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Effects of pioglitazone on subclinical atherosclerosis and insulin resistance in nondiabetic renal allograft recipients

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Abstract

Background. The aim of this study was to evaluate the effect of pioglitazone treatment on the progression of subclinical atherosclerosis and insulin resistance in renal allograft recipients with no preoperative history of diabetes.

Methods. Eighty-three patients without diabetes were randomly assigned to either the pioglitazone group or the control group. Carotid intima–media thickness (IMT), serum adiponectin level and lipid profile were assessed before transplantation and at 12 months after transplantation. Insulin secretory function and insulin resistance were evaluated by the oral glucose tolerance test.

Results. The pioglitazone group showed a significant reduction in the mean and maximum carotid IMT compared with the control group after 12 months (mean carotid IMT, 0.05 ± 0.04 vs −0.03 ± 0.07 mm, $P < 0.001$; maximum carotid IMT, 0.08 ± 0.05 vs −0.05 ± 0.09 mm, $P < 0.001$). Pioglitazone increased the adiponectin level, and the change in adiponectin was negatively correlated with carotid IMT changes. Pioglitazone treatment increased the insulin sensitivity index compared with the control group ($-0.8 \pm 3.1\times10^{-2}$ vs $+1.1 \pm 3.7\times10^{-2}$, $P = 0.036$).

Conclusions. These results suggest that pioglitazone treatment reduces the progression of carotid IMT and improves insulin resistance in renal allograft recipients without a history of diabetes.

Trial Registration. Clinicaltrials.gov Identifier: NCT00598013

Keywords: carotid intima–media thickness; insulin resistance; pioglitazone; renal allograft

Introduction

Cardiovascular disease (CVD) is a common cause of morbidity and mortality after kidney transplantation [1]. The annual risk of CVD is 3.5–5% in renal transplant recipients, 50-fold higher than in the general population [2,3]. The measurement of carotid intima–media thickness (IMT) by high-resolution B-mode ultrasonography is an

easy, reliable and non-invasive method for assessing cardiovascular risk in renal transplant recipients [4–6].

Thiazolidinediones act as insulin sensitizers, leading to improved glycaemic control with reduced insulin requirements in patients with diabetes mellitus. Pioglitazone and rosiglitazone are effective and safe oral agents for post-transplant diabetes mellitus (PTDM), and rosiglitazone improves endothelial function in renal transplant recipients with glucose intolerance [7,8]. Furthermore, pioglitazone has been shown to decrease carotid IMT and improve cardiovascular risk markers, including adiponectin, independently of glycemic control in patients with type 2 diabetes mellitus [9–11]. Recently, hypoadinponectinaemia was shown to be associated with insulin resistance, glucose intolerance and arteriosclerotic risk factors after renal transplantation [12,13].

In light of these data, we investigated the effect of pioglitazone treatment on the progression of carotid IMT and insulin resistance in renal transplant recipients without a history of diabetes.

Materials and methods

Study subjects and study design

This was a prospective, randomized open-label study. Patients on dialysis who received a kidney transplant were eligible to participate in the study if they were ≥18 years old, had no previous history of organ transplantation and were not currently using steroids or other immunosuppressants. Patients were excluded if they had diabetes before transplantation with a fasting glucose ≥7.0 mmol/l or 2-h post-load glucose ≥11.1 mmol/l, symptomatic CVD (coronary artery disease, cerebrovascular disease or peripheral vascular disease), uncontrolled hypertension, severe metabolic or infectious disease, hepatic disease, congestive heart failure, kidney transplantation from a cadaver donor or an unstable condition of the transplanted kidney (serum creatinine ≥2.0 mg/dl and/or blood urea nitrogen ≥30 mg/dl). Patients who were taking a stable dose of statins 2 months prior were allowed to participate in the study. During the study, they maintained their dosage of statin. Statin use was not allowed after patients had entered into the study. The study protocol was approved by the ethics committee of the Yonsei University College of Medicine. All subjects gave informed consent. Participation in this study was proposed to all eligible patients who satisfied the inclusion/exclusion criteria, and no selections were made prior to patient consent. A total of 83 renal allograft recipients who underwent kidney transplantation from November 2004 to September 2006 were enrolled in this study. They were randomly assigned to either the pioglitazone treatment group or the control group according to a computer-generated allocation schedule. The pioglitazone treatment group received 30 mg pioglitazone starting at 2 weeks after renal transplantation for 12 months. All patients were provided counseling regarding diet and exercise. Patients were advised to eat a stable-calorie diet and instructed to maintain the same level of physical activity throughout the study. Patients visited our clinic two times per week for the first month after transplantation, every week for the second and third month, every 2 weeks for the fourth month and monthly thereafter. Vital signs and physical examination, compliance with the study drug and adverse events were assessed at each visit. We measured carotid IMT before and 12 months after renal transplantation. Oral glucose tolerance tests (OGTTs) were performed 1 week before and at 6 and 12 months after transplantation. The insulin secretion and sensitivity index were calculated by OGTT.

