Table 1
Clinical characteristics of the 662 male adolescents and correlation coefficients (r) for HMW adiponectin or leptin

	Mean ± SD	r vs HMW adiponectin	Adjusted for BMI	r vs leptin	Adjusted for BMI
Height (cm)	171.2 ± 5.5	0.051		0.000	
Weight (kg)	61.1 ±8.5	−0.214***	0.037	0.494***	0.021
BMI (kg/m^2)	20.8 ± 2.6	−0.262***	–	0.551***	–
Waist (cm)	72 ± 7	−0.236***	−0.010	0.592***	0.267***
SBP (mm Hg)	115.9 ± 12.1	−0.054		0.202***	0.042
DBP (mm Hg)	63.7 ± 8.0	−0.058		0.123**	0.065
HR (beats/min)	66.8 ± 12.4	−0.029		0.279***	0.296***
TC (mg/dL)	171 ± 27	0.051		0.040	
TG (mg/dL)[a]	52 ± 26	−0.056		0.275***	0.213***
HDL-C (mg/dL)	67 ± 13	0.200***	0.165***	−0.233***	−0.174***
FPG (mg/dL)	86 ± 6	−0.012		0.125***	0.131**
IRI (μU/mL)[a]	4.8 ± 3.3	−0.063		0.437***	0.361***
HOMA-IR[a]	1.04 ± 0.73	−0.061		0.435***	0.363***
TNF-α (pg/mL)[a]	2.50 ± 1.19	−0.071		0.127**	0.133**
IL-6 (pg/mL)[a]	40.96 ± 41.98	−0.028		0.202***	0.233***
MCP-1 (pg/mL)	199.56 ± 65.75	0.020		0.107***	0.094*
Leptin (ng/mL)[a]	1.57 ± 1.48	−0.158***	−0.017	–	–
Adiponectin (μg/mL)[a]	4.18 ± 2.24	–	–	−0.158***	−0.017

Data are expressed as means ± SD.
[a] These parameters were analyzed after logarithmic transformation.
* $P < .05$ by Pearson correlation coefficient.
** $P < .01$ by Pearson correlation coefficient.
*** $P < .001$ by Pearson correlation coefficient.

with increased risks of developing type 2 diabetes mellitus and insulin resistance [9-11], although a recent prospective study revealed that high leptin levels predict a decreased risk of diabetes independently of adiponectin, after adjustment for obesity, hyperinsulinemia, inflammation, and other metabolic components [12].

The purpose of the present study was to clarify the associations of HMW adiponectin and leptin with anthropometric and metabolic parameters, insulin resistance, and proinflammatory cytokines in the general population of Japanese male adolescents, who might still be in the development stage in terms of their adipose tissues and would be less affected by environmental factors, such as medications, smoking, and alcohol consumption, than adults. We compared the results of these adolescents with those of healthy Japanese male adults.

2. Subjects and methods

2.1. Subjects

This study included 662 Japanese male adolescents at Keio high school, 16 to 17 years (16.1 ± 0.2) of age, and 282 Japanese male teachers and employees at Keio university, 30 to 61 years (46.4 ± 9.2) of age, who received annual health checkups. All subjects were asked to fast overnight. None of the adolescents had been prescribed medications for diabetes, hypertension, or dyslipidemia. The proportion of adolescents whose body mass index (BMI) was more than 25.0 kg/m^2 was only 6.0% (40 subjects). Adult subjects with cardiovascular disease, endocrine disease, or significant renal or hepatic disease and those who were taking medications for diabetes, hypertension, or dyslipidemia were excluded from the analyses.

The present study was conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was obtained from each subject after a full explanation of the purpose, procedures, and risks of the study. The protocol was approved by the ethics review committees of the Health Center and the Department of Internal Medicine, Keio University School of Medicine (Tokyo, Japan).

2.2. Clinical variables

Height was measured to the nearest 0.1 cm. Weight was measured in light indoor clothing with shoes removed. Body mass index was calculated as weight in kilograms divided by height in meters squared. Waist circumference was obtained at the navel level while standing with slight expiration.

2.3. Biochemical measurements

Plasma glucose and lipids were assayed by routine automated laboratory methods. The serum insulin concentration was measured by an enzyme immunoassay using a commercially available kit (Tosoh, Tokyo, Japan) with intra- and interassay coefficients ranging from 2.9% to 4.6% and from 4.5% to 7.0%, respectively [2]. The insulin resistance index was calculated based on homeostasis model assessment (HOMA-IR). The serum HMW adiponectin level was measured with an enzyme-linked immunosorbent assay kit (Fujirebio, Tokyo, Japan) [13] with intra- and interassay coefficients ranging from 4.8% to 4.9% and from 3.3% to 6.8%, respectively, as described previously [2]. The serum

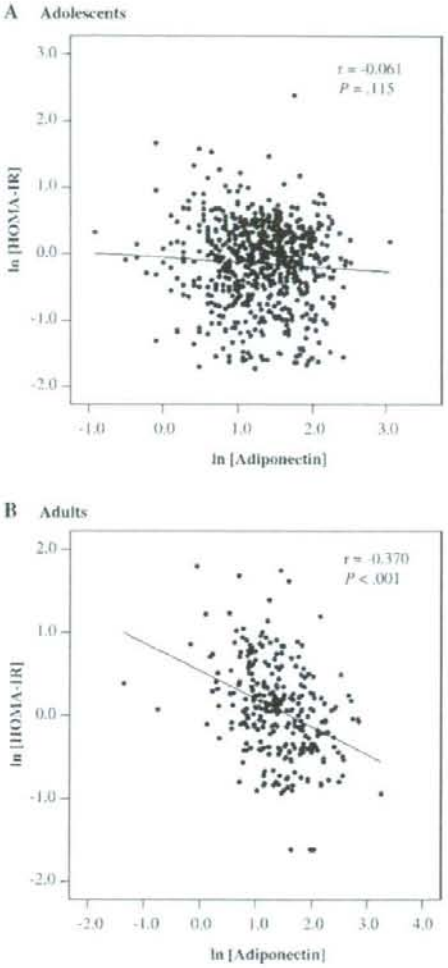

Fig. 1. Relationships between serum HMW adiponectin levels and HOMA-IR in male adolescents (n = 662, A) and male adults (n = 282, B). The HMW adiponectin and HOMA-IR values were log-transformed for statistical analysis. Statistical significance was evaluated by Pearson correlation coefficients.

leptin, tumor necrosis factor (TNF) α, interleukin (IL) 6, and monocyte chemoattractant protein (MCP) 1 levels were measured in adolescent subjects using a LINCOplex kit (Linco Research, St Charles, MO) with intra- and interassay coefficients of 1.4% to 7.9% and <21%, respectively.

2.4. Statistical analysis

All statistical analyses were performed using the SPSS program for Windows (version 12.0; SPSS, Chicago, IL). Relationships between adipokines, HOMA-IR, and other parameters were analyzed by simple and multiple correlations and by stepwise linear regressions. Because serum insulin, HOMA-IR, triglycerides (TG), HMW adiponectin, leptin, TNF-α, and IL-6 were normally distributed after log transformation, the logarithm of these parameters was used for the analyses. All data are expressed as mean ± SD, and $P < .05$ was considered statistically significant.

3. Results

3.1. Relationships between adiponectin and metabolic parameters in adolescents

The anthropometric and metabolic parameters and the proinflammatory cytokine and adipokine levels in male adolescents are shown in Table 1. The serum adiponectin level in adolescents was 4.18 ± 2.24 μg/mL (range, 0.4-21.0 μg/mL). Adiponectin levels in adolescents correlated negatively with BMI and waist circumference. Among metabolic parameters, only high-density lipoprotein cholesterol (HDL-C) correlated significantly with adiponectin ($r = 0.200$, $P < .001$). This correlation was significant even after adjustment for BMI ($r = 0.165$, $P < .001$) or waist circumference ($r = 0.167$, $P < .001$). Adiponectin levels did not correlate with HOMA-IR in adolescents (Fig. 1A).

