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ORIGINAL PAPER

Arterial elasticity and plasma levels of adiponectin and leptin in type 2 diabetic patients treated with thiazolidinediones

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Abstract *Background* Thiazolidinediones (TZDs) improve peripheral insulin sensitivity, but the effect on arterial stiffness is less clear. The aim of the present study was to assess the differential effect of pioglitazone or rosiglitazone on arterial stiffness and plasma levels of adiponectin and leptin in patients with type 2 diabetes mellitus. *Methods* Thirty-five type 2 diabetic subjects were randomly assigned to receive pioglitazone (30 mg/day; $n = 14$), rosiglitazone (4 mg/day; $n = 11$), or placebo (medical nutrition therapy; $n = 10$) for 12 weeks. Changes in plasma glucose, glycosylated hemoglobin, insulin resistance (HOMA-IR), total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, adiponectin, and leptin were evaluated at baseline and after 12 weeks. In parallel, large arterial compliance (C1) and small arterial compliance (C2) were measured at baseline and at the end of treatment period. *Results* At 12 weeks, the rosiglitazone ($P = 0.026$) and pioglitazone ($P = 0.004$) groups had a significant increase from baseline in adiponectin that was not seen in the medical nutrition therapy group. No significant changes in plasma leptin and in C1 and C2 elasticity indexes were observed over the entire study period in any of the treatment groups. *Conclusions* In this study of patients with type 2 diabetes, treatment with TZDs was associated with a significant improvement in adiponectin levels, although no significant effects were seen on leptin levels and arterial elasticity.

Keywords Thiazolidinediones · Diabetes mellitus type 2 · Adipokines · Arterial elasticity

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Introduction

Adipose tissue, in addition to being a fat store, secretes a number of hormones and proteins collectively termed adipokines [1]. Several adipokines, including adiponectin and leptin, have been linked to an increased vascular risk [2]. In most studies, low adiponectin and elevated leptin have been associated with the development of subsequent cardiovascular disorders, and some have shown the associations to be independent of measures of insulin resistance [3, 4]. Notably, previous clinical studies have shown the relationship of arterial elasticity with levels of plasma leptin [5] and adiponectin [6]. Of interest is also the observation that decreased vascular elasticity and storage capacity of the vessels, also known as vascular compliance, has been associated with an increased risk of adverse cardiovascular events [7, 8]. In this regard, a reduction in arterial compliance is a marker for vascular disease and should prompt a more aggressive approach in managing cardiovascular risk factors.

Thiazolidinediones (TZDs) are unique oral agents used in the treatment of diabetes. They work by decreasing insulin resistance at peripheral sites as well as by decreasing hepatic glucose output [9]. TZDs primarily target the peroxisome proliferator activated receptor (PPAR) gamma receptor, which is chiefly expressed in the adipose tissue but which is also found in the liver, pancreas, muscle, and blood vessels [10]. TZDs use has been shown to reduce cardiovascular risk factors by having favorable effects on blood pressure, lipid levels, plasma concentrations of adipokines, and arterial compliance [11].

Several studies have compared the metabolic differences between the two available thiazolidinediones on the market, pioglitazone and rosiglitazone [12]. None of these comparative trials, however, directly addressed findings on arterial compliance. Under these circumstances, we sought to investigate the effect of pioglitazone and rosiglitazone on both large vessels (C1, e.g., aorta) and small vessels (C2, e.g., tissue bed vessels) compliance in patients with type 2 diabetes. We also wanted to compare the metabolic effects of the two thiazolidinediones on plasma levels of adiponectin and leptin.

Subjects and methods

Patients

The study protocol was approved by our Institutional Review Boards and was conducted in accordance with the Declaration of Helsinki. Thirty-five newly diagnosed (duration < 6 months) type 2 diabetic [according to the American Diabetes Association (ADA) criteria] patients were enrolled. Patients were excluded if they had a history of impaired hepatic function (defined as plasma aminotransferase and/or gamma-glutamyltransferase level higher than the upper limit of normal [ULN] for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex), or severe anemia. Patients with serious cardiovascular disease (e.g., New York Heart Association class I–IV congestive heart failure or a history of myocardial infarction or stroke) or cerebrovascular conditions were also excluded. Women who were pregnant or breastfeeding were excluded. Patients were randomly assigned to receive pioglitazone (30 mg/day; $n = 14$), rosiglitazone (4 mg/day; $n = 11$), or placebo (medical nutrition therapy; $n = 10$) for 12 weeks. Medical nutrition therapy was based on ADA recommendations and contained 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/day, and 35 g/day of fiber. All patients provided written informed consent to participate.

