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Early Clinical Detection of Pharmacologic Response in Insulin Action in a Nondiabetic Insulin-Resistant Population

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ABSTRACT

Background: Insulin resistance heightens the risk for type 2 diabetes mellitus and cardiovascular disease. Amelioration of insulin resistance may reduce this risk. The thiazolidinedone class of insulin sensitizers improves insulin action in individuals with insulin-resistant diabetes and nondiabetic individuals. However, there are few reports on the time of onset of such effects independent of reversal of glucotoxicity.
Objective: The goal of our study was to test whether the thiazolidinedione pioglitazone has prominent early metabolic effects that can be detected in an obese, nondiabetic, insulin-resistant population.
Methods: We conducted a randomized, double-blind, placebo-controlled, parallel-group trial in men with nondiabetic insulin resistance using a hyperinsulinemic euglycemic clamp technique (at low and high doses of insulin at 10 and 40 mU/m^2/min, respectively). The patients were given 30 mg daily oral pioglitazone or placebo for 28 days. Patients underwent a baseline clamp before initiation of treatment, and again at 14 and 28 days of treatment.
Results: Compared with placebo, under high-dose hyperinsulinemia, pioglitazone led to significant increases in glucose disposal rates (GDR) of 1.29 mg/kg/min (90% CI, 0.43–2.15; 39%; $P=0.008$) that were detectable at 2 weeks of treatment and persisted at 4 weeks of treatment. Under low-dose hyperinsulinemia, significant increases in GDR of 0.40 mg/kg/min (90% CI, 0.17–0.62; 95%; $P=0.003$) were observed at 4 weeks of treatment. These responses were accompanied by robust suppression of free fatty acids under hyperinsulinemic conditions, and by significant increases in circulating basal total adiponectin at 2 and 4 weeks of treatment.
Conclusions: Significant changes in insulin action across multiple insulin-sensitive tissues can be detected within 2 weeks of initiation of insulin-sensitizing therapy with pioglitazone in obese patients with nondiabetic insulin resistance. ClinicalTrials.gov identifier: NCT01115712.

Introduction

Insulin resistance (IR) is an integral aspect of the pathogenesis of type 2 diabetes mellitus (T2DM) and is independently associated with cardiovascular (CV) risk.[1] Amelioration of IR can be beneficial for both the prevention and treatment of T2DM.[2,3] IR can be partially normalized by exercise and weight loss, with additional contribution from pharmacologic therapy. Thiazolidinediones (TZDs) activate the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ),[4] and TZDs, besides metformin, are generally acknowledged as the only class of oral antihyperglycemic agents with therapeutically relevant effects on peripheral glucose disposal.[5] However, with the emergence of significant concerns about side effects, the use of TZDs has declined[6–8] and there is therefore an urgent need for newer insulin-sensitizing therapies.

The development of novel insulin-sensitizing therapies requires a clear understanding of the tissue site of insulin action of the novel agent, as well as the time of onset of such effects. For practical purposes, it is important to have a simple yet reliable means to perform such interrogation of insulin action early in development, and especially useful to be able to calibrate the novel treatment with the standard of care, which at the time of writing is a TZD. It is of particular interest to ascertain early responses to TZDs in individuals with nondiabetic IR because the interpretation of changes in IR would not be confounded by potential, and possibly variable, alleviation of glucotoxicity.

Various methodologies have been developed for clinical assessment of IR, including the frequently sampled intravenous glucose

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tolerance test (IVGTT)[9] and derivation of IR from an oral glucose tolerance test (OGTT) or meal tolerance test (MTT).[10] However, the gold standard methodology remains the euglycemic clamp under exogenous insulinization.[11] A number of clinical investigations have used the euglycemic clamp to examine the improvement in IR in response to TZD agents.[12–14] These studies have yielded considerable insights into the mechanisms by which PPARγ activation alters IR and glucose homeostasis, but nearly all were conducted after a treatment period of several months, when the response has presumably fully equilibrated.[3,15–17] Few studies have sought to examine leading-edge changes in IR early in the course of treatment with TZD,[18,19] and none have assessed early changes in obese human beings with nondiabetic IR in a controlled setting.