Measurement of carotid IMT

Ultrasonography of the common carotid artery (CCA) was conducted bilaterally by high-resolution B-mode ultrasonography (LOGIQ9, GE Medical Systems, Milwaukee, WI) with a 10-MHz linear transducer by a single sonographer who was unaware of the subject's characteristics, as previously described [14]. A sonographer scanned the left and right CCAs, the carotid bulb and the proximal portion of the internal and external carotid arteries in three planes (anterior oblique, lateral and posterior oblique views) and then focused on the interfaces required to measure IMT. Computer-assisted acquisition, processing, storage of B-mode images and calculation of IMT were performed with Intima Scope software (MediaCross, Tokyo, Japan) as previously described [14,15]. The IMT was measured by using an automated edge-detection algorithm based on significant changes in the density of a section between the lumen and subadventitial structures perpendicular to the vessel wall. The software estimated the length of lines at the lumen–intima interface and the media–adventitia interface on the basis of 30-point pixels per 3 mm obtained from tertiary multiple regression analysis incorporating the least squares method, which was designed to achieve increased accuracy and reproducibility with reduced variability for the measurements of IMT. Two measurements on longitudinal views of both the right and left common carotid arteries were made at the segment 20 mm distal to the carotid bulbs. For each measurement, the average and maximum values were calculated automatically. The average value of IMT was obtained by a computerized calculation from the area detected.

Reading and analysis of the images were conducted at the end of the study by a well-trained physician who was blinded to the identity of the patient, time point and treatment. The intraobserver coefficient of variance was 2.1%.

Measurement of metabolic parameters

Trough cyclosporine (CsA), tacrolimus level, plasma glucose, blood urea nitrogen, creatinine, aspartate aminotransferase (AST) [16] and alanine aminotransferase (ALT) levels were measured at each visit. HbA1c, free fatty acids, lipid profiles, plasma adiponectin, resistin concentration and high-sensitivity C-reactive protein (hsCRP) levels were measured before and 12 months after transplantation.

All samples were obtained in the morning after an overnight fast. Low-density lipoprotein (LDL) cholesterol was calculated according to Friedewald's formula. The hsCRP level was measured as previously described [17]. HbA1C values were determined by high-performance liquid chromatography (Variant II; Greencross, Seoul, Korea). Insulin concentrations were measured using a radioimmunoassay kit (IRMA kit; DAINABOT, Tokyo, Japan). Plasma adiponectin and resistin concentrations were measured using a commercial multiplexed immunoassay kit (Linco Research, Inc., St. Louis, MO, USA).

OGTT-derived insulin secretion and sensitivity index

For assays of plasma glucose and insulin, blood samples were taken at 0, 30, 60, 90 and 120 min after the ingestion of 75 g glucose. The secretion area under the curve (SecrAUC), the first and second phase insulin secretion (Secr1PH, Secr2PH) and the insulin sensitivity index for transplantation (ISI_{TX}) were estimated as previously described [18,19].

$SecrAUC = AUC_{Ins}/AUC_{Gluc}$, $Secr1PH = 1283+(1.829 \times Ins_{30\ min})-(138.7 \times Gluc_{30\ min})+(3.772 \times Ins_{0\ min})$, $Secr2PH = 287+(0.4164 \times Ins_{30\ min})-(26.07 \times Gluc_{30\ min})+(9.226 \times Ins_{0\ min})$ and $ISI_{TX} = 0.208-0.0032 \times BMI\ (kg/m^2)-0.0000645 \times Ins_{120\ min}\ (pmol/l)-0.00375 \times Gluc_{120\ min}\ (mmol/l)$.

PTDM, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were diagnosed according to the American Diabetes Association criteria [20].

Immunosuppression schedule

The main immunosuppressive regimens consisted of calcineurin inhibitors (CsA or tacrolimus) and glucocorticoids. A regimen of calcineurin inhibitors was begun 2 days before the transplantation. Initially, the doses of CsA (10 mg/kg) and tacrolimus (0.3 mg/kg) were administered twice daily. The target trough levels of CsA and tacrolimus were as follows: (1) Months 0–3: 150–300 ng/ml for CsA and 10–20 ng/ml for tacrolimus; (2) Months 3–6: 150–200 ng/ml for CsA and 10–15 ng/ml for tacrolimus; and (3) 6 months: 75–150 ng/ml for CsA and 8–10 ng/ml for tacrolimus. Administration of oral prednisolone at 1 mg/kg/day began 2 days before the transplantation. Methylprednisolone was administered intravenously for the first four postoperative days in a tapered fashion: Day 0, 1 g; Day 1, 500 mg; Day 2, 250 mg; and Day 3, 60 mg. Administration of oral prednisolone began after the fourth post-transplantation day at 30 mg/day