When we separated 171 adolescents (25.8%) whose BMI were more than 22.0 kg/m^2, there was a weak but significant inverse correlation between HMW adiponectin and HOMA-IR ($r = -0.152$, $P = .047$). However, this was not significant after adjustment for BMI.

3.2. Relationships between leptin and metabolic parameters in adolescents

As shown in Table 1, serum leptin levels correlated positively with BMI, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate

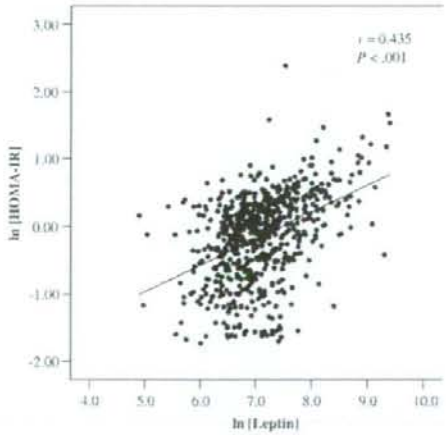

Fig. 2. Relationship between serum leptin levels and HOMA-IR in 662 male adolescents. Leptin and HOMA-IR values were log-transformed for statistical analysis. Statistical significance was evaluated by Pearson correlation coefficients.

Table 2
Clinical characteristics of the 282 male adults and correlation coefficients (r) for HMW adiponectin

	Mean ± SD	r	Adjusted for BMI
Age (y)	46.4 ± 9.2	−0.029	−0.036
Height (cm)	169.7 ± 6.1	−0.041	−0.065
Weight (kg)	66.7 ± 9.1	−0.357**	−0.059
BMI (kg/m^2)	23.2 ± 2.7	−0.387**	–
SBP (mm Hg)	122 ± 16	−0.160**	−0.010
DBP (mm Hg)	77 ± 10	−0.134*	0.035
HR (beats/min)	74 ± 11	−0.049	−0.033
TC (mg/dL)	201 ± 30	−0.090**	−0.053
TG (mg/dL)[a]	122 ± 77	−0.358**	−0.235**
HDL-C (mg/dL)	54 ± 14	0.438**	0.325**
FPG (mg/dL)	94 ± 9	−0.097*	−0.034
IRI (μU/mL)[a]	5.4 ± 3.5	−0.367**	−0.222**
HOMA-IR[a]	1.26 ± 0.83	−0.370**	−0.223**
Adiponectin (μg/mL)[a]	4.84 ± 3.20	–	–

Data are expressed as mean ± SD.
[a] These parameters were analyzed after logarithmic transformation.
* $P < .05$ by Pearson correlation coefficient.
** $P < .001$ by Pearson correlation coefficient.

(HR), TG, fasting plasma glucose (FPG), immunoreactive insulin (IRI), HOMA-IR, TNF-α, IL-6, and MCP-1 and correlated negatively with HDL-C. Correlation of leptin with HR, TG, HDL-C, FPG, IRI, HOMA-IR, TNF-α, IL-6, and MCP-1 was significant even after adjustment for BMI. The correlation between leptin and HOMA-IR was significant even after adjustment for both BMI and waist circumference ($r = 0.343$, $P < .001$) (Fig. 2). Tumor necrosis factor α, IL-6, and MCP-1 correlated with neither BMI nor HOMA-IR.

3.3. Relationships between adiponectin and metabolic parameters in adults

The anthropometric and metabolic parameters and the adiponectin levels in male adults are shown in Table 2. The serum adiponectin level in adults was 4.84 ± 3.20 μg/mL (range, 0.30-26.0 μg/mL). Adult serum adiponectin levels were significantly higher than those in adolescents ($P = .026$), despite adult BMI being significantly higher ($P < .001$). The serum adiponectin levels in adults correlated negatively with BMI, SBP, DBP, total cholesterol (TC), TG, FPG, IRI, and HOMA-IR and correlated positively with HDL-C. The correlations between adiponectin and TG, HDL-C, IRI, and HOMA-IR were significant even after adjustment for BMI.

Table 3A
Stepwise multiple regression analysis for HOMA-IR as the dependent variable in male adolescents (n = 662)

	Standardized β	P	Change in R^2 (%)
Leptin	0.333	<.001	18.8
HR	0.185	<.001	3.8
TG	0.181	<.001	2.6

Variables are shown in the order of entry. Entry of the following variables in regression mode was permitted: BMI, SBP, DBP, HR, TC, TG, HDL-C, HMW adiponectin, and leptin. The HOMA-IR, TG, HMW adiponectin, and leptin were analyzed after logarithmic transformation.

Table 3B
Stepwise multiple regression analysis for HOMA-IR as the dependent variable in male adults (n = 282)

	Standardized β	P	Change in R^2 (%)
BMI	0.320	<.001	25.0
HDL-C	−0.269	<.001	5.9
TC	0.151	<.001	2.9
HR	0.126	<.001	1.4
Adiponectin	−0.111	<.05	0.7

Variables are shown in the order of entry. Entry of the following variables in regression mode was permitted: BMI, SBP, DBP, HR, TC, TG, HDL-C, and HMW adiponectin. The HOMA-IR, TG, HMW adiponectin, and leptin were analyzed after logarithmic transformation.

3.4. Multiple regression analysis with HOMA-IR in adolescents and adults

As shown in Table 3A, stepwise multiple regression analysis revealed leptin, HR, and TG to be significantly correlated with HOMA-IR in adolescents ($R^2 = 0.252$). On the other hand, BMI, HDL-C, TC, HR, and HMW adiponectin correlated significantly with HOMA-IR in adults ($R^2 = 0.359$, Table 3B).

4. Discussion

In this study, we have shown that (1) serum HMW adiponectin levels are decreased and leptin levels are increased with adiposity variables in the general population of male adolescents; (2) HMW adiponectin significantly correlates with HOMA-IR in adults, but not in adolescents; (3) HMW adiponectin correlates positively with HDL-C in adolescents, but not with any other obesity-related metabolic parameters; and (4) leptin is closely associated with HOMA-IR, obesity-related metabolic parameters, and proinflammatory cytokines in adolescents.

It is particularly noteworthy that our observations were made in a general population of adolescents, most of whom were healthy and minimally affected by environmental factors, such as medication, smoking habit, and alcohol consumption.

The present study showed HMW adiponectin to be decreased in accordance with rising levels of adiposity variables while not being significantly associated with insulin resistance (Fig. 1A) or metabolic parameters except for HDL-C in adolescents, whereas a close negative correlation between adiponectin and insulin resistance was observed in adults (Fig. 1B), as we previously reported [2]. Many clinical studies have demonstrated adiponectin to correlate inversely with insulin resistance in adults. Recently, several pediatric studies have shown a negative correlation between total adiponectin and insulin resistance in obese children and adolescents [14-22] and in the general

population of those [23-25], whereas negative data were reported only in 2 studies [26,27]. Our results are not in line with those of former reports. This discrepancy might be attributable to the characteristics of our study subjects. First, our study was performed in a general population in which mean BMI averaged 20.8 kg/m^2. Second, they were in the same grade at high school and their lifestyles were relatively uniform. Third, our subjects were all Japanese. Bush et al [24] reported a positive correlation between adiponectin and insulin sensitivity in adolescents from the general population. In their study, almost half of the subjects were African Americans. Another reason may be related to the form of adiponectin measured: we measured adiponectin by enzyme-linked immunosorbent assay without a denaturing step using a kit capable of detecting only HMW, the more active form of adiponectin [13]. Recently, Araki et al [22] reported that HMW, rather than total, adiponectin levels better reflect insulin resistance and visceral fat area even in obese children. To our knowledge, this is the first report to examine the relationship between HMW adiponectin and insulin resistance in adolescents from the general population.

