2011

Fenofibrate and Insulin Resistance

Corrie E. Paeglow
University of Alabama at Birmingham

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by

CORRIE E. PAEGLOW

EDMOND K. KABAGAMBE, CHAIR
DONNA K. ARNETT
GARY R. CUTTER
FRANK A. FRANKLIN
FERNANDO OVALLE

A DISSERTATION

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Doctor of Public Health

BIRMINGHAM, ALABAMA

2011

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PUBLIC HEALTH

ABSTRACT

Both animal and human models suggest that fenofibrate, a medication widely prescribed to decrease triglycerides, also decreases insulin resistance. In the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) cohort, which included 780 individual who took 160 mg of micronized fenofibrate daily for three weeks, fenofibrate was found to decrease HOMA-IR by -0.24 units, (95% CI: -0.32, -0.14), insulin by -0.65 uU/mL (95% CI: -0.97, -0.34) and glucose by 2.46 mg/dL (95% CI: -2.51, -2.42). In a meta-analysis of 19 studies that included 1,297 individuals without diabetes and 196 individuals with diabetes treated with fenofibrate for three weeks or longer, fenofibrate was found to decrease HOMA-IR by 0.46 units (95% CI: -0.70, -0.22), insulin by 1.23 uU/mL (95% CI: -2.37, -0.09) and glucose concentrations by 2.86 mg/dL (95% CI: -5.01, -0.71).

Change in adiponectin is a potential mediator of the relationship between fenofibrate use and insulin resistance. The relationship between fenofibrate use, changes in adiponectin concentrations and change in HOMA-IR was examined in the GOLDN cohort and significant relationships were found between fenofibrate use and change in adiponectin but not between change in adiponectin and change in HOMA-IR. Thus, changes in adiponectin do not mediate the relationship between fenofibrate use and change in HOMA-IR.

These studies provide support for the hypothesis that fenofibrate use decreases insulin resistance. However, this relationship is not mediated by changes in adiponectin concentrations. Future studies should examine these relationships using a control group so the impact of fenofibrate use on changes in insulin resistance can be more accurately quantified. Further research is also needed to determine if the observed changes in insulin resistance are clinically meaningful.

Keywords: insulin resistance, fenofibrate, adiponectin, meta-analysis

DEDICATION

Gloria Patri, et Filio, et Spiritui Sancto,
sicut erat in principio, et nunc, et semper, et in saecula saeculorum.

ACKNOWLEDGMENTS

I gratefully acknowledge the GOLDN study investigators and steering committee for allowing me to use their data for this dissertation. I also wish to thank my dissertation cmmittee members for their help and encouragement. Special recognition is due to my dissertation committee chair, Edmond Kabagambe, who saw this process through with patience and good cheer. I am proud to be the first doctoral student whose committee he has chaired to graduate, and may I be the first of many. In addition, I would like to acknowledge the contributions of Dr. Jean-Claude Ansquer and Dr. Sang Hak Lee, who kindly provided unpublished data for inclusion in the meta-analysis. Finally, I would like to acknowledge the help of the following individuals: Anna Wallace, Aron Mujumdar, Ben Eisman, Derek Armstrong, May Wallace, Shia Kent and Sophie Zhang.

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Chapter 1

INTRODUCTION

Insulin Resistance

Insulin resistance occurs when insulin is less effective at lowering plasma glucose concentrations as a consequence of reduced glucose uptake by muscle and fat cells (1). The extreme case of insulin resistance is type 2 diabetes where, in the absence of effective treatment, glucose concentrations increase above normal despite higher than normal concentrations of circulating insulin (1) .

Prevalence of Insulin Resistance

There are very few studies that have examined the prevalence of insulin resistance in the United States. According to data from the National Health and Nutrition Examination Surveys (NHANES), the mean population insulin resistance, as measured by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), increased from 2.02 (95% CI: 1.9, 2.1) in 1988-1994 to 2.22 (95% CI 2.15, 2.29) in 1999-2002 and, in the same period, the number of normoglycemic individuals who were insulin resistant increased from 26.2% to 32.2% (2). A more commonly studied outcome is metabolic syndrome, of which insulin resistance is one component. In a nationally representative analysis of NHANES II data, 38.5%

of study participants were found to have metabolic syndrome by the International Diabetes Foundation definition (3).

Even less data exist about the global prevalence of insulin resistance. The limited data that are available indicate that the prevalence is highly varied, from 39.6% of subjects in a clinic-based study in Spain (4) to 11.2% of the participants in the Chennai (India) Urban Population Study (5). These statistics are not representative of entire populations, nor do nationwide estimates of population prevalence exist. It is widely agreed, though, that as the global prevalence of obesity rises in both the developed and developing worlds, the prevalence of insulin resistance and type 2 diabetes will also increase (6).

Insulin Resistance as a Risk Factor

The potential global increase in insulin resistance is of particular concern because insulin resistance is both a precursor to type 2 diabetes (7) and a risk factor for a number of diseases, including: coronary artery disease (8), congestive heart failure (9), colorectal cancer (10; 11) and chronic kidney disease (12). Furthermore, some measure of insulin resistance is a component of all definitions of the metabolic syndrome (13), which has been shown to be a risk factor for many other conditions, most notably all-cause mortality (14; 15) and cardiovascular disease (14-17). It is not clear, though, if insulin resistance alone is responsible for any of these effects or if all components of metabolic syndrome work in concert to result in increased risk, and this remains a matter of debate in the literature (18-21). One analysis (17) that considered both metabolic syndrome overall and its constituent parts found

an increased risk of all-cause mortality (HR = 1.31; 95% CI: 1.06, 1.62) among those with impaired fasting glucose, suggesting that insulin sensitivity may be a risk factor for all-cause mortality independent of metabolic syndrome.

Measurement of Insulin Resistance

The hyperinsulinemic euglycemic clamp method of measuring insulin resistance is widely considered to be the gold standard (1; 22), although it has also been noted that this nomenclature can be misleading (22) as different measures are appropriate for different uses. This method infuses insulin intravenously while simultaneously infusing glucose to maintain homeostasis (23). The subject's blood sugar is checked every 5 to 10 minutes to calibrate the infusion rates to ensure that his/her blood sugar stays within normal boundaries. If the person being tested requires a high level of glucose to maintain his/her blood sugar then s/he is insulin sensitive. If s/he require a low level of glucose to maintain his/her blood sugar than s/he is not insulin sensitive.

This test is invasive, time-consuming and introduces a risk for hypoglycemia, making it less than ideal for epidemiologic studies. To overcome these deficiencies the HOMA-IR model was developed (24) using mathematical modeling that included the following variables: hepatic glucose output and uptake, basal insulin production rate, plasma glucose, insulin half-life, insulin concentration, basal glucose output, and the volume of blood into which the insulin will be secreted, glucose uptake by muscle and glucose uptake by fat and β-

cell response to plasma glucose. After creating the model, Mathews and colleagues (24) developed a simple equation that approximates the results of the more sophisticated models:

HOMA-IR = fasting glucose (mg/dL) * fasting insulin (uU/mL) /405.

This model is widely used in epidemiological studies as it is well-correlated with the measure found using the hyperinsulinemic euglycemic clamp method (r ~ 0.88) (22).

HOMA-IR is not the only model based on fasting plasma insulin and glucose concentrations; other models that are commonly used include the Quantitative Insulin Sensitivity Check Index (QUICKI) (25) and the Matsuda Index (26). QUICKI is proportional to 1/log(HOMA-IR) (27), making the two indexes equally appropriate for use in large epidemiological studies. The Matsuda Index correlates well with other measures of insulin sensitivity (27) but requires an oral glucose tolerance test, making it difficult to use in large scale epidemiological studies.

It is also common in the epidemiological literature to report fasting plasma insulin or glucose concentrations as a measure of insulin resistance (28). It has been noted, however, that insulin action varies significantly even among individuals who are not insulin resistant (29) and thus insulin concentrations should be interpreted in light of the individual's glucose concentration (1). Despite this criticism studies have shown that insulin resistant individuals tend to have higher insulin concentrations than non-insulin resistant individuals while having similar glucose concentrations (30), suggesting that fasting insulin concentrations are a reasonable proxy for insulin resistance. This was further confirmed in a study conducted among a group of Finnish men with varying degrees of insulin resistance that found that fasting

plasma insulin was significantly correlated with insulin resistance as measured by clamp study for all subjects, regardless of whether or not they were normoglycemic (31).

Fasting glucose is a more problematic measure; as is noted above insulin resistant individuals often have higher than normal insulin concentrations but glucose levels that are normal. Fasting glucose concentrations are not often reported as a measure of insulin resistance but, where they are, should be interpreted in light of the associated fasting insulin concentrations.

Fenofibrate

Fenofibrate belongs to the peroxisome proliferator-activated receptor (PPAR)-alpha ligands class of medication and has been widely used in the treatment of dyslipidemia as it is effective in decreasing triglycerides (32-34) and, to a lesser extent, raising HDL (34; 35). Fenofibrate is available in several formulations that are bioequivalent and differ in their dosage based on how highly micronized they are. The American Heart Association has determined that patients with triglyceride levels ≥ 200 mg/dL should be treated for their dyslipidemia (36) and thus are candidates for treatment with fibrates, including fenofibrate. Fibrates have been shown to be highly effective in reducing triglycerides; most people treated with fibrates experience a 25-50% reduction in triglycerides (36) with even larger reductions seen among those who have the highest baseline triglyceride concentrations (37; 38). According to estimates derived from the National Health and Nutrition Examination Survey (NHANES) data from 2003-2006, 8.3% of adults with diagnosed dyslipidemia are taking a fibrate (39),

and the actual percentage is likely to be somewhat higher as this does not include subjects taking combination therapy that included a fibrate (i.e. those taking a fibrate in conjunction with a statin).

Impact of Fenofibrate on Cardiovascular Outcomes

Two large, placebo controlled studies have assessed the impact of fenofibrate on cardiovascular outcomes: 1) the Fenofibrate Intervention and Event Lowering (FIELD) trial (40) and; 2) the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial.

The FIELD Trial

The FIELD trial recruited 9,795 individuals with diabetes from ages 50 to 75, randomized them to 200 mg of daily fenofibrate or placebo and followed them at 6 month intervals for 5 years. The treatment group experienced 11% fewer coronary events than the placebo group, although this difference was not statistically significant (33). The treatment group also had statistically significant 24% reduction in non-fatal myocardial infarction (33).

Although coronary events were the main outcomes of interest, the FIELD trial also considered two other outcomes: diabetic retinopathy (41) and amputation events (42). In the sub-study of diabetic retinopathy, fenofibrate was found to decrease the need for a first instance of laser surgery (HR = 0.69; 95% CI: 0.56, 0.84). While the FIELD study has not published data that included measures of insulin resistance, most likely because patients were on

glucose-lowering medications, this analysis noted that subjects with worse glycemic control were at greater risk for a first instance of laser surgery (41). In the analysis of amputation events, which included all 9,795 original study participants, those treated with fenofibrate had decreased risk of minor amputation (HR = 0.53; 95% CI: 0.44, 0.94) but not of major amputation (HR = 0.93; 95% CI: 0.53, 1.62).

The ACCORD Trial

The ACCORD trial recruited 5,518 individuals with type 2 diabetes who were treated with simvastatin and randomized to either 160 mg of daily fenofibrate or placebo. The average follow-up was 4.7 years, and the primary outcomes of interest were myocardial infarction, stroke or coronary death. Fenofibrate use did not significantly decrease the total number of endpoints (HR = 0.92; 95% CI: 0.79, 1.08). Some commentators, however, have noted that among participants with atherogenic dyslipidemia (i.e., high triglyceride and low HDL concentrations) (n = 941) there was a significant decrease in the composite primary outcome, suggesting that fenofibrate therapy is indeed beneficial in this sub-group (43). This result was confirmed by a recent meta-analysis (44) that found that subjects who had atherogenic dyslipidemia and were treated with fenofibrate had a lower relative risk of vascular events (RR = 0.75; 95% CI: 0.65, 0.86).

Fenofibrate and Insulin Resistance

While the majority of fenofibrate studies have considered lipid and cardiovascular outcomes associated with fenofibrate, there has also been some assessment of the relationship between fenofibrate use and insulin resistance. Both animal (46-49) and human (50-55) studies point to fenofibrate use resulting in decreased insulin resistance.

Fenofibrate and Insulin Resistance Studies in Animals

Choi and colleagues (45) treated rats with fenofibrate and determined that they experienced a statistically significant decrease in their insulin and glucose concentrations. Similar results were found by Guerre-Milo et al (46), who treated insulin resistant mice with fenofibrate and found a statistically significant decrease in blood insulin and glucose concentrations. Lee and colleagues (47) measured HOMA-IR in 10 mice treated with fenofibrate and found that HOMA-IR was lower than in a group of similar mice that were not treated. Fenofibrate's insulin sensitizing effect has not only been shown in rodents, the same relationship has been seen in primates. In one study (48), obese rhesus monkeys treated with 30 mg/kg of fenofibrate twice a day experienced a 40% decrease in fasting insulin concentrations.

Fenofibrate and Insulin Resistance Studies in Humans

While murine models clearly point to fenofibrate use resulting in decreased insulin resistance studies in humans have had mixed results. In a study of 37 men with primary

hyperlipidemia (49), 12 weeks of fenofibrate treatment resulted in a 26.8% decrease in fasting serum insulin levels, representing a decrease in insulin resistance. Cardona and colleagues (50) treated 36 subjects with fenofibrate. Participants were divided into two groups based on genotypic characteristics and one group experienced a 0.66 unit decrease in HOMA-IR and the other a 0.61 unit decrease. Krysiak et al. (51) treated 96 subjects with fenofibrate for 90 days and found a 15.9% ($P < 0.01$) decrease in HOMA-IR among patients with impaired fasting glucose and dyslipidemia. In that same study, patients with impaired glucose tolerance and dyslipidemia experienced a 17.2% ($P < 0.01$) decrease in HOMA-IR. Decreases in HOMA-IR were also seen in studies conducted by Pruski et al. (52) (2.6 unit decrease, $P < 0.001$), Takahashi et al. (53) (0.6 unit decrease, $P < 0.001$) and Wi et al. (54) (0.43 unit decrease, $P < 0.02$).

While the weight of the evidence points to fenofibrate having an impact on insulin resistance there are several studies (55-57) that have not observed this relationship. One study (57) of 37 patients who were overweight or obese and had insulin resistance but not diabetes showed no effect of fenofibrate on insulin resistance. In a more recent publication (55) fenofibrate had no effect on insulin resistance in a group of individuals with metabolic syndrome and insulin resistance, although the sample size was quite small (n = 25). Anderlova and colleagues (56) performed a small study (n = 10) among obese women with type 2 diabetes comparing the effects of fenofibrate to those of rosiglitazone and a calorie-restricted diet and found that fenofibrate did not increase insulin sensitivity.

Causal Pathways Linking Fenofibrate and Insulin Resistance

There are several hypotheses related to the causal pathway between fenofibrate use and decreased insulin resistance. First, in addition to decreasing plasma triglycerides, fenofibrate use has been shown to decrease triglyceride concentrations in muscle in animal studies (47; 58-60). There is also a strong correlation between intramuscular triglyceride concentrations and insulin resistance in both animals (61; 62) and humans (63-67). This suggests that fenofibrate lowers triglyceride concentrations in muscles and this decreases insulin resistance. Studies have demonstrated that the largest decreases in triglycerides after fenofibrate use occur in patients with the highest baseline plasma triglyceride concentrations (38), thus we would expect those with the highest baseline triglyceride concentrations to have the largest decreases in intramuscular triglycerides and thus in insulin resistance.