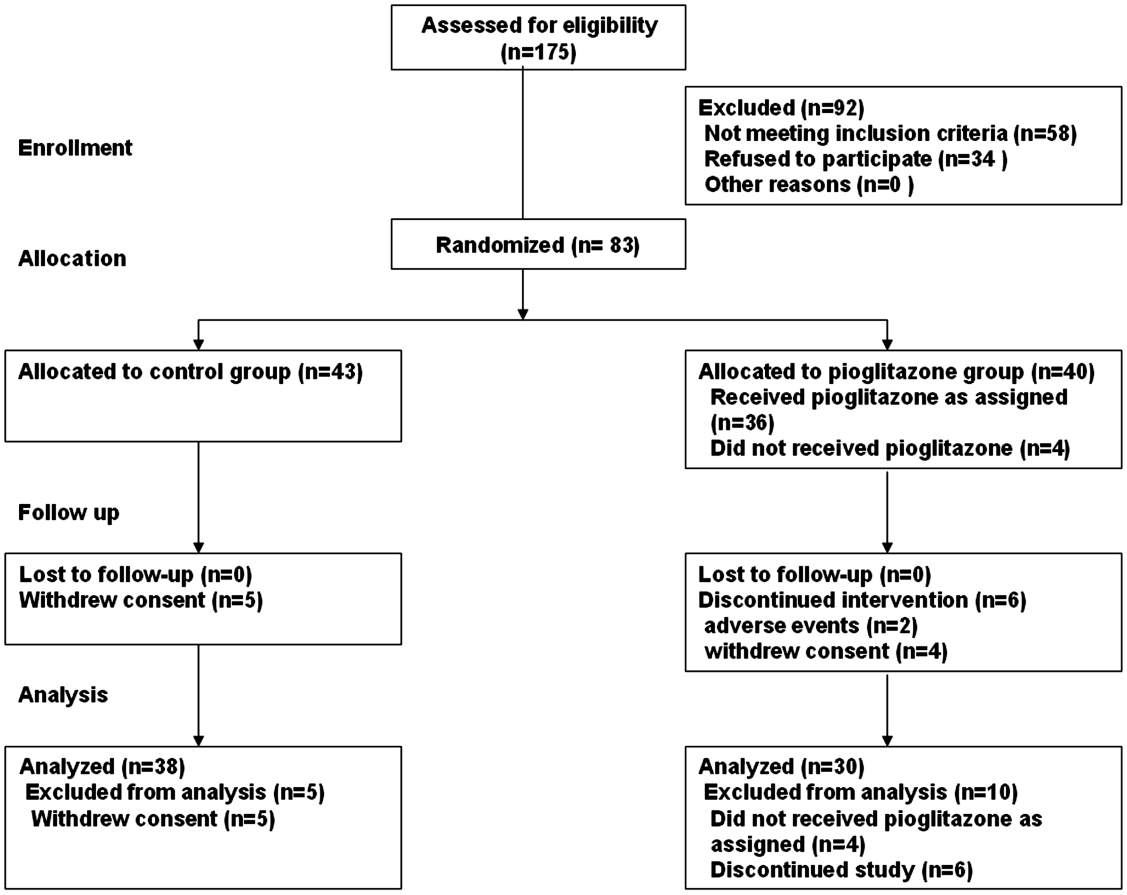


Fig. 1. Flow diagram of the study.

Table 1. Clinical baseline characteristics

	Control (n = 38)	Pioglitazone (n = 30)	P-value
Age (years)	40.4 ± 10.0	38.3 ± 11.1	0.437
Sex (M/F)	22/16	19/11	0.803
Family history of diabetes[a]	1 (2.6%)	1 (3.3%)	1.000
Duration of dialysis (months)	35.8 ± 52.3	22.4 ± 29.3	0.257
HCV infection	0	0	
Living unrelated donor	14 (36.8%)	9 (30.0%)	0.613
IFG or IGT	14 (36.8%)	13 (43.3%)	0.625
Calcineurin inhibitor			0.388
CsA	31 (81.6%)	21 (70.0%)	
Tacrolimus	7 (18.4%)	9 (30.0%)	
Hypertension	36 (94.7%)	26 (86.7%)	0.394
CCB	25 (65.8%)	20 (66.7%)	1.00
ARB or ACEi	10 (26.3%)	6 (20.0%)	0.579
BB	12 (31.6%)	13 (43.3%)	0.448
Antiplatelet therapy	1 (2.6%)	2 (6.7%)	0.579
Statin use	15 (39.5%)	13 (43.3%)	0.807
Acute rejection	1 (2.6%)	2 (6.7%)	0.579

M, male; F, female; HCV, hepatitis C virus; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; CsA, cyclosporine; CCB, calcium channel blocker; ARB, angiotensin receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; BB, beta blocker. [a]Family history of diabetes in a first-degree relative.

and was gradually tapered to 10 mg/day within 1 month. For acute rejection, a total of 2 g methylprednisolone was administered for 5 days. For patients on the triple regimen, the target plasma trough levels of CsA and tacrolimus were lower than for those on the double regimen: 75–100 ng/ml for CsA and 5–10 ng/ml for tacrolimus.

Statistical analyses

We calculated the necessary sample size based on a study of the effects of pioglitazone on carotid IMT in type 2 diabetes patients and a study of changes in carotid IMT during the early post-transplant period [11,21]. Calculations of sample size were based on the change of carotid IMT in both groups (control group = 0.022 ± 0.0437 mm vs pioglitazone group = −0.084 ± 0.1674 mm). It was predicted that a final sample size of 48 participants would be required for 90% power at $P = 0.05$. In anticipation of a 20% dropout rate, we intended to recruit at least 60 participants.

All continuous variables were expressed as mean ± standard deviation (SD). For continuous data, the independent t-test was used. The χ^2 test was implemented for categorical data as appropriate. An independent t-test was used to analyse the differences between the pioglitazone and control groups with regard to changes from baseline to end-point measurements, and changes within the groups were analysed by paired t-tests.

The relationships between IMT and variables likely to have an influence were assessed by univariate regression analysis, followed by multivariate regression analysis to evaluate the independent association of each with IMT. Stepwise multivariate analyses were performed with baseline IMT values and each variable with a P-value <0.20 according to the univariate analysis.

All statistical tests of significance were two-tailed, and P-value <0.05 was considered significant. All analyses were performed using SPSS for Windows (version 12; SPSS, Chicago, IL).

