, low-density, and high-density lipoprotein cholesterol (TC, LDL-C, HDL-C) and also serum triglyceride (TG) concentrations were measured using enzymatic assays.

Measurement of Combined Intimal-Medial Thickness in the Carotid Artery

Intimal-medial thickness (IMT) was measured once during each patient's admission using ultrasonography (SSD-1200; Aloka, Tokyo, Japan) with a linear pulse echoprobe operating at 7.5 MHz (ASU-35WL-7.5). Axial resolution with this probe was better than 0.1 mm. A suitable portion of the right common carotid artery in the neck was scanned longitudinally from an anterioroblique orientation. Using calipers, the IMT was measured at the thickest point in the area scanned (including plaques), and also at points 1 cm proximal and distal to the first. The mean of these 3 thicknesses was taken as the individual's IMT.

Measurement of UAE

UAE was measured by enzyme immunoassay. Albumin values were corrected for urinary Cr concentration.

Ethical Consideration

All subjects gave informed consent to be included in the current study, which was performed according to the guidelines proposed in the Declaration of Helsinki.

Statistical Methods

All data are presented as the mean ± SD. The significance of correlations between 2 variables was determined by simple regression analysis. Comparisons between pretreatment and treatment $\log_{10}$ hsCRP, fibrinogen, BW, girth, and FPG values were examined using paired *t* tests. Multiple regression analysis was performed using stepwise regression analysis with the first hsCRP measured as the dependent variable. Independent variables tested initially were carotid IMT, SBP, DBP, age, gender, duration of diabetes, BMI, FPG, HbA_{1C}, TC/HDL-C ratio, LDL-C, HDL-C, TG and MCP-1. Among these variables, combinations with strong correlation ($R > 0.9$) were not found. Each variable with an F value less than 2 was then excluded.

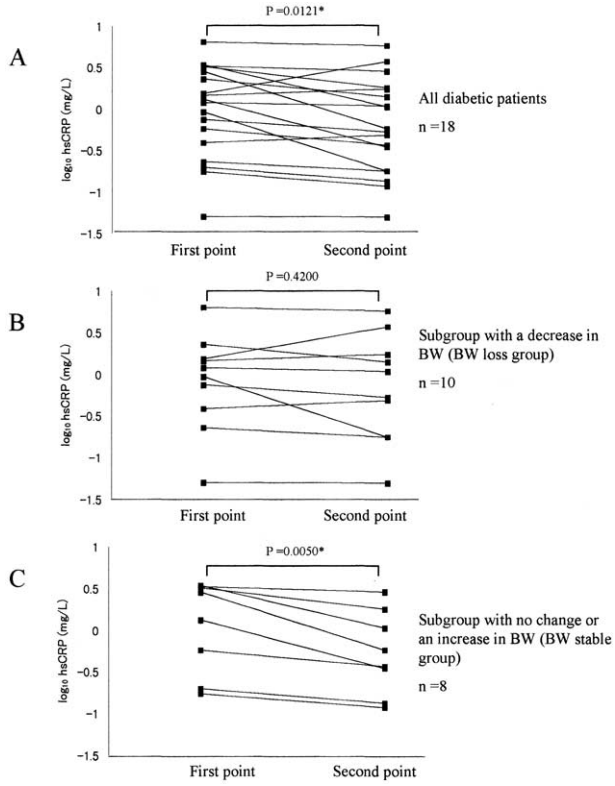

Fig 1. Difference between mean values for $\log_{10}$ hsCRP at the time points (before and after insulin therapy).

Table 2. Changes for hsCRP, Fibrinogen, and MCP-1 Between the Time Points Before and After Insulin Therapy in Total, Male, and Female Patients in All 18 Patients, BW Loss, and No Change or an Increase in BW Groups