Previously, we reported that both BMI and serum leptin levels were lower in adolescents than in adults [28], although the assay method used was different from that of the present study. Herein, we found adiponectin levels in adolescents to be significantly lower than those of adults, despite BMI being significantly lower in adolescents. This observation might be related to the difference in plasma testosterone concentrations because testosterone suppresses adiponectin levels [29] and free testosterone levels are known to increase dramatically during puberty and to decrease with aging.

The positive association between leptin and insulin resistance in adults has been confirmed by many epidemiological studies [30,31]. Whether this is true for adolescents has not been fully elucidated. Huang et al [32] showed plasma leptin levels to be positively associated with insulin resistance independently of BMI in nondiabetic male adolescents with a mean BMI of 26.6 kg/m^2. To our knowledge, there is only one study to find a positive correlation between leptin and insulin resistance in the general adolescent population [27]. Of note, the present study revealed a close positive association between leptin and HOMA-IR (Fig. 2) and many obesity-related metabolic parameters in a general population of Japanese adolescents with a mean BMI of 20.8 kg/m^2. Furthermore, stepwise multiple regression analysis revealed that leptin, but not BMI or waist circumference, significantly correlated with HOMA-IR. This finding suggests that leptin resistance may already exist in such adolescents.

In addition, we demonstrated positive correlations between leptin and proinflammatory cytokines even in this general population of adolescents, although r values were weak. This observation may be compatible with the results of experimental studies that showed that leptin increases the production of TNF-α and IL-6 in macrophages [33] and upregulates MCP-1 expression in hepatic stellate cells [34]. Accumulating evidence suggests that obesity is associated with a state of chronic low-grade inflammation [35]. Cross-sectional studies have demonstrated elevated levels of TNF-α and IL-6 among individuals with obesity [36], and a prospective study has revealed IL-6 to be a determinant of risk for developing type 2 diabetes mellitus [37]. Positive correlations between leptin and proinflammatory cytokines, as observed in the present study, may provide at least one explanation for high leptin levels predicting an increased risk of developing type 2 diabetes mellitus [9-11].

Taken together, our findings suggest that leptin may be a more upstream molecule in the mechanism of fat accumulation–related insulin resistance than adiponectin, at least in male adolescents. Our hypothesis is mentioned below: With the development of adipose tissue, secretion of adiponectin and leptin may increase. In the general population of adolescents, however, only leptin may play a role in preventing fat accumulation–related insulin resistance, which was described in Schmidt et al [12]. In association with leptin resistance, leptin levels may further increase and accelerate the production of proinflammatory cytokines, although proinflammatory cytokine levels in adolescents were not associated with adiposity variables in the present study. In the latter phase of obesity, adiponectin may play an important role as an insulin sensitizer by inhibiting these proinflammatory cytokines, at least in part.

In conclusion, serum leptin is suggested to be a more useful biomarker of fat accumulation–related insulin resistance, inflammation, and metabolic abnormalities than HMW adiponectin in the general population of male adolescents. The inverse correlation between adiponectin and insulin resistance may manifest in the later phase of obesity development.

Acknowledgments

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Effects of Pretreatment with Low-dose Metformin on Metabolic Parameters and Weight Gain by Pioglitazone in Japanese Patients with Type 2 Diabetes

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Abstract

Objective We investigated whether or not "low dose" metformin could prevent weight gain induced by pioglitazone.

Research Design and Methods Sixty-nine patients with type 2 diabetes received 500-750 mg metformin a day for 12 weeks as an observation period before the start of the intervention. After an observation period, inadequately controlled patients (hemoglobin A1c ≥7.5%, n=34) received additional treatment with 15 mg pioglitazone (+P, M+P group). The other patients (n= 35) continued metformin monotherapy (Met group). In addition, another group consisting of 28 patients treated with 15 mg pioglitazone alone (Pio group) was observed. Body mass index (BMI), as well as several clinical parameters of glycemic control and lipid metabolism, was compared before and after 24 weeks of intervention.

Results BMI increased significantly in the Pio group [24.0±3.8 vs. 24.8±4.3 kg/m^2, (mean ± SD), p<0.001], but not in the M+P group (25.1±3.5 vs. 25.3±3.4 kg/m^2, NS) and Met group (24.0±3.3 vs. 24.0±3.5 kg/m^2, NS). In addition to improvement in glycemic control, a significant reduction in the atherogenic index of plasma (AIP), defined as log [TG ×0.0112/HDL-C ×0.02586], was observed in the Pio group (0.06±0.23 vs. -0.04±0.27, p<0.05) and M+P group (0.08±0.24 vs. -0.001±0.252, p<0.01), but not in the Met group.

Conclusion This study indicates potential benefits of the addition of pioglitazone to "low dose" metformin in terms of improvement of glucose and lipid metabolism without weight gain.

Key words: metformin, pioglitazone, combination therapy, weight gain, atherogenic index of plasma (AIP)

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Introduction

Diabetes mellitus is a common metabolic disorder, affecting more than 190 million people worldwide (1, 2). Type 2 diabetes results from the failure of pancreatic beta cells to adequately compensate for obesity and insulin resistance (3). Type 2 diabetes is also a risk factor for macrovascular disease. Patients with type 2 diabetes often have lipid abnormalities, such as hypertriglyceridemia and low high-density lipoprotein cholesterol (HDL-C) level, which are associated with promotion of atherosclerosis. Treatment of dyslipidemia, as well as glycemic control, without weight gain is important in patients with type 2 diabetes to prevent macrovascular disease.

Metformin, a biguanide, and pioglitazone, a thiazolidinedione, are regarded as insulin-sensitizing agents, whereas their target organs and precise mechanisms in increasing insulin sensitivity are different (4). Biguanides primarily improve glycemic control by decreasing hepatic glucose production in the post-prandial state by increasing hepatic insulin sensitivity. Biguanides also enhance insulin-sensitizing effects in peripheral tissues and cause an increase in glucose utilization. In addition, biguanides have been shown to re-

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Table 1. Baseline Characteristics in Metformin-administered Group (Met and M+P)

Characteristics	Metformin-administered cases
n	69
Men/Women	48/21
Age (years)	58.8 ± 8.6
Height (cm)	164.6 ± 9.2
Body weight (kg)	66.9 ± 11.4
BMI (kg/m^2)	24.7 ± 3.3
SBP (mmHg)	129.3 ± 14.5
DBP (mmHg)	76.9 ± 10.3
FPG (mg/dL)	162.5 ± 34.2
HbA1c (%)	7.4 ± 0.8
IRI (μU/mL)	6.0 ± 3.6
Total cholesterol (mg/dL)	209.4 ± 30.1
Triglyceride (mg/dL)	151.7 ± 79.0
HDL-cholesterol (mg/dL)	51.8 ± 15.1

Data are mean ± SD or n.

duce body weight, so they are regarded as the first choice agent for type 2 diabetes with insulin resistance (5-7). However, because the maximum dose of metformin in Japan is 750 mg/day, which is much lower than that in other westernized countries (up to 2,550 mg/day), treatment with metformin alone often results in inadequate glycemic control.

