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Peroxisome Proliferator-Activated Receptor γ (PPAR-γ) Agonist Increases Plasma Adiponectin Levels in Type 2 Diabetic Patients with Proteinuria

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Adiponectin appears to be an important modulator for metabolic and vascular diseases. A case-controlled study was designed to measure plasma adiponectin levels and investigate the effects of rosiglitazone on adiponectin levels in type 2 diabetic patients with proteinuria. Sixty-four patients (mean age, 46.1 ± 4.6 yr; 30 male, 34 female) and 26 healthy volunteers (mean age, 45.3 ± 4.8 yr; 14 male, 12 female) were included. Patients with proteinuria were treated with 4-mg/d rosiglitazone (*n* = 21, 10 males, 11 females) for 4 wk. Adiponectin levels in patients were significantly lower than those of controls (*p* < 0.001). There were significant negative correlations between adiponectin concentrations and insulin levels as well as homeostasis model assessment (HOMA) index in patient's group (*r* = –0.538, *p* < 0.001; *r* = –0.393, *p* = 0.001, respectively). There was also a significant negative correlation between plasma adiponectin concentrations and the degree of proteinuria (*r* = –0.526, *p* = 0.002). Plasma adiponectin levels in patients with proteinuria (*n* = 31; 3.91 ± 2.57 µg/mL) were significantly lower than those without proteinuria (*n* = 33; 10.15 ± 1.97 µg/mL) (*p* < 0.001). After the treatment period, adiponectin levels significantly increased (*p* < 0.001) and proteinuria, plasma insulin, and HOMA indexes significantly decreased in treatment group (*p* < 0.001, *p* < 0.001, *p* < 0.001, respectively). The results suggest that adiponectin is inversely correlated with proteinuria and treatment with peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist rosiglitazone both corrects proteinuria and increases the low adiponectin levels in diabetic patients.

Key Words: Adiponectin; type 2 diabetes mellitus; proteinuria; insulin sensitivity; rosiglitazone.

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Introduction

Proteinuria is a surrogate marker for the development of diabetic nephropathy that is one of the major causes of end-stage renal failure *(1,2).* The presence of proteinuria foretells future cardiovascular events as well *(1,3).* Peroxisome proliferator-activated receptor γ (PPAR-γ) agonists bind to nuclear transcription factors and activate the genes that are involved in insulin action, adipose cell differentiation, and some aspects of lipid metabolism *(4)*. Previous reports have shown that PPAR-γ agonists such as thiazolidinediones (TZD) decrease proteinuria in patients with diabetic nephropathy *(5,6).* How TZDs ameliorate proteinuria and protect against diabetic nephropathy is not clear so far. According to the recent data, this effect may be related to the inhibition of secretion of type-4 collagen *(6),* or adipocytokines such as PAI-1, angiotensin II, and TNF-α *(7,8).*

Adiponectin is a novel protein expressed exclusively in adipocytes *(9,10).* Hypoadiponectinemia is present in obesity, type 2 diabetes mellitus, insulin resistance, and coronary artery disease *(11–13).* The reports imply that adiponectin with its anti-inflammatory properties negatively modulates the process of atherogenesis and may be a predictor of cardiovascular events as well *(11,14,15).* TZDs increase adiponectin levels in subjects with normal and impaired glucose tolerance and in patients with diabetes mellitus *(16–18).*

So far there are two reports that mention high plasma adiponectin levels in patients with proteinuria *(19,20).* However, one expects to find just the opposite, regarding the data about the predictive values of hypoadiponectinemia and proteinuria for the cardiovascular events.

This study was designed in order to answer the following questions: (1) Is there any difference between plasma adiponectin levels of diabetic patients with and without proteinuria? (2) Is there any association between proteinuria and plasma adiponectin levels in diabetic patients? (3) What is the effect of rosiglitazon treatment both on plasma adiponectin levels and proteinuria in type 2 diabetic patients with proteinuria?

