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Effect of Pioglitazone on Progression of Subclinical Atherosclerosis in Non-Diabetic Premenopausal Hispanic Women with Prior Gestational Diabetes

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Abstract

The Pioglitazone in the Prevention of Diabetes (PIPOD) study was a single arm 3-year open-label pioglitazone treatment to determine the effects of pioglitazone in women with prior gestational diabetes mellitus (GDM) who had completed the Troglitazone in the Prevention of Diabetes (TRIPOD) study. Here we report the results on progression of subclinical atherosclerosis, measured by carotid intima-media thickness (CINT) in non-diabetic women. Data were analyzed to compare CINT progression rates during pioglitazone treatment to rates that had been observed during either placebo or troglitazone treatment in the TRIPOD study. Sixty-one women met the entry criteria with mean age of 40 years. In the 30 women who came to PIPOD from the placebo arm of TRIPOD, the CINT rate was 69% lower during pioglitazone treatment than it had been during placebo (0.0031 vs. 0.0100 mm/yr, $p=0.006$). In the 31 women who came to PIPOD from the troglitazone arm of TRIPOD, CINT rate was 38% lower during pioglitazone than it had been during troglitazone, a difference that was not statistically significant (0.0037 vs. 0.0060 mm/year; $p=0.26$). Adjustment for differences in baseline characteristics and potential on-trial confounders did not alter the conclusion but did increase the CINT rates differences slightly. We conclude that treatment with pioglitazone slowed CINT progression in women who had been on placebo in the TRIPOD study and maintained a relatively low rate of progression in women who had been on troglitazone. Pioglitazone slows progression of subclinical atherosclerosis in young Hispanic women at increased risk for type 2 diabetes.

Keywords

Pioglitazone; intima-media thickness; premenopausal; gestational diabetes; atherosclerosis

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INTRODUCTION

Clinical complications of atherosclerosis are the leading cause of death and a major cause of morbidity in people with diabetes mellitus. Clinical events such as myocardial infarction and stroke are the result of two related but separate processes - decades-long development of atherosclerosis, followed by acute arterial occlusion superimposed on the atherosclerosis. Mechanistic studies of thiazolidinedione drugs have provided a fairly broad base of evidence that these drugs could have beneficial effects on atherosclerosis. For example, members of the class have been shown to improve endothelial-dependent vasodilation [1–2], reduce production of PAI-I [3], reduce endothelial proliferation after intimal injury [4], and reduce markers of inflammation [5,6]. Clinically, thiazolidinediones (TZDs) have had little [7] or no [8] beneficial effects on the risk of acute cardiovascular events in cohorts with average ages in the 50–60s or higher. On the other hand, at least three members of the class have been shown to reduce carotid intima-media thickness in individuals with established diabetes [9–14] and in non-diabetic individuals with known coronary disease [15]. Reasons for the apparent dissociation between promising mechanistic and CIMT effects of TZDs and their lack of impact on clinical cardiovascular events remain unexplained, but could be due to a dissociation between antiatherogenic effects of the drugs and their impact on mechanisms for acute arterial occlusion. If that was the case, then early, rather than late use of TZDs would be of potential clinical importance in prevention of clinical atherosclerotic events.

Our group has studied the evolution of both diabetes and atherosclerosis in relatively young but very high risk Hispanic women with a recent history of gestational diabetes. In a cohort of those women whose average age was ~35 years when they entered the Troglitazone in the Prevention of Diabetes (TRIPOD) study, we observed that troglitazone, a TZD no longer available for clinical use, significantly reduced the rate of carotid artery-intima-media thickness (CIMT) progression by 31% compared to placebo [16]. This anti-atherogenic effect in relatively young individuals could represent the real potential for TZDs to alter the natural history of atherosclerosis in high-risk individuals. In the present paper we report the effects of a clinically available TZD, pioglitazone on rates of CIMT progression in women who completed the TRIPOD study.

RESEARCH DESIGN AND METHODS

Subjects

The Pioglitazone in the Prevention of Diabetes (PIPOD) study was a single arm open-label pioglitazone treatment study designed to determine the effects of pioglitazone in women with prior gestational diabetes mellitus (GDM) who had completed the TRIPOD study. Thus, all subjects were initially recruited for the TRIPOD study, the design of which has been published [16–18]. Briefly, Hispanic women of Mexican, Guatemalan or El Salvadoran descent with a recent history of GDM were randomized to troglitazone, 400 mg/d, or placebo. An intravenous glucose tolerance test (IVGTT) was performed prior to randomization to assess baseline insulin sensitivity and pancreatic β -cell function. Fasting glucose was measured at three-month intervals and oral glucose tolerance tests (OGTTs) were performed annually to detect diabetes using American Diabetes Association criteria [19]. Measurements of height, weight, sitting blood pressure, fasting serum lipids and CIMT were performed at the times of OGTTs. Treatment with blinded medication continued in each subject until she developed diabetes or until March 2000 when troglitazone was withdrawn from human use, at which time all subjects stopped study medications.