Another mechanism that has been hypothesized is that high concentrations of free fatty acids result in insulin resistance (67-70). Decreases in free fatty acids have been shown to decrease insulin resistance (68; 71) and increases in free fatty acids have been shown to increase insulin resistance (72). Free fatty acids are also correlated with intramuscular triglycerides (72), which have been associated with insulin resistance. Fenofibrate has been shown to increase β-oxidation of fatty acids (60), thus, it is plausible that fenofibrate use results in decreased free fatty acid concentrations and thus decreased insulin resistance.

A final pathway that has been proposed (73) and seems plausible in light of the current evidence is that fenofibrate use increases blood adiponectin levels and the latter improves insulin sensitivity. Fenofibrate has been shown to increase adiponectin concentrations in animal studies (45; 74; 75) and some (76; 77) but not all (56) studies conducted in humans.

In addition, adiponectin has been shown to be inversely associated with plasma triglycerides (78), and it is possible that adiponectin decreases triglycerides and thus decreases insulin resistance as described above.

Limitations of Current Fenofibrate Studies

There are several factors that may explain why some studies have detected a relationship between fenofibrate and insulin resistance while others have not. All of the studies had small sample sizes, making it possible that they were underpowered to detect any changes that may have occurred. Also, fenofibrate has the greatest effect among those with the highest baseline triglycerides (79), suggesting that variation in each study population's baseline triglyceride levels lead to the differing study results.

Another potential reason studies examining the association between fenofibrate and insulin resistance have been equivocal is that they have not adequately considered the role of genetics. In a large cohort treated with fenofibrate for three weeks, variation at the Apolipoprotein A5 locus (APOA5) was found to be related to fenofibrate response; individuals with the APOA5 56G polymorphism experienced a 35.8% decrease in triglycerides while those without this polymorphism only experienced a 27.9% decrease in triglycerides (80). Several other studies (80-82) that have examined genetics and response to fenofibrate have identified other genes that modulate the degree to which triglycerides change in response to treatment with fenofibrate, including APOA5 (50; 80; 81) and glucokinase regulatory protein (GCKR) (83). Given the relationship between changes in triglyceride levels and insulin resistance dis-

cussed above, these SNPs are also likely to be associated with changes in insulin resistance after fenofibrate use.

Adiponectin

Adiponectin is an adipokine secreted by adipose tissue but, paradoxically, plasma adiponectin concentrations are negatively correlated with body mass index (BMI) (84; 85) and subcutaneous and intra-abdominal body fat (86). In one analysis, non-obese subjects were found to have mean adiponectin concentrations of 8.9 ± 5.4 mg/mL while obese subjects had mean concentrations of 3.7 ± 3.2 mg/mL ($P < 0.0001$) (85). Low adiponectin concentrations are also associated with dyslipidemia; adiponectin concentrations are negatively correlated with triglyceride levels and positively correlated with HDL levels (87).

Adiponectin exists in high-, medium- and low-molecular weight forms in the body. There has been considerable debate in the literature about the most appropriate way to model adiponectin with respect to insulin resistance: either as a ratio of high-molecular-weight adiponectin (HMWA) to total adiponectin or as total adiponectin. Some studies (88-92) have suggested that a ratio of HMWA to total adiponectin is more closely correlated to glucose intolerance than total adiponectin, while others (93; 94) have found little difference between the two measures.

Adiponectin is crucial in regulating glucose uptake and insulin sensitivity in humans (95; 96), and studies support a relationship between adiponectin, fenofibrate use and insulin resistance.

Fenofibrate and Adiponectin

In humans, the evidence clearly points to fenofibrate use resulting in increases in adiponectin. In one crossover study of 46 patients treated with 200 mg/day of fenofibrate for 8 weeks, adiponectin levels rose by 12% ($P = 0.02$) (76). In a study of 53 individuals with hypertriglycedemia – the group for which treatment with fenofibrate is indicated – randomized to fenofibrate or placebo therapy, adiponectin were 17% in the treatment group than in the placebo group (97) . Some studies, however, have not seen an impact of fenofibrate use on adiponectin concentrations. In a randomized trial among HIV-positive individuals (n = 41) adiponectin concentrations decreased after fenofibrate use (98) and in another study of fenofibrate use among individuals without diabetes (n = 4) fenofibrate had no effect on adiponectin concentrations (99). Both studies were relatively small, however, thus it is possible that they lacked sufficient statistical power to detect a relationship.

Adiponectin and Insulin Resistance

Animal models suggest a strong relationship between insulin resistance and adiponectin concentrations; rhesus monkeys that are obese and with diabetes have lower adiponectin concentrations than monkeys who are normal weight and do not have diabetes (100). To assess the temporality of this relationship the monkeys' adiponectin levels were followed from normoglycemia to insulin resistance to diabetes, with decreases in adiponectin observed at each step. More evidence for a causal relationship is supplied by two studies conducted in mice, where insulin resistant mice who were given a combination of adiponectin and leptin

became normoglycemic (101; 102). In addition, mice bred to be deficient in adiponectin were found to have greater insulin resistance than wild type mice (103). This relationship showed a dose-response effect, with homozygous adiponectin deficient mice having greater insulin resistance than heterozygous adiponectin deficient mice (103).

Among humans, adiponectin levels have been shown to be inversely correlated with HOMA-IR (104) and fasting plasma glucose (96). In addition, individuals with diabetes or impaired glucose tolerance typically have lower adiponectin concentrations than normoglycemic individuals (87; 105). There is also ample evidence that a low adiponectin concentration is a risk factor for extreme insulin resistance or type 2 diabetes. This relationship has been seen in multiple populations, including subjects in the Atherosclerosis Risk in Communities (ARIC) study (HR for highest vs. lowest quartile of adiponectin = 0.18; 95% CI: 0.11, 0.27) (106), the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (OR highest vs. lowest quartile = 0.3; 95% CI: 0.2, 0.7) (107), a cohort of 1,792 Japanese (OR highest vs. lowest tertile = 9.32; 95% CI: 1.05, 83.1)(108) and individuals investigated in a case-control study of 140 Pima Indians (IRR high vs. low adiponectin = 0.65; 95% CI: 0.43, 0.92) (109). The reproducibility and consistency of these results strongly support a relationship between adiponectin and insulin resistance. In addition, changes in adiponectin have been shown to be inversely correlated with changes in insulin sensitivity as measured by QUICKI (97), adding further evidence of a relationship between adiponectin concentrations and insulin resistance.

Areas for Future Research

While previously conducted research suggests that fenofibrate use decreases insulin resistance and adiponectin may mediate the relationship between fenofibrate and insulin resistance, significant gaps in the literature remain. First, many of the studies assessing the effect of fenofibrate have not specifically considered changes in insulin resistance after fenofibrate use. In particular, the two largest fenofibrate trials, ACCORD and FIELD, have not reported any measures of insulin resistance. Studies that have reported measures of insulin resistance are generally quite small and in many cases the study population included individuals who have diabetes, a population that is likely using a medication that affects insulin resistance. Diabetes medication use renders interpretation of insulin challenging. Finally, few studies have considered adiponectin as a potential mediator between fenofibrate use and insulin resistance.

This dissertation will fill these gaps by conducting a meta-analysis looking at fenofibrate and insulin resistance and then examining the relationship between fenofibrate and insulin resistance and then fenofibrate, adiponectin concentrations and insulin resistance in a large (n = 780) cohort of subjects treated with fenofibrate.

EFFECT OF FENOFIBRATE ON INSULIN RESISTANCE IN THE GOLDN COHORT

CORRIE E. PAEGLOW, EDMOND K. KABAGAMBE, MIKE Y. TSAI, ROBERT J. STRAKA, GARY R. CUTTER, FRANK A. FRANKLIN, FERNANDO OVALLE, DONNA K. ARNETT

In preparation for *Diabetes and Metabolic Syndrome*

Format Adapted for Dissertation

Abstract

Objective

To assess the short-term effect of fenofibrate on insulin resistance and to identify variables associated with change in insulin resistance.

Methods

Men and women (n = 780, age 47.4 ± 15.9) in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) family study were treated with 160 mg of fenofibrate once daily for three weeks. Insulin, glucose and lipids were measured before and after treatment. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from these data. Change in HOMA-IR was calculated as post-treatment minus pre-treatment HOMA-IR value. Generalized Estimating Equations (GEE) were used to test whether fenofibrate is associated with a significant change in HOMA-IR in the total sample and within tertiles of baseline HOMA-IR. To identify variables associated with response to fenofibrate, we added covariates to the GEE models.

Results

Fenofibrate use resulted in significant absolute changes ($P < 0.05$) in HOMA-IR (-0.24; 95% CI: -0.32, -0.14). The mean (95% CI) change in HOMA-IR in the 1st, 2nd and 3rd tertile was -0.13 (-0.05, -0.22), -0.10 (-0.19, -0.01) and -0.72 (-0.96, -0.49), respectively. Of the variables tested for their effect on change in HOMA (including age, gender, BMI, PA, alcohol use and smoking), only BMI ($P < 0.01$) was significantly associated with change in HOMA-IR, but this was only seen among participants in the middle tertile of baseline HOMA-IR.

Conclusion

Fenofibrate significantly reduces insulin resistance and this effect is most pronounced among individuals with elevated baseline HOMA-IR values.

Background

Fenofibrate, a Peroxisome Proliferator Activated Receptor-alpha agonist, is effective in lowering triglycerides (1) and in decreasing cardiovascular events, but not in decreasing all-cause mortality (1-3). In both the Fenofibrate Intervention and Event Lowering (FIELD) trial (2) and a meta-analysis assessing the risk of cardiovascular death among fibrate users (3), fenofibrate use was not found to decrease cardiovascular mortality. Whether fenofibrate has clinical benefits other than lowering triglycerides and improving other lipid fractions in humans is a subject of much discussion (4-6). One potential benefit of fenofibrate use is a reduction in insulin resistance, as hypertriglyceridemia is associated with increased insulin resistance (IR) (7-11). This benefit has been demonstrated in animal studies (12-14) and also in some (15-22) but not all (23-25) human studies. Other benefits reported for fenofibrate – such as reduction in the number of non-traumatic amputations (26) and the number of patients who needed laser treatment for retinopathy (27) – may be in part due to reductions in insulin resistance. Published studies on fenofibrate and insulin resistance-related phenotypes have been very small (mostly < 50 participants), used varying doses and formulations of fenofibrate and yielded inconsistent findings. Some studies have included patients on treatment with insulin sensitizing medications, making it impossible to separate the potential insulin sensitizing effects of fenofibrate from those of other medications. In addition, the two largest long-term studies on fenofibrate use, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study (28) and the FIELD study (29), did not report insulin resistance as an outcome, most likely because most participants had diabetes and were on treatment. Thus, there is paucity of evidence on the relationship between fenofibrate and insulin resistance.

One challenge in determining fenofibrate's effect on IR is the difficulty of assessing IR. The hyperinsulinemic euglycemic clamp method is the gold standard for measuring IR (30; 31) but is invasive and time-consuming, and thus difficult to use in epidemiological investigations. To overcome these difficulties the HOMA-IR approach based on fasting plasma and glucose concentrations was developed (32). The HOMA-IR model is widely used in epidemiological studies of insulin resistance and it correlates well with more objective measures such as the hyperinsulinemic euglycemic clamp method (r ~0.88) (31; 32).

In this report we examined the relationship between fenofibrate use and change in insulin resistance in the GOLDN study, a cohort with 780 individuals who were treated with a 160 mg daily dose of fenofibrate for three weeks and had data collected about their metabolic parameters both before and after treatment.

Methods

The details of the GOLDN study have been reported in detail elsewhere (33). Briefly the study recruited patients in three generation pedigrees from Minneapolis, MN and Salt Lake City, UT, sites chosen because they are likely to be largely genetically homogeneous. Before commencing treatment with fenofibrate, all patients were asked to discontinue use of their lipid-lowering medications for 3 weeks. At the baseline visits, participants completed questionnaires related to their medical history and lifestyle factors, had anthropometric measures taken and gave a fasting blood sample that was analyzed for metabolic parameters, including glucose, insulin and lipid concentrations. After this initial visit participants began

three weeks of treatment with 160 mg of micronized fenofibrate once daily. After completing three weeks of treatment they returned to the study site and provided another fasting blood sample.

Laboratory Measurements

Details about the laboratory measurements are fully described elsewhere (33). In short, all samples were centrifuged within 20 minutes of collection and stored at −70 °C to ensure they were frozen. For each analyte, specimens from each participant were assayed in the same batch to eliminate inter-assay imprecision. Fasting glucose was measured using the hexokinase-mediated reaction on a Hitachi 911 analyzer (Roche Diagnostics), while fasting insulin was measured using the human insulin specific RIA kit (Linco Research, St. Charles, MO). Triglycerides were measured using a glycerol blanked enzymatic method (Trig/GB, Roche Diagnostics Corporation, Indianapolis, IN) and cholesterol was measured with a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics Corporation) on the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation). For HDL-cholesterol, the non-HDL-cholesterol was first precipitated with magnesium/dextran. LDL-cholesterol was measured by a homogeneous direct method (LDL Direct Liquid Select™ Cholesterol Reagent, Equal Diagnostics, Exton, PA) (33).

Statistical Analyses

All data management and analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). From 845 participants with data on insulin and glucose before and after treatment, we excluded the following participants as measures of HOMA-IR are known to be

unreliable in these populations: those who reported a diagnosis of diabetes (n = 22) or were taking a medication used to treat diabetes (n = 43). We also intended to exclude those subjects who had insulin concentrations ≥ 58 uU/mL or ≤ 2.88 uU/mL post-treatment; and those who had glucose concentrations ≥ 360 mg/dL or post-treatment glucose concentrations ≤ 63 mg/dL, however no subjects who met the previous criteria did not meet this criteria.

HOMA-IR was calculated both at baseline and follow-up using the formula: HOMA-IR = fasting glucose (mg/dL) * fasting insulin (uU/mL)/405 (32). The absolute change in HOMA-IR was calculated by subtracting the pre-treatment value from the post-treatment value.

Demographic and anthropometric characteristics were calculated by tertiles of baseline HOMA-IR, and the significance of the differences between tertiles tested using ANOVA for continuous variables and chi-square tests for categorical variables. Absolute changes in insulin, glucose and HOMA-IR values were the main outcomes of interest, and as a preliminary assessment of these changes we created scatter plots of change in HOMA-IR, insulin and glucose by their respective baseline values. We also examined change in HOMA-IR, insulin and glucose in the entire cohort and among tertiles of their respective baseline values.

GEE models that adjusted for pedigree were used to test whether the changes in insulin, glucose and HOMA-IR values in the total sample and by tertile of baseline concentration were significantly different from zero. Since these tests are analogous to the paired t-test, they were not adjusted for any covariates. The GEE models were refitted after adding covariates to determine whether there are variables that determine response to fenofibrate with regard to HOMA-IR, insulin and glucose. The covariates tested were gender, age, BMI and lifestyle factors such as current drinking, current smoking and physical activity. Gender, cur-

rent drinking and smoking were modeled as categorical variables while BMI and the average number of reported hours of computer or television use on a weekday (used as a proxy for physical activity) were modeled as continuous variables.