Table 1
Clinical and Laboratory Features of the Patient and Control Groups

	Patients (n = 64)	Controls (n = 26)	p
Age (yr)	46.0 ± 4.6	45.3 ± 4.8	0.532†
Sex (M/F)	30/34	14/12	0.556‡
Blood urea (mg/dL)	39.26 ± 5.22	38.69 ± 5.06	0.633†
Serum creatinine (mg/dL)	0.90 ± 0.13	0.89 ± 0.35	0.900§
Total protein (g/dL)	6.81 ± 0.42	6.96 ± 0.41	0.127†
Serum albumin (g/dL)	4.04 ± 0.31	4.00 ± 0.25	0.536†
Systolic BP (mmHg)	128.48 ± 10.54	125.73 ± 8.48	0.642†
Diastolic BP (mmHg)	82.64 ± 2.96	82.00 ± 2.66	0.324†
Total Cholesterol (mg/dL)	206.03 ± 22.67	202.07 ± 16.62	0.360†
Triglyceride (mg/dL)	128.82 ± 14.71	125.46 ± 11.83	0.260†
LDL Cholesterol (mg/dL)	118.06 ± 10.77	115.07 ± 9.54	0.201†
HDL Cholesterol (mg/dL)	41.75 ± 5.43	43.30 ± 3.53	0.181§
BMI (kg/m^2)	26.41 ± 1.77	26.10 ± 1.78	0.459†
Insulin (μIU/mL)	13.86 ± 6.28	6.02 ± 1.41	<0.001§
HbA_{1c} (%)	8.64 ± 1.82	4.02 ± 1.04	<0.001§
HOMA	4.66 ± 1.11	1.27 ± 0.39	<0.001§
FPG (mg/dL)	136.75 ± 22.41	86.30 ± 10.69	<0.001§
Adiponectin (μg/mL)	7.15 ± 3.84	11.96 ± 2.00	<0.001§

BP, blood pressure (mmHg); BMI, body mass index; FPG, fasting plasma glucose; HOMA, homeostasis model assessment.
†Independent samples t test; §Mann–Whitney U test; and ‡Chi-square test.

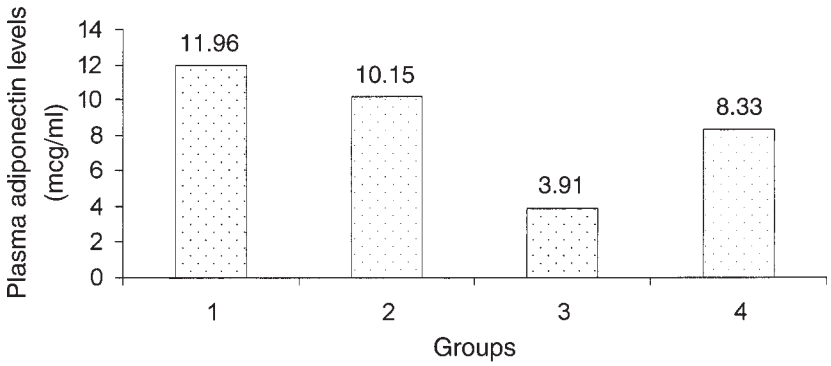

Fig. 1. Plasma adiponectin levels of control subjects and patients before and after treatment.

Results

The characteristics of patients and control group are shown in Table 1. There were no significant differences between patients and control group in terms of age, body mass index (BMI), serum urea, creatinine, total protein, albumin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, systolic blood pressure, and diastolic blood pressure. Basal insulin levels, fasting blood glucose levels, HbA_{1c}, and HOMA indexes of the patients were significantly higher than those of control subjects, as expected ($p < 0.001$ for all).

Plasma Adiponectin Concentrations in Patients with and without Proteinuria

Plasma adiponectin levels of patients with proteinuria were significantly lower than those of patients without proteinuria, meanwhile both were significantly lower than those of controls ($p < 0.001$ for all) (Table 1, Fig. 1). There were significant negative correlations between adiponectin concentrations and insulin levels as well as HOMA indexes in patients' group ($r = -0.538$, $p < 0.001$; $r = -0.393$, $p = 0.001$, respectively). There was also a moderately significant negative correlation between plasma adiponectin concentrations and the degree of proteinuria ($r = -0.526$, $p = 0.002$) (Fig. 2).