Subjects in the TRIPOD study were asked to return for an OGTT, IVGTT, CIMT and associated blood pressure, lipids and morphometric measurements approximately eight months after study medications were stopped. For women whose hemoglobin A_{1C} was <7%, this visit constituted

the baseline visit for the PIPOD study. The present report of the effect of pioglitazone on CIMT is based on the 61 women who did not develop diabetes during the TRIPOD study and who had at least one follow-up CIMT measurement during the PIPOD study. All participants gave written informed consent for participation in both of these Institutional Review Board-approved studies.

Study Protocol

Subjects who agreed to enroll in the PIPOD study received dietary advice and were advised to walk for 30 minutes three times each week, as they had been at annual visits during the TRIPOD study. They were started on pioglitazone, 30 mg/d for two months. Since there was no clinical evidence of fluid retention at this dose, the dose was increased to 45 mg/d for the remainder of a 3-year treatment period for each subject. Follow-up visits were scheduled every two months during the first year and every three months thereafter. Fasting glucose was measured at each visit. OGTTs and measurements of CIMT, sitting blood pressure and serum lipids were performed annually. Subjects remained on treatment unless the A_{1C} exceeded 7%, at which time final testing was performed. Medication was stopped for women who became pregnant and resumed after delivery and completion of breastfeeding. OGTT and CIMT testing for those subjects were done at least four months after delivery and at least one month after completion of breastfeeding.

Clinical Testing Protocols

Height was measured with a stadiometer. Weight was measured on a calibrated beam balance. Blood pressure was measured in triplicate with a Dinamap (Critikon, Inc. Tampa, FL) after subjects had been sitting with legs dangling for ≥ 5 minutes.

OGTTs and IVGTTs were performed on separate days and initiated between 7–9 am, after an 8–12 hour overnight fast. For OGTTs, subjects drank 75 grams of dextrose. Venous blood was sampled from an indwelling catheter before and 30, 60, 90 and 120 minutes after the dextrose ingestion. For IVGTTs, dextrose (300 mg/kg body weight) was injected into an antecubital vein. Tolbutamide (Orinase Diagnostic, Pharmacia & Upjohn, Peapack, NJ), 125 mg/m² body surface area, was injected 20 minutes later. Twenty-two arterialized venous blood samples were drawn and placed on ice before and for up to 240 min after the dextrose injection. For both tests, plasma was separated within 20 minutes and stored at -80°C .

High resolution B-mode carotid artery ultrasounds for the purpose of measuring the intima-media thickness were obtained using a Toshiba SSH 140A (Toshiba Corp., Tokyo, Japan) ultrasound system with a linear array 7.5 MHz transducer as described previously [20,21]. In brief, the common carotid artery (CCA) was first imaged in cross section with the jugular vein juxtaposed above the carotid artery. The scanhead was then rotated 90 degrees around the central image line, maintaining the jugular vein stacked above the CCA while obtaining a longitudinal view of both vessels. The proximal portion of the carotid bulb was included in all images as an anatomical reference point for standardization of IMT measurements. The electrocardiogram signal and ultrasound images were simultaneously recorded on SVHS videotape. Minimum gain necessary for clear visualization of structures was used and all initial instrumentation settings were recorded and used in subsequent scans. Each individual's baseline image was used as an on-line guide for follow-up examinations on a split-screen system designed for repeat image acquisition for longitudinal studies (Patents 2005, 2006) as part of the standardization protocol. Ultrasound examinations and image processing were conducted without knowledge of TRIPOD treatment status. The computer system and software program (Prowin, Patents 2005, 2006) used to measure CIMT utilized automated boundary detection to locate the lumen-intima and media-adventitia echo boundaries at sub-pixel resolution [22,23]. The CIMT measurement consisted of an average of 70 to 100 individual

measurements made along a 1 cm distance in the far wall of the right distal CCA. The coefficient of variation for CIMT was 2.5% within and between operators [16].

Laboratory Methods

Glucose was measured by glucose oxidase (YSI Glucose Analyzer, Yellow Springs Instruments, Yellow Springs, OH). Insulin was measured by a radioimmunoassay (Linco Research, St. Charles, MO) that provided <0.2% cross-reactivity with proinsulin. Serum total cholesterol, total triglycerides (TG), and high-density lipoprotein (HDL)-cholesterol concentrations were determined by enzymatic assays and standardized to the Centers for Disease and Control and Prevention using the Lipid Research Clinic Protocol. HDL-cholesterol concentrations were measured after apolipoprotein B-containing lipoproteins were precipitated in whole plasma with heparin manganese chloride. Low-density lipoprotein (LDL)-cholesterol concentrations were estimated using the Friedewald equation [24].

Data Analysis

Whole body insulin sensitivity (S_I) was calculated from IVGTTs using the Bergman minimal model [25]. The acute insulin response to intravenous glucose (AIRg, the incremental insulin area between 0–10 min after the glucose injection) was used as a combined measure of β -cell mass [26] and function [27]. The product of AIRg and S_I (the “disposition index” or “DI”) was used as a measure of β -cell compensation for insulin resistance [28,29]. Areas under glucose and insulin curves were calculated using the trapezoid rule. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by the square of height in meters.