The goal of the present study was to appraise the onset of clinically relevant changes in IR in response to treatment with the TZD agent pioglitazone (PIO) using a 2-step euglycemic hyperinsulinemic clamp at 2 and 4 weeks of treatment in obese men with nondiabetic IR, with potential application of these findings as a benchmark for the evaluation of novel insulin-sensitizing therapies. Prior studies suggest that measurable changes can occur within an interval of 12 weeks[20–23]; however, none of these reported data earlier in the course of treatment. Two trials of antihyperglycemic therapy in T2DM[18,19] and a single uncontrolled trial in a small group of nonobese patients without diabetes suggested improvements as early as 3 weeks of treatment.[24] Our findings demonstrate for the first time in obese volunteers with nondiabetic IR in a randomized, double-blind, placebo-controlled setting, that improvement of IR can be clearly detected within 2 weeks of initiation of treatment with PIO. Furthermore, we demonstrate that this improvement is measurable across multiple key tissues involved in the pathogenesis of IR, including adipose, hepatic, and skeletal muscle tissues, suggesting that interorgan crosstalk likely originating from adipose tissue is already evident and is measurable systemically very early in the course of treatment.

Methods

Study Participants

All patients were overweight or obese (body mass index >28 kg/m^2 and ≤ 38 kg/m^2) men without diabetes by clinical history and fasting glucose measurement and normotensive by cuff measurements per the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI).[25] Participants also had normal cholesterol levels per the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP III) criteria,[26] and were not taking any medications routinely. All patients provided informed consent for the trial.

Study Drugs

The 30 mg PIO (Takeda Pharmaceuticals USA Inc., Deerfield, Illinois) or placebo was administered as a daily oral dose for 28 consecutive days.

Protocol

This was a randomized, double-blind, placebo-controlled study with a parallel-group design for treatment groups. The study (Protocol 170) was conducted between 2009 and 2010 at a Phase I clinical research unit (ICON Development Solutions, San Antonio, Texas) with approval from the local ethics review committee (IntegReview Ethical Review Board, San Antonio, TX). The trial was conducted in accordance with principles of Good Clinical Practice. Patients meeting study entry criteria underwent a hyperinsulinemic euglycemic clamp at baseline and were randomly allocated to 1 of 2 treatments (30 mg PIO or placebo). Patients underwent a second hyperinsulinemic euglycemic clamp after 14 days of treatment and a third hyperinsulinemic euglycemic clamp after 28 days of treatment. A total of 38 patients completed the baseline clamp procedure, and 31 and 29 patients completed the Day 14 and Day 28 clamp procedures, respectively.

The euglycemic clamp was performed in 2 steps each lasting approximately 180 minutes: a low-dose portion with insulin infusion rates of 10 mU/m^2/min followed by a high-dose portion with infusion rates of 40 mU/m^2/min. The clamp procedures were performed using the method described by DeFronzo et al[27] with target plasma glucose levels of approximately 90 mg/dL. Samples for measurement of insulin and free fatty acids (FFA) were obtained at baseline and at steady state of each step of the clamp.

The key end points of the trial were the effects of PIO compared with placebo on insulin sensitivity, measured as average changes in glucose disposal rates (GDR) from baseline at 28 days and 14 days corrected for body weight (M) at steady state of the clamp during the high- and low-dose portions. Additional end points included M/SSPG (M normalized to plasma glucose at steady state); M/I (M normalized to plasma insulin at steady state); circulating levels of fasting insulin, FFA, adiponectin, and retinol binding protein 4 (RBP4); and insulin-induced suppression of FFA. All measurements were performed using commercially available assays.