Table 2. Comparison of the cumulative dose of prednisolone, plasma tacrolimus, CsA trough level and dose during the study period

	Control	Pioglitazone	*P*-value
Cumulative steroid dose (g/1 year)	(*n* = 38)	(*n* = 30)	
	6.20 ± 0.65	6.48 ± 1.00	0.170
Plasma tacrolimus trough level (ng/ml)	(*n* = 7)	(*n* = 9)	
1 month	11.9 ± 5.1	12.0 ± 3.0	0.968
3 months	11.8 ± 3.5	10.8 ± 3.4	0.579
6 months	9.1 ± 2.4	10.4 ± 1.9	0.266
9 months	8.5 ± 2.4	9.4 ± 3.7	0.597
12 months	7.7 ± 0.8	8.1 ± 3.9	0.763
Plasma tacrolimus dose (ng/ml)			
1 month	9.1 ± 2.9	11.1 ± 4.0	0.274
3 months	8.6 ± 3.3	8.7 ± 3.2	0.954
6 months	7.3 ± 3.0	7.6 ± 3.2	0.866
9 months	7.0 ± 3.1	7.6 ± 4.2	0.763
12 months	6.4 ± 2.8	6.8 ± 3.8	0.809
Plasma CsA trough level (ng/ml)	(*n* = 31)	(*n* = 21)	
1 month	180.2 ± 59.0	198.9 ± 41.2	0.342
3 months	144.9 ± 41.7	158.1 ± 30.9	0.348
6 months	140.9 ± 41.5	159.7 ± 37.1	0.216
9 months	119.3 ± 32.2	140.4 ± 29.0	0.080
12 months	128.1 ± 53.9	129.5 ± 28.2	0.934
Plasma CsA dose (ng/ml)			
1 month	253.6 ± 51.8	283.9 ± 69.1	0.201
3 months	232.1 ± 44.3	258.9 ± 73.8	0.257
6 months	226.8 ± 47.5	233.9 ± 63.3	0.738
9 months	221.4 ± 45.8	219.6 ± 50.2	0.922
12 months	203.7 ± 69.0	216.1 ± 53.4	0.601

Data are expressed as mean ± SD. CsA, cyclosporine.

Results

Subjects and safety

The disposition of patients in the trial is shown in Figure 1. Four subjects who were assigned to the pioglitazone group withdrew consent before receiving the drug. Five subjects in the control group refused to undergo a follow-up OGTT at 6 months after transplantation. In the pioglitazone group, four subjects withdrew from the study for personal reasons, three within 1 week and one 4 weeks after the start of the study. One withdrew after 5 weeks of treatment because of mild lower-extremity oedema; another subject withdrew after 5 months of treatment because of *Pneumocystis carinii* pneumonia. Another two patients in the pioglitazone group developed mild lower-extremity oedema but did not discontinue pioglitazone. No other clinical or laboratory adverse events were associated with the use of pioglitazone.

The two groups were similar with respect to baseline clinical characteristics (Table 1). There was no significant difference in CsA or tacrolimus levels, doses or the cumulative dose of prednisolone over 1 year between the control and pioglitazone groups (Table 2). There was no significant difference in creatinine, AST and ALT levels between both groups (Table 3). The rate of acute rejection during the 12-month period for the control group and pioglitazone group was 10.5% and 13.3%, respectively ($P = 0.724$).

Changes in metabolic parameters and adipocytokines after treatment

In both groups, the levels of fasting plasma glucose, HbA1c, total cholesterol, high-density lipoprotein (HDL) cholesterol and LDL cholesterol increased, while the levels of hsCRP decreased at 1 year after transplantation (Table 4). Compared to the control group, the pioglitazone group had significantly lower increases of triglyceride, free fatty acid (FFA) and LDL cholesterol levels (Table 4).

Adiponectin levels declined in the control group and increased in the pioglitazone group after transplant ($P < 0.001$). Levels of resistin were lower in both groups compared with levels before transplantation, but the differences of resistin were not significant in either group.

Changes in carotid IMT

Subjects in the control group showed higher maximum carotid IMT but no significant changes in mean carotid IMT during the 12-month study period (Table 4). The mean and maximum carotid IMT of the pioglitazone group decreased 0.05 ± 0.04 and 0.08 ± 0.05 mm, respectively, from baseline after 12 months ($P < 0.001$ for both).

As shown in Table 5, a univariate regression analysis found that pioglitazone treatment and changes in adiponectin are associated with changes in carotid IMT ($P < 0.05$). Multivariate regression analysis showed that pioglitazone treatment is an independent factor associated with carotid IMT changes after adjusting for baseline IMT and changes in adiponectin, ISI_{TX} and FFA (Table 6). When we repeated the multivariate analysis using the same variables except for pioglitazone treatment, the change in adiponectin was the only significant factor associated with carotid IMT changes.

Table 3. Comparison of hepatic function and graft function during the study period

	Control (*n* = 38)			Pioglitazone (*n* = 30)			*P* [b]-value (absolute change, between groups)
	Initial	After 1 year	*P*[a]-value	Initial	After 1 year	*P*[a]-value	
AST	16.0 ± 6.2	18.1 ± 3.9	0.060	16.6 ± 7.1	19.4 ± 5.2	0.063	0.559
ALT	16.7 ± 8.9	20.2 ± 8.9	0.063	15.6 ± 8.5	17.4 ± 7.2	0.337	0.450
Creatinine	1.30 ± 0.29	1.35 ± 0.40	0.401	1.41 ± 0.26	1.33 ± 0.36	0.144	0.113

Data are expressed as mean ± SD. AST, aspartate aminotransferase; ALT, alanine aminotransferase. [a]*P*-values are for the comparison of variables from the beginning (2 weeks after transplantation) to the end point (1 year) in each group. [b]*P*-values are for the comparison of the absolute change in each variable between the pioglitazone and control groups.