On the other hand, thiazolidinediones improve glycemic control by increasing insulin sensitivity in the periphery partly via modulating the transcription of genes that play important roles in glucose metabolism (8). Activation of peroxisome proliferator-activated receptor (PPAR)-γ by thiazolidinediones has been shown to improve not only glycemic control but also the lipid profile (9-11). We have reported that thiazolidinediones modulate body fat distribution (12, 13) and have anti-atherosclerotic effects through increasing plasma adiponectin level (13). However, weight gain has been reported after treatment with thiazolidinediones in general (10, 11, 13-15).

Both biguanides and thiazolidinediones are widely used for the treatment of type 2 diabetes with underlying insulin resistance. Combination therapy with the two agents is expected to overcome the disadvantages of each of the two agents, with the expectation of better improvement in glycemic and lipid metabolism without weight gain. In this study, we evaluated the effects of additional treatment with pioglitazone for 24 weeks on glycemic and lipid metabolism and body weight in Japanese patients with type 2 diabetes who had inadequate control with "low dose" metformin as the first choice, compared with 15 mg pioglitazone monotherapy.

Materials and Methods

Subjects and protocol

A total of 69 Japanese type 2 diabetic patients (48 men and 21 women; mean ± SD age 58.8±8.6 years), attending our outpatient diabetic clinics at Keio University Hospital or Saiseikai Central Hospital, were enrolled. All of them were naïve to oral hypoglycemic agents. Before starting metformin (500 or 750 mg/day) monotherapy for 12 weeks in the observation period, all 69 subjects received dietary instructions from nutritionists, using a meal-exchange plan. The ideal dietary caloric intake for each patient was calculated as the ideal body weight (kilograms)×25 kcal/kg. Baseline characteristics (at -12 weeks) of all participants are shown in Table 1. At the start (0 week) of intervention, the patients who had inadequate control (HbA1c ≥7.5%) received additional treatment with 15 mg pioglitazone (M+P group: n=34, mean ± SD age 58.6±8.0 years; BMI 25.1±3.5 kg/m^2), while other patients (with HbA1c level <7.5%) continued metformin monotherapy (Met only group (Met): n=35, mean ± SD age 59.0±9.2 years; BMI 24.0±3.3 kg/m^2).

Another group consisting of 28 patients with type 2 diabetes, who received pioglitazone monotherapy (Pio only group (Pio): n=28, mean ± SD age 60.3±12.4 years; BMI 24.0±3.8 kg/m^2), was observed. They were also naïve to oral hypoglycemic agents. After receiving the same dietary instructions as above, the patients received 15 mg/day pioglitazone alone for 24 weeks.

Parameters including glycemic and lipid profiles were compared between the start and after 24 weeks. The study protocol is shown in Fig. 1. All patients gave informed con-

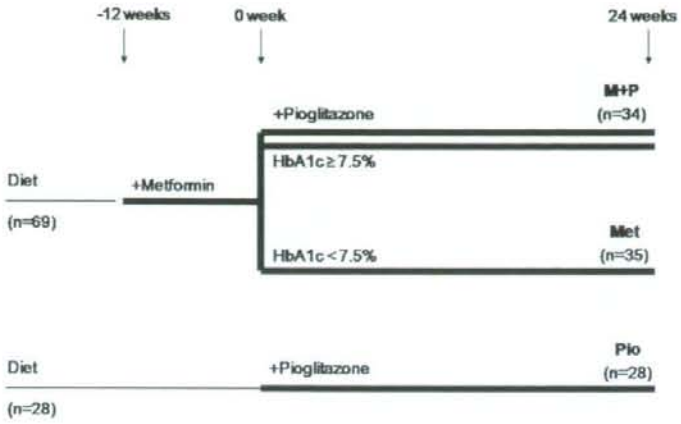

Figure 1. **Study protocol. After a 12-week observation period with metformin monotherapy, patients who had inadequate control (HbA1c ≥7.5%) received additional treatment with 15 mg pioglitazone (+P, M+P group). Patients with hemoglobin A1c <7.5% continued metformin monotherapy (Met group). Another 28 patients received treatment with 15 mg pioglitazone monotherapy for 24 weeks (Pio group).**

sent and the study protocol was approved by institutional review board.

Measurements

Blood pressure was determined in the sitting position after a 10-minute rest. Body weight was measured at the clinic under the same conditions for each patient. Blood samples were taken from each subject before breakfast in the early morning, after overnight bed rest. Fasting plasma glucose (FPG) was determined by the glucose oxidase method. Hemoglobin A1c (HbA1c) was determined by high-performance liquid chromatography (Toso, Tokyo, Japan). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were measured enzymatically by an autoanalyzer (Hitachi, Tokyo, Japan).

The atherogenic index of plasma (AIP) was calculated according to the following equation: AIP=log [TG ×0.0112/ HDL-C ×0.02586], with units for TG and HDL-C of mg/dL.

Statistical analysis

All results are presented as mean ± S.D. Differences in these parameters between the start and after 24 weeks were analyzed using Wilcoxon's matched-pair signed-rank test. Differences in each parameter among groups at each time point were analyzed using Kruskal-Wallis test. A p value less than 0.05 was considered statistically significant. Statistical analyses were carried out using StatView 5.0 software (SAS Institute, Cary, NC).

Results

At the start (0 week) of the intervention, there were no statistically significant differences in parameters, except for FPG (168.9±27.2 mg/dL in M+P vs. 154.2±37.6 mg/dL in Met vs. 169.3±25.9 mg/dL in Pio, $p<0.05$) and HbA1c (7.9±0.8% in M+P vs. 7.1±0.9% in Met vs. 8.1±0.6% in Pio, $p<0.0001$), among the three groups.

Changes in parameters between 0 week and after 24 weeks in all groups are shown in Table 2a-c. There was no change in blood pressure between 0 week and after 24 weeks in all groups. Figure 2 shows the changes in body weight among the groups. While the Pio group showed a significant increase in body weight (63.0±13.7 vs. 65.2±14.8 kg, $p<0.001$), the M+P group (69.1±11.8 vs. 69.7±11.9 kg, not significant (NS)) and Met group (64.2±10.9 vs. 64.3± 11.3 kg, NS) showed a slight but non-significant increase in body weight. And, the changes in body weight (body weight at 24 weeks - body weight at 0 week) did not depend on sexual distinction [+1.8±3.0 kg ($p<0.05$ for body weight at 0 week vs. at 24 weeks) in men and +2.6±1.3 kg ($p<0.01$) in women in the Pio group, +0.8±2.0 kg (NS) in men and -0.2±3.1 kg (NS) in women in the M+P group, +0.5±2.2 kg (NS) in men and - 0.5±1.6 kg (NS) in women in the Met group]. Data-driven post-hoc analyses showed that body weight at -12 weeks (time point for initiation of metformin) was 69.2±11.9 kg in the M+P group and 64.8±10.5 kg in the Met group.

With regard to glycemic control, FPG decreased significantly in the Pio group (169.3±25.9 vs. 140.2±23.1 mg/dL, $p<0.001$) and M+P group (168.9±27.2 vs. 150.4±31.6 mg/dL, $p<0.01$) after 24 weeks, but not in the Met group (154.2±37.6 vs. 148.2±31.8 mg/dL, NS). HbA1c also decreased significantly in the Pio group (8.1±0.6 vs. 6.9± 0.8%, $p<0.0001$) and M+P group (7.9±0.8 vs. 7.3±0.7%, $p<0.001$), but not in the Met group (7.1±0.9 vs. 7.0±1.0%, NS).