Statistical Methods

For comparison of the treatment groups with respect to the change from baseline for each end point, a constrained longitudinal data analysis method was used. This model assumes a common mean across treatment groups at baseline and a different mean for each treatment at each of the postbaseline time points. In this model, the response vector consists of baseline and the values observed at each postbaseline time point; that is, M at baseline, 14 days, and 28 days for the low- and high-dose portions of the clamp. Time is treated as a categorical variable so that no restriction is imposed on the trajectory of the means over time. The analysis model was adjusted for baseline glucose level and baseline insulin level. An unstructured covariance matrix was used to model the correlation among repeated measurements. A closed-testing procedure was employed to test the 2 primary hypotheses whereby the difference between 30 mg PIO and placebo with respect to the change in M from baseline at 28 days was tested first, and only if this hypothesis was met was the hypothesis at 14 days tested. This ensured that the overall type I error rate was controlled at the 0.05 1-sided level. One-sided confidence testing was used because the direction of the PIO effect on M is known.[28]

Further analysis was conducted to assess the reproducibility of M, insulin, and M normalized to plasma insulin at steady state (M/I) for the low- and high-dose insulin infusion clamps. Data from patients in the placebo group were used in this analysis and reproducibility was assessed over 3 time points: baseline (Day 0), Day 14, and Day 28. The concordance correlation coefficient (CCC) was computed to assess reproducibility.

Results

Overweight and obese patients with nondiabetic IR were randomly assigned in a double-blind manner to PIO and placebo (PLB) treatment groups. As shown in Table I, the 2 groups were well matched for demographic and metabolic variables, including

Table I
Baseline demographic and metabolic characteristics of the study groups.

Characteristic	30 mg Pioglitazone (n = 19)		Placebo (n = 17)		*P* value (2-sided)*
	Mean	SEM	Mean	SEM	
Age, y	28.6	1.9	30.5	1.6	0.454
Body mass index	32.5	0.6	32.3	0.7	0.646
Systolic blood pressure, mm Hg	120.5	2.5	120.8	2.2	0.932
Diastolic blood pressure, mm Hg	79.3	1.9	77.8	1.6	0.556
Total cholesterol, mg/dL	181.1	8.1	195.2	9.5	0.265
Triglyceride, mg/dL	183.0	20.1	171.4	10.7	0.624

* *P* value is from a 2-sample *t* test.

obesity ($P > 0.05$). Each patient manifested IR during a baseline clamp and the groups were well matched ($P > 0.05$) for M at baseline, as shown in Table II. During all the clamp studies, plasma glucose was maintained at a target level of 90 mg/dL. Steady state plasma insulin levels during each of the 2 steps of the clamp studies were matched across groups at baseline, 2 weeks of treatment, and 4 weeks of treatment. Clamp data are shown in **Figures** 1 and 2 and presented in Table II.

During PIO treatment, M increased significantly from baseline and in comparison to PLB treatment, with these increases becoming evident at 2 weeks of treatment. At 2 weeks of PIO treatment, M increased by 40% ($P = 0.045$) during the 40 mU/m^2/min step of the insulin infusion. Although glucose disposal also improved over 2 weeks during the 10 mU/m^2/min infusion, the change in M was not statistically significant. Suppression of plasma FFA in response to insulin infusion was greater with PIO treatment than with placebo at 2 weeks during infusion with 10 and 40 mU/m^2/min insulin ($P < 0.05$). At 4 weeks of treatment, the improvement in M during PIO treatment was evident and significant during both the 10 mU/m^2/min infusion step (95% increase; $P=0.003$) and the 40 mU/m^2/min infusion step (39% increase; $P = 0.008$). At 4 weeks of treatment, the greater suppression of plasma FFA with PIO compared with placebo was evident only during the 10 mU/m^2/min insulin infusion. Supplementary analyses of M normalized to plasma insulin at steady state (M/I) and M normalized to plasma glucose at steady state (M/SSPG) yielded similar results (data not shown).