Study design

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, calculation of body mass index (BMI), assessment of glycemic control (HbA_{1c}, fasting plasma glucose and insulin levels [FPG, and FPI, respectively], and homeostasis model assessment [HOMA] index), plasma leptin, plasma adiponectin and arterial elasticity indices. BMI was calculated by the investigators as weight in kilograms divided by the square of height in meters. The estimate of insulin resistance was calculated using the HOMA index, with the following formula: insulin resistance = FPI (μU/ml) × FPG (mmol/l)/22.5 (normal if <2.5, presence of insulin-resistance if ≥ 2.5). All measurements were repeated after 12 weeks of TZDs therapy.

Biochemical analysis

Venous blood samples were drawn in all patients between 8:00 a.m. and 9:00 a.m. We used plasma obtained by addition of Na_2-EDTA, 1 mg/ml, and centrifuged at 3,000*g* for 15 min at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at −80°C for less than 3 months. HbA_{1c} level was measured using high-performance liquid chromatography (DIAMAT, Bio-Rad Laboratories, Inc., Hercules, CA), with intra- and inter-assay coefficients of variation (CsV) of <2%. Plasma glucose was assayed using a glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and inter-assay CsV < 2%. Plasma insulin was assayed with Phadiaseph Insulin Radioimmunoassay (Pharmacia, Uppsala, Sweden) using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and inter-assay CsV, 4.6 and 7.3%, respectively). Leptin concentrations were assessed in duplicate by commercially available ELISA kits (TiterZyme EIA kit, Assay Designs, Inc., Ann Arbor, MI, USA) according to the manufacturer's protocol. The intra-assay CsV was 4.5% and the inter-assay CsV was 6.5%. Adiponectin level was determined using ELISA kits (B-Bridge International, Inc., Sunnyvale, CA, USA). The intra-assay CsV were 3.6% for low and 3.3% for high control samples, while the inter-assay CsV were 3.2% for low and 7.3% for high control samples.

Large vessels and small vessels compliance

Following approximately 5–10 min of supine rest, large artery (C1) and small artery (C2) elasticity indices were obtained by an HDI/Pulswave™ CR-000344 Cardiovascular Profiling System (Hypertension Diagnostic, Inc., Eagan, MN, USA). An appropriate-sized blood pressure cuff was wrapped around the upper left arm, and a rigid

plastic wrist stabilizer was placed on the right wrist to minimize movement of the radial artery during the measurement. With the right forearm resting in a supine position, an Arterial Pulsewave™ Sensor was placed on the skin directly over the radial artery at the point of the strongest pulse. The sensor was adjusted to the highest relative signal strength, and the C1 and C2 measures were obtained during 30 s of blood pressure waveform collection. This device can measure the decay in diastolic pressure in the large arteries, and the decay in the reflective waves of the small arteries. All measurements were averaged over three continuous 30-s trials. C1 and C2 measurements were measured at baseline and following 12 weeks of TZDs treatment.

Data analysis

Baseline characteristics are given as mean ± standard deviation or counts, as appropriate. Chi-square testing was used for categorical data. Unpaired *t*-test analysis was performed to compare the general characteristics between the three study groups (controls, patients treated with rosiglitazone and patients treated with pioglitazone) at baseline. One-sample paired *t*-tests were performed for within-group comparisons between baseline and post-treatment values. Linear mixed models were used to detect potential interactions, which might influence the relation between treatment and change in the study variables (including age and gender). Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS, Inc., Chicago, IL, USA). In all statistical analyses, a two-tailed $P < 0.05$ was considered statistically significant.

Results

The general characteristics of the study participants are depicted in Table 1. A total of 35 patients (18 males, 17 females; mean age: 55.2 ± 7.7 years) were enrolled in the study. The characteristics of the patient population at the initial period of the study were similar in the three treatment groups with regard to age ($P = 0.64$) and gender ($P = 0.32$). Baseline body mass index, glycemic control values, lipid variables, adipokines, and arterial elasticity did not differ significantly across the three study groups.

Body mass index

There was a significant reduction in the mean BMI value of the subjects in the medical nutrition therapy group. No mean BMI change was observed after 12 weeks in either TZDs group (Table 2).