Table 2 shows clinical and laboratory values of patients comparing those with proteinuria to those without proteinuria. There were no differences between these subgroups with respect to age, sex, BMI, blood urea, creatinine, total protein, albumin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride levels, FPG, HbA_{1c}, and duration of diabetes. Patients with proteinuria had significantly lower

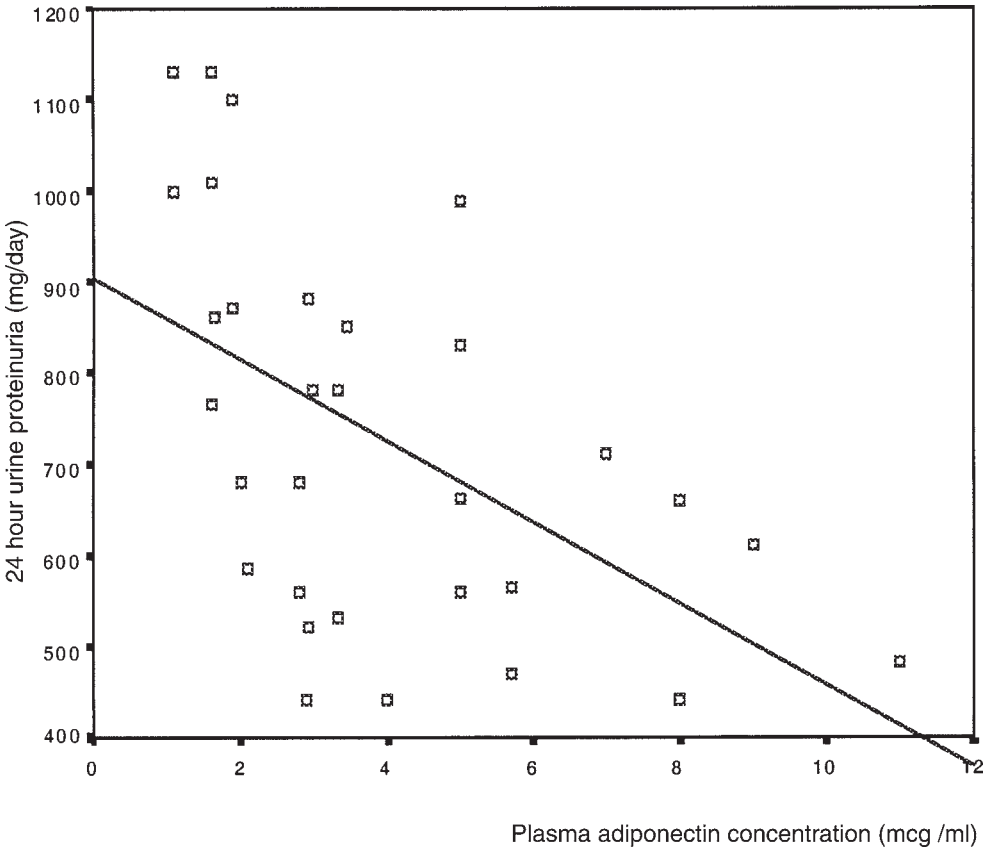

Fig. 2. Correlation between adiponectin and 24-h urine proteinuria before treatment *(r* = –0.526, *p* = 0.002).

Table 2
Characteristics of Patients According to Proteinuria

	No protenuria (n = 33)	Proteinuria (n = 31)	p
Age (yr)	46.1 ± 5.6	45.9 ± 3.4	0.852§
Sex (M/F)	18/15	16/15	0.969‡
Blood urea (mg/dL)	40.48 ± 3.93	37.96 ± 6.23	0.061§
Serum creatinine (mg/dL)	0.90 ± 0.13	0.89 ± 0.14	0.783†
Total protein (g/dL)	6.93 ± 0.42	6.69 ± 0.38	0.121†
Serum albumin (g/dL)	4.02 ± 0.32	4.06 ± 0.30	0.577†
Systolic BP (mmHg)	129.96 ± 10.74	127.70 ± 7.63	0.421†
Diastolic BP (mmHg)	82.18 ± 3.35	83.12 ± 2.43	0.200†
Total Cholesterol (mg/dL)	204.90 ± 25.42	207.29 ± 19.66	0.676§
Triglyceride (mg/dL)	127.21 ± 17.15	130.54 ± 11.61	0.364§
LDL Cholesterol (mg/dL)	118.12 ± 8.77	118.00 ± 11.72	0.965†
HDL Cholesterol (mg/dL)	42.84 ± 6.02	40.58 ± 4.54	0.093†
BMI (kg/m^2)	26.00 ± 1.53	26.84 ± 1.93	0.061†
Insulin (μIU/mL)	9.83 ± 2.24	18.14 ± 6.39	<0.001§
HOMA	3.29 ± 0.68	6.04 ± 1.37	0.001§
HbA_{1c} (%)	8.78 ± 1.57	8.50 ± 2.07	0.549†
FPG (mg/dL)	136.60 ± 24.20	136.90 ± 20.74	0.958†
Duration of diabetes (mo)	43.42 ± 7.01	45.87 ± 8.92	0.193§
Adiponectin (μg/mL)	10.15 ± 1.97	3.91 ± 2.57	<0.001§