In response to PIO, circulating adiponectin increased by ~50% at Week 2 and by ~80% at Week 4 ($P < 0.01$ compared with placebo) (Table III). There was not a significant correlation between changes in adiponectin and changes in M. Whereas the clamp data provided clear support for the notion that the effects of PIO on insulin sensitivity can be measured as early as 2 weeks after treatment initiation, most of the fasting parameters measured revealed little change (Table III). Fasting glucose did not change from baseline in either group across the study period, whereas fasting levels of insulin decreased slightly (~25%) in the PIO group, although this difference was significant only at 2 weeks of treatment. Fasting levels of plasma FFA and retinol binding protein 4 were not different between the groups at baseline, 2 weeks of treatment, or 4 weeks of treatment.

An ancillary goal was to assess the reproducibility of M in the PLB-treated group across a relatively brief interval of 4 weeks of treatment. Taking into consideration the baseline, 2-week, and 4-week clamp determinations in the PLB group, there was an observed CCC of 0.70 and 0.73 for M values obtained during the

Table II
Glucose, insulin, glucose disposal rate (GDR), and free fatty acid (FFA) levels at Day 0, 14, and 28 during steady-state conditions for the low- and high-dose conditions of a hyperinsulinemic euglycemic clamp.

Variable	Day	30 mg Pioglitazone		Placebo		*P* value*
		Mean	SEM	Mean	SEM	
Steady state glucose (mg/dL): Low dose	0	90.5	0.4	89.8	0.2	–
	14	89.6	0.1	89.9	0.3	–
	28	89.9	0.2	89.7	0.2	–
Steady state glucose (mg/dL): High dose	0	89.2	0.4	89.9	0.6	–
	14	90.3	0.9	90.0	0.4	–
	28	89.2	0.7	89.9	0.6	–
Steady state insulin (mcIU/mL): Low dose	0	34.5	1.3	32.0	1.8	
	14	31.7	1.6	33.4	2.8	–
	28	29.9	1.7	32.1	2.0	–
Steady state insulin (mcIU/mL): High dose	0	106.1	3.4	105.5	3.9	–
	14	104.2	4.2	109.6	5.7	–
	28	104.1	4.2	105.5	4.4	–
GDR (mg/kg/min): Low dose	0	0.74	0.11	0.93	0.21	–
	14	0.94	0.11	0.83	0.15	0.069
	28	1.12	0.16	0.64	0.11	0.003
GDR (mg/kg/min): High dose	0	4.85	0.46	6.16	0.57	–
	14	5.8	0.63	5.86	0.60	0.045
	28	6.27	0.53	5.52	0.50	0.008
FFA (mmol/L): Low dose	0	0.182	0.015	0.180	0.030	
	14	0.123	0.012	0.147	0.016	0.028
	28	0.102	0.013	0.159	0.017	0.009
FFA (mmol/L): High dose	0	0.048	0.003	0.046	0.008	
	14	0.038	0.004	0.042	0.006	0.031
	28	0.035	0.004	0.047	0.007	0.064

* *P* value is a 1-sided *P* value from a constrained longitudinal data analysis model for comparing the change from baseline for the given end point between the 2 groups.

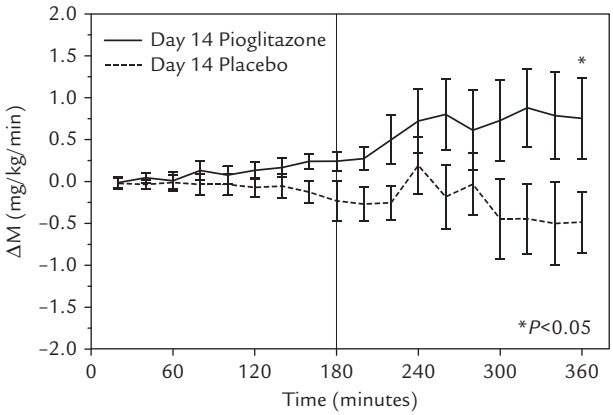

Figure 1. Means and SE for change (Δ) from baseline in glucose disposal rate per kilogram body weight (mg/kg/min) (M) across the entire duration of the clamp performed at Day 14 of treatment with 30 mg pioglitazone or placebo. Insulin was administered at a low dose (10 mU/m^2/min) from 0 to 180 minutes, and at a high dose (40 mU/m^2/min) from 180 to 360 minutes.