Table 4. Changes in clinical and metabolic parameters in the control and pioglitazone groups

	Control (n = 38)			Pioglitazone (n = 30)			P [b]-value (absolute change, between groups)
	Before	After 1 year	P[a]-value	Before	After 1 year	P[a]-value	
Body weight (kg)	58.6 ± 13.5	60.9 ± 10.1	0.175	59.7 ± 10.5	61.2 ± 10.2	0.070	0.600
BMI (kg/m^2)	21.4 ± 4.2	22.3 ± 2.8	0.155	21.2 ± 2.4	21.7 ± 2.4	0.057	0.593
Systolic BP (mmHg)	136.6 ± 17.0	125.7 ± 12.0	0.002	134.6 ± 10.9	124.7 ± 12.8	0.001	0.805
Diastolic BP (mmHg)	85.9 ± 9.4	79.3 ± 10.9	0.005	85.2 ± 9.0	74.4 ± 7.8	<0.001	0.141
FPG (mmol/l)	4.61 ± 0.58	5.34 ± 0.75	<0.001	4.61 ± 0.52	5.21 ± 0.96	0.002	0.349
Fasting insulin (pmol/l)	40.9 ± 33.8	82.4 ± 36.8	<0.001	39.2 ± 24.1	58.4 ± 25.1	0.282	0.024
Glucose AUC (mmol/l×2h)	30.8 ± 6.6	30.0 ± 5.2	0.479	31.1 ± 5.5	30.6 ± 8.6	0.786	0.910
Insulin AUC (pmol/l×2h)	1341 ± 541	2050 ± 1319	0.003	1300 ± 729	1268 ± 728	0.842	0.009
HbA1c (%)	4.96 ± 0.50	5.63 ± 0.52	<0.001	4.95 ± 0.56	5.65 ± 0.53	<0.001	0.809
Total cholesterol (mmol/l)	4.11 ± 0.91	5.22 ± 0.85	<0.001	4.08 ± 0.79	5.05 ± 0.73	<0.001	0.527
Triglyceride (mmol/l)	1.89 ± 1.05	2.42 ± 1.91	0.136	2.16 ± 1.22	1.61 ± 0.70	0.012	0.010
HDL cholesterol (mmol/l)	1.04 ± 0.24	1.62 ± 0.36	<0.001	1.15 ± 0.31	1.87 ± 0.46	<0.001	0.186
LDL cholesterol (mmol/l)	2.48 ± 0.74	3.35 ± 0.83	<0.001	2.38 ± 0.56	2.74 ± 0.58	0.014	0.018
FFA (μEq/l)	326 ± 212	540 ± 231	0.001	447 ± 241	440 ± 174	0.875	0.004
hsCRP (mg/l)	3.02 ± 7.22	0.21 ± 0.35	0.043	4.72 ± 9.49	0.48 ± 1.08	0.022	0.520
Adiponectin (μg/ml)	32.35 ± 10.70	20.40 ± 11.74	0.001	24.86 ± 10.48	73.06 ± 35.91	<0.001	<0.001
Resistin (ng/ml)	50.50 ± 25.58	25.83 ± 11.62	<0.001	41.39 ± 39.39	18.10 ± 8.04	0.014	0.884
Mean carotid IMT (mm)	0.54 ± 0.06	0.57 ± 0.10	0.062	0.53 ± 0.09	0.49 ± 0.08	<0.001	<0.001
Maximum carotid IMT (mm)	0.65 ± 0.07	0.70 ± 0.13	0.010	0.67 ± 0.10	0.59 ± 0.09	<0.001	<0.001

Data are expressed as mean ± SD. BP, blood pressure; BMI, body mass index; FPG, fasting plasma glucose; AUC, area under the curve; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid; IMT, intima–media thickness. [a]*P*-values are for the comparison of variables from baseline (before transplantation) to the end point (1 year) in each group. [b]*P*-values are for the comparison of the absolute change in each variable between the pioglitazone and control groups.

Changes in glucose tolerance, insulin sensitivity and secretion

The prevalence of PTDM was lower at 12 months after renal transplantation than at 6 months, but there was no difference in the prevalence of PTDM between the two groups (P = 0.494; Figure 2).

Although there was no significant difference in the mean total glucose concentration during OGTT (AUC_{glc}) in either group, pioglitazone lessened the increase of mean total insulin concentration during OGTT (AUC_{Ins}) after transplantation (Table 4, Figure 3). In the control group, the estimated first phase insulin secretion (from 1052 ± 437 to 1649 ± 887, $P < 0.001$), second phase insulin secretion (from 3522 ± 2130 to 6462 ± 4253, $P < 0.001$) and Sec_{AUC} (from 44 ± 17 to 68 ± 37, $P < 0.001$) increased significantly 1 year after transplantation. These secretory function indices did not change significantly in the pioglitazone group. Pioglitazone treatment increased the ISI_{TX} level in comparison with that of the control group (pioglitazone vs control $-0.8 \pm 3.0 \times 10^{-2}$ vs $+1.1 \pm 3.7 \times 10^{-2}$, $P = 0.036$).

