

Letter to the Editor

A randomized, placebo-controlled, double-blinded, crossover trial of pioglitazone for severe asthma*To the Editor:*

The peroxisome proliferator-activated receptor (PPAR) family of nuclear receptor transcription factors regulate metabolism, inflammation, and immunity.¹ Pharmacological agents that activate PPAR- γ , such as thiazolidinediones (TZD), sensitize cells to insulin and are used to treat diabetes. PPAR- γ is also expressed in the lung where it has anti-inflammatory effects.² Based on the protective effects of PPAR- γ agonists in murine asthma models,^{2,3} we performed a clinical trial of the TZD, pioglitazone, to assess whether it might be beneficial for severe asthmatic patients who are persistently symptomatic despite treatment with high doses of inhaled corticosteroids with or without oral prednisone. The objective of this randomized, double-blind, placebo-controlled, 2-period, crossover study was to assess whether the primary outcome measure of the Juniper asthma quality of life questionnaire score (AQLQ) would be significantly higher at the end of the pioglitazone treatment period as compared to placebo in patients with severe asthma.

This study of pioglitazone hydrochloride in severe asthma was an investigator-initiated study that was conducted at the National Institutes of Health Clinical Research Center (Bethesda, Md) between October 2009 and June 2016. Protocol 09-H-0244 was approved by the National Heart, Lung, and Blood Institute Institutional Review Board and all participants provided informed consent. Subjects whose baseline asthma status did not change during a 4-week run-in period (eg, absence of unscheduled health care visits for asthma care and no change in maintenance asthma medications) were randomized to either pioglitazone or matching placebo (see Fig E1 in this article's Online Repository at www.jacionline.org). Pioglitazone, 30 mg daily, was administered for the initial 2 weeks of the first treatment phase, followed by 45 mg daily for an additional 14 weeks. This was followed by a 4-week washout period. Subjects were assessed for clinical stability during a second 4-week run-in period prior to crossing over to the second treatment phase to receive the other agent. Therefore, each subject received both placebo and study drug at different times during the study. Detailed methods are reported in this article's Online Repository at www.jacionline.org.

Of the 59 subjects screened for the study, 34 met inclusion criteria for participation and 16 subjects underwent randomization (Fig 1). The study was terminated early due to difficulty in recruiting additional subjects to achieve a sample size of 26 completers. The demographics for the 12 subjects who were enrolled, randomized, and completed the study are shown in Table E1 in this article's Online Repository at www.jacionline.org. Subjects were predominantly white with elevated body mass index, moderately severe airflow obstruction, and poor asthma control. All subjects had a history of allergy and were receiving high doses of inhaled corticosteroids. Five subjects were also receiving an inhaled long-acting β_2 -agonist, 2 subjects were receiving oral prednisone, and 4 subjects were receiving an inhaled long-acting β_2 -agonist plus oral prednisone. Eleven subjects were receiving a leukotriene-modifying agent, while 5 subjects were

receiving omalizumab. Two subjects who were randomized withdrew from the study for personal reasons, while study drug administration was discontinued in 2 subjects due to adverse events of pedal edema and presumptive angioedema. Details of adverse events are reported in this article's Online Repository at www.jacionline.org and Table E2.

There was no difference regarding the prespecified primary outcome measure of the Juniper AQLQ score at the end of the pioglitazone as compared to the placebo treatment period (4.7 ± 1.3 vs 4.6 ± 1.3 , $P = .677$) (Fig 2). Similarly, no difference in the Juniper AQLQ score was observed by period ($P = .456$). A similar analysis was performed using a mixed-effects method to model correlation within subjects with a random intercept, which yielded the same conclusion. There were also no differences in prespecified secondary outcome measures regarding asthma symptoms or control (Fig 2), airflow obstruction and inflammation (details are reported in the Online Repository at www.jacionline.org and Figs E2-E4), except for a significantly lower peripheral blood lymphocyte count after the pioglitazone as compared to the placebo treatment periods (2.1 ± 0.8 vs 2.6 ± 1.1 thousand/ μ L, $P = .002$).