Variable	Patients	First Point	Second Point	*P* Value
Log_{10} CRP (mg/L) (n)				
All patients	Total (18)	−0.025 ± 0.557	−0.213 ± 0.571	.0121*
	Men (7)	0.061 ± 0.442	−0.105 ± 0.487	.2637
	Women (11)	−0.080 ± 0.633	−0.282 ± 0.631	.0212*
BW loss group	Total (10)	−0.089 ± 0.581	−0.1638 ± 0.642	.4200
	Men (3)	0.146 ± 0.056	0.281 ± 0.270	.3988
	Women (7)	−0.189 ± 0.682	−0.354 ± 0.673	.1512
No change or an increase in BW group	Total (8)	0.055 ± 0.554	−0.275 ± 0.503	.0050*
	Men (4)	−0.002 ± 0.614	−0.395 ± 0.405	.0529
	Women (4)	0.112 ± 0.574	−0.155 ± 0.622	.0987
Fibrinogen (mg/dL) (n)				
All patients	Total (18)	362.0 ± 84.0	379.7 ± 76.9	.2826
	Men (7)	314.3 ± 78.7	354.7 ± 107.6	.2867
	Women (11)	392.7 ± 75.3	395.5 ± 48.9	.8243
BW loss group	Total (10)	379.1 ± 100.4	411.3 ± 59.8	.2484
	Men (3)	309.3 ± 98.3	427.7 ± 82.5	.1515
	Women (7)	409.0 ± 91.8	404.3 ± 53.8	.7987
No change or an increase in BW group	Total (8)	340.6 ± 56.8	340.1 ± 80.9	.9732
	Men (4)	318.0 ± 76.8	300.0 ± 96.5	.2589
	Women (4)	363.2 ± 16.3	380.3 ± 40.9	.5348
MCP-1 (pg/mL) (n)				
All patients	Total (18)	290.3 ± 95.2	272.2 ± 76.3	.1679
	Men (7)	259.4 ± 77.9	245.1 ± 56.0	.5610
	Women (11)	309.9 ± 103.3	289.4 ± 84.7	.2078
BW loss group	Total (10)	303.6 ± 99.4	288.8 ± 77.7	.4352
	Men (3)	246.0 ± 37.6	253.3 ± 36.7	.8797
	Women (7)	328.3 ± 109.5	304.0 ± 87.9	.2660
No change or an increase in BW group	Total (8)	273.6 ± 93.5	251.4 ± 74.0	.2613
	Men (4)	269.5 ± 104.4	239.0 ± 72.5	.3502
	Women (4)	277.8 ± 97.4	263.8 ± 84.3	.6420

Abbreviations: R, Pearson's correlation coefficient; n, the number of the patients; hsCRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; BW loss group, subgroup of patients with a decrease in BW between the time points; no change or an increase in BW group, subgroup of patients with no change or an increase in BW.

*$P < .05$ is defined as statistical significance.

Multiple regression analysis was performed among independent variables selected according to F values. A *P* value less than .05 was accepted as indicating statistical significance.

RESULTS

In all 18 diabetic patients, a significant decrease of mean values for log_{10} hsCRP 14 days after insulin therapy (second time point) was found, compared with those before initiation of insulin therapy (first time point) ($P = .0121$; Fig 1A). Although a significant decrease of log_{10} hsCRP was also detected between the first and second time points in 11 female diabetic patients ($P = .0212$), a decrease did not achieve a statistical significance in 7 male diabetic patients ($P = .2637$). While some decrease in log_{10} hsCRP between the time points occurred in the BW loss group (subgroup of patients with a decrease in BW between the time points, n = 10), statistical significance was not attained ($P = .4200$; Fig 1B). On the other hand, a highly significant decrease in log_{10} hsCRP occurred in the BW stable group (subgroup of patients with no change or an increase in BW, n = 8) ($P = .0050$; Fig 1C).

There were no significant differences between mean values for plasma fibrinogen at the first and the second points in 7 male and 11 female patients, as well as in all 18 patients. Significant differences were not obtained between mean values for plasma fibrinogen at the time points in both BW loss and BW stable groups.

Although a tendency of decrease of mean values for MCP-1 was found between the first and second time points in the 11 female diabetic patients, as well as in all 18 diabetic patients, these decreases did not achieve statistical significance. No significant differences in mean values for MCP-1 between the time points were found in 7 male diabetic patients. Significant decreases were not detected between mean values for MCP-1 at the time points in both BW loss and BW stable groups. These data are listed in Table 2.