Changes in BMI are associated with changes in insulin resistance, thus we assessed changes in weight in the cohort. Subjects with unrealistically large weight changes (i.e. change in BMI ≥ 5 kg/m^2 within 3 weeks of treatment) were excluded from this analysis as their results are likely a product of measurement error. To test whether the change in weight was significantly different from zero, we used GEE that adjusted for pedigree as a random effect.

Next we sought to determine whether compliance to fenofibrate treatment may have confounded the results. Participants were considered compliant if pill counts at the end of the study revealed that the study participant had taken more than 75% of the pills. In this sensitivity analysis we restricted the cohort to only those who were compliant with fenofibrate treatment (n = 743) and re-assessed the associations. Finally, we also performed a sensitivity analysis that only included individuals with baseline triglyceride concentrations ≥ 200 mg/dL (n = 79). Previous work demonstrated that response to fibrates is greatest among patients with high baseline concentrations of triglycerides (34; 35).

Results

Demographic and metabolic characteristics of the participants at baseline are presented in **Table 1**. Participants in the highest tertile of HOMA-IR were significantly ($P < 0.05$) older and had a higher BMI than those in the lower two tertiles.

Figures 1, 2 and **3** show scatter plots of change in HOMA-IR, insulin and glucose by their respective baseline values. **Figures 4, 5 and 6** and **Table 2** show change in HOMA-IR, insulin and glucose in the total sample and by tertile of their respective baseline concentrations. Insulin and glucose decreased significantly from baseline to post-treatment ($P< 0.0001$ for both). In stratified analysis insulin decreased in the two highest tertiles but increased slightly in the lowest tertile while glucose decreased in all three tertiles. HOMA-IR decreased among those in the two highest tertiles of baseline HOMA-IR, and this change was significant in both tertiles (highest tertile -0.77; $P < 0.0001$, middle tertile -0.09; $P = 0.04$). Participants in the lowest tertile of baseline HOMA-IR experienced a significant increase in HOMA-IR (0.13; $P = 0.001$).

Next, we tested whether there are variables that are associated with response to fenofibrate as it pertains to insulin, glucose and HOMA-IR. In the most basic model, which did not include any covariates other than adjustment for familial relationships, use of fenofibrate resulted in a statistically significant 0.24 unit decrease in HOMA-IR (95% CI: -0.32, -0.14). In a multivariate model, none of the covariates added to the model – including age, gender, BMI, physical activity and current smoking and alcohol use – were significantly associated with response to fenofibrate in the total sample. There was, however, an association between BMI and change in HOMA-IR in the middle tertile ($P = 0.01$) in which those with higher BMIs experienced smaller changes in HOMA-IR concentrations.

Use of fenofibrate also resulted in a 0.65 uU/mL decrease in insulin concentrations that was statistically significant (95% CI: -0.90, -0.34). There was a significant association between age and change in insulin levels ($P = 0.01$), although when the analysis was stratified by baseline insulin concentrations this relationship was only seen in the middle tertile

(lowest tertile, $P = 0.13$, middle tertile $P = 0.03$, highest tertile $P = 0.12$). There was no association between BMI and response to fenofibrate overall ($P = 0.12$), however such a relationship was observed in the middle tertile (lowest tertile, $P = 0.07$, middle tertile $P = 0.001$, highest tertile $P = 0.27$). In the middle tertile, those with a higher BMI experienced greater changes in insulin concentrations.

Glucose concentrations were also modified by fenofibrate use; glucose decreased 2.46 mg/dL (95% CI: -2.51, -2.42) in the entire cohort. This change was modified by age in the entire cohort ($P = 0.01$), with older subjects undergoing smaller changes in glucose concentrations. In the lowest tertile of baseline glucose, gender ($P = 0.02$) and current smoking ($P = 0.01$) were significantly associated with change in glucose. Women experienced smaller changes in glucose than men and smokers experienced larger changes in glucose than non-smokers. In the highest tertile of baseline glucose there was a significant association between BMI and physical activity ($P = 0.01$) and change in glucose, with those having a higher BMI experiencing larger changes in glucose concentrations and those who were more physically active also experiencing larger changes. In the middle tertile those who were more physically active experienced smaller changes in glucose ($P = 0.01$).

We also assessed changes in BMI across the study period. Overall, the cohort experienced a statistically significant ($P < 0.0001$) 0.16 kg/m^2 increase in BMI.

The results of the sensitivity analyses mirrored those of the main analysis. Among those who were compliant with fenofibrate treatment the intercept-only model showed a 0.24 unit decrease in HOMA-IR (95% CI: -0.33, -0.14). Those with elevated baseline triglyceride concentrations (n = 79) experienced a significant 0.61 unit decrease in HOMA-IR (95% CI: -0.86, -0.36).

Discussion

This analysis supports the hypothesis that fenofibrate use results in improvements in insulin resistance in general and has the most benefit among those with higher baseline concentration of HOMA-IR, insulin or glucose. This difference in response by tertile is likely because the body will resist changes to metabolic parameters if they are already in the normal range but will respond if the parameters are outside of normal values. This is also significant as those in the highest tertile of HOMA-IR had average triglyceride concentrations of 187 mg/dL (25^{th}, 75^{th} percentile: 107, 225) making many of the patients in this tertile candidates for fenofibrate therapy. In addition, the sensitivity analysis that was restricted to patients with baseline triglyceride concentration $\geq$ 200 mg/dL demonstrated that the effect of fenofibrate on change in HOMA-IR was larger in this group compared to those with lower baseline triglyceride concentrations. Thus, this analysis supports the conclusion that fenofibrate decreases insulin resistance among the population for which its use is currently clinically indicated.

It is possible, however, that the changes we observed are attributable to changes in weight rather than fenofibrate use; weight loss also results in decreased insulin resistance. We found a small but significant increase in BMI, however, an increase in weight would be associated with a decrease in insulin sensitivity, thus these weight changes are likely not a factor in our results.

Our results are consistent with previous research that has shown that fibrates have the greatest effect in reducing triglycerides in those with the highest triglyceride concentrations prior to initiating treatment(34; 35) In addition to decreasing plasma triglycerides fenofibrate has been shown to decrease triglyceride concentrations in muscle in animal studies (36-39),

and intramuscular triglycerides concentrations are positively correlated with insulin resistance in humans (7-11). Fibrates have also been shown to reduce circulating free fatty acids (40), thus our findings are consistent with the hypothesis that high concentrations of free fatty acids result in insulin resistance (41) and that lowering free fatty acids decreases insulin resistance (42).

The reduction in insulin resistance seen among those using fenofibrate stands in contrast to the effect of statins on insulin resistance; statins – particularly atorvastatin, rosuvastatin and simvastatin – have been shown to increase the risk of insulin resistance (43) and type 2 diabetes (44). Theoretically, then, combining fenofibrate with a statin would be expected to be beneficial in terms of decreasing insulin resistance, but to our knowledge no studies have assessed the impact of combined fenofibrate and statin therapy on insulin resistance outcomes. This includes the largest trial of fenofibrate and simvastatin, the ACCORD study (28). The FIELD study also did not report any insulin resistance outcomes, however, indirect measures of improved insulin resistance, such retinopathy and amputations, were favorable (26; 27). Future research should examine whether concomitant use of fenofibrate and statins decrease both LDL and triglyceride concentrations while not resulting in the increase in insulin resistance seen among users of some statins. However, this is likely to be difficult to implement given that many patients initiate therapy for diabetes before beginning therapy for dyslipidemia.

In addition to decreasing HOMA-IR concentrations, fenofibrate use also decreased insulin and glucose concentrations. This is consistent with other studies that have considered fenofibrate's effect on glucose (17; 22; 45-48) and insulin (15; 17; 20; 22; 45; 48; 49).

This study has a number of strengths. The large sample size afforded ample statistical power to detect differences before and after treatment and enabled analyses stratified by baseline HOMA-IR. Stratification with meaningful statistical power within groups was previously impossible given the small sample sizes of published studies on this topic. In addition, most of the participants were compliant with their treatment as determined by pill counts, an objective measure of adherence to therapy. Blood samples were taken in accordance with one established protocol and the laboratory analyses were done at one site, thus minimizing the impact of variability between laboratories. Furthermore, this study is unique in that only one lipid-lowering medication was used, making it possible to examine the effect of this drug independent of other lipid-lowering medications.

This study had some limitations, including a lack of randomization and lack of a comparison group that is not taking fenofibrate. It is possible that changes in all three measures in insulin resistance are due in part to regression to the mean effects that cannot be quantified in this cohort. Future studies should examine specifically the relationship between fenofibrate use and insulin sensitivity using a control group that is not treated with fenofibrate so that this relationship can be more accurately characterized. Ethical challenges may prevent such a study, however; withholding fenofibrate from patients who have high triglyceride levels would be unethical.

The other limitation of this analysis is the difficulty of assessing the clinical importance of the changes in HOMA-IR, insulin and glucose concentrations found after fenofibrate use. Although fenofibrate use did decrease HOMA-IR by 0.24 units, this is a modest reduction and while it is statistically significant the clinical relevance of this is yet to be deter-

mined. Although, HOMA-IR values of ≥ 3 are considered indicative of insulin resistance (50), currently there is no broad consensus as to what level of HOMA-IR denotes insulin resistance or diabetes, making it particularly difficult to interpret the clinical significance of this change. Future research focused specifically on establishing these cut-points will be vital in helping to understand the implications of this analysis.

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Table 1. Demographic and anthropometric characteristics of included participants

		Tertile of HOMA-IR		
	All participants (n=780)	**1 (n=259)**	**2 (n= 261)**	**3 (n= 260)**
Age, y	47.4 (15.9)	45.4 (15.0)	46.3 (16.6)	50.64 (15.43)
BMI, kg/m^2	28.2 (5.32)	24.85 (3.60)	28.02 (4.48)	31.65 (5.37)
Average timespent on TV/Computer, hr/week	2.66 (1.91)	2.33 (1.92)	2.54 (1.79)	3.11 (1.94)
Female, %	49.5	57.9	47.89	57.3
Current smokers, %	7.82	10.04	8.43	5.00
Current alcohol users, %	50.5	57.9	51.0	42.69

Numbers are presented as mean (s.d.) or percent

Except for smoking, all variables showed statistically significant ($P<0.05$) differences in their distribution across tertiles of baseline HOMA-IR.

Table 2. Change in HOMA-IR, insulin and glucose

	Treatment or *P*	All participants (n = 780)	Tertile of Baseline HOMA-IR		
			1	2	3
			(n = 259)	(n =261)	(n =260)
HOMA-IR	Pre-treatment	3.43 (2.32)	1.80 (0.37)	2.87 (.33)	5.61 (2.87)
	Post-treatment	3.19 (2.13)	1.94 (.69)	2.77 (.82)	4.84 (2.82)
	P	< 0.0001	0.001	0.04	< 0.0001
Insulin	Pre-treatment	13.65 (7.59)	7.88 (1.60)	11.86 (1.61)	21.19 (8.63)
	Post-treatment	12.99 (6.92)	8.49 (2.70)	11.67 (3.21)	18.78 (8.41)
	P	< 0.0001	0.0002	0.17	< 0.0001
Glucose	Pre-treatment	99.20 (13.86)	92.78 (7.39)	98.46 (8.27)	106.32 (19.03)
	Post-treatment	96.86 (13.52)	91.72 (7.32)	96.16 (8.29)	102.67 (19.15)
	P	< 0.0001	0.0004	< 0.0001	< 0.0001