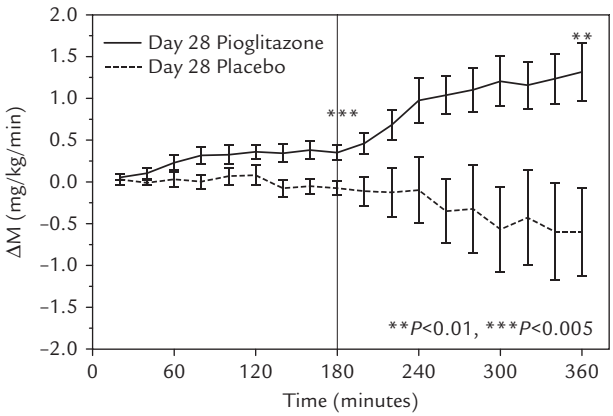

Figure 2. Means and SE for change (Δ) from baseline in glucose disposal rate per kg body weight (mg/kg/min) (M) across the entire duration of the clamp performed at Day 28 of treatment with 30 mg pioglitazone or placebo. Insulin was administered at a low dose (10 mU/m^2/min) from 0 to 180 minutes, and at a high dose (40 mU/m^2/min) from 180 to 360 minutes.

low-dose and the high-dose insulin infusion, respectively (**Figure 3A**). The insulin levels during the low-dose and the high-dose insulin infusion in the PLB-treated group were comparable on all 3 occasions (**Figure 3B**).

Discussion

The goal of our trial was to determine the onset of clinically relevant changes in insulin action in various insulin-sensitive tissues in response to 2- and 4-week treatment with PIO in the setting of a placebo-controlled trial in obese volunteers with nondiabetic IR. The key finding of this trial was the observation of a substantial improvement in glucose disposal under hyperinsulinemia in obese individuals with nondiabetic IR who were otherwise healthy as early as after 2 weeks of PIO treatment. This was accompanied by a concomitant significant improvement in suppression of FFA under hyperinsulinemia and a significant increase in basal adiponectin levels. These effects persisted at 4 weeks of treatment, with evidence of suppression of endogenous glucose production and no change in basal fasting glucose, FFA, or other lipid parameters.

Although diet and exercise remain the foundation of treatment of T2DM, especially for alleviation of IR, the development of novel pharmacologic treatments that alleviate IR could be of substantial

Table III
Body weight, fasting glucose, fasting insulin, fasting free fatty acid (FFA), receptor binding protein 4 (RBP4), and adiponectin at Day 0, 14, and 28.

Variable	Day	30 mg Pioglitazone		Placebo		*P* value*
		Mean	SEM	Mean	SEM	
Body weight, kg	0	100.9	2.8	96.9	3.4	–
	14	99.1	3.0	96.9	3.7	0.173
	28	100.6	3.0	97.7	4.1	0.231
Fasting glucose, mg/dL	0	97.6	1.7	99.0	1.5	–
	14	96.6	1.1	98.1	1.5	0.303
	28	96.3	1.4	98.6	1.7	0.408
Fasting insulin, mcIU/mL	0	18.4	1.8	15.2	1.9	–
	14	14.2	1.6	14.9	1.8	0.005
	28	13.9	1.7	15.0	1.7	0.074
Fasting FFA, mmol/L	0	0.48	0.018	0.50	0.031	–
	14	0.42	0.033	0.46	0.039	0.272
	28	0.45	0.031	0.49	0.032	0.307
RBP4, μg/mL	0	9.62	0.922	8.48	0.691	–
	14	9.95	0.983	8.30	0.960	0.227
	28	11.15	1.266	9.68	0.743	0.491
Adiponectin, μg/mL	0	10.27	0.816	11.22	1.539	–
	14	15.90	2.097	9.67	0.690	0.003
	28	18.38	2.840	10.91	0.913	0.007

* *P* value is a 1-sided *P* value from a constrained longitudinal data analysis model for comparing the change from baseline for the given end point between the 2 groups.