BP, blood pressure (mmHg); BMI, body mass index; FPG, fasting plasma glucose; HOMA, homeostasis model assessment.

†Independent samples *t* test; §Mann–Whitney *U* test; and ‡Chi-square test.

Table 3
The Comparisons of the Parameters in the Proteinuric Patients after the Intervention Period

	Before treatment	After treatment	p*
Total protein (g/dL)	6.69 ± 0.38	6.75 ± 0.29	0.868
Serum albumin (g/dL)	4.06 ± 0.30	3.92 ± 0.34	0.517
Systolic BP (mmHg)	127.70 ± 7.63	125.16 ± 6.19	0.312
Diastolic BP (mmHg)	83.12 ± 2.43	82.12 ± 4.18	0.734
Total Cholesterol (mg/dL)	207.29 ± 19.66	202.76 ± 16.21	0.302
Triglycerides (mg/dL)	130.54 ± 11.61	124.18 ± 13.45	0.442
LDL Cholesterol (mg/dL)	118.00 ± 11.72	117.55 ± 11.78	0.714
HDL Cholesterol (mg/dL)	40.58 ± 4.54	46.67 ± 4.69	0.041
BMI (kg/m^2)	26.84 ± 1.93	25.88 ± 2.64	0.811
Insulin (μIU/mL)	18.14 ± 6.39	12.67 ± 2.84	<0.001
HOMA	6.04 ± 1.37	4.00 ± 0.42	<0.001
HbA_{1c} (%)	8.50 ± 2.07	8.11 ± 2.34	0.788
FPG (mg/dL)	136.90 ± 20.74	127.87 ± 19.75	0.034
24 hour urine proteinuria (mg/d)	728.06 ± 213.38	310.67 ± 131.28	<0.001
Adiponectin (μg/mL)	3.94 ± 2.51	8.33 ± 3.21	<0.001

BP, blood pressure (mmHg); BMI, body mass index; FPG, fasting plasma glucose; HOMA, homeostasis model assessment.

*Paired t test.

adiponectin levels than those without proteinuria (3.91 ± 2.57 μg/mL vs 10.15 ± 1.97 μg/mL, $p < 0.001$, respectively). Patients without proteinuria also had lower levels of adiponectin than those of controls (10.15 ± 1.97 μg/mL vs 11.96 ± 2.00 μg/mL vs $p < 0.001$, respectively)

PPAR-γ Agonist Increases Plasma Adiponectin Concentrations

After the treatment period, adiponectin concentrations significantly increased in the patients group taking rosiglitazone (3.91 ± 2.51 μg/mL before treatment, and 8.33 ± 3.21 μg/mL after treatment, $p < 0.001$). PPAR-γ agonist also reduced plasma insulin levels after the intervention period (18.14 ± 6.39 IU/mL before treatment and 12.67 ± 2.84 μIU/mL after treatment, $p < 0.001$) (Table 3). Furthermore, HOMA indexes, which reflect insulin resistance, were also significantly decreased after rosiglitazone therapy (6.04 ± 1.37 vs 4.00 ± 0.42, $p < 0.001$) (Table 3). Additionally, the degree of proteinuria was also decreased after the intervention period (728.06 ± 213.38 mg/d vs 310.67 ± 131.28 mg/d, $p < 0.001$) (Table 3). The administration of rosiglitazone for 4 wk did not change serum albumin, total cholesterol, and triglyceride concentrations. There was also no difference between two groups according to HbA_{1c} and BMI, after intervention (Table 3).