Figure 1. Change in HOMA-IR by baseline HOMA-IR

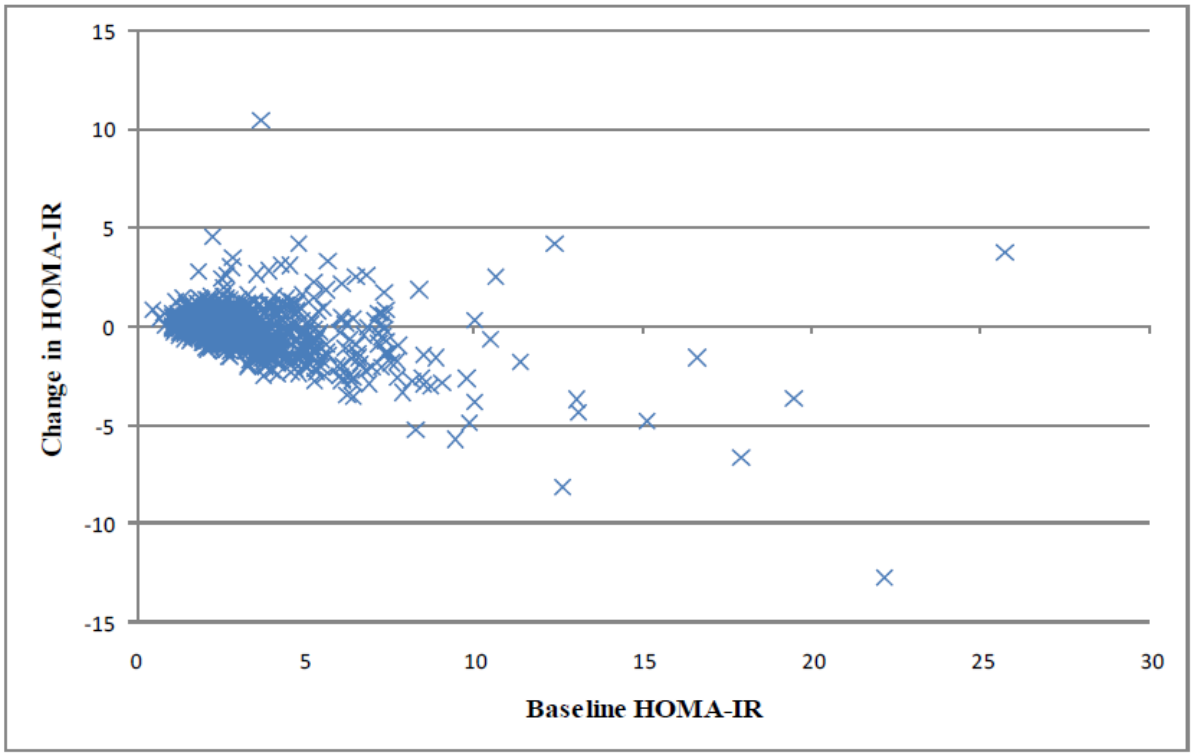


Figure 2. Change in insulin by baseline insulin

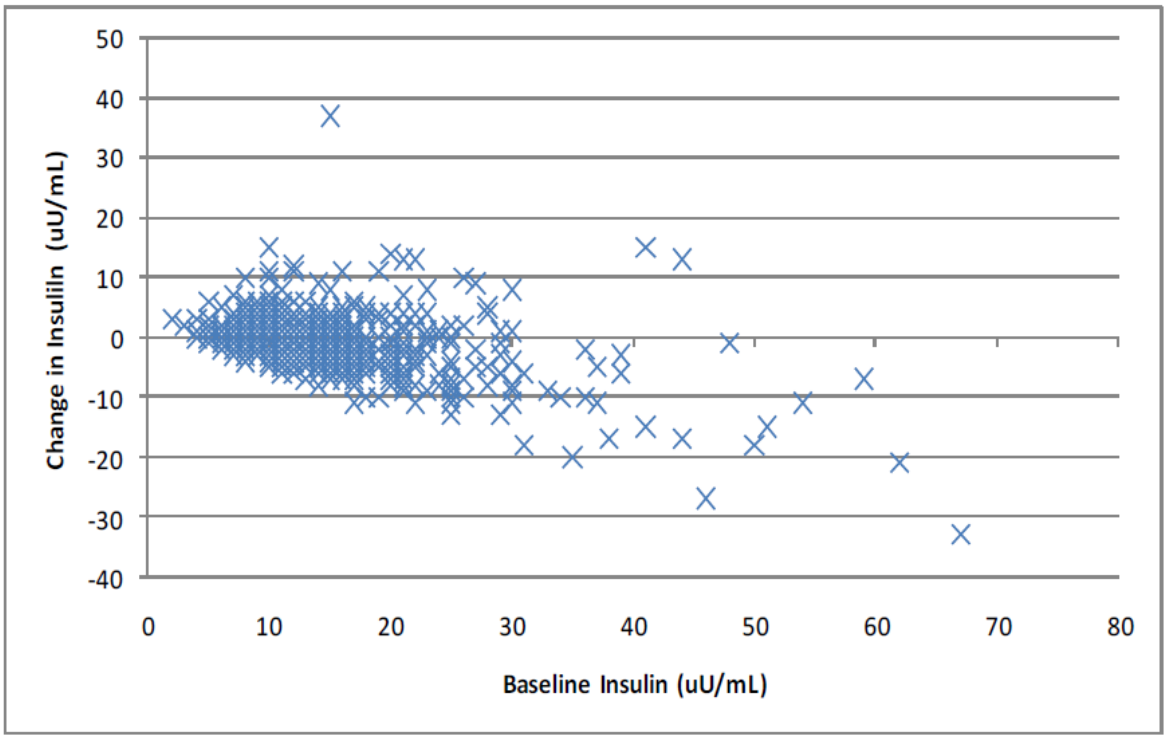


Figure 3. Change in glucose by baseline glucose

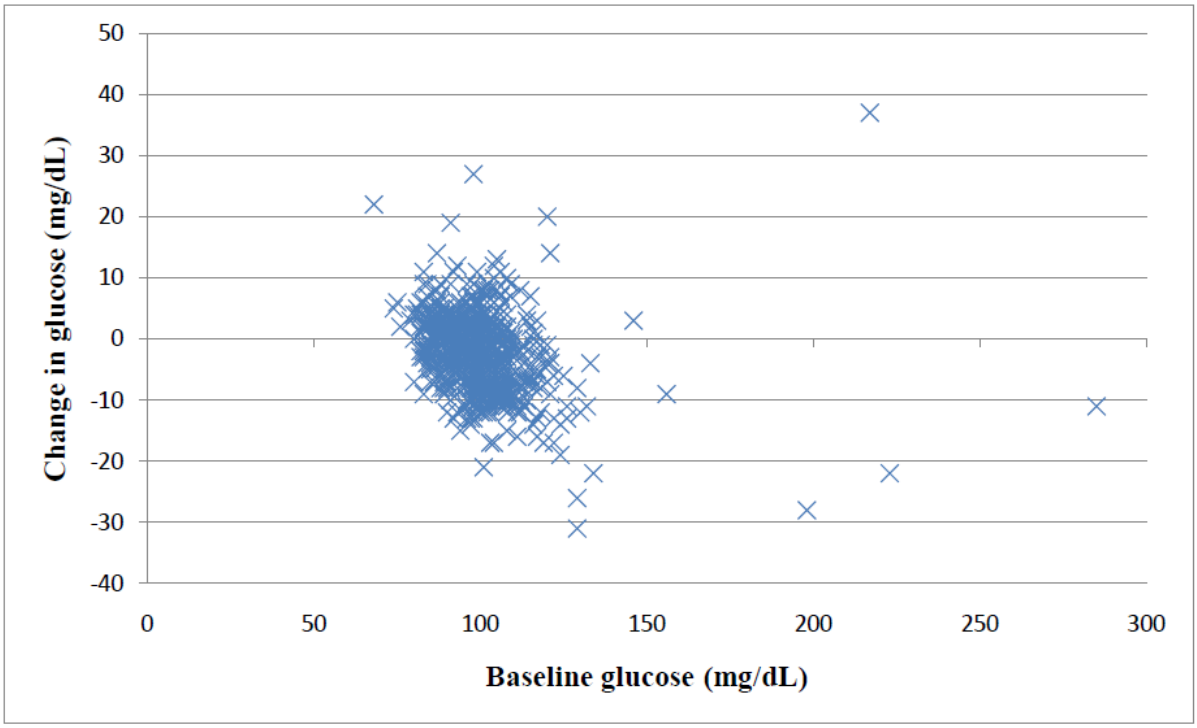


Figure 4. Change in HOMA-IR overall and by baseline tertile of HOMA-IR

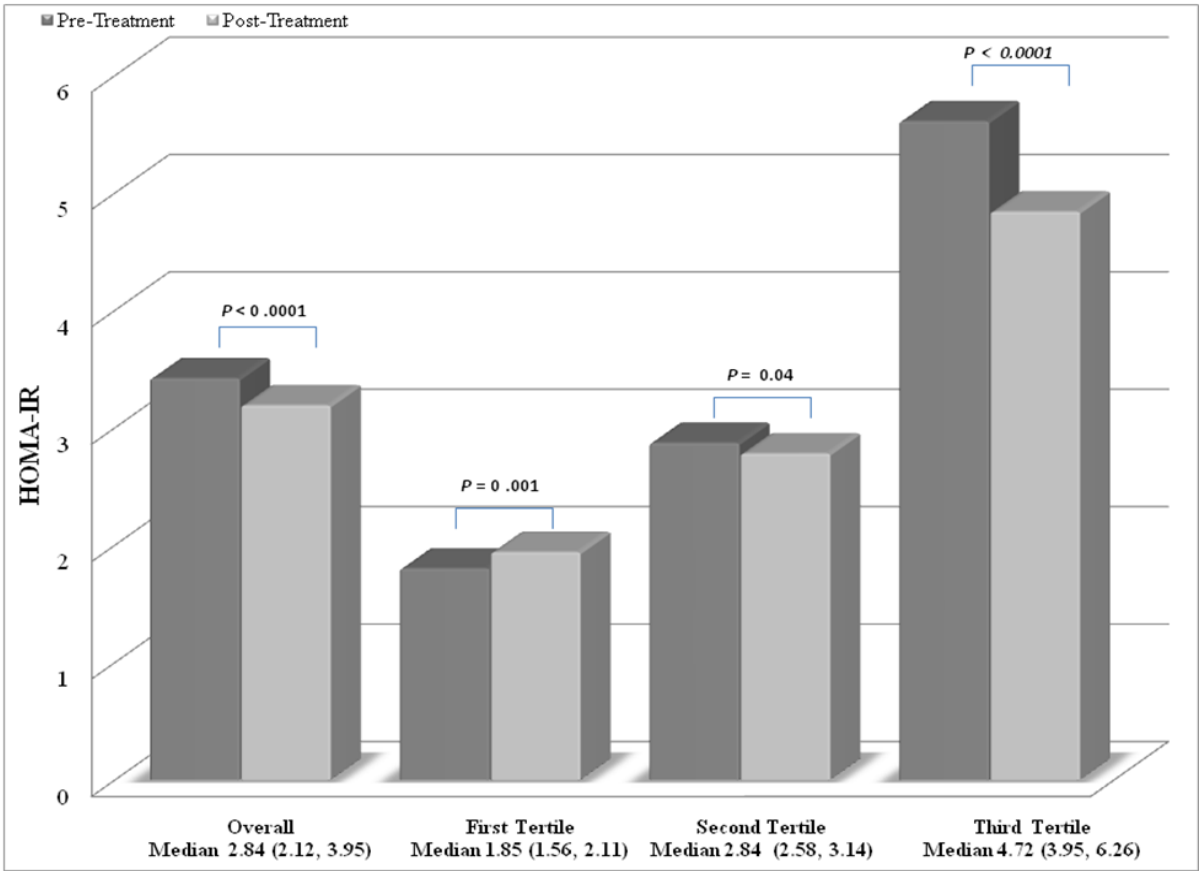


Figure 5. Change in insulin overall and by baseline tertile of insulin

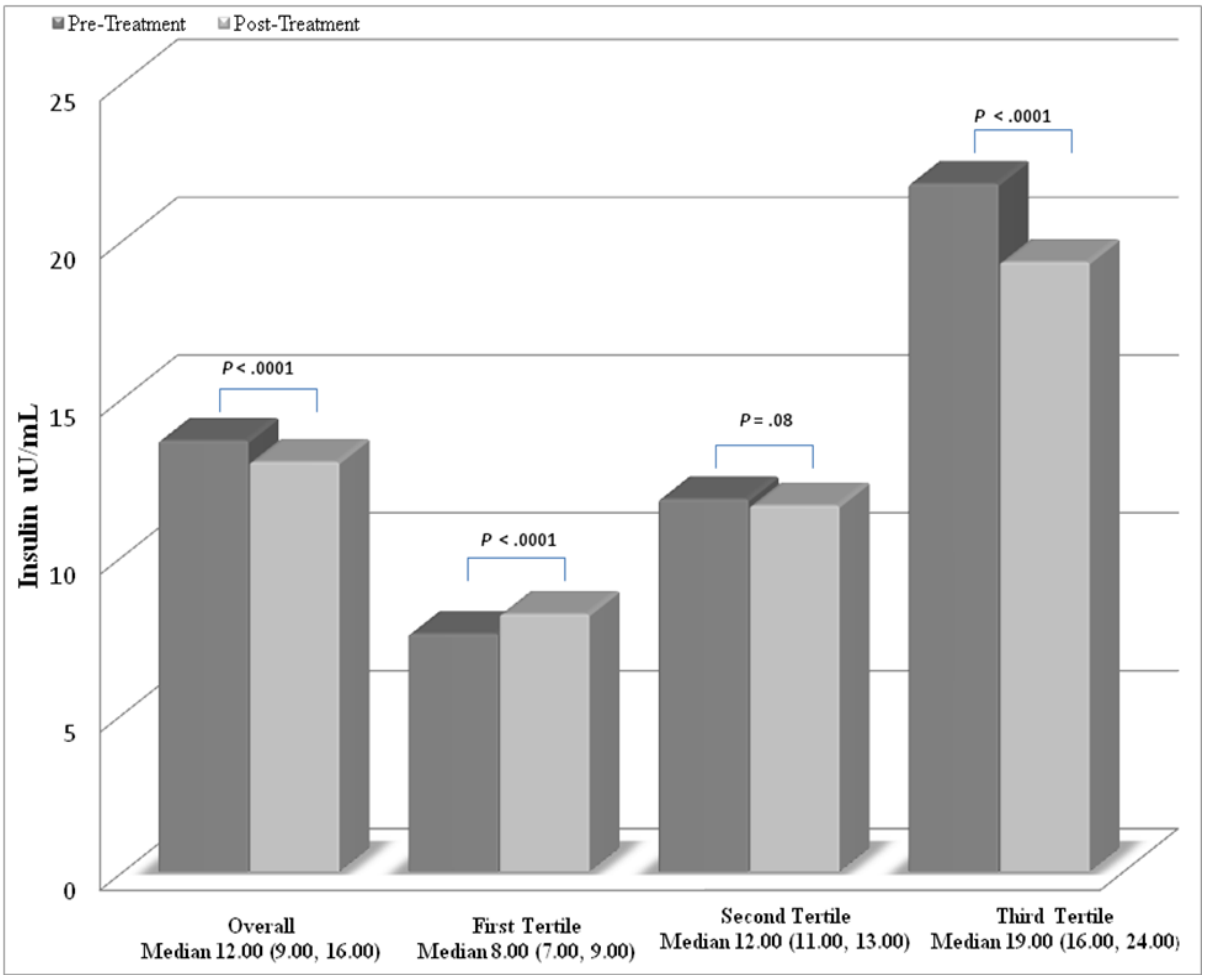
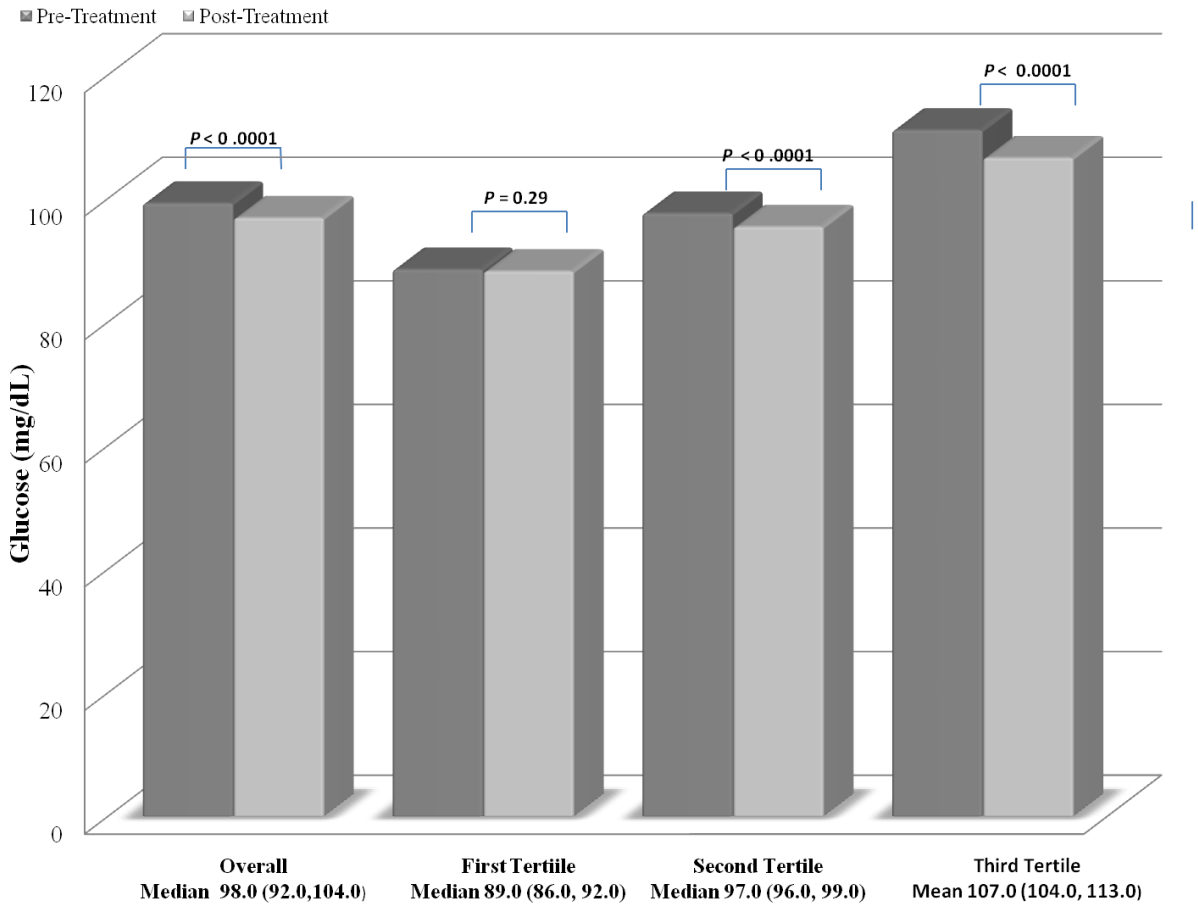


Figure 6. Change in glucose overall and by baseline tertile of glucose



EFFECT OF FENOFIBRATE ON INSULIN RESISTANCE: A META-ANALYSIS

CORRIE E. PAEGLOW, SHIA T. KENT, MIKE Y. TSAI, ROBERT J. STRAKA, GARY R. CUTTER, FRANK A. FRANKLIN, FERNANDO OVALLE, DONNA K. ARNETT, EDMOND K. KABAGAMBE

In preparation for *Diabetes and Metabolic Syndrome*

Format Adapted for Dissertation

Abstract

Objective

Whether fenofibrate, a drug commonly used to lower triglycerides, improves insulin resistance is still under debate. We conducted a meta-analysis to determine whether fenofibrate decreases insulin resistance (IR).