Average CIMT progression rates were estimated by regressing CIMT on follow-up time using linear random coefficients mixed-effect modeling. Both intercept and slope were specified as random effects to take into account individual baseline and slope differences. Linear trend in CIMT over the treatment period was tested and no significant deviations were found. No assumption of homogeneity of variances between groups or study periods was made. Group differences in CIMT rates were tested by examining the interaction between group and follow-up time in the mixed-effects model.

Given that all subjects were assigned to pioglitazone in the PIPOD study, we chose as the main analysis for this report a comparison of CIMT progression rates during PIPOD to rates previously observed during TRIPOD in each of the two subgroups of PIPOD subjects, those who had been randomized to placebo during TRIPOD and those who had been randomized to troglitazone. The non-independence of TRIPOD and PIPOD periods in each subject was accounted for in the mixed-effects model by specifying periods nested within subjects. CIMT progression rates were compared between TRIPOD and PIPOD treatment periods in three ways: (a) unadjusted, (b) adjusted for TRIPOD and PIPOD baseline differences in other characteristics and (c) adjusted for TRIPOD and PIPOD baseline differences as well as on-trial differences in potential confounders. To consider the impact of small sample size on significance, we elected to include variables that differed with a p-value ≤ 0.40 in the adjusted analyses. Baseline characteristics tested for such a difference were age; BMI; OGTT glucose and insulin areas; IVGTT S_I , AIRg and DI; systolic and diastolic blood pressure; and serum lipids. Testing was done with paired t-test, Wilcoxon signed rank test and repeated analysis of variance where log transformation was applied to OGTT insulin area, S_I , AIRg, DI and triglycerides. Consistent results were observed and p-values from paired t-tests are reported. Because IVGTTs were not performed annually during the trial, potential on-trial confounders considered for use in adjustments were OGTT glucose and insulin areas, blood pressure, lipids and weight gain. They were compared between TRIPOD and PIPOD periods as average rates of change using random coefficients mixed-effect modeling, as described above. To estimate the adjusted CIMT rates in each study, covariates were centered using the mean value of PIPOD

baseline in the adjusted analysis. SAS (SAS, Inc., Cary, NC) was used to perform all the analyses, and PROC MIXED was used for the mixed-effects model analyses.

Data are presented in tables and text as means and standard deviations or standard errors in their original scales. All reported p values are two-sided. A p-value of 0.05 was accepted as statistically significant.

RESULTS

Subjects

Of 82 women who completed TRIPOD on their assigned treatment without diabetes and, thus, were eligible for enrollment in PIPOD, 68 enrolled and had a baseline CIMT measurement. Sixty one women had at least one annual follow-up CIMT measurement to qualify them for inclusion in this CIMT analysis, none of them were pregnant during PIPOD period. Compared to the 21 women not included in the analysis (14 with no baseline CIMT measurement, seven with no follow-up measurement), the 61 women reported hereafter had slightly higher insulin sensitivity (S_I : $2.42 \pm 1.83 \times 10^{-4}$ vs. $1.60 \pm 0.97 \times 10^{-4} \text{ min}^{-1} \text{ per uU/ml}$, $p=0.06$) and slightly better β -cell function (disposition index: 931 ± 602 vs. 533 ± 423 , $p=0.07$). The two groups did not differ significantly in age, BMI, OGTT glucose or insulin levels, blood pressure or fasting lipid levels (all $p>0.14$). Of the 61 women who had at least one follow-up CIMT measurement, 31 were from the TRIPOD troglitazone group and 30 were from the TRIPOD placebo group. Mean insulin sensitivity in both groups was below the level of $4.45 \times 10^{-4} \text{ min}^{-1} \text{ per uU/ml}$ that we observed in 32 Hispanic women with BMI $<25 \text{ kg/m}^2$, normal glucose tolerance, and no history of GDM (Xiang, unpublished).

Baseline Characteristics

(Table 1): In the 31 women who had been randomized to troglitazone in TRIPOD, CIMT at the beginning of PIPOD had increased slightly, but not significantly compared to the beginning of TRIPOD. BMI, HDL and LDL cholesterol had increased significantly and total triglycerides had decreased significantly. Parameters of glucose and insulin metabolism had not changed significantly. In the 30 women who were randomized to placebo in TRIPOD, CIMT at the beginning of PIPOD was significantly higher than it had been at the start of TRIPOD. So were the OGTT insulin area and HDL cholesterol levels, while the acute insulin response and disposition index were significantly lower than they had been at the start of TRIPOD.