Patients were classified into 2 groups with an equal number of them by values for log_{10} hsCRP, fibrinogen, and MCP-1 at the first time point; With 0.03 mg/L of log_{10} hsCRP, 360 mg/dL of fibrinogen, and 250 pg/mL of MCP-1, the patients were divided into a low-value group (n = 9) and a high-value group (n = 9), respectively. As for mean values for hsCRP, fibrinogen, and MCP-1 at the first points, significant differences were found between these groups ($P = .00007$, $P = .0010$, $P = .0011$, respectively). There were no significant differences for

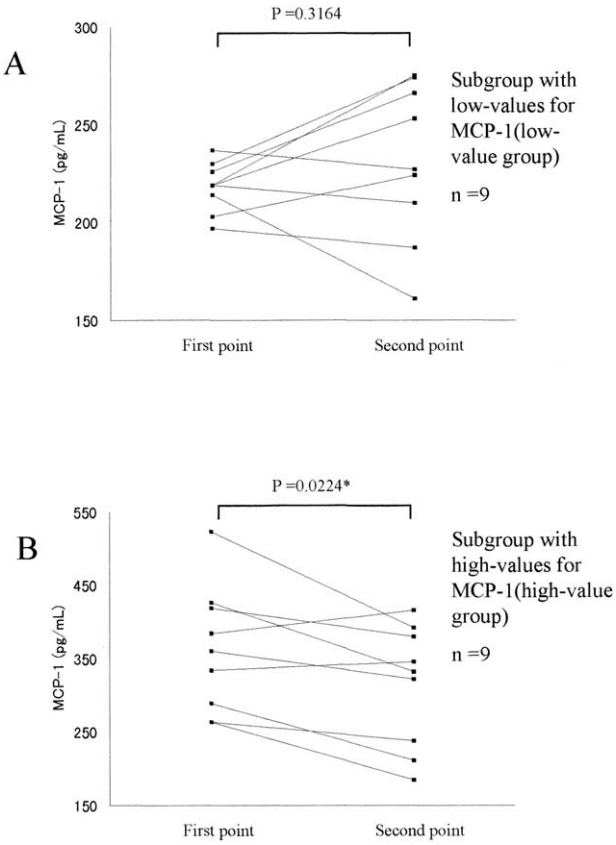

Fig 2. Difference between mean values for MCP-1 at the time points (before and after insulin therapy) in low- and high-value groups.

$\log_{10}$ hsCRP, as well as fibrinogen between the time points in both low- and high-value groups. A significant decrease of MCP-1 between the time points was obtained in the high-value group, while no decrease was observed in the low- value group ($P = .0224$, .3364, respectively; Fig 2A and B).

The mean values for FPG at the first and second time points in all 18 patients were 215.6 ± 64.3 and 129.8 ± 32.1 mg/dL ($P = .00002$).

In this study, patients were also divided into 2 groups with an equal number of them by FPG values at the first point, ie, low-FPG group (n = 9) and high-FPG group (n = 9). The mean values for FPG in these groups at the first point were 165.1 ± 25.2 and 266.0 ± 49.2 mg/dL ($P = .00005$). A significant decrease for $\log_{10}$ hsCRP was observed between the time points in low-FPG group, while there was no significant decrease between the time points in high-FPG group ($P = .0234$, .1584, respectively). No significant decreases in MCP-1, as well as fibrinogen, were detected between the time points in both low- and high-FPG groups.

No significant difference was noted between smokers' group (n = 5) and nonsmokers' group (n = 13) in mean values for $\log_{10}$ hsCRP and fibrinogen, as well as MCP-1 at the first time point. A significant decrease of $\log_{10}$ hsCRP between the time points was found in nonsmokers, while no significant decrease was obtained in smokers ($P = .0030$, $P = .7524$, respectively). On the other hand, there were no significant differences in mean values for fibrinogen, as well as MCP-1, between the time points in both smokers' and nonsmokers' groups. These data are presented in Table 3.

Mean values for BW and waist girth in the 18 patients at the first time point were 58.8 ± 14.4 kg and 83.7 ± 13.9 cm; at the second time point, these were 58.2 ± 13.8 kg and 83.1 ± 13.6 cm (no significant differences).

In the 18 patients, a significant positive correlation was detected between $\log_{10}$ hsCRP at the first time point and IMT in the carotid artery ($P = .0420$), as well as BMI ($P = .0011$). Although $\log_{10}$ hsCRP at the first time point showed some tendency to correlate with girth, serum TC, LDL-C, and TG, these relationships were not statistically significant. $\log_{10}$ hsCRP at the first time point did not correlate with age, duration of diabetes, BW, FPG, HbA_{1C}, or HDL-C. In addition, no significant associations were detected between $\log_{10}$ hsCRP at the first time point and SBP, DBP, R-R interval coefficient of heart rate variation (CV_{RR}), or $\log_{10}$ UAE. No significant correlation was noted between $\log_{10}$ hsCRP and fibrinogen or MCP-1 at the first point. Coefficients and *P* values for correlations between $\log_{10}$ hsCRP and various parameters according to simple regression analysis are shown in Table 4.