Methods

Two investigators searched the literature to identify human studies where fenofibrate was administered and insulin resistance-related phenotypes (HOMA-IR, fasting glucose or fasting insulin) were reported. The results of these studies were combined using a random effects meta-analysis to determine the effect of fenofibrate on insulin resistance-related phenotypes. The analysis also stratified studies by diabetes status to determine if fenofibrate had the same effect in individuals with diabetes and those without diabetes.

Results

Nineteen studies qualified for inclusion in the meta-analysis, representing 196 individuals with diabetes and 1,297 individuals without diabetes. Fenofibrate use resulted in statistically significant declines in HOMA-IR, insulin and glucose. Overall, fenofibrate was associated with 0.46 unit decrease (95% CI: -0.32, -0.14) in HOMA-IR concentration, 1.23 uU/mL decrease (95% CI: -0.97, -0.34) in insulin concentration and 2.86 mg/dL (95% CI: -5.01, -0.71) decrease in glucose concentration. In stratified analyses, individuals without diabetes showed statistically significant declines in all the three insulin resistance-related phenotypes: HOMA-

IR -0.44 (95% CI: -0.65, -0.23), insulin -1.32 uU/mL (95% CI: -2.22, -0.41), glucose -2.91 mg/dL (95% CI: -3.88, -1.95) while those with diabetes experienced non-statistically significant changes in insulin of -1.60 uU/mL (95% CI: -6.95, 3.76) and glucose of -4.19 mg/dL (95% CI: -20.64, 12.26). Only one study measured HOMA-IR among individuals with diabetes and this study found a statistically significant decrease in HOMA-IR of -2.62 (95% CI: -4.53, -0.69)

Conclusion

These results show that fenofibrate use resulted in a small but significant reduction in insulin resistance. Further studies with more objective measures of insulin resistance, such as oral glucose tolerance test, are needed to further characterize the effect of fenofibrate on insulin resistance.

Background

Fenofibrate, a Peroxisome Proliferator Activated Receptor-alpha agonist, is effective in lowering triglycerides (1) and in decreasing cardiovascular events, but not in decreasing all-cause mortality (1-3). Whether fenofibrate has clinical benefits other than lowering triglycerides is a subject of much discussion (3-5). One potential benefit of fenofibrate use is a reduction in insulin resistance (IR) as hypertriglyceridemia is often associated with increased insulin resistance (6-10). This benefit has been demonstrated in animal studies (11-13) and also in some (14-21) but not all (22-24) human studies. Other reported benefits of fenofibrate – including reduction in the number of non-traumatic amputations (25) and the number of patients who needed laser treatment for retinopathy (26) – following fenofibrate treatment in the Fenofibrate Intervention and Event Lowering (FIELD) trial, may be due to a reduction in insulin resistance. Published studies on fenofibrate and insulin resistance-related phenotypes have been very small (mostly <50 participants), used varying doses and formulations of fenofibrate and yielded inconsistent findings.

Currently, there is not enough accumulated evidence to state definitively whether fenofibrate decreases insulin resistance. The literature in this area has several limitations, chief among them that many fenofibrate trials did not assess insulin resistance. To our knowledge, neither of the largest fenofibrate trials undertaken to date, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) (27) and the FIELD study (28), reported insulin resistance as an outcome. In addition, some studies included patients being treated for type 2 diabetes with an insulin sensitizing medication, making it impossible to separate the potential insulin sensitizing effects of fenofibrate from those of other medications. In this meta-analysis will

aggregate the results from various studies, while appropriately stratifying by diabetes status, and thus elucidate the relationship between fenofibrate use and insulin resistance.

One final challenge in assessing the relationship between fenofibrate use and insulin resistance is that there are several measures of insulin resistance that are widely reported in the epidemiologic literature, making it difficult to aggregate results across studies. Limiting a meta-analysis to any one measure would result in much of the relevant literature being excluded, thus we examined three insulin resistance-related phenotypes: homeostatic model assessment of insulin resistance (HOMA-IR), fasting insulin, and fasting glucose. All three measures are commonly used to assess insulin resistance.

Methods

Literature Search

Two investigators (CEP and STK) searched PubMed and Google Scholar between August 19, 2010 and March 2011 for human fenofibrate clinical trials that measured insulin and glucose before and after fenofibrate and were published in English. Search terms that were used included "fenofibrate and insulin resistance", "fenofibrate and glucose", "fenofibrate and insulin" and "fenofibrate and clinical trial." After identifying the initial groups of studies the investigator searched their citations to identify other potentially relevant papers. ClinicalTrials.gov and the Agency for Health Care Research and Quality (AHRQ) clinical trials registry were also searched to identify relevant papers. Unpublished data from one

study, the GOLDN trial, was also included in the analysis. In total, 60 papers were identified for possible inclusion in the study.

The two investigators who conducted the initial literature review (CEP, STK) independently reviewed all the papers to determine whether they violated the exclusion criteria, which were:1) Did not have a mean and standard deviation reported for at least one of the following at baseline and follow-up: insulin, glucose or HOMA-IR; 2) Fewer than 10 subjects; 3) Less than 3 weeks of treatment with fenofibrate; 4) Greater than 10% drop-out rate among those treated with fenofibrate; 5) Subjects have insulin resistance secondary to trauma, non-alcoholic fatty liver disease or treatment for HIV/AIDS; and 6) Study used a crossover design but did not include a washout period between treatment arms.

If the study reported baseline or follow-up measures of insulin resistance but not both baseline and follow-up measures the investigators made an effort to contact the study authors and request the missing value(s). If the two investigators differed in their assessments of whether the study violated the exclusion criteria they discussed the matter until they reached consensus on whether the paper should be included. Although they had planned to consult a third investigator if they could not reach a consensus this proved unnecessary as the two investigators were able to reach a consensus in every case.

Results Abstraction

After identifying the relevant body of literature two investigators (C.E.P and S.T.K) independently abstracted the data to be used in the analysis. One investigator (C.E.P) compared the data abstraction tables and if any discrepancies were noted the original abstractors discussed them until they reached a consensus.

Statistical Analysis

The vast majority of studies that met the inclusion criteria were pre-/post-analyses of patients beginning fenofibrate treatment and did not include a control group. Although some studies included a comparison group often this group was taking an alternative insulin-sensitizing medication. Thus, we only abstracted the results for groups that were taking fenofibrate and reported pre- and post-treatment measures of insulin resistance. This somewhat limits the statistical power of the analysis, as baseline and follow-up values tend to be positively correlated, an attribute that would result in overestimating the variance of the difference in the pre and post-treatment measures. While this could bias the results towards the null, given the available data a comparison of pre- and post-treatment measurements is the most appropriate way to undertake this meta-analysis.

In some studies (16; 31; 32) the results were provided by sub-groups and no overall measures were provided. We contacted the authors to obtain the aggregate data, however as they did not respond we calculated pooled means and standard deviations for the entire study population. This was not done for one study (31) as the results were stratified by diabetes status, thus each group – individuals with diabetes and individuals without diabetes – was included separately in the analysis to enable the calculation of measures stratified by diabetes

status. One study (33) reported data that was collected from family groups, and the standard deviation corrected for the correlation within family groups was used in this analysis.

All analyses were conducted after harmonizing units of measurement to conventional units. When a study reported results using standard units they were converted using the conversion factors recommended by the American Medical Association (34). The baseline and follow-up measures were included in a mean difference model that weighted each study using the inverse variance method. We used a random effects model, as there is evidence that response to fenofibrate is modulated by a number of factors, including genetics (16; 35-37) and baseline triglyceride concentrations (1). The random effects model assumes there is no fixed effect across populations and therefore is appropriate for this analysis.

As it is difficult to interpret the impact of fenofibrate in individuals with diabetes who may be talking other medications that impact insulin sensitivity and lipids we stratified our analyses by diabetes status and reported both overall and strata-specific results. We also conducted a sensitivity analysis by excluding one study (33) that had a much larger sample size than the other studies to see if excluding this study altered the results. All analyses were conducted using the Cochrane Review's Review Manager software, version 5 (38). All plots were created using MixPro 2.0.

Results

Nineteen studies (14; 16; 18-21; 23; 31-33; 39-48) qualified for inclusion in the meta-analysis, representing 196 individuals with diabetes and 1,297 individuals without diabetes. **Table 1** gives detailed information about the studies that were included in the meta-analysis. After reviewing the papers to see if the met the inclusion criteria the inter-rater reliability be-

tween the two investigators was calculated and found to be good (κ = 0.63; 95% CI 0.39, 0.86). The studies that were included in this meta-analysis generally included subjects who were obese, had metabolic syndrome, or had some type of dyslipidemia. The smallest dose of fenofibrate given to participants was 145 mg/day and the doses went as high as 300 mg/day. The average length of fenofibrate treatment was 80 days, although this ranged from a low of 21 days to a high of 180 days.

HOMA-IR

Figure 1 shows the relation between fenofibrate and HOMA-IR. Use of fenofibrate resulted in a small but statistically significant decrease in HOMA-IR (*P* for overall effect = 0.0002). Overall HOMA-IR decreased by 0.46 units (95% CI: -0.70, -0.22). Among individuals without diabetes, the decrease in HOMA-IR was 0.44 units (95% CI: -0.65, -0.23). Only one study (18) reported HOMA-IR for individuals with diabetes, and the observed decrease in HOMA-IR was 2.61 units (95% CI:-4.53, -0.69). We observed significant heterogeneity across studies: $Tau^2 = 0.05$; $Chi^2 = 15.22$, df = 7 (*P* = 0.03); $I^2 = 54\%$.

Figure 2 is an exclusion sensitivity plot to determine whether any one study unduly influenced the analysis. No study was found to have undue influence, however we also conducted a sensitivity analysis by excluding one study (33) that had a much larger sample size than the others. Excluding this study caused the point estimate to change slightly but still indicted a statistically significant decrease in HOMA-IR (-0.52; 95% CI: -0.78, -0.25).

Insulin

Figure 3 shows the relation between fenofibrate use and fasting insulin concentrations. Overall, fenofibrate use resulted in a statistically significant decrease in insulin concentrations (-1.23; 95% CI: -2.37, -0.09). We observed a significant decrease in insulin concentrations only among individuals without diabetes (-1.32 uU/mL; 95% CI: -2.22, -0.41). Individuals with diabetes experienced a small, but statistically insignificant, increase in insulin concentrations (1.60 uU/mL; 95% CI: -6.95, 3.76). Significant heterogeneity across studies was observed: $Tau^2 = 3.41$; $Chi^2 = 95.98$, df = 15 ($P < 0.00001$); $I^2 = 84\%$.

Figure 4 shows an exclusion sensitivity plot for insulin. No study appeared to unduly influence the results of the analysis. In addition, the results of the largest study were excluded in a sensitivity analysis. This resulted in small change in the point estimates for all subjects (-1.32; 95% CI: -2.62, -0.02) and subjects without diabetes (-1.42; 95% CI: -2.43, -0.41). As the excluded analysis did not include diabetic subjects the estimates for diabetic subjects did not change.

Glucose

Figure 5 shows the relation between fenofibrate use and fasting glucose concentrations. Overall, glucose decreased by 2.86 mg/dL after treatment with fenofibrate (95% CI: -5.01, -0.71). In analyses stratified by diabetes status, no statistically significant difference was observed among individuals with diabetes (-4.19 mg/dL; 95% CI: -20.64, 12.26) but individuals without diabetes experienced a statistically significant decrease in glucose concentrations (-2.91 mg/dL; 95%

CI: -3.88, -1.95). We observed significant heterogeneity across studies: $Tau^2 = 10.00$; $Chi^2 = 52.63$, df = 17 ($P < 0.00001$); $I^2 = 68\%$.

When the results of the largest study (33) were excluded the point estimate changed slightly but still indicted a statistically significant decrease in glucose among all subjects (-2.91 mg/dL; 95% CI: -5.44, -0.38) and those without diabetes (-3.13 mg/dL; 95% CI: -4.26, -2.00). As the excluded analysis did not include diabetic subjects the estimates for diabetic subjects did not change.

Assessment of Heterogeneity

The initial analysis demonstrated significant heterogeneity between studies in all the three insulin resistance phenotypes, thus we sought to assess potential sources of this heterogeneity. Four potential sources – dose of fenofibrate, length of treatment, baseline value of the parameter of interest and baseline triglyceride values – were plotted against the mean difference in the parameter of interest. These plots suggested that individuals who begin with the highest baseline value of the given parameter experienced the greatest change, and this may be a significant source of heterogeneity. We tested whether variations in baseline values contributed significantly to the heterogeneity between studies in a fixed effects meta-regression mixed model using change in HOMA-IR, insulin or glucose as the dependent variable and baseline HOMA-IR, glucose, insulin or triglycerides as the independent variable. The only significant association found was between baseline triglycerides and change in insulin (parameter estimate -0.057, $P = 0.04$). This magnitude of effect is quite small and no other factors were found to be significantly associated with heterogeneity between studies,

suggesting that the majority of the heterogeneity is due to factors that were not measured in the studies included in the current analyses.

Discussion

Fenofibrate use resulted in significant decreases in HOMA-IR, insulin and glucose concentrations. Regardless of which measure of insulin resistance was considered, subjects experienced a decrease in that measure. For individuals without diabetes these changes were statistically significant, while for those subjects with diabetes the change was not significant. The lack of a significant effect among individuals with diabetes may be because of the relatively small number of individuals with diabetes (n = 196) compared to those with diabetes (n = 1,297) in the analysis.

The exclusion sensitivity plots showed that these results are robust to the exclusion of any study; in each case removing any of the studies would not significantly alter the results. This was further confirmed by the sensitivity analysis that excluded the largest study and showed that excluding this study did not significantly alter the relationship between fenofibrate use and any of the measures of insulin resistance.

It is important to note that the statistical method used, while the most appropriate for the available data, cannot account for the fact that the pre- and post-treatment measurements are correlated. However, the pre/post analysis also eliminates the impact of potential non-time varying confounders, making it more likely that we are observing a true relationship.

The literature on fenofibrate use to date has been largely inconclusive, with some studies showing a decrease in insulin resistance and others showing no effect. One limitation of the current body of evidence is that most studies have a small sample size; with the exception of the GOLDN study none of the studies included in this analysis had more than 80 subjects. Given the modest effect sizes we found in this analysis it is possible that studies that had a null result lacked the statistical power necessary to detect the small changes in HOMA-IR, insulin and glucose. This analysis was able to detect these differences because of its much larger sample size.

Another key strength of this study is that it excluded *a priori* poor quality studies. Studies with fewer than 10 subjects or less than 3 weeks of follow-up were excluded, and studies that included subjects with type 2 diabetes were analyzed separately. The separate analysis of studies including subjects with type 2 diabetes is particularly important; measures of HOMA-IR, glucose and insulin are unreliable in this population, making the results of such studies difficult to interpret.