Table 2a. Changes in Glycemic and Lipid Parameters with 24-week Additional Treatment with Pioglitazone to Metformin (M+P group) [n=34 (25 Men, 9 Women)]

Parameters	0 week	24 weeks	p
Age (years)	58.6 ± 8.0		
Height (cm)	165.8 ± 10.1		
Body weight (kg)	69.1 ± 11.8	69.7 ± 11.9	NS
BMI (kg/m^2)	25.1 ± 3.5	25.3 ± 3.4	NS
SBP (mmHg)	132.9 ± 17.3	133.7 ± 12.9	NS
DBP (mmHg)	77.9 ± 10.7	78.0 ± 8.8	NS
FPG (mg/dL)	168.9 ± 27.2	150.4 ± 31.6	$p < 0.01$
HbA1c (%)	7.9 ± 0.8	7.3 ± 0.7	$p < 0.001$
TC (mg/dL)	208.9 ± 27.6	212.5 ± 26.8	NS
TG (mg/dL)	147.4 ± 58.5	131.7 ± 49.5	$p < 0.05$
HDL-C (mg/dL)	50.8 ± 12.6	55.0 ± 15.4	$p < 0.01$
AIP	0.08 ± 0.24	-0.001± 0.252	$p < 0.01$
AST (IU/L)	24.7 ± 13.4	24.6 ± 9.9	NS
ALT (IU/L)	33.4 ± 26.2	29.3 ± 18.3	NS

Data are shown as mean ± SD. Parameters at 0 week and after 24 weeks of treatment were compared by Wilcoxon's matched-pair signed-rank test.

Concerning lipid profile, all groups showed a mild but non-significant increase in TC level. A significant decrease in TG level was found in the M+P group (147.4±58.5 vs. 131.7±49.5 mg/dL, $p<0.05$), but not in the Pio group (141.8±59.7 vs. 128.3±58.5 mg/dL, NS) and Met group (149.9±90.6 vs. 143.9±66.4 mg/dL, NS). HDL-C level increased significantly in the Pio group (50.6±11.5 vs. 56.2± 13.9 mg/dL, $p<0.01$) and M+P group (50.8±12.6 vs. 55.0± 15.4 mg/dL, $p<0.01$), but not in the Met group (53.7±17.4 vs. 53.9±18.0 mg/dL, NS). Consequently, a significant reduction in the atherogenic index of plasma (AIP) was observed in the Pio group (0.06±0.23 vs. -0.04±0.27, $p<0.05$) and M+P group (0.08±0.24 vs. -0.001±0.252, $p<0.01$), but not in the Met group (0.04±0.30 vs. 0.04±0.26, NS).

All participants completed the trial, and none of the patients experienced adverse events such as hypoglycemia or pre-tibial edema.

Discussion

In the present study, additional treatment with pioglitazone onto "low dose" metformin in patients whose diabetes had been inadequately controlled with metformin alone improved not only glycemic control but also lipid metabolism, with no gain in weight.

Both pioglitazone and metformin have been shown to improve glycemic control in clinical practice. The present study showed the efficacy of pioglitazone for glycemic control in not only patients naive to glucose-lowering medication, but also patients inadequately controlled with metformin monotherapy.

Concerning lipid metabolism, a significant increase in HDL-cholesterol level was observed in pioglitazone-treated patients (M+P and Pio groups), even at a lower dose of 15 mg. While the decrease in triglyceride level was significant in the M+P group, the decrease did not reach statistical significance in the Pio group. Because lowering the triglyceride level and increasing the HDL-cholesterol level have been shown to prevent macroangiopathy (16), the effect of pioglitazone on lipid metabolism should be noted.

Recently, AIP has been proposed as an indicator of risk of macroangiopathy (17, 18). Predominance of small dense low-density lipoprotein (LDL) in plasma is associated with an increase in risk of macroangiopathy (19, 20). AIP was reported to be negatively correlated with the LDL particle size. So, it has been proposed that patients with higher AIP have a higher risk of macroangiopathy than those with lower AIP. In the present study, pioglitazone significantly decreased AIP when used as monotherapy or in combination with metformin. These results are consistent with other stud-

Table 2b. Changes in Glycemic and Lipid Parameters with 24-week Metformin Monotherapy (Met Group) [n=35 (23 Men, 12 Women)]

Parameters	0 week	24 weeks	p
Age (years)	59.0 ± 9.2		
Height (cm)	163.4 ± 8.3		
Body weight (kg)	64.2 ± 10.9	64.3 ± 11.3	NS
BMI (kg/m^2)	24.0 ± 3.3	24.0 ± 3.5	NS
SBP (mmHg)	130.4 ± 15.9	131.0 ± 15.0	NS
DBP (mmHg)	78.1 ± 8.5	79.2 ± 9.8	NS
FPG (mg/dL)	154.2 ± 37.6	148.2 ± 31.8	NS
HbA1c (%)	7.1 ± 0.9	7.0 ± 1.0	NS
TC (mg/dL)	210.6 ± 27.4	211.5 ± 29.6	NS
TG (mg/dL)	149.9 ± 90.6	143.9 ± 66.4	NS
HDL-C (mg/dL)	53.7 ± 17.4	53.9 ± 18.0	NS
AIP	0.04 ± 0.30	0.04 ± 0.26	NS
AST (IU/L)	24.5 ± 16.4	26.5 ± 19.3	NS
ALT (IU/L)	27.6 ± 16.3	28.5 ± 18.3	NS

Data are shown as mean ± SD. Parameters at 0 week and after 24 weeks of treatment were compared by Wilcoxon's matched-pair signed-rank test.

ies indicating its efficacy as monotherapy (21) and as combination therapy (21-23). Our finding might be related to the results in the prospective pioglitazone clinical trial in macrovascular events (PROactive) study (24), which showed that pioglitazone reduced macrovascular events in patients with type 2 diabetes, who are at risk of developing macroangiopathy.

Pioglitazone is generally prescribed as second or third choice among the oral hypoglycemic agents. While it is controversial whether pioglitazone should be prescribed as the first choice or used in the early stage of diabetes (25), the pioglitazone monotherapy group showed an impressive improvement in glycemic and lipid metabolism in our study. HbA1c level at 24 weeks was lowest in the Pio group among the three groups, even though it was highest at the start of intervention. From the standpoint of prevention of macroangiopathy, pioglitazone could be prescribed as the first choice for patients with type 2 diabetes who are naïve to oral hypoglycemic agents, although the problem of weight gain needs to be overcome.

Previous studies also have shown that pioglitazone monotherapy has beneficial effects on glucose and lipid profiles (9-11). On the other hand, weight gain due to pioglitazone is a major clinical concern because it is frequently associated with major side effects (10, 11, 13-15). It is suggested that several mechanisms could contribute to weight gain in patients treated with pioglitazone. First, fluid retention leading to peripheral edema, which is a well-known symptom, with the use of pioglitazone might be associated with weight gain. Chen et al (26) showed that GI262570 (farglitazar), a PPAR-γ agonist, increased sodium reabsorption in the distal nephron in rats, while its precise mechanisms remain unknown (27). Secondly, thiazolidinediones stimulate PPAR-γ on adipocytes and promote adipocyte differentiation, especially of subcutaneous fat cells, leading to an increase in the number of small fat cells (28) and in the total amount of subcutaneous fat (12, 13).

To prevent weight gain with the clinical use of thiazolidinediones, several approaches have been attempted. Majima et al (29) showed that 7.5 mg (half dose) pioglitazone significantly improved glucose and lipid metabolism with less weight gain, although a study limitation was that the subjects were all female. Strowig et al (30) showed that weight gain was avoided when "higher dose" metformin therapy (2,000 mg/day) preceded the addition of thiazolidinedione therapy, and the finding is consistent with the results of our study, although there was a difference in the dose of metformin. Because metformin may cause a reduction of caloric intake and loss of adipose tissue (5), weight gain might be minimized if metformin is administered before the addition of pioglitazone.