Table 5. Univariate regression analyses with the change in carotid IMT as a dependent variable and clinical parameters as independent variables

Variables	Δ Mean carotid IMT		Δ Maximum carotid IMT	
	β	P	β	P
Age	0.001	0.430	0.001	0.494
Sex[a]	−0.005	0.794	−0.007	0.814
Aspirin use[b]	0.013	0.787	0.042	0.554
Acute rejection[b]	0.031	0.337	0.007	0.877
Pioglitazone treatment[b]	0.075	<0.001	0.133	<0.001
Δ Systolic BP	<0.001	0.749	−0.001	0.367
Δ Triglyceride	9.065	0.237	9.945	0.369
Δ LDL cholesterol	<0.001	0.605	<0.001	0.678
Δ FFA	5.050	0.175	3.945	0.462
Δ hsCRP	0.001	0.460	0.001	0.547
Δ Adiponectin	−0.001	0.011	−0.001	0.001
Δ Resistin	<0.001	0.500	<0.001	0.383
Δ SecrAUC	8.856	0.776	<0.001	0.670
Δ ISI_{TX}	−0.453	0.138	−0.577	0.185

Δ indicates change of each parameter between baseline and 12 months. IMT, intima–media thickness; BP, blood pressure; LDL, low-density lipoprotein; FFA, free fatty acid; SecrAUC, secretion area under the curve; ISI_{tx}, insulin sensitivity index for transplantation. [a]0 = male; 1 = female. [b]0 = no; 1 = yes.

Discussion

The major findings of the present study are that pioglitazone treatment may reduce the progression of carotid IMT

Table 6. Multiple linear regression analyses with the change in carotid IMT as a dependent variable and clinical parameters as independent variables

Variable	Δ Mean carotid IMT		Δ Maximum carotid IMT	
	β	P	β	P
Step 1				
Pioglitazone treatment[a]	0.091	0.002	0.149	<0.001
Step 2				
Δ Adiponectin	−0.001	0.016	−0.002	0.001

In Step 1, stepwise multivariate analyses were performed with pioglitazone treatment, baseline IMT, Δ adiponectin, Δ ISI_{TX} and Δ FFA as independent variables. In Step 2, stepwise multivariate analyses were performed with baseline IMT values, Δ adiponectin, Δ ISI_{TX} and Δ FFA as independent variables. Δ indicates change of each parameter between baseline and 12 months. [a]0 = no; 1 = yes.

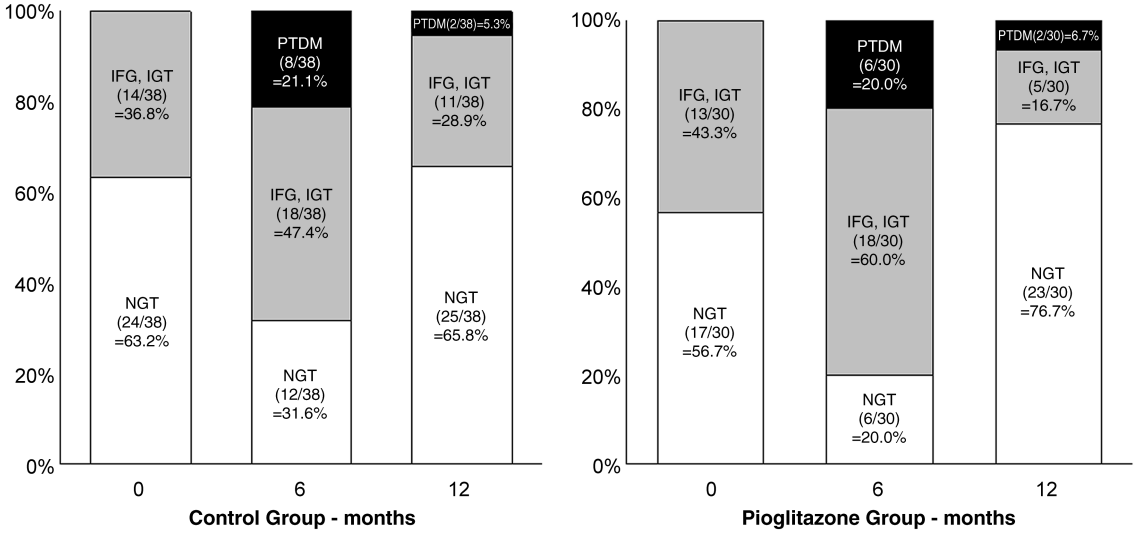


Fig. 2. Change in the glucose tolerance category from baseline to 12 months after renal transplantation. NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance, PTDM, post-transplant diabetes mellitus.

and that it may improve insulin resistance in renal allograft recipients without a history of diabetes.

It is estimated that CVDs (primarily atherosclerosis and coronary artery disease) are responsible for 40–55% of all deaths after kidney transplantation [22]. Carotid IMT has been established as an early marker of atherosclerosis and has been associated with the prevalence and incidence of CVD. Carotid IMT in transplant patients is significantly greater in the short period after renal transplantation than in healthy individuals [23]. The rate of carotid IMT increase is high during the first 6 months after transplantation, even in asymptomatic patients without major cardiovascular risk factors [21]. Although our observed baseline carotid IMT was relatively thin compared to that of previous studies because of the large proportion of young renal allograft patients in our study, the control group showed a significant increase in maximum carotid IMT 1 year after transplantation compared with that at baseline [21,23]. In contrast, patients receiving pioglitazone had a thinner carotid IMT at 12 months than at baseline.