In addition, the correlations between the absolute changes of adiponectin and fasting insulin or HOMA-IR or HDL or levels of proteinuria were calculated ($r = –0.504$, $p = 0.004$; $r = –0.340$, $p = 0.062$; $r = 0.508$, $p = 0.004$; $r = –0.576$, $p = 0.001$, respectively). The association between the absolute changes of plasma adiponectin levels and the level of proteinuria is depicted in Fig. 3.

The correlations between the percentage changes of adipnectin and fasting insulin or HOMA-IR or HDL or levels of proteinuria were also calculated ($r = –0.306$, $p = 0.095$; $r = –0.330$, $p = 0.070$; $r = 0.321$, $p = 0.079$; $r = –0.434$, $p = 0.015$, respectively). The association between the percentage changes plasma adiponectin levels and the level of proteinuria is depicted in Fig. 4.

Discussion

The results of the present study imply that (1) patients with type 2 diabetes mellitus who have proteinuria had lower adiponectin levels than the ones without proteinuria or healthy controls; (2) plasma adiponectin concentrations were inversely correlated with insulin levels, HOMA indexes, and the magnitude of proteinuria; (3) 4 wk of rosiglitazone treatment increased plasma adiponectin concentrations and decreased proteinuria with accompanying reduction in insulin levels and HOMA indexes.

PPAR-γ agonists, such as thiazolidinediones (TZD), not only improve the insulin resistance but also ameliorate dyslipidemia, prevent the procoagulant activity, and inhibit the inflammatory response *(4).* Furthermore, several reports indicate that TZDs decrease proteinuria in patients with diabetic nephropathy *(5,6).* How TZDs improve proteinuria and protect against diabetic nephropathy is not clear so far. PPAR-γ receptors are expressed on glomerules *(21).* Also, the presence of a polymorphism of the gene encoding the PPAR-γ2 receptor, Pro12Ala, was reported to protect from the development of overt proteinuria *(22).* Moreover, benefits of troglitazone regime on serum type IV collagen concentrations, elevated in diabetic nephropathy, are reported