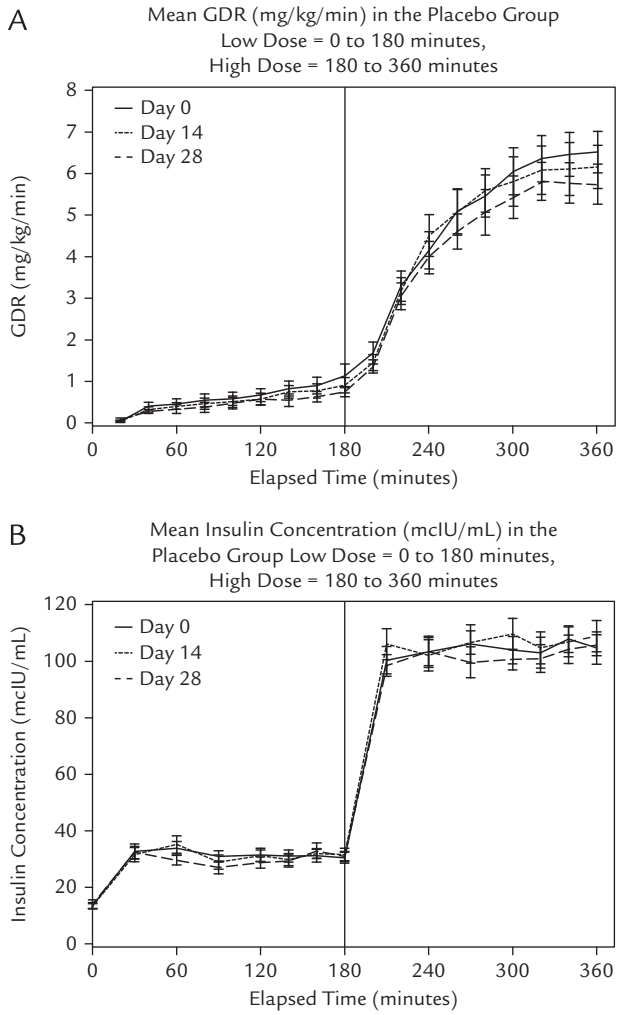

Figure 3. (A) Mean glucose disposal rate (GDR) (mg/kg/min) in the placebo group. (B) Mean insulin concentration (mcIU/mL) in the placebo group.

value in addressing the growing epidemic of T2DM. Identifying new targets for amelioration of IR and choosing those most likely to translate preclinical efficacy in rodents into efficacy in human beings is recognized to be highly challenging because the pathogenesis of IR remains controversial and is probably multifactorial.[29,30] Perhaps as a consequence of this complexity of IR and uncertainty over the molecular causes of IR, the clinical translation of novel targets that are typically identified in rodent models of diabetes and obesity seems fraught by failures. There is no facile solution for this challenge, but our study addresses 1 aspect that we posit to be crucial, namely provision of a relevant rationale and a simple yet reliable paradigm to efficiently and rapidly test the therapeutic effects of a novel insulin-sensitizing mechanism. We used a 2-step hyperinsulinemic euglycemic clamp, a well-described methodology, to assess early changes in IR evoked by PIO, a TZD, and the standard of care for pharmacologic treatment of IR. Two dose levels of insulin infusion, low and high, were used as windows of sensitivity to assess changes in adipose tissue and liver at the low dose, and to more clearly probe changes in glucose disposal within peripheral tissues, notably skeletal muscle, at the high dose. Significant improvements in IR were observed in response to PIO therapy, with changes evident at 2 weeks of treatment and persisting, possibly even further improving, over 4 weeks of treatment. From a practical perspective, as well as from intent to achieve some clarity in assessing pharmacodynamic response in a relatively small number of patients, this 2- to 4-week window is quite efficient and useful. Although it is possible that not all novel mechanisms for treating IR will demonstrate a similar early pattern of changes that we observed for a TZD, presumably the temporal pattern of improvement seen with novel mechanisms for IR in preclinical studies will shed light on a potential time course that can be anticipated for clinical improvement and, therefore, whether assessment and comparison to a TZD benchmark at 2 weeks of treatment seems rational. In addition, knowing the magnitude of improvement in insulin action at each of the key tissues may provide insight into the integrated therapeutic effect that might be anticipated from a novel insulin sensitizer.