Degree of change between 2 time points in hsCRP, fibrinogen, MCP-1, BW, waist girth, and FPG was defined as the ratio of the value at the second point to the value at the first point (abbreviation: hsCRP ratio, fibrinogen ratio, MCP-1 ratio, BW ratio, waist girth ratio, and FPG ratio). No significant correlation was noted between hsCRP ratio and fibrinogen ratio, MCP-1 ratio, BW ratio, waist girth ratio, or FPG ratio ($R = 0.3061$, $P = .2166$; $R = 0.2937$, $P = .2368$; $R = -0.4118$, $P = .0900$; $R = -0.1926$, $P = .4430$; $R = 0.0182$, $P = .7488$, respectively).

BMI and FPG showed significant associations by stepwise regression analysis with $\log_{10}$ hsCRP at the first time point ($P = .0004$, $P = .0419$, respectively; Table 5).

DISCUSSION

Recently, insulin has been found to inhibit NFκB in vascular endothelial cells and mononuclear cells and to have an acute anti-inflammatory effect, as suggested by a decrease of proinflammatory cytokines.[19,20] However, it has not been evident whether insulin therapy can rapidly reduce serum concentrations of CRP, as well as proinflammatory cytokines, although Yudkin et al[22] have recently demonstrated the reduction of hsCRP by insulin in a relative long-term observation (16 weeks). We therefore compared serum hsCRP concentrations before and in a relative short-term (2 weeks) after initiation of insulin therapy in patients with poorly controlled type 2 diabetes. A statistically significant decrease in hsCRP was seen with insulin therapy, with only 3 of 18 patients showing any increase in hsCRP. These results were compatible with those shown by Yudkin et al.[22] If the short-time decreases in serum hsCRP concentrations shown in our study also persist over the long-term as years, insulin therapy may have direct antiatherogenic effects that are independent of lowering the plasma glucose concentration. However, a more detailed analysis is required before this hypothesis is accepted fully.

Table 3. Changes for hsCRP, Fibrinogen, and MCP-1 Between the Time Points Before and After Insulin Therapy in Low- and High-Value Groups, Low- and High-FPG Groups, and Nonsmoking and Smoking Groups

Variable	First Point	Second Point	*P* Value
Log_{10} CRP (mg/L) (n)			
Low-value group (9)	−0.457 ± 0.436	−0.623 ± 0.408	.0608
High-value group (9)	0.408 ± 0.220	0.197 ± 0.384	.1027
Low-FPG group (9)	−0.157 ± 0.670	−0.346 ± 0.630	.0231*
High-FPG group (9)	0.107 ± 0.413	−0.080 ± 0.505	.0847
Nonsmoking (13)	−0.111 ± 0.602	−0.385 ± 0.552	.0030*
Smoking (5)	0.201 ± 0.380	0.233 ± 0.469	.7524
Fibrinogen (mg/dL) (n)			
Low-value group (9)	304.3 ± 50.9	347.7 ± 85.1	.1355
High-value group (9)	419.7 ± 70.1	411.7 ± 55.0	.6131
Low-FPG group (9)	329.4 ± 68.8	351.4 ± 84.1	.4315
High-FPG group (9)	394.6 ± 88.7	407.9 ± 60.8	.5063
Nonsmoking (13)	384.5 ± 77.1	382.3 ± 59.56	.8446
Smoking (5)	303.4 ± 78.9	372.8 ± 120.0	.1943
MCP-1 (pg/mL) (n)			
Low-value group (9)	218.2 ± 12.5	230.8 ± 40.1	.3164
High-value group (9)	362.3 ± 86.2	313.5 ± 83.1	.0224*
Low-FPG group (9)	266.4 ± 76.8	237.4 ± 60.7	.0847
High-FPG group (9)	314.1 ± 110.0	306.9 ± 77.3	.7351
Nonsmoking (13)	308.5 ± 105.9	287.7 ± 80.9	.1853
Smoking (5)	243.0 ± 32.0	231.8 ± 47.8	.6935

NOTE. The patients were classified into 2 groups with an equal number of them for log_{10} hsCRP, fibrinogen, and MCP-1 (low- and high-value group, respectively). The patients were divided into 2 groups with an equal number of them by FPG values at the first point (low- and high-FPG group, respectively).