One potential form of bias in meta-analyses is publication bias; studies that show null or unexpected results are less likely to be published than studies that find significant, expected results. To investigate the impact of publication bias we created **Fig 7, 8, and 9** for HOMA-IR, insulin and glucose, respectively. The plot for HOMA-IR does not appear to be symmetrical, suggesting that the results may be influenced by publication bias. There are other potential explanations for this as well; the outlier to the extreme left represents the only study that reported HOMA-IR for subjects with diabetes. Subjects with diabetes will have high HOMA-IR at baseline, thus giving them more potential to experience larger changes in

HOMA-IR. It is also possible that the asymmetrical nature of the plot is attributable to an overall scarcity of research examining fenofibrate use and HOMA-IR and not that the existing research has remained unpublished. Finally, HOMA-IR is not the only measure of insulin resistance thus it is possible that other studies have reported different measures and thus were not included in this analysis. In short, making any judgment about the impact of publication bias on these results would require more information.

The funnel plots for insulin and glucose are more symmetrical, although they each have one outlier. In both cases the outlier arises from the same study (49). The subjects included in this study had diabetes, and at baseline their mean glucose concentrations were 174 mg/dL and their insulin concentrations were 37.1 uU/mL. In both cases these values were the highest baseline values of all the studies. This suggests that these subjects experienced the largest changes in insulin and glucose because they began with the highest values which, in turn, suggests that it is not publication bias that is causing the funnel plots to be asymmetrical. Thus, publication bias is not likely to be the cause of the significant changes we see in insulin and glucose values.

While this analysis has demonstrated that fenofibrate decreases insulin resistance it is difficult to assess the clinical significance of a 0.46 unit decrease in HOMA-IR. To our knowledge there are no studies that have investigated the relationship between HOMA-IR and risk of diabetes-related sequelae among individuals with diabetes, nor is there a clear relationship between HOMA-IR and diabetes risk. This makes understanding the true clinical significance of these results somewhat difficult.

These results may have clinical implications, however, for the use of combined statin and fenofibrate therapy for dyslipidemia. It is well established that some statins, another commonly prescribed class of drugs used to treat dyslipidemia, increase insulin resistance (50) while fenofibrate decreases insulin resistance. Thus, prescribing a statin and fenofibrate in concert might result in the insulin sensitizing effects of fenofibrate ameliorating the insulin desensitizing effects of statins. While previous studies have considered the combined effect of fenofibrate and statins on changes in lipids (51) and cardiovascular outcomes (52) to our knowledge none have examined the combined effects on insulin resistance, making this an important area for future research. Ethical challenge may prevent such a study, however; withholding fenofibrate from patients who have high triglyceride levels would be unethical.

The major limitation of this study is that the statistical analysis cannot account for the correlation between pre- and post-treatment measurements. However, this biases results towards the null, and our results were statistically significant despite this bias to the null. This may suggest that the observed inverse association between fenofibrate use insulin resistance may be large than the estimate from the current analyses.

Conclusion

This meta-analyses shows that use of fenofibrate leads to a small but statistically significant decrease in insulin resistance as measured by HOMA-IR, fasting blood glucose and fasting blood insulin. These results suggest that fenofibrate could have important clinical uses not only in treating hypertriglycedemia but also in minimizing statin-related insulin resistance in

cases of combined fenofibrate-statin therapy. Future studies are needed to determine the degree of change in HOMA-IR that is clinically meaningful and to determine whether combination therapy with a statin and fenofibrate can offset the insulin desensitizing effects of some statins.

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Table 1. Studies included in the meta-analysis

Author	Year	n	Study Population	Dose (mg/day)	Length of Therapy (days)
Anderlova et al.	2007	10	Obese women with type 2 diabetes	200	90
Ansquer et al.	2009	60	LDL ≥ 160 mg/dL, triglycerides ≤ 150 mg/dL and ≥ 405 mg/dL and at least 2 components of metabolic syndrome	145	70
Cardona et al.	2009	50	Patients with metabolic syndrome and "an important increase in fasting triglycerides"	160	90
Cree et al.	2007	19	Volunteers ages 65-72 with total cholesterol < 300 mg/dL	160	60
Damci et al.	2004	31	Type 2 diabetics with triglycerides ≤ 250 mg/dL and ≥ 405 mg/dL	250	90
Haluzik et al.	2009	11	Obese women with type 2 diabetes	200	90
Hodgson et al.	2002	18	Type 2 diabetics with dyslipidemia	200	84
Idzior-Walus et al.	2000	44	Patients with dyslipidemia and metabolic syndrome	200	84
Jastrezebeska et al.	2009	64 non-diabetic/64 diabetic	Patients with metabolic syndrome	200	60
Kilcarslan et al.	2008	25	Patients with metabolic syndrome	200	56
Koh et al.	2006	44	Patients with triglycerides ≥ 150 mg/dL	200	60
Krysiak et al.	2010	96	Patients with mixed dyslipidemia that was not controlled after 3 months of dietary treatment	267	90
Oki et al.	2007	11	Patients with triglycerides ≥ 150 mg/dL	150, then increased to 300	90
Paeglow et al.	2011	780	Subjects recruited in 3 generation pedigrees	160	21
Pruski et al	2009	31	Type 2 diabetics with mixed dyslipidemia	267	30
Takahashi et al.	2007	26	Patients who had primary gout as defined by American Rheumatism Association	300	180
Tan et al.	2001	35	Type 2 diabetics with HbA1C < 9%	200	180
Wi et al.	2011	80	Patients with TG 150-499 mg/dL, HDL < 45 mg/dL and LDL < 130 mg/dL	160	112
Yong et al.	1997	23	Patients with HDL < 34.75 mg/dL	300	180