Changes in CIMT during the PIPOD Study

The median follow-up time on PIPOD was 3.0 years (range: 1.0 – 3.3 years). The CIMT progression rate for all subjects, estimated from random coefficients mixed-models, was $0.0034 \pm 0.0010 \text{ mm/yr}$ ($p=0.008$ vs. no change). CIMT progression rates during PIPOD did not differ appreciably between women who had been on troglitazone and women who had been on placebo in the TRIPOD study (0.0037 ± 0.0010 vs. $0.0031 \pm 0.0017 \text{ mm/yr}$, respectively; $p=0.67$). Comparison of the CIMT progression rates between TRIPOD and PIPOD periods by initial TRIPOD randomization status is shown in Figure 1. For the TRIPOD troglitazone group, the CIMT progression rate during the PIPOD study was 38% lower, but not significantly lower than the rate during the TRIPOD period ($0.0037 \pm 0.0010 \text{ mm/yr}$ vs. $0.0060 \pm 0.0018 \text{ mm/yr}$; difference, -0.0023 mm/yr ; 95% confidence interval [CI], -0.0062 to 0.0016 mm/yr ; $p=0.26$). By contrast, in the TRIPOD placebo group, the CIMT progression rate during PIPOD was 69% lower and significantly lower than the rate during TRIPOD ($0.0031 \pm 0.0017 \text{ mm/yr}$ vs. $0.0100 \pm 0.0017 \text{ mm/yr}$; difference, -0.0069 mm/yr ; 95% CI, -0.0116 to -0.0022 mm/yr ; $p=0.006$).

Adjustment for baseline differences between the TRIPOD and PIPOD studies that are shown in Table 1 with $p \leq 0.40$ slightly increased these CIMT rates differences. In the TRIPOD

troglitazone group, the adjusted CIMT rates were 0.0039 mm/yr during pioglitazone treatment and 0.0066 mm/yr during troglitazone treatment (difference, -0.0027 mm/yr; 95% CI, -0.0070 to 0.0016 mm/yr; $p=0.23$), after adjustment for baseline differences in age, BMI, OGTT glucose area, HDL-cholesterol and triglycerides. In the TRIPOD placebo group, the corresponding adjusted CIMT rates were 0.0028 mm/yr during pioglitazone treatment and 0.0107 mm/yr during placebo treatment (difference, -0.0079 mm/yr; 95% CI, -0.0130 to -0.0028 mm/yr; $p=0.004$) after adjustment for baseline differences in age, BMI, OGTT insulin area, SI, AIRg and HDL-cholesterol.

The impact of treatments on some potential modifiers of CIMT progression is shown in Table 2. For the TRIPOD troglitazone group, on-trial changes from baseline were similar in the TRIPOD and PIPOD treatment periods. Further adjustment for on-trial change differences in OGTT glucose area, OGTT insulin area, systolic blood pressure and LDL-cholesterol (all $p\leq 0.40$) further increased the difference in CIMT progression rate between PIPOD and TRIPOD (49% lower in PIPOD), with the difference remaining statistically insignificant (0.0036 vs. 0.0071 mm/yr; difference, -0.0035 mm/yr; 95% CI, -0.0080 to 0.0010 mm/yr; $p=0.13$). For the TRIPOD placebo group, weight gain was slightly greater during PIPOD than during TRIPOD ($p=0.06$) and OGTT insulin area fell in PIPOD but essentially unchanged in TRIPOD ($p=0.02$ between studies). After additional adjustment for these two on-trial differences plus diastolic blood pressure, LDL-cholesterol and triglycerides that reached $p\leq 0.40$, the difference in CIMT rate was 79% lower during pioglitazone treatment in the PIPOD study than it had been during placebo treatment in the TRIPOD study (0.0023 vs. 0.0110 mm/yr; difference, -0.0087 mm/yr; 95% CI, -0.0142 to -0.0032 mm/yr; $p=0.003$).

Five women developed diabetes during the PIPOD study period, 2 from the TRIPOD troglitazone group and 3 from the TRIPOD placebo group. Excluding these five individuals has no important impact on the pattern of CIMT differences between TRIPOD and PIPOD studies. CIMT progression remained lower, but not significantly so in women who had been on troglitazone in the TRIPOD study (0.0038 vs. 0.0070 mm/yr; $p=0.21$). CIMT progression remained lower during PIPOD than TRIPOD in women who had been on placebo in the TRIPOD study (0.0011 vs. 0.0107 mm/yr; $p=0.001$).

DISCUSSION

There are two main findings in this report. First, pioglitazone treatment was associated with a significant reduction in the rate of progression of CIMT compared to rates that had been observed in the same individuals during placebo treatment in the TRIPOD study. The reduction was slightly increased after adjustment for baseline differences in age, BMI, insulin sensitivity, insulin secretion, lipids and difference in on-trial weight change, insulin secretion, blood pressure and lipids. The difference remained significant after exclusion of three subjects who developed diabetes during PIPOD. This finding provides strong evidence for an effect of pioglitazone to change the natural history of CIMT progression in our relatively young subjects at high-risk for type 2 diabetes. In the absence of a parallel control group in PIPOD, we cannot rule out the possibility that slowing of CIMT progression would have been the natural history of CIMT progression in the absence of treatment. However, such a natural history would be at odds with data demonstrating that CIMT increases with increasing age in the absence of interventions [30].

The second main finding was a persistently low CIMT progression rate in women who had been on troglitazone treatment in TRIPOD and pioglitazone in PIPOD, even after the adjustment for differences in baseline age, BMI, glucose, lipids and differences in on-trial glucose, insulin secretion, blood pressure and lipids. The pattern was unchanged after excluding two subjects who developed diabetes during PIPOD. There is no way to exclude a

lingering effect of troglitazone as the explanation for their low CIMT progression rate during the PIPOD study. However, two observations speak against such an explanation. First, the CIMT progression rate during PIPOD was 49% lower than the rate that was observed during troglitazone treatment. Second, the progression rates in PIPOD were essentially the same in the former troglitazone and the former placebo groups from TRIPOD. Based on these findings, we conclude that pioglitazone reduced CIMT progression rates below what would be expected in the absence of treatment in young Hispanic women at high risk for type 2 diabetes. Whether this effect will lead to a reduction in the risk of acute cardiovascular events in 1–2 decades remains unknown.