Although several clinical studies generated initial evidence suggesting that the TZD class of PPAR- γ agonist medications might be beneficial for asthma,^{4,5} results from subsequent randomized clinical trials have either showed no effect or only a modest improvement in prespecified primary end points.⁶⁻⁹ Here, we assessed the role of pioglitazone in severe asthma. Our cohort of severe asthmatics was characterized by a history of allergy, obesity, moderately severe airflow obstruction, and elevated FeNO consistent with airway inflammation. We show that treatment with pioglitazone for 4 months was not different than placebo regarding the prespecified, primary outcome measure of the Juniper AQLQ score. Although our study was terminated prematurely, had we continued until we had achieved 26 completers, we still would have had little chance of obtaining a significant difference between the 2 treatment arms based on a conditional power of 0.007. There were also no differences between pioglitazone and placebo regarding prespecified secondary end points, except for a reduction in peripheral blood

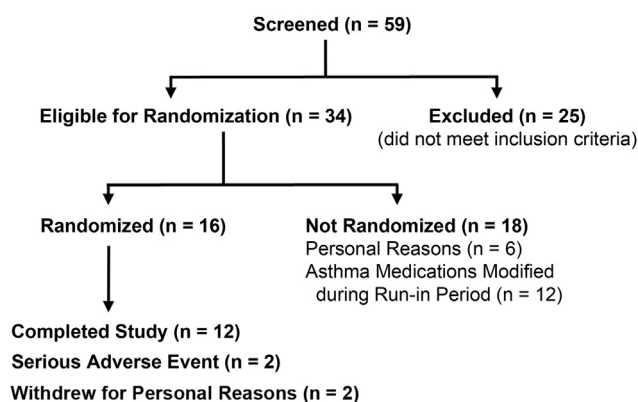


FIG 1. Flow of subjects through the study showing number of participants screened, randomized, withdrawn, and completed. Participants who were excluded did not meet the prespecified inclusion criteria.