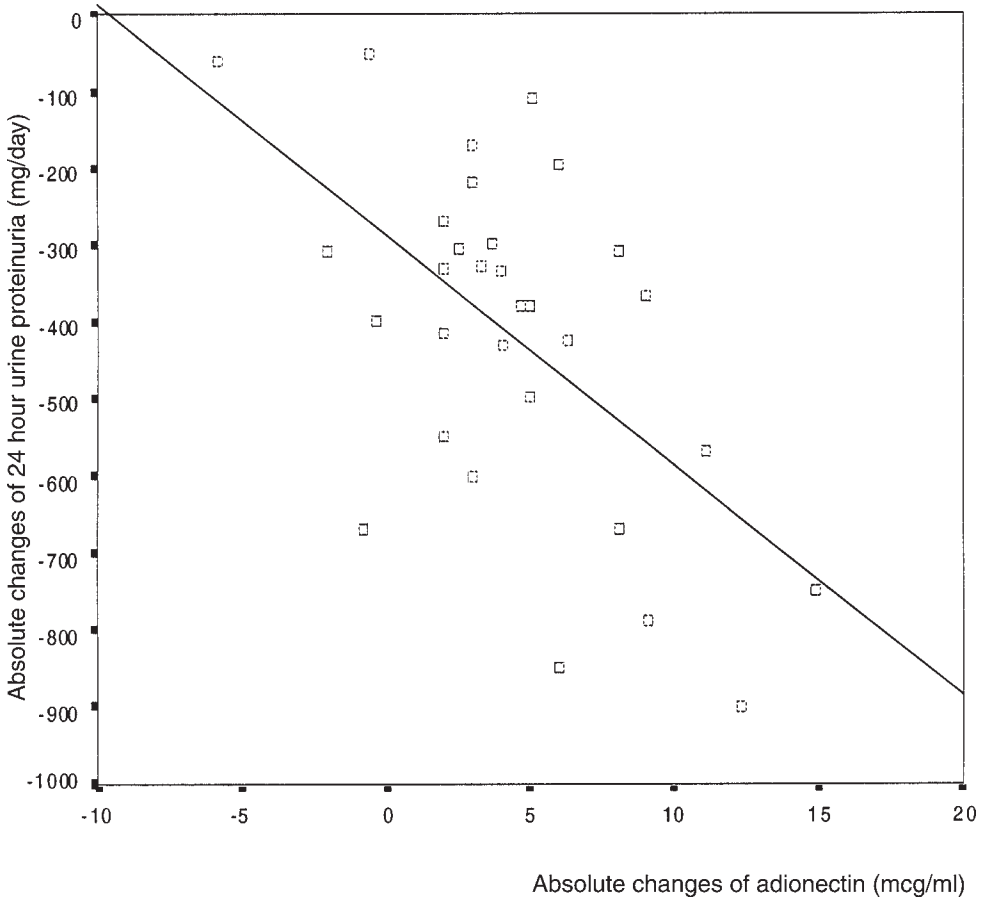

Fig. 3. Correlation of absolute changes between adiponectin and 24-h urine proteinuria after rosiglitazone therapy ($r = -0.576$, $p = 0.001$).

(6). From these data, the direct effects of TZDs on glomerules may be speculated to protect from nephropathy. Yet, the highest concentrations of PPAR-γ receptors are found in adipocytes *(23)* and most of the systemic effects of TZDs are attributed to their effects on adipose tissue rather than the direct effects on muscle, liver, or other tissues *(24,25)*. The inhibition of PPAR-γ receptors on adipose tissue may prevent the generation of these culprit adipose tissue proteins such as PAI-1, angiotensin II, and TNF-α, and perform renoprotective effects *(6–8)*.

Adiponectin, one of the recently defined adipocyte-derived proteins, has a negative association with insulin resistance, endothelial dysfunction, and coronary artery disease *(26)*. So far there is not enough data about the relation between diabetic proteinuria and plasma adiponectin levels. Koshimura and colleagues reported elevated plasma adiponectin levels in patients with diabetic nephropathy *(15)*. Zoccali et al. reported increased adiponectin levels in a group of proteinuria patients who have risk factors other than diabetes mellitus *(20)*. However, both studies involved patients who have impaired renal functions. Renal failure was reported to be associated with increased plasma adiponectin levels by other studies as well *(27)*. Up to now there has been no data about the plasma adiponectin levels in proteinuria with normal renal functions. Hypoadiponectinectinemia is speculated to be a novel predictor of cardiovascular events *(26)*. Proteinuria as well is an omen for the development of atherosclerotic disease in both diabetics and nondiabetics *(3,28, 29)*. To us, high plasma adiponectin level in proteinuria is not expected. Therefore, we aimed to investigate any association between diabetic proteinuria and plasma adiponectin levels, this time in patients with normal renal functions. According to results, plasma adiponectin levels were lower in diabetic patients and the presence of proteinuria was associated with a further decrease. Adiponectin levels were inversely correlated both with insulin resistance and with the degree of proteinuria.

The negative association of plasma adiponectin levels with proteinuria in type 2 diabetes is a novel observation. However, regarding the data about the relation between adiponectin and cardiovascular events, both low adiponectin levels in diabetic proteinuria and the association between proteinuria and adiponectin levels are sensible. Another reasonable result of the study is the increment of plasma adiponectin levels and improvement of proteinuria after 4 wk of rosiglitasone treatment. The positive effect of rosiglita-