The improvement in IR observed in our study at 2 weeks and 4 weeks of treatment with PIO complements the findings of several clinical studies on changes in IR following several months of TZD treatment in populations with T2DM. Interestingly, in this group of overweight and obese men with nondiabetic IR, an approximately 40% improvement in IR was observed during the high-dose insulin infusion (40 mU/m^2/min) at 2 weeks; this was sustained at 4 weeks of treatment and was similar to the improvement in IR observed after several months of TZD treatment in other studies.[15,23,31] This similarity, in response, supports the rationale and robustness of the paradigm and methods employed in the current trial, namely the use of a simple 2-step hyperinsulinemic euglycemic clamp in patients with nondiabetic IR performed early in the course of treatment. The mechanism by which TZDs improve IR through activation of PPARγ receptors, mainly in adipocytes and preadipocytes,[12,19] remains incompletely understood. At 2 weeks of PIO treatment, in addition to the improvement in glucose disposal, there was a notable improvement in insulin-mediated suppression of plasma FFA and an increase in plasma adiponectin levels. Adiponectin is a biomarker of TZD action in adipose tissue[32,33] and, together with the increased suppression of plasma FFA, signifies changes in adipose tissue IR; the concordance in these changes makes our findings particularly robust.

These changes in adipose tissue metabolism, together with the increase in glucose disposal observed during the high-dose insulin infusion, suggest crosstalk between adipose tissue and skeletal muscle in mediating the early improvement in IR. In contrast, although FFA levels were nearly completely suppressed during the low-dose insulin infusion at 2 weeks, the corresponding M values during this step of the clamp were unchanged from basal and across groups. However, by 4 weeks of PIO treatment, M values during the low-dose insulin infusion had nearly doubled ($P < 0.01$), indicating progressive improvement in an aspect of IR that was not evident at 2 weeks of treatment. Our interpretation is that this improvement from 2 to 4 weeks of PIO treatment that becomes manifest during the low-dose insulin infusion represents progressive improvement in hepatic IR. Although a more precise delineation of the respective contributions of improvements of IR in the liver and the periphery could perhaps have been attained using glucose isotope tracer methodology during the clamp studies, our findings are consistent with those reported in T2DM after 3 weeks of PIO treatment.[19] It should be noted that the low-dose insulin infusion of 10 mU/m^2/min, which was chosen to assess changes in suppression of endogenous glucose production, provided circulating levels of insulin sufficient to suppress FFA and at least partially suppress endogenous glucose production while remaining lower than the exposures required to robustly stimulate peripheral glucose disposal (> 2-fold augmentation over fasting levels). In contrast, circulating insulin levels showed a 5-fold augmentation over fasting levels during the high-dose insulin infusion of 40 mU/m^2/min, which was associated with a 40% increase in M. Taken together, these findings suggest that the doses of exogenous insulin selected and the levels of hyperinsulinemic exposures achieved in this trial successfully provide insight into the magnitude and temporal profile of changes in insulin action at adipose, hepatic, and skeletal muscle tissues in a population with IR in a single experiment.