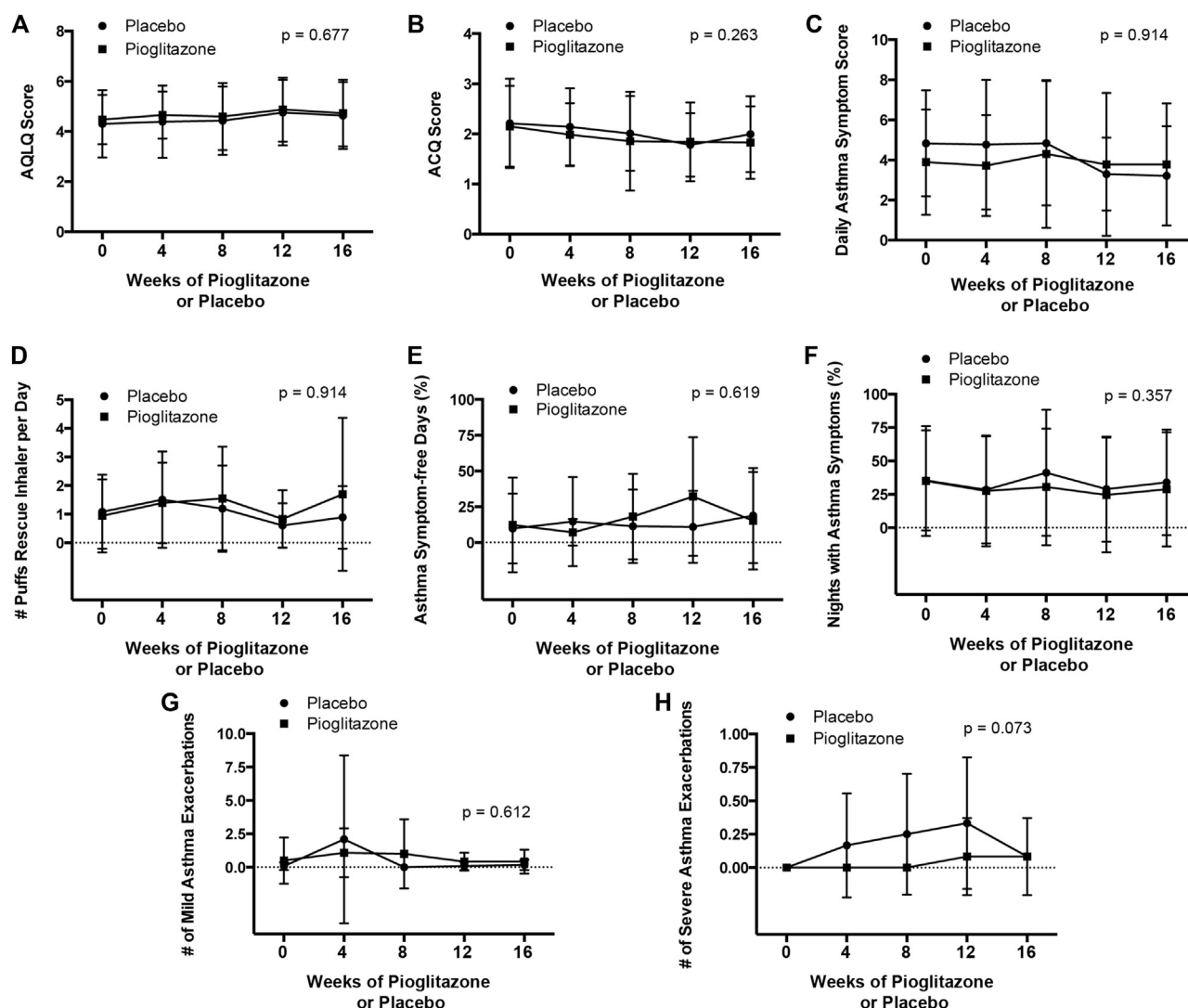


FIG 2. Comparison of pioglitazone and placebo treatment periods for the primary end point of AQLQ score and secondary end points of asthma symptoms and control. **A**, Juniper AQLQ score. **B**, Juniper asthma control questionnaire (ACQ) score. **C**, Daily asthma symptom score. **D**, Number of puffs of rescue β_2 -agonist inhaler per day. **E**, Asthma symptom-free days (%). **F**, Nights with asthma symptoms (%). **G**, Number of mild asthma exacerbations. **H**, Number of severe asthma exacerbations. Data for daily asthma symptom score, rescue β_2 -agonist inhaler use, asthma symptom-free days, and nights with asthma symptoms are averages for each period based on the days that subjects reported data. *P* values are shown for differences between the pioglitazone and placebo treatment periods.

lymphocytes in the pioglitazone treatment arm. We cannot, however, exclude the possibility that a difference between the 2 treatment arms might have been detected if the duration of the treatment period had been longer.

Two of 14 subjects experienced severe adverse reactions secondary to pioglitazone. These included significant peripheral edema in 1 subject and presumptive angioedema in another. Peripheral edema is a recognized adverse effect of pioglitazone therapy that occurs because of fluid retention. Although angioedema is not known to be an adverse effect of pioglitazone, individuals with a known hypersensitivity should not receive this medication. Other potential serious side effects related to pioglitazone, such as congestive heart failure, hypoglycemia, hepatotoxicity, urinary bladder tumors, fractures, or decreased visual acuity due to macular edema did not occur.

Our results suggest that pioglitazone is not safe to use in severe asthmatic patients as 14% of subjects experienced pioglitazone-related severe adverse events that necessitated discontinuation of the study medication. Furthermore, although our sample size was small, pioglitazone did not improve the primary end point of the AQLQ score. Taken collectively with the results from other clinical trials of TZDs, the absence of a positive treatment effect, as well as the potential for significant side effects, suggest that no further clinical trials of the PPAR- γ agonist, pioglitazone, should be performed for severe asthma.

We are most grateful for the participation of the research subjects in this study, as well as their primary physicians. We are also very appreciative of the staffs of the National Heart, Lung, and Blood Institute Pulmonary Function Laboratory, the National Cancer Institute Cytopathology Laboratory, Dr Oleh Hnatiuk, and Dr Rita Volochayev for their contributions to this study.