There are two general mechanisms that could explain the low CIMT progression rates that were observed during pioglitazone treatment. The first general mechanism involves direct effects of PPAR- γ activation in the arterial wall [4,31]. The present study provides no evidence for or against that mechanism. The second general mechanism is indirect, i.e., a change in some circulating factor or factors that modify atherosclerosis. We could not identify an impact of glucose, insulin, standard lipids, blood pressure or body weight on differences in CIMT rates between the PIPOD and TRIPOD studies, suggesting that alterations in these known risks for CIMT progression do not explain the CIMT reduction associated with pioglitazone treatment. We came to a similar conclusion for troglitazone [16], so the findings are consistent between these two thiazolidinedione drugs in our study cohort. Alterations in circulating factors that we did not measure, such as LDL particle size [32], adiponectin levels [33,34], inflammatory markers [5,35,36] and/or plasminogen activator inhibitor-1 [3] remain as possible mediators of the effects of pioglitazone on CIMT progression.

The major strength of this study is the evaluation of the pioglitazone effect on atherosclerosis progression in a relatively young population (mean PIPOD study entry age was 40 years) who were insulin resistance and at risk for cardiovascular disease [37], but not diabetic at baseline and without clinical cardiovascular disease. Other groups have reported the effects of pioglitazone on CIMT. To our knowledge, all of these studies were in older individuals with type 2 diabetes. Koshiyama et al. [9] reported a significant reduction in CIMT in type 2 diabetic patients (mean age of 62 years) during 3–6 months of pioglitazone treatment compared to placebo treatment. Langefeld et al. [10] reported a significant reduction in CIMT in type 2 diabetic patients (mean age of 63 years) treated with pioglitazone as compared to glimepiride for 24-week, the reduction of CIMT was independent of glycemic control. Similarly, Mazzone et al. [13] demonstrated that pioglitazone slowed CIMT progression compared to glimepiride in type 2 diabetic patients (mean age of 60 years) over an 18-month treatment period. Pioglitazone has also been shown to reduce CIMT in normotensive type 2 diabetic patients with nephropathy [11]. Our results extend these findings to a relatively young population and demonstrate the potential for pioglitazone to modify atherosclerosis progression at a relatively young age and early stage of the disease. We recognize that there are two important limitations to this study: no randomized parallel control group in evaluating the pioglitazone effect and a relatively small sample size. However, our study provides the first results that demonstrate the anti-atherogenic effect by a clinically available TZD in a relatively young population who are at risk for cardiovascular disease. Further randomized controlled trials with a large sample size in young populations who are at risk for cardiovascular disease are needed to confirm our results. Longer follow-up time is needed to evaluate the ultimate effect on risk reduction of clinical cardiovascular events.

In summary, we found that treatment with pioglitazone slowed CIMT progression in women who had been on placebo treatment in the TRIPOD study and maintained a relatively low rate of CIMT progression in women who had been on troglitazone. The impact of pioglitazone on CIMT was not explained by alterations in body weight, blood pressure or circulating concentrations of glucose, insulin, or standard lipids. These findings indicate that pioglitazone

slows progression of subclinical atherosclerosis by a mechanism that remains to be determined. The effect was observed in relatively young premenopausal women who were free of diabetes and clinical evidence of cardiovascular disease, demonstrating the potential for modification of atherosclerosis at a relatively young age and early stage of atherosclerosis.