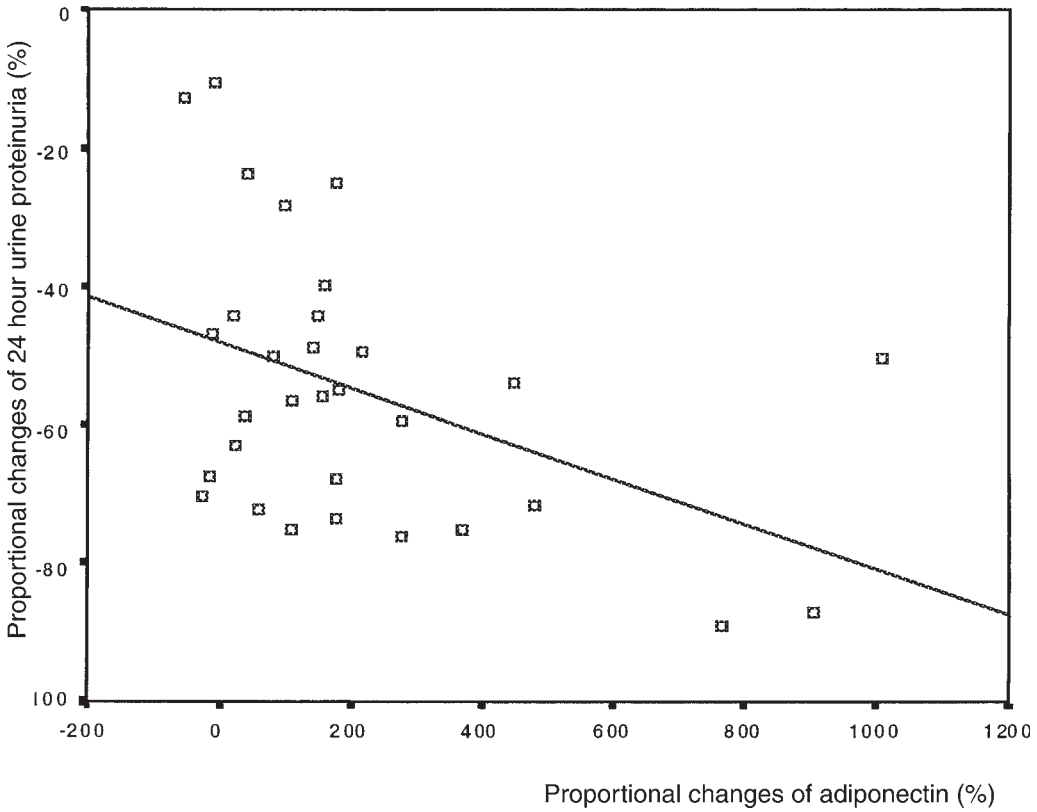

Fig. 4. Correlation of proportional changes between adiponectin and 24-h urine proteinuria after rosiglitazone therapy ($r = -0.434$, $p = 0.015$).

zone on plasma adiponectin levels is not a novel finding *(17, 30).* There are several mechanisms to explain the increment of adiponectin levels after the rosiglitazone treatment.

The improvement of insulin resistance is probably the primary reason, as the relation between the insulin resistance and plasma adiponectin levels are well reported *(31),* whereas Faraj et al. report that improved insulin sensitivity is predicted by the increase in adiponectin levels *(32).* Although some reports do not support the idea that insulin sensitizers have positive effects on adiponectin levels *(31,33),* a recent report from Pajvani et al. imply that the TZD induced effect on insulin sensitivity may not be related with the total amount of plasma adiponectin but its high-molecular-weight portion instead *(34).* Another mechanism may be the direct effects of PPARγ agonists on adiponectin promoter *(16).* By now we do not exactly know how rosiglitazone treatment increased plasma adiponectin levels, and the present study is not designed to answer such a question. Also, it is not clear that the improvement of plasma adiponectin levels have an effect on the improvement of proteinuria or vice versa. As the research was designed as a case-control study, it is hard to predict the cause and effect relationship. Future prospective studies with greater patient numbers are recommended to establish the direct relationship of the plasma adiponectin levels with diabetic complications and to investigate the effects of PPARγ agonists on adiponectin levels in type 2 diabetic patients with proteinuria.

In summary, the results of the present study suggest that (1) plasma adiponectin levels are lower in type 2 diabetes mellitus especially in patients with proteinuria than the age, BMI, and sex matched controls; (2) also, the levels are lower in diabetic patients with proteinuria than the ones without proteinuria; (3) rosiglitazone treatment improves plasma adiponectin levels while decreasing the proteinuria.

Methods

Patients

One hundred and eleven consecutive patients of newly diagnosed or previously uncontrolled type 2 diabetes mellitus (DM) referred to the outpatient clinics of Gülhane Medical School were recruited for study purposes. Patients were evaluated by standard physical examination, chest X-ray, baseline electrocardiogram, exercise electrocardiogram, two-dimensional echocardiography, and routine clinical laboratory tests, including liver and kidney function tests and 24-h urinary protein measurements.