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Online Repository

A Randomized, Placebo-controlled, Double-blinded, Crossover Trial of Pioglitazone for Severe Asthma

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Online Methods

Study Design

This study was registered at clinicaltrials.gov as NCT00994175. The Food and Drug Administration also approved an Investigational New Drug Application for this study. Pioglitazone and matching placebo were prepared by the NIH Clinical Center Pharmacy, which also performed the randomization. A random numbers table was used by NIH Clinical Center Pharmacy to generate the random allocation of subjects to either sequence 1 (placebo first followed by pioglitazone) or sequence 2 (pioglitazone first followed by placebo), which was maintained in a randomization table and subjects were assigned sequentially by NIH Clinical Center Pharmacy. Study investigators enrolled the subjects, but were blinded regarding the randomization allocation. Medication bottles, which did not indicate whether the contents were pioglitazone or placebo, were prepared by the NIH Clinical Center Pharmacy, so that both study investigators and participants were blinded to the assignment. Study subjects were evaluated monthly and continued to receive the study drug during asthma exacerbations. The subject's local physicians managed their asthma medications and treated exacerbations without input from the study investigators. Subjects were monitored monthly for pioglitazone-related side effects, such as anemia, elevations in liver function tests, and fluid retention. Study drug usage, which was monitored by monthly pill counts, showed compliance rates of $89.1\% \pm 8.9\%$ during the pioglitazone treatment phase and $88.6\% \pm 12.3\%$ during the placebo treatment phase.

Eligibility Criteria

Severe asthmatics, between 18 and 75 years of age, who were persistently symptomatic and required use of a rescue β -agonist inhaler $> 2x$ per week despite treatment with high-dose inhaled corticosteroids (e.g., equivalent to $\geq 1,000 \mu\text{g}$ daily of fluticasone propionate inhalation powder) or oral corticosteroids were eligible to participate^{E1}. All subjects had a documented history of reversible airflow obstruction, as defined by a positive response to an inhaled bronchodilator or a positive methacholine bronchoprovocation challenge, as well as a left ventricular ejection fraction of $\geq 50\%$ by echocardiography. Exclusion criteria included known hypersensitivity to pioglitazone, an asthma exacerbation requiring treatment with additional oral corticosteroids in the previous 6 weeks, cigarette smoking within the previous 12 months or a prior history of > 20 cumulative pack-years, receipt of other investigational therapies within 1 month of the screening visit, presence of another lung disease other than asthma, diabetes mellitus, infection with the human immunodeficiency virus, congestive heart failure, greater than 2^+ pedal edema on physical examination, hemoglobin $< 6.8 \text{ mmol/l}$ for males and $< 6.2 \text{ mmol/l}$ for females, active liver disease or abnormal liver function tests (> 2 times the upper limit of normal), history of bladder or colon cancer, any cancer not in remission, breast feeding, and use of medications that can potentially interact with pioglitazone

(gemfibrozil, atazanavir, ritonavir, rifampin, carbamazepine, phenobarbital, phenytoin, rifapentine, secobarbital, amiodarone, paclitaxel, replaglinide, ketoconazole, atorvastatin, fosphenytoin, itraconazole, trimethoprim, thioridazine).

Study Outcome Measurements

The primary hypothesis was that the Juniper Asthma Quality of Life Questionnaire (AQLQ) score would be significantly improved at the end of the pioglitazone treatment period as compared to the placebo treatment period^{E2}. Secondary outcome measurements included the Juniper Asthma Control Questionnaire (ACQ) score, daily asthma symptom score, rescue inhaler utilization (# puffs/day), asthma symptom-free days, nights with asthma symptoms, asthma exacerbations (mild and severe), pre- and post-bronchodilator FEV₁, blood inflammatory cell counts (eosinophils, neutrophils, lymphocytes, monocytes and basophils), serum IgE levels, and exhaled nitric oxide levels (FeNO)^{E3-E5}. A mild asthma exacerbation was defined as at least one of the following on two consecutive days as compared to baseline; (a) a reduction in the morning peak expiratory flow rate of $\geq 20\%$, (b) the need for > 3 additional puffs of rescue medication in a 24-hour period, and (c) an increase in nocturnal awakenings caused by asthma symptoms^{E4, E6}. A severe asthma exacerbation was defined as an episode of worsening asthma symptoms requiring oral corticosteroids (≥ 20 mg per day above basal dosing), or intravenous corticosteroids, or an unexpected health care visit⁶. Although an additional pre-specified secondary end-point was the number of sputum inflammatory cells, samples that were suitable for analysis were obtained from only 3 study participants. Therefore, these results were not included in the analysis. Home peak expiratory flow and home FEV₁ measurements using a Piko-1 expiratory flow monitor (nSpire Health, Longmont, CO) were available for only 5 subjects and these were also not included.