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Reference List

1. Suzuki M, Takamisawa I, Yoshimasa Y, Harano Y. Association between insulin resistance and endothelial dysfunction in type 2 diabetes and the effects of pioglitazone. *Diabetes Res Clin Pract* 2007;76(1):12–7. [PubMed: 17007957]
2. Esposito K, Ciotola M, Carleo D, Schisano B, Saccomanno F, Sasso FC, Cozzolino D, Assaloni R, Merante D, Ceriello A, Giugliano D. Effect of rosiglitazone on endothelial function and inflammatory markers in patients with the metabolic syndrome. *Diabetes Care* 2006;29(5):1071–6. [PubMed: 16644639]
3. Kruszynska YT, Yu JG, Olefsky JM, Sobel BE. Effects of troglitazone on blood concentrations of plasminogen activator inhibitor 1 in patients with type 2 diabetes and in lean and obese normal subjects. *Diabetes* 2000;49(4):633–9. [PubMed: 10871202]
4. Hsueh WA, Law RE. PPARgamma and atherosclerosis: effects on cell growth and movement. *Arterioscler Thromb Vasc Biol* 2001;21:1891–1895. [PubMed: 11742860]
5. Haffner SM, Greenberg AS, Weston WM, Chen H, Williams K, Freed MI. Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. *Circulation* 2002;106:679–684. [PubMed: 12163427]
6. Hanefeld M, Marx N, Pfützner A, Baurecht W, Lübken G, Karagiannis E, Stier U, Forst T. Anti-inflammatory effects of pioglitazone and/or simvastatin in high cardiovascular risk patients with elevated high sensitivity C-reactive protein: the PIOSTAT study. *J Am Coll Cardio* 2007;49(3):290–7.
7. Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005;366:1279–89. [PubMed: 16214598]
8. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007;356(24):2457–71. [PubMed: 17517853]
9. Koshiyama H, Shimono D, Kuwamura N, Minamikawa J, Nakamura Y. Inhibitory effect of pioglitazone on carotid arterial wall thickness in type 2 diabetes. *J Clin Endocrinol Metab* 2001;86:3452–3456. [PubMed: 11443224]
10. Langefeld MR, Forst T, Hohberg C, Kann P, Lubben G, Konrad T, Fullert SD, Sachara C, Pfützner A. Pioglitazone decreases carotid intima-media thickness independently of glycemic control in patients with type 2 diabetes mellitus - Results from a controlled randomized study. *Circulation* 2005;111:2525–2531. [PubMed: 15883215]
11. Nakamura T, Matsuda T, Kawagoe Y, Ogawa H, Takahashi Y, Sekizuka K, Koide H. Effect of pioglitazone on carotid intima-media thickness and arterial stiffness in type 2 diabetic nephropathy patients. *Metabolism* 2004;53:1382–1386. [PubMed: 15375799]
12. Hodis HN, Mack WJ, Zheng L, Li Y, Torres M, Sevilla D, Stewart Y, Hollen B, Garcia K, Alaupovic P, Buchanan TA. Effect of peroxisome proliferator-activated receptor gamma agonist treatment on subclinical atherosclerosis in patients with insulin-requiring type 2 diabetes. *Diabetes Care* 2006;29(7):1545–53. [PubMed: 16801577]

13. Mazzone T, Meyer PM, Feinstein SB, Davidson MH, Kondos GT, D'Agostino RB, Perez A, Provost JC, Haffner SM. Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in type 2 diabetes. *JAMA* 2006;296(21):2572–81. [PubMed: 17101640]
14. Minamikawa J, Tanaka S, Yamauchi M, Inoue D, Koshiyama H. Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes. *J Clin Endocrinol Metab* 1998;83:1818–120. [PubMed: 9589700]
15. Sidhu JS, Kaposzta Z, Markus HS, Kaski JC. Effect of rosiglitazone on common carotid intima-media thickness progression in coronary artery disease patients without diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2004;24:930–934. [PubMed: 15001452]
16. Xiang AH, Peters RK, Kjos SL, Ochoa C, Marroquin A, Goico J, Tan S, Wang C, Azen S, Liu C-R, Liu C-H, Hodis HN, Buchanan TA. Effect of thiazolidinedione treatment on progression of subclinical atherosclerosis in premenopausal women at high risk for type 2 diabetes. *J Clin Endocrinol Metab* 2005;90:1986–1991. [PubMed: 15623809]
17. Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, Ochoa C, Tan S, Berkowitz K, Hodis HN, Azen SP. Preservation of pancreatic B-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk Hispanic women. *Diabetes* 2002;51:2769–2803.
18. Azen SP, Berkowitz K, Kjos S, Peters R, Xiang A, Buchanan TA. TRIPOD: A randomized placebo-controlled trial of troglitazone in women with prior gestational diabetes mellitus. *Controlled Clinical Trials* 1998;19:217–231. [PubMed: 9551285]
19. American Diabetes Association: Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2004;27(Suppl 1):S5–S10. [PubMed: 14693921]
20. Hodis HN, Mack WJ, LaBree L, Mahrer PR, Sevanian A, Liu CR, Liu CH, Hwang J, Selzer RH, Azen SP. Vitamin E supplementation in healthy individuals reduces LDL oxidation but not atherosclerosis: the Vitamin E Atherosclerosis Prevention Study (VEAPS). *Circulation* 2002;106:1453–1459. [PubMed: 12234947]
21. Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, Selzer RH, Liu CR, Liu CH, Azen SP. for the Estrogen in the Prevention of Atherosclerosis Trial Research Group. Estrogen in the Prevention of Atherosclerosis: a randomized, double-blind placebo-controlled trial. *Ann Intern Med* 2001;135:939–953. [PubMed: 11730394]
22. Selzer RH, Mack WJ, Lee PL, Kwong-Fu H, Hodis HN. Improved common carotid elasticity and intima-media thickness measurement from computer analysis of sequential ultrasound frames. *Atherosclerosis* 2001;154:185–193. [PubMed: 11137099]
23. Selzer RH, Hodis HN, Kwong-Fu H, Mack WJ, Lee PL, Liu CR, Liu CH. Evaluation of computerized edge tracking for quantifying intima-media thickness of the common carotid artery from B-mode ultrasound images. *Atherosclerosis* 1994;111:1–11. [PubMed: 7840805]
24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502. [PubMed: 4337382]
25. Pacini G, Bergman RN. MINMOD: A computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed* 1986;23:113–122. [PubMed: 3640682]
26. Kjemis LL, Kirby BM, Welsh EM, Veldhuis JD, Straume M, McIntyre SS, Yang D, Lefebvre P, Butler PC. Decrease in beta-cell mass leads to impaired pulsatile insulin secretion, reduced postprandial hepatic insulin clearance, and relative hyperglucagonemia in the minipig. *Diabetes* 2001;50:2001–2012. [PubMed: 11522665]
27. Vague P, Moulin J-P. The defective glucose sensitivity of the B-cell in noninsulin dependent diabetes: improvement after twenty hours of normoglycemia. *Metabolism* 1982;31:139–142. [PubMed: 7043166]
28. Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose disposition in man. Measurement of insulin sensitivity and beta-cell sensitivity from the response to intravenous glucose. *J Clin Invest* 1981;68:1456–1467. [PubMed: 7033284]
29. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte D Jr. Quantification of the relationship between insulin sensitivity