Abbreviations: hsCRP, high-sensitivity C-reactive pritein; MCP-1, monocyte chemoattractant protein-1; n, number of patients.

*$P < .05$ is defined as statistical significance.

IL-6 and TNF-α are produced not only by vascular endothelial cells and mononuclear cells, but also by adipose tissue.[23,24] Accordingly, hsCRP may have been reduced in the present study, at least in part, by decreases in body fat during the 2-week interval studied as suggested by decreased BW. In a study of a large number of subjects, Festa et al[25] noted a strong correlation between hsCRP and BMI (reflecting overall adiposity) or girth (reflecting visceral adiposity). In our current study, although patient numbers were small, log_{10} hsCRP correlated closely with BMI and tended to correlate with girth. The correlation of the former was higher rather than that between log_{10} hsCRP and IMT in the carotid artery, which like hsCRP, is considered as a marker for systemic atherosclerosis. Thus, body fat clearly influences hsCRP.

However, no significant change occurred in BW or girth with 2 weeks of insulin therapy, and hsCRP ratio did not correlate significantly with BW ratio or girth ratio. Further, a significant decrease in log_{10} hsCRP occurred in patients with no decrease in BW between the time point, as well as in the 18 patients considered together. In the current study, we adopted BW instead of waist girth for patients' grouping because of its better reproducibility. Interestingly, degrees of the decrease in the BW stable group were larger rather than those in all 18 patients. Therefore, the decrease of hsCRP with insulin therapy appears unlikely to have resulted wholly from decreases in body fat.

Another possible influence on hsCRP is plasma glucose. As expected, FPG was significantly reduced by insulin therapy. FPG also showed weak, but significant, association with log_{10} hsCRP by stepwise regression analysis. Furthermore, we found

Table 4. Correlation With Various Factors and Serum hsCRP in 18 Diabetic Patients

Variable	Log_{10} hsCRP	
Age (yr)	$r = 0.3175$	$P = .1992$
Duration (yr)	$r = -0.0056$	$P = .9825$
BMI (kg/m^2)	$r = 0.7048$	$P = .0011$*
BW (kg)	$r = 0.3700$	$P = .1307$
Waist girth (cm)	$r = 0.4287$	$P = .0758$
FPG (mg/dL)	$r = 0.2570$	$P = .3033$
HbA_{1C} (%)	$r = 0.3008$	$P = .2251$
TC (mg/dL)	$r = 0.4388$	$P = .0685$
LDL-C (mg/dL)	$r = 0.4486$	$P = .0618$
HDL-C (mg/dL)	$r = -0.1219$	$P = .6299$
TG (mg/dL)	$r = 0.4354$	$P = .0709$
SBP (mm Hg)	$r = 0.2291$	$P = .3605$
DBP (mm Hg)	$r = 0.1412$	$P = .5764$
CV_{RR} (%)	$r = 0.0209$	$P = .9344$
Log_{10} UAE (mg/g · Cr)	$r = -0.0548$	$P = .8288$
IMT (mm)	$r = 0.4837$	$P = .0420$*
Fibrinogen (mg/dL)	$r = 0.1160$	$P = .6467$
MCP-1 (pg/mL)	$r = 0.1458$	$P = .5637$

Abbreviations: hsCRP, high sensitivity C-reactive protein; BMI, body mass index; BW, body weight; FPG, fasting plasma glucose; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure; CV_{RR}, coefficient of variance of heart rate variations; UAE, urinary albumin excretion; IMT, combined intimal-medial thickness in the carotid artery; MCP-1, monocyte chemoattractant protein-1.

*$P < .05$ is defined as statistical significance.

Table 5. Stepwise Regression Analysis With Log_{10} hsCRP as the Dependent Variable

Independent Variables	β	SEM	F	*P*
BMI (kg/m^2)	0.6924	0.0194	20.7704	.0004*
FPG (mg/dL)	0.3352	0.0013	5.0127	.0419*
TC/HDL-C ratio	0.2885	0.0804	3.6472	.0739
R^2 for the model: 68.9%				

NOTE. Variables with F values less than 2 were excluded from this multiple regression analysis.