Sample Size

We assumed that a sample size of 26 study participants would allow us to detect a difference in the AQLQ score of 0.5 between the pioglitazone and the placebo treatment periods, with 85% power at a two-sided significance level of 0.05, assuming a standard deviation of 1.02 and correlation of repeated measurements of $r=0.7$. A change in the AQLQ score of 0.5 was selected as this has been shown to represent the minimal clinically important difference^{E7}. An independent Data Safety and Monitoring Board (DSMB) reviewed the progress and safety of the study. A decision was made to suspend enrollment after 12 subjects had completed the study because the study investigators and the DSMB concluded that difficulty in recruiting additional subjects made it unlikely that the projected sample size of 26 completers would be achieved within a reasonable time. Data from the 12 completers were then analyzed.

Statistical Analysis

The primary analysis was performed on an intention-to-treat basis. All subjects are analyzed according to their treatment group assignment regardless of actual treatment received. The paired t-test was used to test the primary hypothesis of difference in AQLQ score within each subject between pioglitazone and placebo treatments. The primary analysis was not adjusted for any covariates. In addition, we performed a secondary analysis of the primary endpoint using a mixed effects method to model correlation within subjects with a random intercept. We derived a conditional power estimate to assess the probability of a significant difference in the AQLQ score between the two arms had we continued to full recruitment of 26 subjects. The secondary endpoints were analyzed in a similar way as the analyses described above. In addition, binary responses were compared using the Prescott's test.

Online Results

Pre-specified Secondary End-points

There were no differences between the pioglitazone and placebo treatment periods regarding airflow obstruction, as measured by the pre- and post-bronchodilator FEV₁ (Figure E2), or measures of type 2 inflammation and allergy, such as peripheral blood eosinophils, FeNO, and serum IgE levels (Figure E3). There were also no differences in peripheral blood counts of neutrophils, basophils, and monocytes between the pioglitazone and placebo treatment periods (Figure E4).

Adverse Effects

Two subjects had the study medication discontinued during the pioglitazone treatment arm. One subject developed 4+ pedal edema with a concomitant 4 kg weight gain. The other subject developed facial and lip edema consistent with a presumptive diagnosis of angioedema. Both adverse events fully resolved following discontinuation of the study medication. One additional subject experienced a Grade 4 increase in serum creatine phosphokinase that was noted at the week 16 visit of the pioglitazone treatment period, at which time the subject had completed both phases of the study. This episode was thought to possibly reflect an intensified personal exercise program and the serum creatine phosphokinase subsequently returned to baseline at a follow-up visit. No subject had to discontinue the study medication during the placebo arm. Additional adverse effects related to the pioglitazone and placebo treatments that occurred in two or more subjects are shown in Table E2. There was no difference in the weight of the study subjects at the end of the pioglitazone and placebo treatment periods (96.7 ± 15.1 vs. 96.1 ± 12.9 kg, $p = 0.65$).

Online References

- E1. Proceedings of the ATS workshop on refractory asthma: Current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am J Respir Crit Care Med* 2000; 162:2341-51.
- E2. Juniper EF, Guyatt GH, Epstein RS, Ferrie PJ, Jaeschke R, Hiller TK. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. *Thorax* 1992; 47:76-83.
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Online Repository Tables

Table E1. Baseline demographics of the subjects who completed the study.