Concerning combination therapy with pioglitazone and

Table 2c. Changes in Glycemic and Lipid Parameters with 24-week Pioglitazone Monotherapy (Pio Group) [n=28 (16 Men, 12 Women)]

Parameters	0 week	24 weeks	p
Age (year)	60.3 ± 12.4		
Height (cm)	161.4 ± 8.8		
Body weight (kg)	63.0 ± 13.7	65.2± 14.8	p < 0.001
BMI (kg/m^2)	24.0 ± 3.8	24.8 ± 4.3	p < 0.001
SBP (mmHg)	128.1 ± 16.0	129.6 ± 18.4	NS
DBP (mmHg)	76.8 ± 11.6	76.4 ± 9.6	NS
FPG (mg/dL)	169.3 ± 25.9	140.2 ± 23.1	p < 0.001
HbA1c (%)	8.1 ± 0.6	6.9 ± 0.8	p < 0.0001
TC (mg/dL)	210.8 ± 31.1	212.3 ± 21.3	NS
TG (mg/dL)	141.8 ± 59.7	128.3 ± 58.5	NS
HDL-C (mg/dL)	50.6 ± 11.5	56.2 ± 13.9	p < 0.01
AIP	0.06 ± 0.23	-0.04± 0.27	p < 0.05
AST (IU/L)	21.5 ± 10.8	20.2 ± 6.9	NS
ALT (IU/L)	26.0 ± 24.7	20.0 ± 15.7	p < 0.01

Data are shown as mean ± SD. Parameters at 0 week and after 24 weeks of treatment were compared by Wilcoxon's matched-pair signed-rank test.

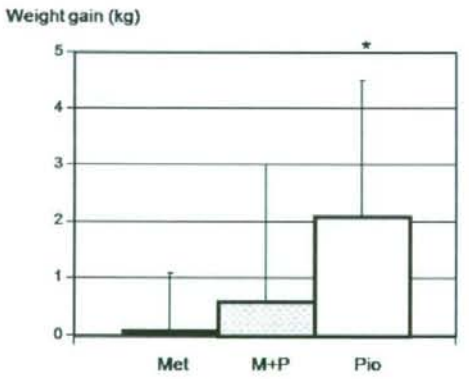

Figure 2. Increases in body weight after 24 weeks in each group. Values are mean ± SD. *p <0.001. Weight gain in each group was calculated as (body weight at 24 weeks - body weight at 0 week). Body weights at 0 week and at 24 weeks were compared by Wilcoxon's matched-pair signed-rank test. Met: Metformin monotherapy, M+P: Metformin + pioglitazone, Pio: Pioglitazone monotherapy.

metformin, several studies have been reported. Kendall et al (31) reported the beneficial effects of a combination of pioglitazone and "higher dose" of metformin (1,500-2,550 mg/day) on glucose and lipid metabolism in comparison with a combination of muraglitazar, a dual (α/γ) PPAR agonist, and metformin. They observed a slight increase in body weight (+0.6 kg/24 weeks) in the pioglitazone + metformin group. Matthews et al (22) also reported the efficacy of combination therapy of pioglitazone and metformin. They showed that weight gain appeared to have stabilized by 52 weeks. Our findings also showed a slight, but not significant weight gain by additional treatment with pioglitazone onto "low dose" metformin. One of the problems in the use of metformin is that the frequency of digestive disturbance increases when the dose of metformin exceeds 1,000 mg/day (32). So, the finding that "low dose" metformin (500-750 mg/day), i.e., the maximum dose of metformin used in Japan, was effective in preventing weight gain by pioglitazone is clinically important. The differences in magnitude of weight gain among the several reports and our observation might be due to differences in baseline characteristics, dose of medication, study duration or race.

There are several limitations to the current study. First, the present study was an open-label prospective observational study, but not a double-blind, randomized, controlled trial. It is difficult to directly assess the impact of metformin on pioglitazone-induced weight gain in comparable groups with this study design. Second, the size of the cohort was small for comparison when we calculated the required sam-

ple size using the effect size based on our previous study (13), a level of statistical significance (α) of 0.05, and the statistical power (1-β) of 0.80. Despite the limitations of this study, we believe that the results of the present study are useful in clinical practice.

In conclusion, additional treatment with pioglitazone onto "low dose" metformin might be effective for improvement of glycemic control, TG and HDL-C levels without weight gain, leading to prevention of the development of macrovascular disease in patients with type 2 diabetes. However, this requires confirmation with a larger number of subjects and a longer study period. Also, the effects of a different order of combination therapy, i.e., additional treatment with "low dose" metformin onto pioglitazone, on glycemic and lipid metabolism and body weight should be elucidated in further studies.

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Effects of 12-Month Valsartan Therapy on Glycation and Oxidative Stress Markers in Type 2 Diabetic Subjects With Hypertension

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SUMMARY

Although it has been reported that angiotensin II receptor blockers inhibited the formation and accumulation of advanced glycation endproducts (AGEs) *in vitro* and *in vivo*, whether they can do so clinically is not clear. We investigated the effects of 12-month valsartan therapy on various markers of inflammation, glycation, and oxidation in type 2 diabetic subjects with hypertension.

We started 40 mg/day valsartan treatment in 15 type 2 diabetic patients with hypertension. In 6 patients, the dose of valsartan was increased to 80 mg/day after 6 months and maintained until 12 months. Metabolic parameters including BMI and serum high molecular weight (HMW)-adiponectin, high-sensitivity C-reactive protein (hs-CRP) as an inflammation marker, AGEs, paraoxonase activity, platelet-activating factor (PAF)-acetylhydrolase activity, and urine 8-isoprostane levels were measured at baseline and after 6 and 12 months of treatment. Urine microalbumin level and carotid artery intima-media thickness (IMT) were also measured.

Even after valsartan therapy, the blood pressure levels of the patients were not decreased significantly. Serum AGEs and urine 8-isoprostane levels decreased at both 6 and 12 months ($P < 0.05$ for both), although other metabolic and oxidative markers were unchanged. Though urine microalbumin levels tended to be decreased after 6 and 12 months of valsartan treatment, the changes were not significant. Mean IMT at 12 months was not changed from the baseline value. In conclusion, the findings suggest that treatment with valsartan, even at a low dose, may ameliorate some glycation and oxidative stress markers independently of an effect on blood pressure in hypertensive type 2 diabetic subjects. (Int Heart J 2008; 49: 681-689)

Key words: Angiotensin II receptor blocker, Advanced glycation endproducts, Oxidative stress markers, Type 2 diabetes mellitus

THE prevalence of type 2 diabetes mellitus is increasing worldwide[1] as well as in Japan,[2] predisposing the patients to a high risk of developing diabetic microan-

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giopathy and atherosclerotic vascular diseases (ischemic heart disease, cerebrovascular disease, and peripheral artery disease). It is well-known that when diabetic subjects become hypertensive, the risks of developing microangiopathy and macroangiopathy are much higher than in nondiabetic subjects.[3,4]

As for nephropathy, it has been shown that an angiotensin II receptor blocker (ARB), valsartan, has renoprotective effects in patients with type 2 diabetes mellitus, independently of blood pressure.[5] However, the detailed mechanisms of such effects remain unclear. Advanced glycation endproducts (AGEs) have been focused on as one of the causal candidates, because AGEs are formed by glycation and oxidation in the Maillard reaction both *in vitro* and *in vivo*, and their levels are increased in diabetic subjects.[6,7] Also, an association between AGEs and diabetic microangiopathy has been reported, and intervention to reduce AGEs is considered an important strategy in treating diabetic nephropathy.[8]

Miyata, *et al* reported that ARBs or angiotensin-converting enzyme inhibitors (ACE-Is) decreased AGE formation *in vitro* via radical scavenging and transition metal chelation,[9] and it has been shown that some ARBs reduced renal AGE accumulation and proteinuria in diabetic rodents *in vivo*[10-12] to a degree similar to that of an ACE-I.[13] Sebekova, *et al* reported that treatment with the ACE-I ramipril for 2 months significantly decreased the fluorescent AGE level in 12 subjects with nondiabetic nephropathy.[14]

In the present study, we investigated the effects of valsartan treatment in Japanese type 2 diabetic subjects for up to 12 months, on various markers of glycation and oxidation as well as metabolic parameters. We also measured serum high molecular weight (HMW)-adiponectin and high-sensitivity C-reactive protein (hs-CRP) as metabolic and inflammatory markers, urine microalbumin level as a marker of nephropathy, and carotid artery intima-media thickness (IMT) to assess the degree of atherosclerosis.