Table 1 Baseline characteristics of patients enrolled in the study ($n = 35$)

	Baseline
Age (years)	55.2 ± 7.7
Gender (males/females)	18/17
BMI (kg/m^2)	29.1 ± 2.4
FPG (mg/dl)	144.2 ± 19.7
HbA1c (%)	6.90 ± 1.3
TC (mg/dl)	207.7 ± 39.9
HDL-C (mg/dl)	49.9 ± 10.4
LDL-C (mg/dl)	112.7 ± 33.3
TG (mg/dl)	177.6 ± 89.9
HOMA-IR	3.64 ± 1.23
Adiponectin (μg/ml)	16.5 ± 4.4
Leptin (ng/ml)	17.1 ± 10.4
LAEI (ml/mmHg × 100)	12.4 ± 3.2
SAEI (ml/mmHg × 100)	4.20 ± 1.9

BMI: body mass index, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, HOMA-IR: homeostasis model assessment-insulin resistance, LAEI: large arterial elasticity index, SAEI: small arterial elasticity index

Glycemic control

Significant decreases in FPG were observed after 12 weeks ($P < 0.05$) in the medical nutrition therapy group as well as in both TZDs groups (both *P*-values < 0.001). A significant reduction of HbA_{1c} was observed after 12 weeks both in the pioglitazone and rosiglitazone groups ($P = 0.003$ and $P = 0.019$, respectively), whereas no HbA_{1c} change was obtained in the medical nutrition therapy group after 12 weeks compared to baseline values. A significant improvement in HOMA index was obtained at 6 months in both TZDs groups (both *P*-values < 0.05) but not in the medical nutrition therapy group (Table 2).

Lipid variables

No significant changes in total cholesterol, HDL-cholesterol and LDL-cholesterol were observed in the three study groups at the 12-week assessment when compared with the baseline. A significant decrease in TG ($P = 0.004$) was observed with pioglitazone treatment after 12 weeks compared with the baseline values (Table 2).

Adipokines measurements

At 12 weeks, the rosiglitazone ($P = 0.026$) and pioglitazone ($P = 0.004$) groups had a significant increase from baseline in adiponectin that was not seen in the medical nutrition therapy group. This effect appeared to be enhanced more

Table 2 Baseline characteristics and parameter changes at 12 weeks in the three study groups

	Control ($n = 10$)			Pioglitazone ($n = 14$)			Rosiglitazone ($n = 11$)		
	Baseline	12 weeks	*P*	Baseline	12 weeks	*P*	Baseline	12 weeks	*P*
BMI (kg/m^2)	29.2 ± 2.3	28.2 ± 1.7	0.013	29.3 ± 2.9	29.2 ± 3.1	ns	28.3 ± 4.09	27.9 ± 4.3	ns
FPG (mg/dl)	137.9 ± 26.9	119.3 ± 25	0.028	164 ± 46.6	116 ± 14.8	0.001	137.5 ± 30.1	110 ± 8.1	0.001
HbA1c (%)	6.39 ± 1.1	6.45 ± 0.7	ns	7.82 ± 1.7	6.6 ± 0.6	0.003	7.0 ± 1.07	6.2 ± 0.4	0.019
TC (mg/dl)	188.3 ± 33.09	188.6 ± 22	ns	223.07 ± 36.9	212.9 ± 36	ns	200.3 ± 41.4	192 ± 43	ns
HDL-C (mg/dl)	50.2 ± 13.7	47.5 ± 10	ns	49.6 ± 6.3	47.5 ± 10	ns	48.8 ± 7.8	47 ± 9.5	ns
LDL-C (mg/dl)	107.3 ± 32.1	116 ± 20	ns	131.3 ± 31.3	116 ± 20	ns	121.3 ± 34.8	118 ± 38.5	ns
TG (mg/dl)	152.6 ± 97.7	125 ± 52	ns	207.6 ± 100.9	125 ± 52	0.004	148.8 ± 62.1	135 ± 69	ns
HOMA-IR	3.14 ± 1.08	2.5 ± 1.7	ns	4.59 ± 2.29	2.6 ± 1.8	0.026	3.5 ± 1.6	2.1 ± 1.3	0.011
Adiponectin (μg/ml)	18.7 ± 4.4	10.4 ± 2.8	ns	14.5 ± 8.5	19.8 ± 10.4	0.004	14.2 ± 2.4	20.1 ± 12.1	0.026
Leptin (ng/ml)	19.6 ± 13.9	15.5 ± 9.7	ns	14.7 ± 9.2	10.9 ± 8.8	ns	14.5 ± 14.5	12 ± 12	ns
LAEI (ml/mmHg × 100)	11.9 ± 2.9	14.5 ± 5	ns	12.3 ± 4.8	12.3 ± 2.6	ns	14.5 ± 4.5	14.9 ± 4	ns
SAEI (ml/mmHg × 100)	4.08 ± 2.2	4.5 ± 2.2	ns	4.6 ± 1.7	4.5 ± 2.2	ns	3.7 ± 1.3	4.5 ± 2.4	ns

BMI: body mass index, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, HOMA-IR: homeostasis model assessment-insulin resistance, LAEI: large arterial elasticity index, SAEI: small arterial elasticity index, ns: not significant

with pioglitazone than with rosiglitazone ($P = 0.031$). No gender interaction was present on the effect of either rosiglitazone or pioglitazone on plasma adiponectin level. No significant changes in plasma leptin values in any of the treatment groups were observed over the entire study period.