Age (years)	53.4 $\pm$ 11.5
Female/Male	7/5
Ethnicity (White/Black/Asian)	10 / 1 / 1
BMI	33.5 $\pm$ 4.2
Prior Pack-Years of Cigarette Smoking	0.6 $\pm$ 1.7
Age at Asthma Onset	21.4 $\pm$ 17.3
Duration of Asthma (years)	32 $\pm$ 15.9
# of Subjects with prior Asthma-related Respiratory Failure	2
# of Severe Asthma Exacerbations in the Prior Year	3.4 $\pm$ 2.6
Pre-bronchodilator FEV ₁ (liters)	2.07 $\pm$ 0.47
Pre-bronchodilator FEV ₁ (% predicted)	69.4 $\pm$ 9.8
Pre-bronchodilator FEV ₁ /FVC (%)	60.7 $\pm$ 12.5
Post-bronchodilator FEV ₁ (liters)	2.40 $\pm$ 0.39
Post-bronchodilator FEV ₁ (% predicted)	80.1 $\pm$ 12.4
Post-bronchodilator FEV ₁ /FVC (%)	62.3 $\pm$ 13.3
FeNO (ppb)	49.3 $\pm$ 54.5
Blood Eosinophils (cells/ μ l)	309.2 $\pm$ 217.7
Serum IgE (IU/ml)	207.9 $\pm$ 241.2
Asthma Quality of Life Questionnaire Score (Juniper AQLQ)	4.3 $\pm$ 0.9
Asthma Control Questionnaire Score (Juniper ACQ)	2.2 $\pm$ 0.5

Data presented as mean $\pm$ standard deviation.

Table E2. Reported adverse events occurring in two or more subjects.

<u>Adverse Event</u>	<u>Placebo</u> <u>(n = 12)</u>	<u>Pioglitazone</u> <u>(n = 14)</u>
Abdominal Cramping	1	1
Angioedema	1	1
Back Discomfort	1	1
Bronchitis	3	1
Myalgias	1	1
Pedal edema	1	3
Urinary Tract Infection	2	3

Online Figure Legends

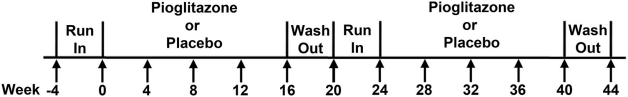
Figure E1. Design of this randomized, double-blinded, placebo-controlled, two period, crossover study of pioglitazone for the treatment of severe asthma.

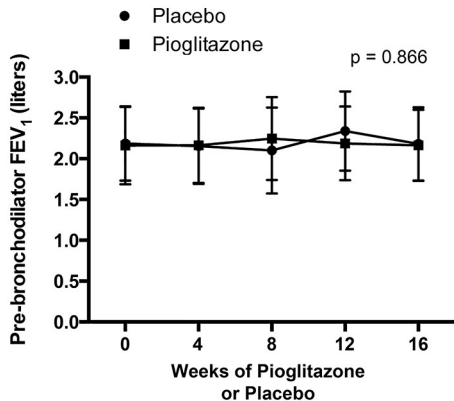
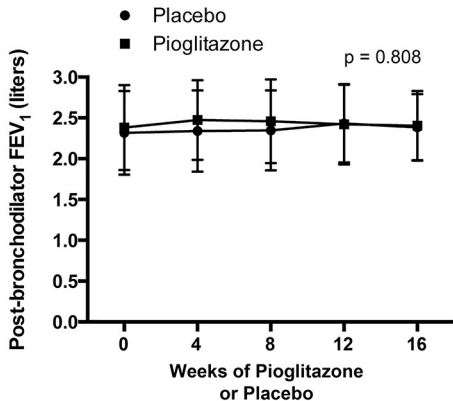
Subjects whose asthma remained stable during a 4 week run-in period were randomized to receive either pioglitazone or matching placebo. Pioglitazone was administered at a dose of 30 mg daily for the initial 2 weeks of the first treatment phase, followed by a dose escalation to 45 mg daily for weeks 3 through 16. Following a 4 week washout period, subjects again were assessed for clinical stability during a second 4 week run-in period prior to crossing over to the second treatment phase to receive the other agent.