- and B-cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 1993;42:1663–1672. [PubMed: 8405710]
30. Howard G, Sharett AR, Heiss G, Evans GW, Chambless LE, Riley WA, Burke GL. for the ARIC Investigators. Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. *Stroke* 1993;24:1297–1304. [PubMed: 8362421]
 31. Wakino S, Kintscher U, Kim S, Yin F, Hsueh WA, Law RE. Peroxisome proliferator-activated receptor γ ligands inhibit retinoblastoma phosphorylation and G1/S transition in vascular smooth muscle cells. *J Biol Chem* 2000;275:159–163. [PubMed: 10617600]
 32. Shand B, Scott R, Connolly S, Clarke R, Baker J, Elder P, Frampton C, Yeo J. Comparative study on the efficacy of pioglitazone in Caucasian and Maori-Polynesian patients with poorly controlled type 2 diabetes. *Diabetes Obes Metab* 2007;9(4):540–7. [PubMed: 17587396]
 33. Satoh N, Ogawa Y, Usui T, Tagami T, Kono S, Uesugi H, Sugiyama H, Sugawara A, Yamada K, Shimatsu A, Kuzuya H, Nakao K. Antiatherogenic effect of pioglitazone in type 2 diabetic patients irrespective of the responsiveness to its antidiabetic effect. *Diabetes Care* 2003;26:2493–2499. [PubMed: 12941708]
 34. Pajvani UB, Hawkins M, Combs P, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA, Scherer PE. Complex distribution, not absolute amount of adiponectin correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 2004;279:12152–12162. [PubMed: 14699128]
 35. Ghanim H, Garg R, Aljada A, Mohanty P, Kumbkarni Y, Assian E, Hamouda W, Dandona P. Suppression of nuclear factor- κ B and stimulation of inhibitor κ B by troglitazone: evidence for an anti-inflammatory effect and a potential antiatherosclerotic effect in the obese. *J Clin Endocrinol Metab* 2001;86:1306–1312. [PubMed: 11238525]
 36. Sidhu JS, Cowan D, Kaski JC. The effects of rosiglitazone, a peroxisome proliferator-activated receptor- γ agonist, on markers of endothelial cell activation, C-reactive protein, and fibrinogen levels in non-diabetic coronary artery disease patients. *Journal of the American College of Cardiology* 2003;42:1757–1769. [PubMed: 14642684]
 37. Lauenborg J, Mathiesen E, Hansen T, Glumer C, Jorgensen T, Borch-Johnsen K, Hornnes P, Pedersen O, Damm P. The prevalence of the metabolic syndrome in a danish population of women with previous gestational diabetes mellitus is three-fold higher than in the general population. *J Clin Endocrinol Metab* 2005;90:4004–10. [PubMed: 15840755]

Figure 1.

CIMT progression rates during TRIPOD and PIPOD treatment periods for women who completed TRIPOD without diabetes and participated in PIPOD with at least one follow-up CIMT. Rates are presented as mean and SE by their initial TRIPOD randomization status: TRIPOD troglitazone group (N=31) and TRIPOD placebo group (N=30). P-values are for differences between TRIPOD and PIPOD study periods, by random coefficients mixed-effect modeling.