Arterial elasticity

No significant changes in both C1 large artery and C2 small artery elasticity indices were observed in any of the treatment groups when compared with the baseline (Table 2).

Discussion

In patients with type 2 diabetes, the main aims are to prevent the emergence of insulin resistance, to maintain a favourable lipid profile, and to control cardiovascular risk factors in order to minimize cardiovascular complications and improve patient's prognosis. This study examined the effect of TZDs treatment on arterial elasticity and plasma adipokines levels in newly diagnosed type 2 diabetic patients. To the best of our knowledge, no direct comparison has been carried out on the effect of pioglitazone and rosiglitazone on arterial elasticity indices have been carried out. The results of our study showed that both drug regimens had a similar positive effect on plasma levels of adiponectin after 12 weeks of treatment, whereas no effect was seen on leptin concentrations or indices of arterial elasticity.

Adiponectin, also known as ACRP30, is a 30 kDa protein abundantly and selectively expressed in white adipose tissue. Its role in insulin resistance and atherosclerosis has been well established, and hypoadiponectinemia has been independently associated with adverse cardiovascular events [13]. Our data are consistent with previous studies showing that PPAR gamma activators are able to increase adiponectin plasma level [14, 15]. Previous studies have shown that TZDs may induce synthesis of adiponectin presumably via PPAR gamma activation involving a post-transcriptional mechanism [16]. Adiponectin exhibits a prominent vasoprotective function. In our study, TZDs therapy significantly increased adiponectin levels without a corresponding change in arterial elasticity. Thus, it is possible that TZDs therapy is directly altering adiponectin levels independent of measures of vascular elasticity.

In the present study, we also detected plasma leptin levels in newly diagnosed type 2 diabetic patients before and after 12 weeks of TZDs treatment. Leptin, a 167-amino-acid protein, is expressed almost exclusively in adipose tissue, from which it is secreted into the circulation [17]. High leptin levels have been linked to cardiovascular risk in diabetes, and drugs used in the treatment of type 2 diabetes have been shown to modulate levels of this adipokine [18]. However, no significant variation was seen in circulating concentrations of leptin over the entire study period. This result in line with previous data showing no significant effects of TZDs on plasma levels of this adipokine [19]. Notably, in our study, no significant effect of TZDs on BMI was seen. Since plasma leptin concentrations are strongly related to BMI [17], the lack of effect of pioglitazone and rosiglitazone on plasma leptin levels was expected in our cohort.

As a further aim of our study, we examined the effect of TZDs therapy on arterial elasticity indices in newly diagnosed type 2 diabetic patients. A growing body of evidence

has suggested that arterial elasticity might serve as a surrogate end point for estimation of success of treatment in patients at high risk for adverse cardiovascular events [7, 8]. However, no significant changes in arterial elasticity of large and small arteries in our patients with diabetes were seen. These data are in contrast with recent studies showing an improved arterial elasticity after long-term treatment with rosiglitazone in type 2 diabetic patients or in subjects with the metabolic syndrome [20, 21]. The lack of improvement in arterial elasticity as observed in our study might be explained by the possible absence of short-term benefit of TZDs on arterial compliance. Nonetheless, our data need to be confirmed in randomized, placebo-controlled studies.

Several limitations are inherent in the present study. The present investigation was designed as an exploratory pilot project. The primary outcome measures in this study was arterial elasticity and adipokines levels. Thus, the study was not powered to detect changes in the clinical outcome as a result of TZDs treatment in type 2 diabetic. Greater numbers and longer treatment duration are required to evaluate this possibility fully. However, our findings provide a rationale for such a study. Another limitation is that the pulse contour analysis as used in our study is a noninvasive measure of large and small arterial elasticity, and an invasive measure would be more precise. We recognize that the most widespread noninvasive technique to assess endothelial function is flow-mediated dilation (FMD). However, FMD is time-consuming, the equipment is very expensive, and it requires an experienced examiner [22]. Other techniques available to evaluate arterial elasticity are ultrasound measurement, magnetic resonance imaging, and indirect measures such as pulse pressure. Finally, it is worth noting that the treatment period in our study was short. Thus, it cannot be excluded that longer observation time would yield significant results with regard to the effects of thiazolidinediones on arterial elasticity.

Conclusions

In summary, in this study of patients with type 2 diabetes, treatment with TZDs was associated with a significant improvement in adiponectin levels, although no significant effects were seen on leptin levels and arterial elasticity. Further trials are necessary to clarify the possible long-term differences between pioglitazone and rosiglitazone on both large artery and small artery elasticity indices.

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