METHODS

Subjects: Fifteen type 2 diabetic outpatients with hypertension being treated by the Department of Internal Medicine, Keio University Hospital, Tokyo, participated in this study. The patients included 14 men and 1 woman. As shown in Table I, the mean age was 63.2 ± 7.8 (SD) years and the mean duration of diabetes was 8.1 ± 3.8 years. Body mass index (BMI) was 24.8 ± 4.0 kg/m^2 and the fasting plasma glucose was 151 ± 15 mg/dL. We recruited subjects whose diet therapy or medication had not been changed for at least 3 months and whose glycemic control was relatively stable for this period (mean hemoglobin A_{1C} 6.7 ± 0.6%).

Table I. Subject Profiles at Baseline

		Range
n (male/female)	14/1	
Age (years)	63.2 ± 7.8	46-74
Duration of diabetes (years)	8.1 ± 3.8	2-16
Height (cm)	165.7 ± 4.7	159.0-177.0
Weight (kg)	68.2 ± 11.4	48.0-90.0
Body mass index (kg/m^2)	24.8 ± 4.0	18.1-33.9
Systolic blood pressure (mmHg)	149 ± 15	122-182
Diastolic blood pressure (mmHg)	86 ± 11	67-109
Fasting plasma glucose (mg/dL)	151 ± 15	118-177
Hemoglobin A_{1c} (%)	6.7 ± 0.6	5.6-7.6
Microvascular complications (retinopathy, nephropathy, neuropathy)	4, 2, 2	
Macrovascular complications (coronary, cerebral, peripheral)	0, 2, 0	
Current smoking	10 (66.7%)	
Therapy for type 2 diabetes (diet only, oral hypoglycemic agent, insulin)	0, 14, 1	
Statin therapy	3 (20.0%)	

Data are *n* or mean ± SD.

Five patients were being treated with a calcium channel blocker and one with a beta-blocker at baseline, but none with an ACE-I, ARB, or diuretic. Antihypertensive agents being taken by the patients other than valsartan were not changed during the study. All patients were treated with oral hypoglycemic agents, but one with insulin. Ten patients were current smokers, and three had been treated with an HMG-CoA reductase inhibitor (Table I).

The present study was conducted according to the principles expressed in the Declaration of Helsinki, 1964 and the Declaration of Tokyo, 1975, as revised in 1983. Informed consent was obtained from each subject after full explanation of the purpose, nature, and risk of all procedures used. The protocol was approved by the ethical review committee of the Department of Internal Medicine, School of Medicine, Keio University, Tokyo. Some of the study subjects also participated in one of our previous studies.[15] However, we extended the follow-up period from 6 months to 12 months, and we examined serum HMW-adiponectin and hs-CRP levels, as well as measuring carotid artery mean IMT as a marker of atherosclerosis.

Treatment with 40 mg/day of valsartan in the morning was started. In 6 out of 15 patients, the dose of valsartan was increased to 80 mg/day (40 mg tablet twice daily) after 6 months and maintained at that level until 12 months.

Measurements: Various metabolic parameters, namely serum AGEs, paraoxo-

nase activity, platelet-activating factor acetylhydrolase (PAF-AH) activity, and urine 8-isoprostane levels were measured at baseline and 6 and 12 months after initiation of treatment.

BMI was calculated as weight in kg divided by height in meters squared. Systolic blood pressure and diastolic blood pressure were measured with subjects in the sitting position after resting for at least 5 minutes. The blood samples were collected around 9 AM in the morning after an overnight fast. Plasma glucose, serum low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, and uric acid levels were assayed by routine automated laboratory methods, as described previously.[16-18]

HMW-adiponectin was measured using a commercially available kit (HMW Adiponectin ELISA Kit, Fujirebio Inc., Tokyo). This ELISA system does not need a denaturing step, and the monoclonal antibody (IH7) is reported to react specifically with the HMW form of adiponectin.[19] The dilution curve was parallel to the standard curve. Intra- and interassay coefficients of variation (CV) were 2.4-3.0% and 4.2-5.1%, respectively. Serum hs-CRP level was measured by nephelometry, using a latex particle-enhanced immunoassay (N Latex CRP II, Dade Behring, Tokyo) with both intra- and interassay CV of < 5.0%. The assay could detect 0.005 mg/dL of CRP.

Serum paraoxonase activity was measured by colorimetric assay, as previously described,[20] and PAF-AH activity was also measured by colorimetric assay (PAF acetylhydrolase assay kit, Cayman, Ann Arbor, MI, USA). Serum AGEs were measured by ELISA as previously described,[7] and the intra- and interassay CV of this ELISA system were 4.8-10.2% and 3.5-6.2%, respectively. Urine-8-isoprostane was measured by ELISA (8-isoprostane EIA kit, Cayman). Creatinine clearance (Ccr) was calculated using the Cockcroft-Gault equation.

Assessment of common carotid artery IMT: Ultrasonography of the carotid arteries was performed, as described previously,[21,22] using an echotomographic system (LOGIC S6 and LOGIC 7, GE Yokogawa Medical System Inc., Tokyo) with a linear transducer (mid-frequency range of 7-12 MHz). Scanning of the extracranial carotid arteries in the neck was performed bilaterally in 3 different longitudinal projections (anterior-oblique, lateral, and posterior-oblique) and in the transverse projections. This allowed the common carotid artery, carotid bulb, and internal carotid artery to be scanned bilaterally. All of the images were photographed.

The common carotid artery IMT was defined as the distance from the leading edge of the first echographic line to the leading edge of the second echographic line on the scans, with the first line representing the collagen-containing upper layer of the tunica adventia. In each longitudinal projection, the site of the greatest IMT thickness was detected by scanning along the vessel from the com-

mon carotid artery to the internal carotid artery. Three measurements of the IMT on both sides were performed at the site of greatest thickness and 2 other points (1 cm proximal and 1 cm distal to this site) for each patient. The highest value of the 6 averaged values of IMT (3 from the right side and 3 from the left side) was used as the representative mean IMT for each patient. A few specialized physicians performed all the scans and IMT measurements.

Statistical analysis: Statistical analyses were performed using the SPSS® program for Windows (version 15.0-J, SPSS Japan Inc., Tokyo). P values < 0.05 were considered to denote statistical significance. The Wilcoxon signed-rank test was used to compare the serum HMW-adiponectin level and carotid artery IMT before and 12 months after valsartan treatment. Analysis of variance (ANOVA) followed by the Dunnett test was used to compare the other parameters before to 6 and 12 months after valsartan treatment. Because serum triglycerides, HMW adiponectin, hs-CRP and AGEs levels, and urine 8-isoprostane level were normally distributed after logarithmic transformation, we used logarithms of these data in the analyses.