Table 1
Comparison of TRIPOD and PIPOD baseline characteristics^a

Variable	TRIPOD Baseline	PIPOD Baseline	p-value ^b
TRIPOD Troglitazone Group (n=31)			
Age (yrs)	35.6 (6.4)	40.1 (6.3)	<0.0001
CIMT (mm)	0.586 (0.071)	0.607 (0.065)	0.07
BMI (kg/m ²)	29.8 (5.4)	31.5 (5.9)	<0.0001
OGTT glucose area (mg/dl × min × 10 ⁻³) ^c	18.6 (2.1)	17.8 (3.3)	0.12
OGTT insulin area (uU/ml × min) ^c	9993 (7172)	11115 (6838)	0.54
Insulin sensitivity (S _i ; min ⁻¹ per uU/ml × 10 ⁻⁴) ^d	2.33 (1.52)	2.59 (2.27)	0.42
Acute insulin response (AIRg; uU/ml × min) ^e	475 (293)	495 (331)	0.65
Disposition index (S _I × AIRg) ^f	933 (566)	1037 (651)	0.37
Systolic blood pressure (mmHg)	114.1 (11.8)	113.4 (11.9)	0.78
Diastolic blood pressure (mmHg)	70.6 (7.4)	70.5 (7.0)	0.91
HDL cholesterol (mg/dl)	37.1 (9.2)	45.7 (11.1)	<0.0001
LDL cholesterol (mg/dl)	109.4 (29.7)	118.8 (32.2)	0.06
Triglycerides (mg/dl)	134.2 (69.9)	108.4 (58.6)	0.03
TRIPOD Placebo Group (n=30)			
Age (yrs)	35.3 (7.1)	39.7 (7.2)	<0.0001
CIMT (mm)	0.599 (0.111)	0.638 (0.142)	0.001
BMI (kg/m ²)	29.2 (4.1)	29.8 (3.7)	0.09
OGTT glucose area (mg/dl × min × 10 ⁻³) ^c	18.1 (1.6)	18.3 (2.6)	0.70
OGTT insulin area (uU/ml × min) ^c	10040 (5275)	12058 (7197)	0.03
Insulin sensitivity (S _i ; min ⁻¹ per uU/ml × 10 ⁻⁴) ^d	2.83 (2.22)	2.23 (1.29)	0.11
Acute insulin response (AIRg; uU/ml × min) ^e	646 (550)	440 (308)	0.04
Disposition index (S _I × AIRg) ^f	1213 (643)	830 (548)	0.004
Systolic blood pressure (mmHg)	115.5 (11.1)	114.2 (14.8)	0.51
Diastolic blood pressure (mmHg)	70.0 (8.5)	70.1 (10.1)	0.98
HDL cholesterol (mg/dl)	36.7 (7.6)	44.3 (10.1)	0.0004
LDL cholesterol (mg/dl)	112.5 (26.3)	115.1 (29.2)	0.53
Triglycerides (mg/dl)	135.4 (88.5)	130.4 (72.1)	0.68

^aData (mean(sd)) are from women who did not developed diabetes during TRIPOD and returned for at least one follow-up annual CIMT during PIPOD

^bby paired t-test

^c75 gm oral glucose tolerance test; areas are total calculated by trapezoid rule

^dminimal model analysis of intravenous glucose tolerance test (IVGTT) results

^eincremental insulin area during first 10 min after glucose injection

^fS_I × AIRg, a measure of β-cell compensation for insulin resistance

To convert values from mg/dl to mmol/l, multiply by: (a) 0.0551 for glucose, (b) 0.02586 for cholesterol, and (c) 0.01130 for triglycerides. To convert values for insulin from uU/ml to pmol/l, multiply by 6.0.

Table 2
Comparison of on-trial changes in potential mediators of CIMT progression between TRIPOD and PIPOD study periods^a

Variable	TRIPOD On-trial	PIPOD On-trial	p-value ^b
<u>TRIPOD Troglitazone Group (n=31)</u>			
Weight (kg/yr)	0.9 (0.2)	0.7 (0.3)	0.63
OGTT glucose area (mg/dl × min × 10 ⁻³ per yr) ^c	-0.6 (0.1)	-0.4 (0.2)	0.32
OGTT insulin area (uU/ml × min per yr) ^c	-748 (200)	-1412 (360)	0.21
Systolic blood pressure (mmHg/yr)	0.1 (0.5)	-0.9 (0.6)	0.32
Diastolic blood pressure (mmHg/yr)	-0.6 (0.3)	-1.0 (0.6)	0.59
HDL cholesterol (mg/dl/yr)	2.7 (0.4)	2.7 (0.4)	0.85
LDL cholesterol (mg/dl/yr)	0.2 (1.0)	-2.1 (1.5)	0.24
Triglycerides (mg/dl/yr)	-4.4 (2.3)	-4.7 (2.8)	0.73
<u>TRIPOD Placebo Group (n=30)</u>			
Weight (kg/yr)	0.2 (0.2)	0.9 (0.3)	0.06
OGTT glucose area (mg/dl × min × 10 ⁻³ per yr) ^c	-0.1 (0.1)	-0.3 (0.2)	0.67
OGTT insulin area (uU/ml × min per yr) ^c	-9 (224)	-986 (311)	0.02
Systolic blood pressure (mmHg/yr)	-0.6 (0.5)	-0.3 (0.7)	0.72
Diastolic blood pressure (mmHg/yr)	-0.2 (0.4)	-0.8 (0.6)	0.40
HDL cholesterol (mg/dl/yr)	2.3 (0.3)	1.9 (0.5)	0.47
LDL cholesterol (mg/dl/yr)	-0.1 (0.9)	1.5 (1.4)	0.31
Triglycerides (mg/dl/yr)	3.6 (3.5)	-1.2 (3.9)	0.40

^aData are mean (SE) in rate of change for each study period

^bfrom mixed-effect model

^c75 gm oral glucose tolerance test; areas are total calculated by trapezoid rule

To convert values from mg/dl to mmol/l, multiply by: (a) 0.0551 for glucose, (b) 0.02586 for cholesterol, and (c) 0.01130 for triglycerides. To convert values for insulin from uU/ml to pmol/l, multiply by 6.0.