The fact that the improvement in M during the low-dose insulin infusion was evident at 4 weeks of treatment but not at 2 weeks, whereas the suppression of FFA and increase in M during the high-dose insulin infusion remained stable between 2 and 4 weeks of treatment, likely signifies progressive improvement in hepatic IR. We did not measure changes in body composition during these brief studies and there was no weight loss among our patients. But even in the context of TZD-induced weight gain, a decrease in hepatic fat content during TZD treatment has been observed.[33,34] In a separate study, we observed a 20% decrease in hepatic fat content in obese men after 4 weeks of PIO treatment, although changes at 2 weeks of treatment were not assessed (unpublished data).

Apart from the changes in plasma adiponectin induced by PIO treatment in these men without diabetes, there were no changes in fasting levels of plasma glucose, insulin, or FFA, indicating the utility of a dynamic testing platform, like the hyperinsulinemic clamp, in assessing changes in IR. Furthermore, the feasibility of employing individuals without diabetes was demonstrated, thereby obviating a confounding potential effect of reversing glucotoxicity. It is worth noting that the baseline insulin sensitivity of the study participants, which was confirmed by baseline clamp determinations, was efficiently and accurately predicted using standard clinical parameters, as outlined in the Methods section.

Finally, the findings in placebo-treated study participants, who also underwent 3 separate clamp measurements across an approximately 4-week interval (and in the absence of diet or activity interventions) fills a void in the literature concerning reproducibility. Technical parameters upon which clamp measurements are closely dependent, namely clamped levels of glucose and insulin, were tightly matched across the 3 clamp studies in the placebo group. The parameter of reproducibility (observed CCC, 0.70–0.77) is quite good, and in view of the technical consistency we are confident that the residual variance probably represents biological fluctuation in IR in these individuals even across this relatively short interval of time.

Conclusions

Our findings demonstrate for the first time in a randomized, double-blind, placebo-controlled trial in obese volunteers with nondiabetic IR that clearly interpretable evidence of amelioration of IR can be detected across adipose, hepatic, and skeletal muscle tissues as early as after 2 weeks of treatment with an insulin sensitizer. PIO provided a good benchmark for detecting therapeutically relevant improvements in IR and its efficacy was evident in the clamp studies at 2 weeks by improvements in stimulation of glucose disposal and suppression of FFA, as well as increases in circulating adiponectin, with further improvement in the suppression of endogenous glucose production at 4 weeks of treatment. Taken together, these data suggest that tissue crosstalk associated with detectable improvements in insulin sensitivity occurs early during treatment, setting the stage for further work to tease out the underlying molecular mechanisms and interactions. Furthermore, because these data were acquired in the setting of a controlled trial design using an approved antihyperglycemic agent as a probe and a simple yet reliable experimental design and methodology, a paradigm such as the one presented here can be readily applied in early clinical development. This may be a very useful and highly informative antecedent to support investment in advancing novel insulin sensitizers into further phases of development.

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S. S. Shankar, R. R. Shankar, R. A. Railkar, C. R. Beals, H. O. Steinberg, and D. E. Kelley participated in conception and design or planning of study. S. S. Shankar reported acquisition of the data. S. S. Shankar, R. R. Shankar, R. A. Railkar, C. R. Beals, H. O. Steinberg, and D. E. Kelley participated in analysis of data. S. S. Shankar, R. R. Shankar, C. R. Beals, and D. E. Kelley contributed to interpretation of results. S. S. Shankar, R. R. Shankar, R. A. Railkar, and H. O. Steinberg participated in drafting of the manuscript. All authors reviewed and revised the manuscript for important intellectual content. All authors approved the final submitted version of manuscript.

Conflicts of Interest

All authors were employees of Merck Sharp & Dohme Corp, a subsidiary of Merck & Co., Inc., Kenilworth, NJ, at the time that work for this study was performed and may own stock or stock options with Merck. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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