Results

The change in each parameter before to after the treatment is shown in Tables II and III. Even after valsartan treatment (40-80 mg/day), the blood pressure level of the patients did not change during the study. In 6 patients, to whom 40 mg/day of valsartan was added in the evening from 6 months to 12 months, blood pressure levels were still not changed at both 6 and 12 months (from 148 /

Table II. Changes in Metabolic Parameters Between Before and After Valsartan Treatment (40-80 mg/day) in 15 Type 2 Diabetic Subjects

	Baseline	6 months	12 months	P
Body mass index (kg/m^2)	24.8 ± 4.0	25.8 ± 3.3	25.3 ± 3.7	NS
Systolic blood pressure (mmHg)	149 ± 15	145 ± 22	146 ± 20	NS
Diastolic blood pressure (mmHg)	86 ± 11	84 ± 12	86 ± 13	NS
Hemoglobin A_{1C} (%)	6.7 ± 0.6	6.8 ± 0.9	7.0 ± 1.0	NS
LDL-cholesterol (mg/dL)	133 ± 19	132 ± 21	123 ± 20	NS
HDL-cholesterol (mg/dL)	52 ± 11	50 ± 11	49 ± 10	NS
Triglycerides (mg/dL)	137 ± 69	145 ± 64	154 ± 71	NS
Uric acid (mg/dL)	5.9 ± 1.0	6.1 ± 0.9	6.1 ± 1.2	NS
HMW adiponectin (μg/mL)	5.6 ± 2.3	-	5.7 ± 2.0	NS
hs-CRP (mg/dL)	0.81 ± 0.39	0.91 ± 0.59	1.11 ± 0.89	NS

Values are the mean ± SD. **$P < 0.01$ versus baseline by Dunnett test. NS indicates not significant; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HMW, high molecular weight; and hs-CRP, high sensitivity C-reactive protein.

Table III. Changes in Markers of Glycation, Oxidation, and Diabetic Complications Between Before and After Valsartan Treatment (40-80 mg/day) in 15 Type 2 Diabetic Subjects

	Baseline	6 months	12 months	*P*
AGEs (unit/L)	2.71 ± 0.62	2.23 ± 0.36 *	2.29 ± 0.28 *	0.021
Paraoxonase activity (unit/L)	239 ± 68	230 ± 74	233 ± 60	NS
PAF-AH (mmol/L/minute)	20.1 ± 5.1	18.9 ± 5.7	17.3 ± 5.7	NS
Urine 8-isoprostane (ng/gCr)	347 ± 215	190 ± 116*	205 ± 122*	0.025
Creatinine (mg/dL)	0.86 ± 0.14	0.87 ± 0.14	0.89 ± 0.16	NS
Calculated Ccr (mL/minute)	86.7 ± 26.7	88.4 ± 27.2	84.7 ± 24.2	NS
Urine microalbumin (mg/gCr)	167 ± 258	114 ± 218	128 ± 247	NS
Carotid artery mean IMT (mm)	0.81 ± 0.18	-	0.86 ± 0.20	NS

Values are the mean ± SD. *$P < 0.05$ versus baseline by Dunnett test. NS indicates not significant; AGEs, advanced glycation endproducts; PAF-AH, platelet-activating factor acetylhydrolase; Ccr, creatinine clearance; and IMT, intima-media thickness.

88 mmHg to 158 / 91 and 160 / 95 mmHg, respectively, $P > 0.6$). BMI and HbA1c level increased slightly during the study, though the increases were not significant. Serum paraoxonase activity and PAF-AH levels were also unchanged. In contrast, serum AGE levels significantly decreased at both 6 and 12 months (from 2.71 ± 0.62 unit/L to 2.23 ± 0.36 and 2.29 ± 0.28 unit/L, respectively, $P < 0.05$ for both) after valsartan treatment (Table III). The urine 8-isoprostane level also decreased significantly at both 6 and 12 months (from 347 ± 215 ng/gCr to 190 ± 116 and 205 ± 122 ng/gCr, respectively, $P < 0.05$ for both).

Neither the serum creatinine level nor calculated Ccr changed during the study. The urine microalbumin level was slightly decreased after 6 and 12 months of valsartan treatment (from 167 ± 258 mg/gCr to 114 ± 218 and 128 ± 247 mg/gCr, respectively), however, the changes were not significant. Carotid artery mean IMT was not changed at 12 months compared with the baseline value (from 0.81 ± 0.18 mm to 0.86 ± 0.20 mm, $P = 0.22$).

There were no significant correlations between the changes in AGEs or urine 8-isoprostane levels with the changes in urine microalbumin levels, calculated Ccr, or IMT.

Discussion

JNC-7[23] and JSH 2004[24] both recommended that the target blood pressure in patients with type 2 diabetes mellitus should be below 130/80 mmHg. Although we increased the dose of valsartan to 80 mg/day from 6 months to 12 months in 6 out of 15 patients, mean blood pressure was not decreased. One of the reasons why valsartan treatment in this study could not decrease mean blood pressure might have been the increase in BMI during the study period, or that the

relatively low-dose valsartan treatment might have been insufficient in diabetic subjects to achieve the target blood pressure in the morning before taking the medication.

It has been reported that the ARBs olmesartan[9-11] and candesartan[12] inhibited the formation and accumulation of AGEs *in vitro*[9] and in diabetic animal models.[10-12] However, whether they can do so clinically is not clear. In our study, valsartan treatment significantly decreased the urine 8-isoprostane level as well as serum AGEs. However, other markers did not change during the study. Although the detailed mechanism(s) of the effects of valsartan on AGEs *in vivo* are unclear, the antioxidative effect of valsartan might be one of the mechanisms. Recently, Monacelli, *et al* described the effects of valsartan on AGEs and oxidative stress markers in 12 type 2 diabetic subjects.[25] They found that 3 to 6-month valsartan treatment (80-160 mg/day) significantly decreased blood pressures, plasma pentosidine and carboxymethyhl-lysine (CML) concentrations, both of which are major components of AGEs, and urine 15-F2t-isoprostanes levels.

In the present study, we have shown that relatively low-dose valsartan therapy (40-80 mg/day) decreased serum AGE and urine 8-isoprostane levels, and also slightly reduced the urine microalbumin level for an extended period of 12 months. It is noteworthy that these beneficial effects were observed even if the blood pressure did not decrease significantly. Although the lack of a significant reduction in blood pressure was unexpected, these findings suggest valsartan therapy has some kind of blood pressure-independent effect. Although the change in AGE level was not associated with the change in urine microalbumin level or calculated Ccr in our study, a significant positive correlation between renal pentosidine content and proteinuria has been reported in a rat model.[10,11] Further longitudinal studies are needed to clarify whether the lowering of serum AGE levels affects the clinical outcome of diabetic microangiopathy.

Accumulating evidence suggests that oxidative stress may play a crucial role in the development of atherosclerosis.[26-28] For example, it has been reported that circulating levels of 8-isoprostane were an independent risk factor associated with coronary artery disease in a Chinese population (odds ratio 2.47, $P < 0.001$),[26] and were associated with peripheral artery disease.[27,28] In this study, we have shown that valsartan therapy decreased urine 8-isoprostane levels up to 12 months in type 2 diabetic subjects with hypertension, despite the fact that the blood pressure levels were unchanged. Although carotid artery mean IMT did not decrease after 12 months of treatment in this study, there is a possibility that valsartan treatment of a longer period might inhibit the progression of atherosclerosis via suppression of oxidative stress in type 2 diabetic subjects. Further longitudinal studies of much longer duration with different sex, ethnicity, and/or ARB would facilitate the understanding of this important issue.