Figure 1. Flow diagram of studies included in the meta-analysis

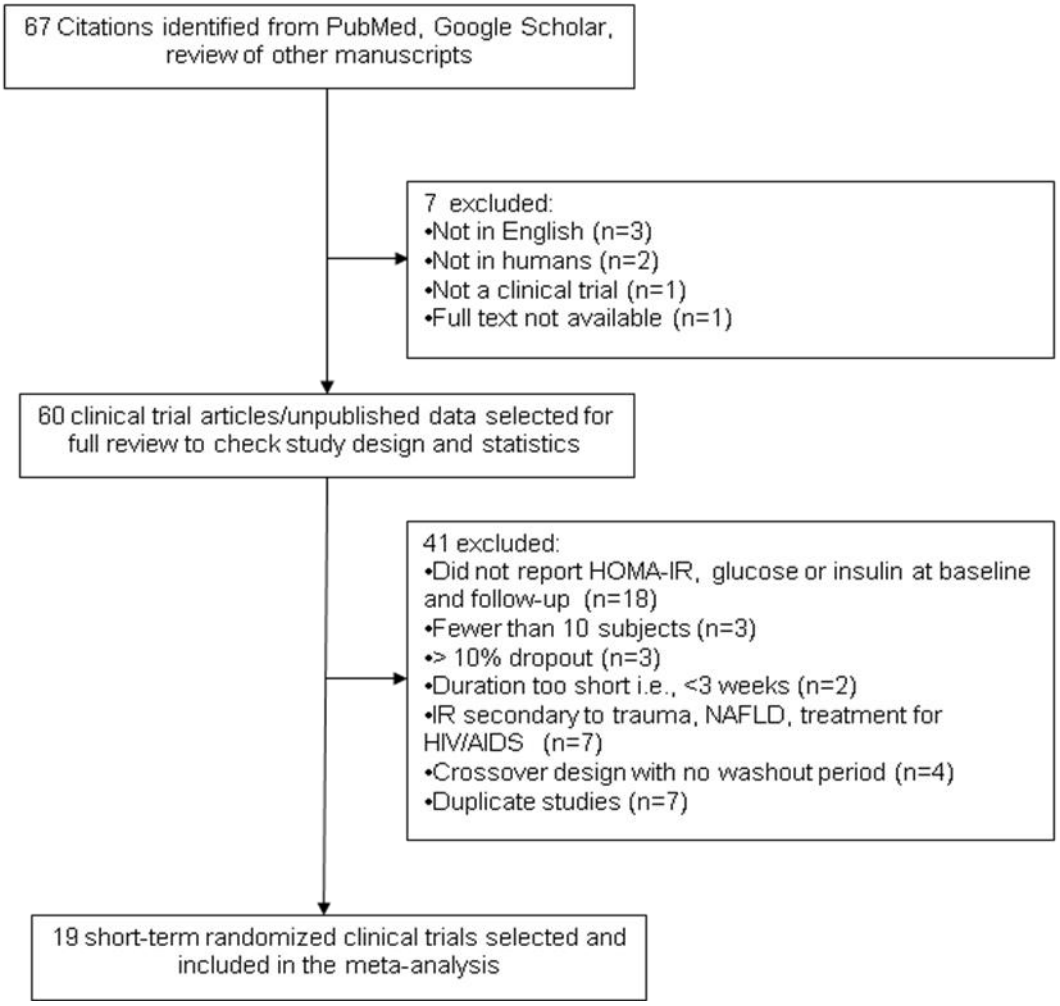


Figure 2. Forest plot of change in HOMA-IR among all subjects

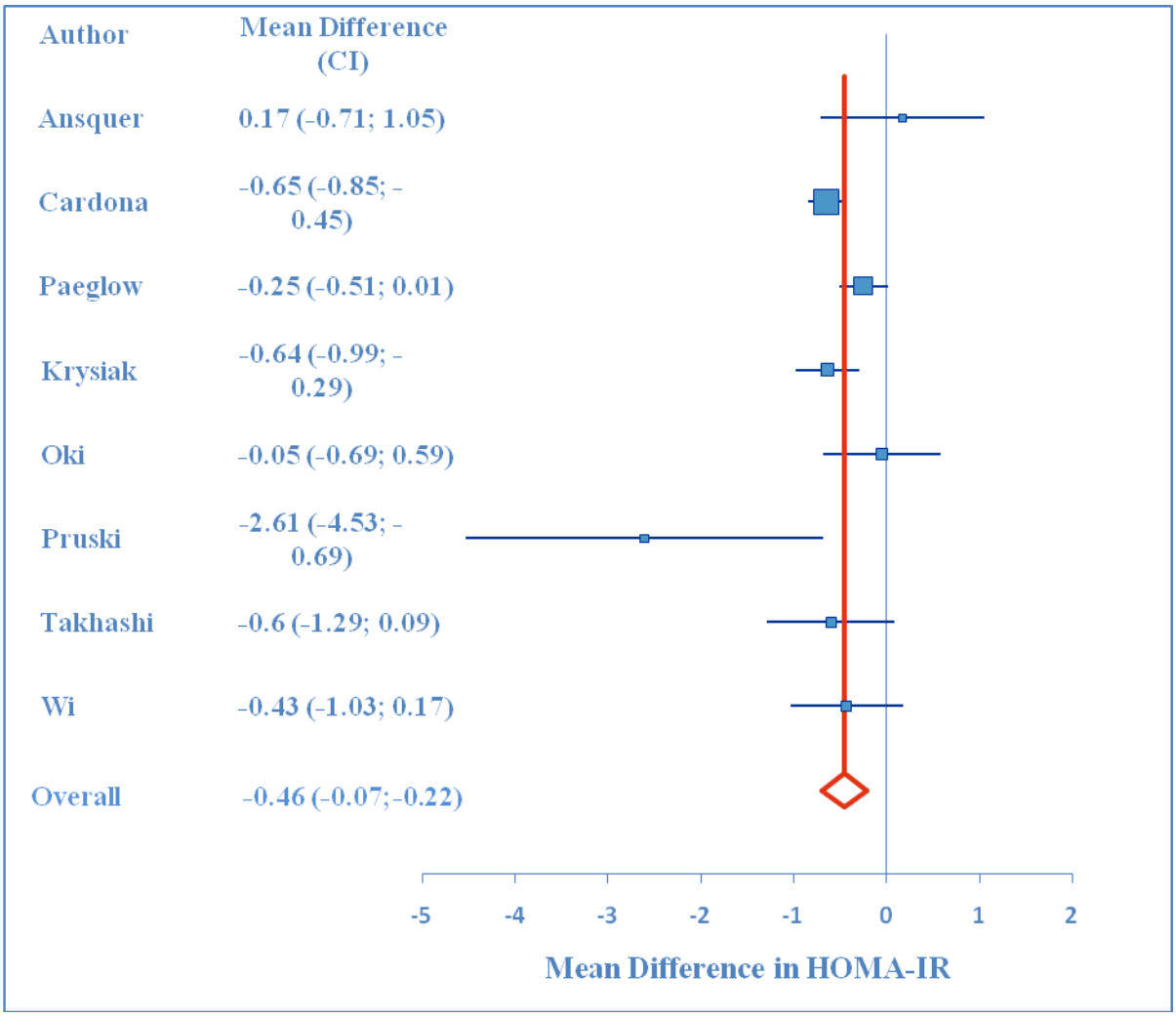


Figure 3. HOMA-IR exclusion sensitivity plot

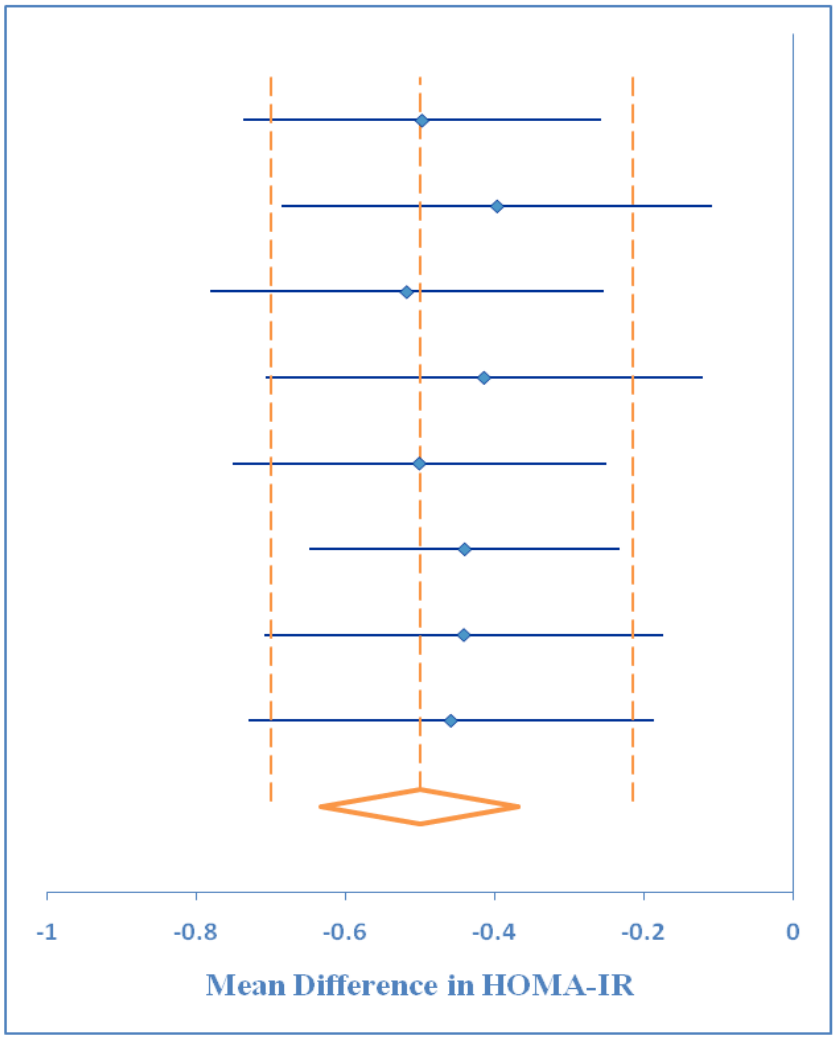


Figure 4. Forest plot of change in insulin among all subjects

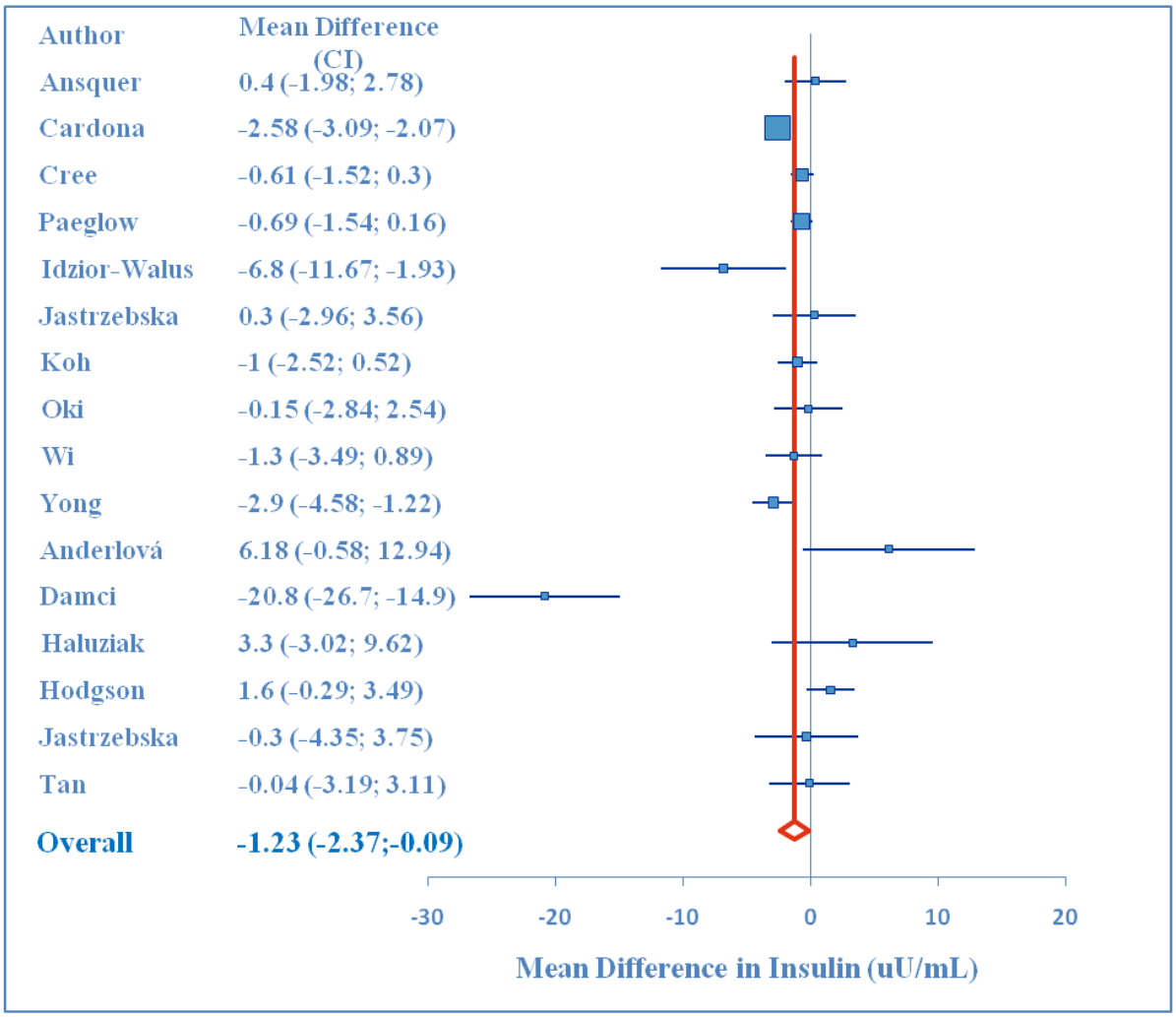


Figure 5. Insulin exclusion sensitivity plot

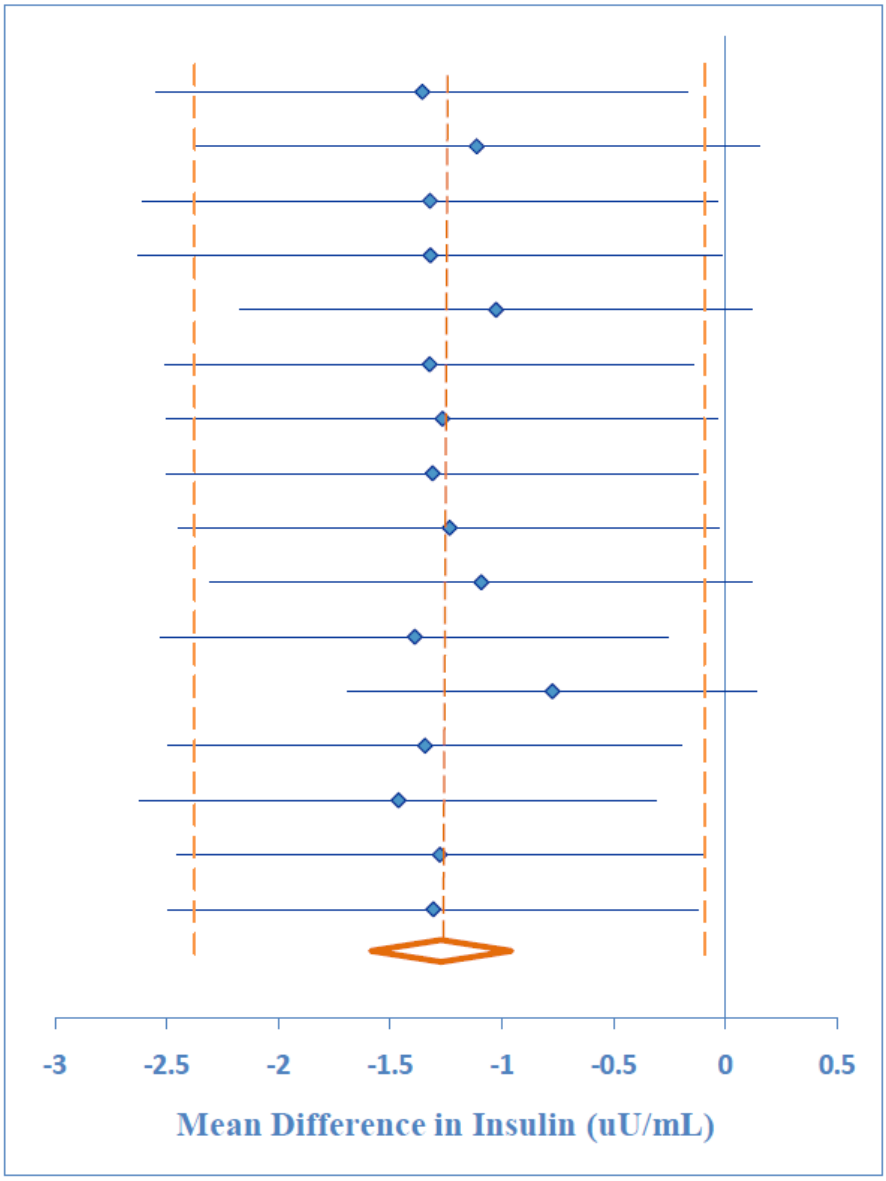


Figure 6. Forest plot of change in glucose among all subjects

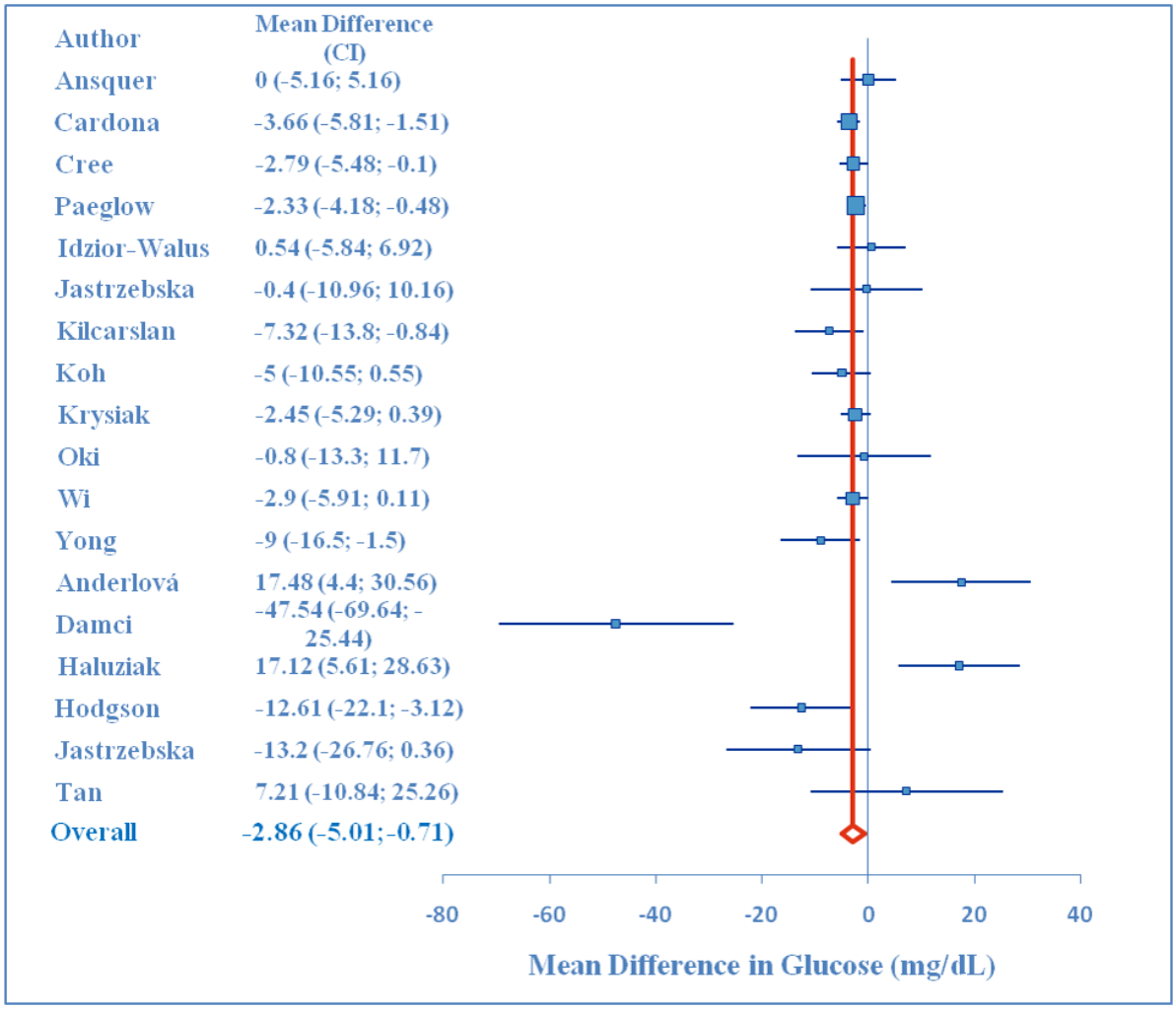


Figure 7. Glucose exclusion sensitivity plot

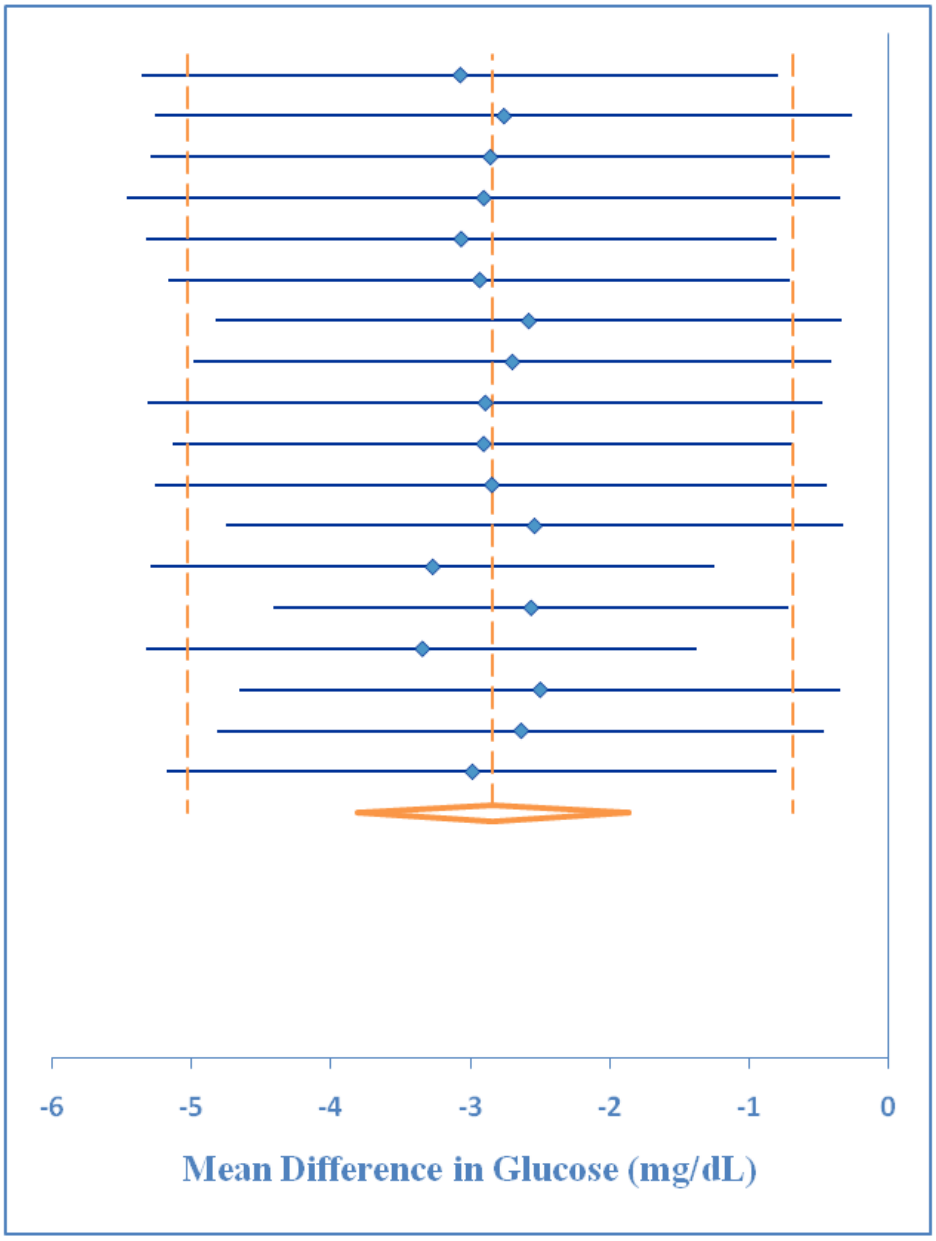


Figure 8. HOMA-IR funnel plot

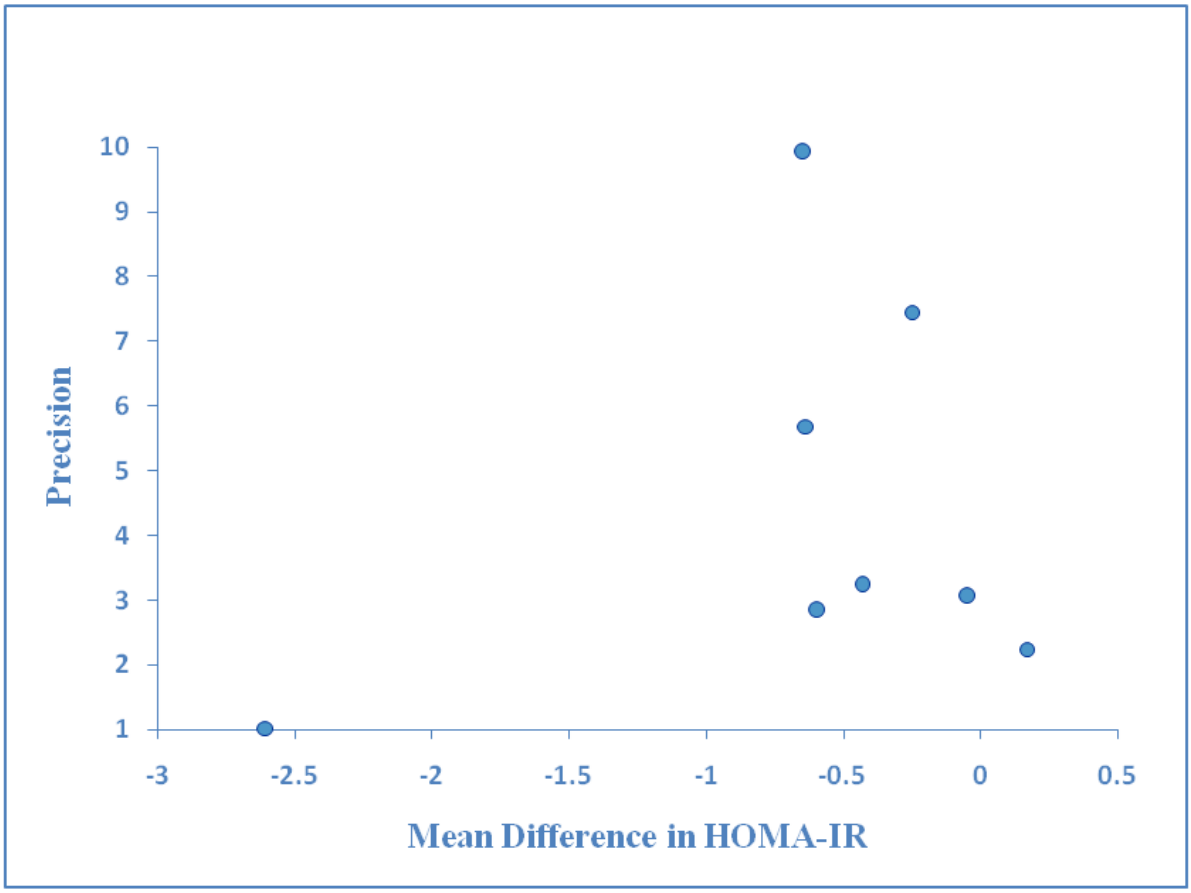


Figure 9. Insulin funnel plot

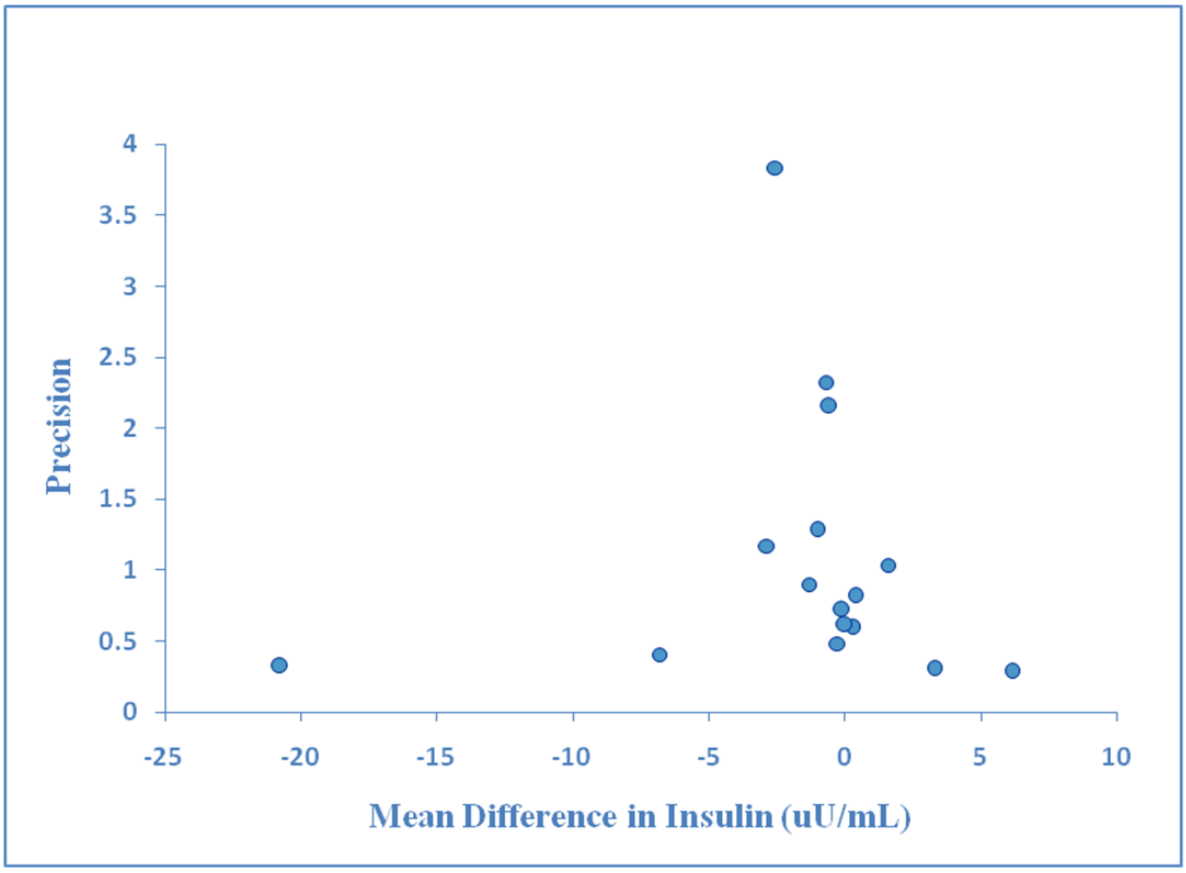
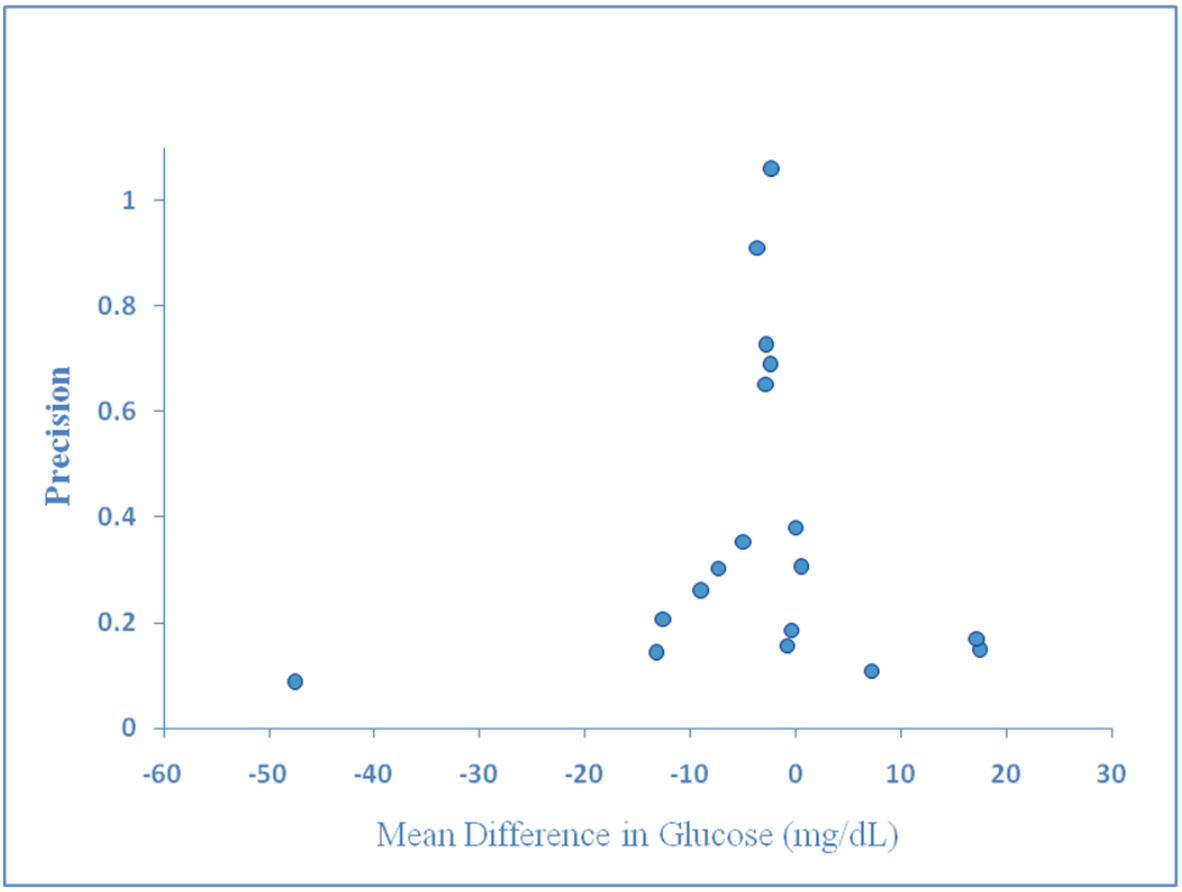


Figure 10. Glucose funnel plot



IS ADIPONECTIN A MEDIATOR BETWEEN FENOFIBRATE USE AND DECREASED INSULIN RESISTANCE?

CORRIE E. PAEGLOW, EDMOND K. KABAGAMBE, MIKE Y. TSAI, ROBERT J. STRAKA, GARY R. CUTTER, FRANK A. FRANKLIN, FERNANDO OVALLE, DONNA K. ARNETT

In preparation for *Diabetes and Metabolic Syndrome*

Format Adapted for Dissertation

Abstract

Objective

To assess whether the relationship between fenofibrate use and insulin resistance is mediated by changes in adiponectin concentrations.

Methods

Men and women (n = 780, age 47.4 ± 15.9) in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) family study were treated with 160 mg of fenofibrate once daily for three weeks. Lipids, insulin and glucose were measured before and after treatment. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from these data and used as a measure of insulin resistance. GEE models were used to test the relationships between fenofibrate use, change in adiponectin concentrations and change in HOMA-IR, and these results used to determine whether change in adiponectin is a mediator of this relationship. This analysis was repeated in a randomly selected group of unrelated participants to further confirm the findings.

Results

Fenofibrate use resulted in significant decreases ($P < 0.0001$) in HOMA-IR (-0.24; 95% CI: -0.32, -0.14) and significant ($P < .0001$) unexpected decreases in adiponectin (-365 ng/mL; 95% CI: -458, -272). There was, however, no association ($P = 0.91$) between change in adi-

ponectin concentration and change in HOMA-IR. The results in the unrelated sub-cohort also showed no association between change in adiponectin and change in HOMA-IR (Pearson r = -0.07, P = 0.39), further confirming that change in adiponectin does not mediate this relationship.

Conclusion

We did not find evidence to support the notion that adiponectin is a mediator in the relationship between fenofibrate use and change in HOMA-IR. As this is inconsistent with results from some previous studies, further research that includes measures of expression of adiponectin receptors are needed to provide additional insight into this relationship.

Background