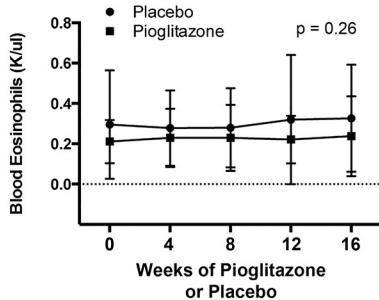
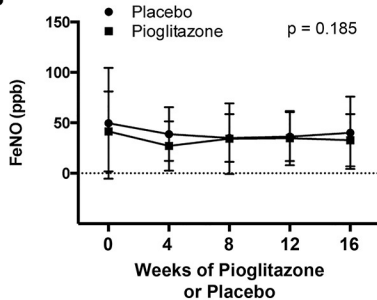
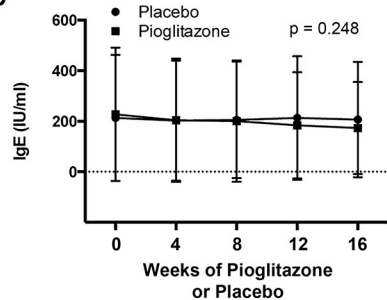
Figure E2. Comparison of pioglitazone and placebo treatment periods for the secondary end-points of pre- and post-bronchodilator FEV₁ (liters). Panel A. Pre-bronchodilator FEV₁, $p = 0.866$. Panel B. Post-bronchodilator FEV₁, $p = .808$.

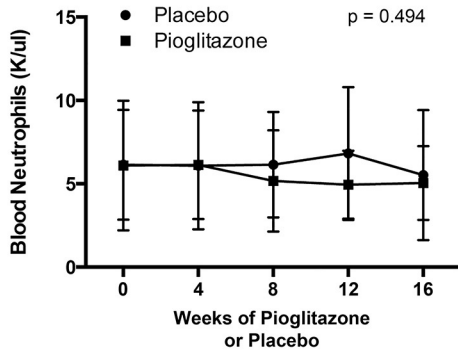
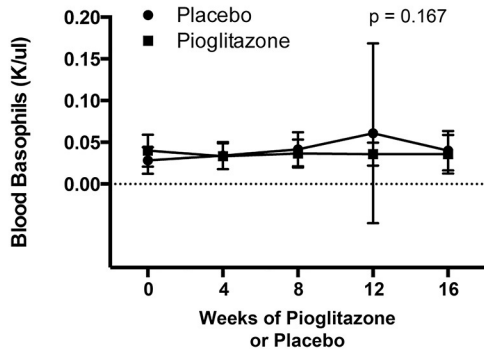
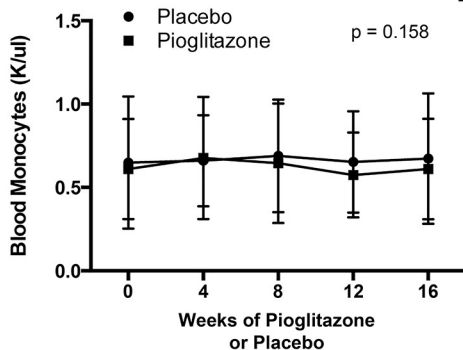
Figure E3. Comparison of pioglitazone and placebo treatment periods for the secondary end-points of eosinophilic inflammation and allergy. Panel A. Blood eosinophils (K/ μ l), $p = 0.26$. Panel B. FeNO (ppb), $p = 0.185$. Panel C. Serum IgE (IU/ml), $p = 0.248$. FeNO results are shown for the 11 subjects who were able to successfully perform testing.

Figure E4. Comparison of pioglitazone and placebo treatment periods for the secondary end-points of blood inflammatory cells. Panel A. Blood neutrophils (K/ μ l), $p = 0.494$. Panel B. Blood basophils (K/ μ l), $p = 0.167$. Panel C. Blood monocytes (K/ μ l), $p = 0.158$. Panel D. Blood lymphocytes (K/ μ l), $p = 0.002$.



A**B**

A**B****C**

A**B****C****D**