The exclusion criteria were as follows: Being under any drug treatment other than sulfonylureas. Hypertension (systolic blood pressures ≥ 140 mmHg and/or diastolic blood

pressures ≥ 90 mmHg), BMI > 30 kg/m^2, coronary heart disease (patients with ischemic ST- T alterations and voltage criteria for LVH on electrocardiogram, and with history of revascularization), elevated liver enzymes (AST or ALT levels ≥ 40U/L), renal failure (serum creatinine levels ≥ 1.3 mg/dL), dyslipidemia (patients with total cholesterol levels higher than 240 mg/dL and triglyceride levels higher than 150 mg/dL).

After the first analysis, 64 patients were eligible for this study (30 males, 34 females, mean age of 46.1 ± 4.6 yr). The duration of diabetes after initial diagnosis was 44.64 ± 7.9 mo. The control group consisted of 26 healthy subjects who were matched to the patients with respect to age, sex, and BMI (14 males, 12 females, mean age of 45.3 ± 4.8 yr). They underwent comprehensive physical and laboratory evaluation to ascertain that they had no hypertension, metabolic, hepatic, or renal diseases. The control subjects also had no family history of hypertension and diabetes mellitus. All subjects gave informed consent for participating in the study. The local ethics committee of Gulhane School of Medicine approved the study.

Study Protocol

After an overnight fast, venous blood samples were obtained from all subjects. In addition to the metabolic panel, plasma adiponectin concentrations, HbA_{1c}, basal insulin value, and insulin-resistance score (HOMA-IR) were measured. For the evaluation of proteinuria, 24-h urine was collected from each patient. After the first analysis, patients were divided into two groups on the basis of proteinuria. Patients with proteinuria (>500 mg/d) were allocated to one study group and patients without proteinuria (<150 mg/d) to another group. The patients with levels less than 500 mg/d and more than 150 mg/d were not enrolled.

In order to evaluate the effect of rosiglitazone on plasma adiponectin concentrations, patients with proteinuria were given a peroxisome proliferator-activated receptor-γ (PPAR-γ) (rosiglitazone 4 mg/d) for 4 wk. The effect of PPAR-γ on insulin sensitivity and proteinuria were also investigated. After the intervention period, blood samples were obtained for assay of plasma adiponectin concentrations, HbA_{1c}, and insulin resistance scores (HOMA-IR). Twenty-four hour urine samples were also collected to determine proteinuria.

Laboratory Procedures

After an overnight fast, venous blood samples were drawn and promptly centrifuged, and the plasma was stored at –20°C until adiponectin assay was performed. Plasma adiponectin concentrations were measured in duplicate by RIA (Human Adiponectin RIA Kit, Linco Research, Inc., St. Charles, MO). All samples were run in the same assay.

Fasting plasma glucose, blood urea, serum creatinine, total protein, serum albumin, total cholesterol, HDL cholesterol, and triglycerides were determined by enzymatic colorimetric method with Olympus AU 600 auto analyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). LDL cholesterol was calculated by Friedewald's formula. HbA_{1c} was measured by inhibition of latex agglutination, using a DCA 2000 analyzer (Bayer, Elkhart, IN). Proteinuria was calculated as the mean of three 24-h urine collections. Twenty-four hour proteinuria was detected by trichloroacetic acid. The serum basal insulin value was determined by the coated-tube method (DPC-USA). In particular, an insulin-resistance score (HOMA-IR) was computed with the formula: (HOMA-IR) = Fasting plasma glucose (FPG) (mg/dL) × immunoreactive insulin (IRI) (μU/mL)/405 *(35).*

Data Analysis

Results are reported as the mean ± standard deviation (SD). The Levene's test was used to evaluate the homogenity of variance. Differences between diabetic and controls groups were tested for significance by independent samples *t* test, Mann–Whitney U test, and chi-square test, as appropriate. The relationship between variables was analyzed by Pearson's correlation. Differences between before and after roziglatazone therapy were tested by paired *t* test. Differences and correlations were considered significant at $p < 0.05$.

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