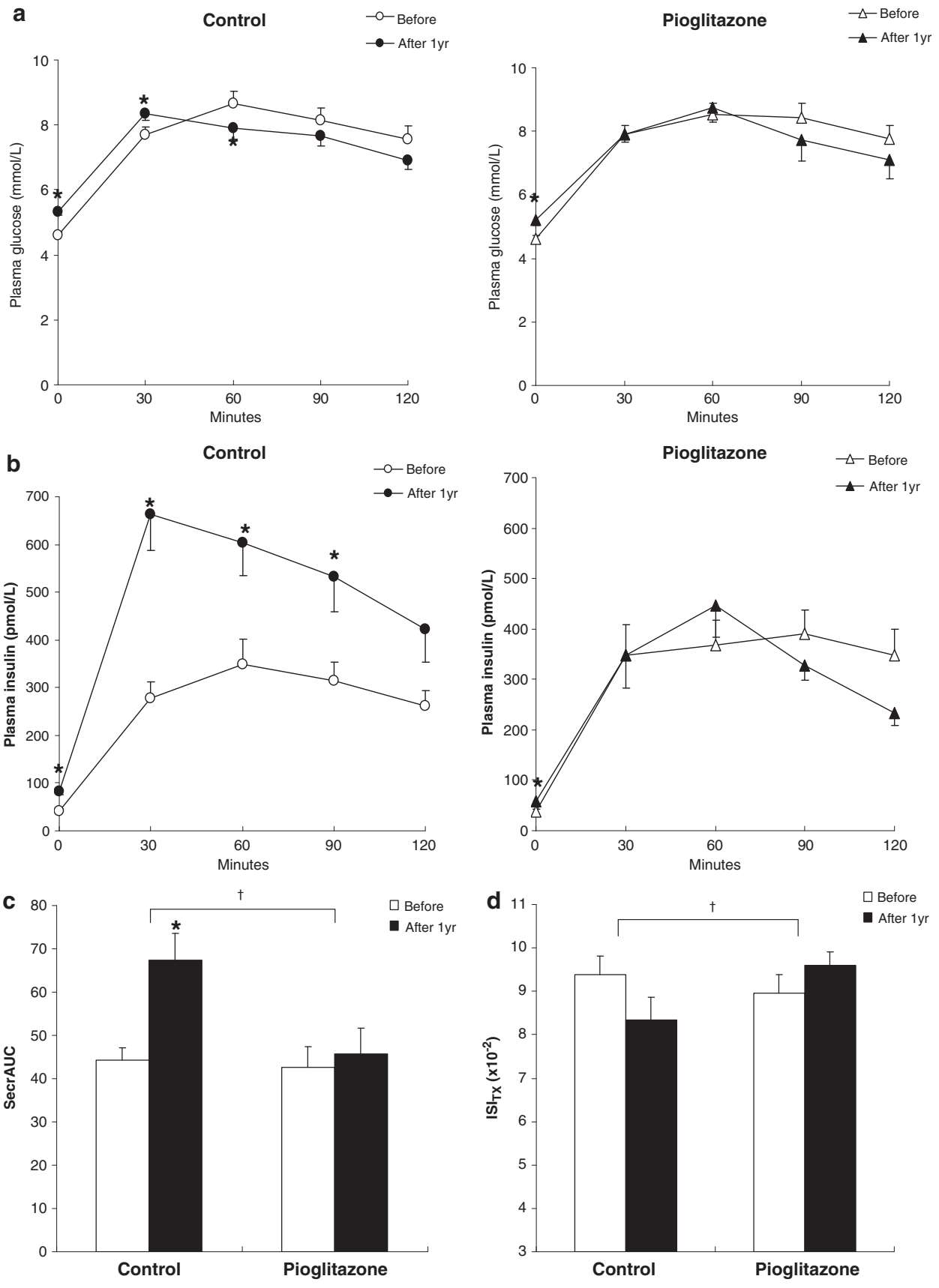
Until now, clinical trials with statin drugs and homocysteine-lowering therapy have shown a regression of carotid IMT in renal transplant recipients [16,24]. However, to our knowledge, no previous studies have examined the effects of pioglitazone on carotid IMT in renal transplant recipients. The observed beneficial effect of pioglitazone on carotid IMT is consistent with the findings of the studies of type 2 diabetes patients [9,11]. Moreover, Nissen *et al.* reported recently that treatment with pioglitazone resulted in a significantly lower rate of coronary atherosclerosis progression than glimepiride in patients with type 2 diabetes and coronary artery disease [25].

Pioglitazone increased the adiponectin level, and the change in adiponectin was negatively correlated with carotid IMT changes in our study. Pioglitazone treatment was the only independent parameter associated with changes in carotid IMT in the multivariate analysis. However, when the multivariate analysis was repeated using the same variables except for pioglitazone treatment, which was omitted to exclude the effects of pioglitazone on the other variables, the change in adiponectin was an independent factor associated with changes in carotid IMT. Based on these results, we believe that pioglitazone may improve carotid IMT by increasing adiponectin in renal allograft recipients. Adiponectin has insulin-sensitizing, anti-atherosclerotic and anti-inflammatory properties. Adiponectin decreases the expression of adhesion molecules in endothelial cells in response to inflammatory stimuli (including tumor necrosis factor-α), suppresses cytokine production in macrophages and inhibits the proliferation of monocytes [26].