Abbreviations: β, standardized regression coefficient; SEM, standard error of the mean; F, F value; *P, P* value; R^2, coefficient of determination; BMI, body mass index; FPG, fasting plasma glucose; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol.

*$P < .05$ is defined as statistical significance.

the significant decrease for hsCRP between the time points in patients with relatively low FPG at the first point, while no significant decrease was detected in patients with relatively high FPG at the first point. Therefore, although speculatively, it might be possible that the hyperglycemic state can weaken the anti-inflammatory effect of insulin and influence hsCRP consequently because of an activation of NFκB caused by hyperglycemia.[26] However, pretreatment log_{10} hsCRP did not correlate with FPG or HbA_{1C}, and no significant correlation was seen between hsCRP ratio and FPG ratio. In addition, Dandona et al[20] found that insulin inhibits NFκB in mononuclear cells independently of plasma glucose concentrations. Thus, the decrease of hsCRP in the current study probably was not solely a result of decreased plasma glucose concentrations.

Considering various findings together, we believe that insulin therapy had a direct clinical acute anti-inflammatory affect that was, at least partly, responsible for decreases in hsCRP independently of plasma glucose concentrations.

We measured fibrinogen as another acute-phase reactant. Contrary to our expectation, no significant association could be found between log_{10} CRP and fibrinogen on admission. In addition, fibrinogen did not decrease significantly with insulin therapy. As a coagulant, fibrinogen may be influenced in a complex manner by factors quite different from those affecting hsCRP. Accordingly, we believe that assessing atherosclerosis by plasma fibrinogen concentration alone can be misleading.

We also investigated serum MCP-1, which are key chemokine for atherogenesis. Although MCP-1 as chemokines acts locally, we hypothesized that not only MCP-1 expression in local inflammatory portions, but also serum MCP-1 concentrations, may be reduced if insulin shows an anti-inflammatory effect for systemic vascular endothelial cells. In the current study, MCP-1 tended to decrease with insulin therapy, although it was not significant. Especially, the significant decrease for MCP-1 was found in a group with relative high value at first time point. Although it is difficult to explain this result fully, insulin therapy might act more effectively for patients with stronger local inflammations, as suggested by elevation of serum MCP-1. However, with respect to influence for MCP-1 of insulin, a more detailed analysis should be required.

In the current study, we verified the gender effect for the results. We found a significant decrease for log_{10} hsCRP between the time points in female patients unlike male patients. Accordingly, although the reason is fully unknown, a decrease of hsCRP by insulin might be predominant in women rather than in men. In addition, we also investigated whether smoking has effects on the results. A significant decrease for hsCRP and a tendency of decrease for MCP-1 was detected in the nonsmoking group, while no significant decrease was obtained in the smoking group. Therefore, probably, smoking might weaken the anti-inflammatory effect of insulin by the effect for vascular endothelial cells. However, also in these issues, more detailed analyses should be required.

In the current study, limitations for the study designs and interpretations of the results must be pointed out. First, the administered sulfonylureas were immediately suspended after initiation of insulin therapy, but we could not provide washout periods for these drugs. Therefore, in patients treated with sulfonylureas, the influence of these drugs might have remained at the first measured point. This is one of the limitations in our study. Second, we must point out that the decrease in CRP noted after insulin therapy was small, although it was statistical significant. Therefore, we believe an additional study with a large number of subjects and in a more long-term observation should be performed to analyze the influence of insulin therapy on CRP more exactly. Furthermore, although Yudkin et al[22] have found that insulin therapy had no effect on IL-6, which is one of the major determinants of hsCRP, we also should have measured IL-6 as an additional test.

In conclusion, we measured serum hsCRP concentrations before and during insulin therapy to assess possible acute anti-inflammatory effects. Over approximately 2 weeks, log_{10} hsCRP decreased significantly. Because hsCRP ratio did not correlate with BW ratio, girth ratio, or FPG ratio, insulin therapy may have reduced hsCRP, at least partly, by direct anti-inflammatory effects. Concentrations of hsCRP reflect chronic inflammatory states, but like cytokines, CRP also may have an atherogenic effect in itself. Therefore, insulin therapy should be studied for the long-term.

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