It appears that both the pioglitazone group and control group showed reductions in hsCRP. These results are consistent with those of previous studies [27,28]. End-stage renal disease (ESRD) *per se* has been considered pro-inflammatory with an ongoing acute phase response. When renal function improves with a functioning renal transplantation, it is expected that inflammation may be resolved or improved.

Uraemia is typically associated with impaired glucose metabolism. Insulin resistance and hyperinsulinaemia have been demonstrated in patients with chronic renal failure without clinical diabetes [29]. In contrast, spontaneous hypoglycaemia is a complication in both nondiabetic and diabetic patients with renal failure. While the evidence regarding the importance of impaired glucose tolerance before transplantation is conflicted, a systemic review of the literature suggests that immunosuppressive drugs account for 74% of the risk for PTDM development [30]. Nonetheless, when resolving insulin resistance caused by uraemia after renal transplantation, the use of immunosuppressive drugs was an important factor in the development of PTDM.

We hypothesized that pioglitazone would reduce the incidence of PTDM by improving insulin resistance, which



contributes to the development of glucose intolerance following renal transplantation. Pioglitazone enhanced insulin sensitivity, thus reducing the demand for insulin secretion in renal allograft patients. Our results are consistent with the theory that a class effect of thiazolidinediones reduces the glucose-stimulated insulin secretion rate and increases insulin clearance in nondiabetic, insulin-resistant individuals by enhancing insulin sensitivity [31]. Voytovich *et al.* showed that 4 weeks of rosiglitazone treatment increased insulin sensitivity but did not change the SecrAUC and estimated first and second phase insulin secretion rates in renal allograft recipients with glucose intolerance [8]. These results are similar to those obtained in our study; however, the fasting glucose and glucose AUC were significantly reduced after rosiglitazone treatment. It is possible that the subjects of this study comprised seven recipients with PTDM and three with IGT.

We were unable to find any protective effects of pioglitazone on the development of PTDM in our study. Based on previous studies, impaired insulin secretion is the important factor in the development of PTDM [32–34]. Our control group showed significantly increased insulin secretion indices (SecrAUC, Secr1PH and Secr2PH) at 1 year after transplantation, indicating that our control group compensated relatively well for insulin action defects and remained normoglycaemic until 1 year after transplantation. Thus, the incidence of PTDM in the control group may be low and we cannot find the difference of the incidence of PTDM in both groups. We believe that, after long-term follow-up, the control group may have an increased incidence of PTDM compared with the pioglitazone group. Therefore, we cannot exclude the possibility that the absence of a protective effect of pioglitazone on the development of PTDM is due to the recent low incidence of PTDM, the small number of subjects and the short follow-up duration in this study.

Pioglitazone, which does not induce CYP3A4, is known to have no significant impact on kidney function and tacrolimus levels or doses in renal transplant recipients [7,35]. Moreover, recent studies have shown that thiazolidinedione administration was associated with some benefits in an animal model of chronic CsA nephrotoxicity [36,37]. We found that pioglitazone did not have any relevant effect on CsA or tacrolimus levels or doses. Renal and hepatic function also remained unchanged during pioglitazone treatment. Three subjects in the pioglitazone group developed mild lower-extremity oedema.

Our study has some limitations. First, our study population had normal carotid IMT at baseline, and the change in carotid IMT after pioglitazone treatment was relatively small. Second, pioglitazone may have a direct effect on arterial tension, so carotid IMT may be affected by wall tension. Measuring the carotid intima–media area may be helpful to rule out this effect. Third, dialysis and ESRD can alter glucose metabolism, so it may cause errors to include or exclude diabetic patients during enrollment [38]. Fourth, the glucose-lowering effect of pioglitazone was not definitively found in renal allograft recipients. Finally, the sample size was small and the follow-up period was relatively short. Despite such limitations, we believe that our results are valuable in showing the protective effect of pioglitazone on cardiovascular risk in nondiabetic renal allograft recipients.

In conclusion, pioglitazone treatment decreased the progression of subclinical atherosclerosis and insulin resistance in nondiabetic renal allograft recipients. The beneficial effects of pioglitazone may help to diminish the risk of post-transplant CVD. Larger controlled trials of longer duration are warranted to assess the preventive effect of pioglitazone on atherosclerosis, glucose intolerance and safety.

Conflict of interest statement. None declared. The results presented in this paper have not been published previously in whole or part, except in abstract form.

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Fig. 3. Changes in plasma glucose (**a**) and insulin (**b**) concentrations during a 75-g oral glucose tolerance test (OGTT), estimated insulin secretion (**c**) and insulin sensitivity (**d**). Panels a and b—control group before transplantation (white circle), 1 year after transplantation (black circle); pioglitazone group before transplantation (white triangle), 1 year after transplantation (black triangle). $^{*}P < 0.05$, compared with the value before transplantation at each time point by a paired *t*-test. Panels c and d—before transplantation (white bar); 1 year after transplantation (black bar). $^{*}P < 0.05$, compared with the value before transplantation by a paired *t*-test, $^{\dagger}P < 0.05$, compared with the difference between the pioglitazone and control group with regard to changes from baseline to end-point measurements by an independent *t*-test. Data are expressed as mean ± SE. See equations in text for additional details.

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