Fenofibrate is widely prescribed to decrease triglycerides. One potential benefit of fenofibrate use beyond triglyceride reduction is a reduction in insulin resistance, as hypertriglyceridemia is often associated with increased insulin resistance (IR) (1-5). This benefit has been demonstrated in animal studies (6-8) and in some (9-16), but not all (17-19), human studies. The insulin sensitizing effects of fenofibrate have also been demonstrated in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) family study where HOMA-IR decreased the most among individuals with the highest baseline HOMA-IR levels (20). This finding has been further confirmed through a meta-analysis of short-term studies on fenofibrate and insulin resistance (21).

One challenge in assessing the relationship between fenofibrate and insulin resistance is the lack of a clear mechanism by which fenofibrate might impact insulin resistance. One pathway that has been proposed (22) is that fenofibrate increases plasma adiponectin levels, which in turn increases insulin sensitivity. This pathway is plausible given that adiponectin concentrations are inversely associated with plasma triglycerides (23) and hypertriglycedemia is associated with insulin resistance (1-5).

Adiponectin is a hormone secreted by adipose tissue that, paradoxically, is inversely correlated with percent body fat (24). Among humans, adiponectin levels are inversely correlated with HOMA-IR (25) and, in one study, fasting plasma glucose (24). Changes in adiponectin have also been shown to be inversely correlated with changes in insulin resistance (26). In addition, individuals with diabetes or impaired glucose tolerance typically have lower adiponectin concentrations than normoglycemic individuals (27; 28). There is also evi-

dence that low adiponectin concentrations are a risk factor for extreme insulin resistance or type 2 diabetes. This relationship has been seen in several populations, including participants in the Atherosclerosis Risk in Communities (ARIC) study (HR for highest vs. lowest quartile = 0.18; 95% CI: 0.11, 0.27) (29), participants in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (OR highest vs. lowest quartile = 0.3; 95% CI: 0.2, 0.7) (30), a cohort of 1,792 Japanese (OR highest vs. lowest tertile = 9.32; 95% CI: 1.05, 83.1)(31) and participants in a case-control study of 140 Pima Indians (IRR high vs. low adiponectin = 0.63; 95% CI: 0.43, 0.92) (32).

Adiponectin's impact on insulin resistance has been demonstrated in animal studies (6; 33; 34), and further confirmed in studies where giving adiponectin to insulin resistant mice has improved their insulin sensitivity (35) . Some human studies have also shown an association between increased adiponectin levels secondary to fenofibrate use and decreased insulin resistance (26). Other studies, however, have shown no change in adiponectin levels after fenofibrate use (36). We will investigate whether change in adiponectin mediates the relationship between fenofibrate use and decrease in insulin resistance in the GOLDN study, a large cohort of subjects treated with fenofibrate.

Methods

Study population

The GOLDN study recruited subjects in three generation pedigrees and in two centers, Minneapolis, MN and Salt Lake City, UT, chosen because they are likely to be largely

genetically homogeneous. At the baseline visits subjects completed questionnaires related to their medical history and lifestyle factors, had anthropometric measures taken and gave a fasting blood sample that was analyzed for metabolic parameters, including glucose, insulin and lipids. After this initial visit subjects began three weeks of treatment with 160 mg of micronized fenofibrate daily. After completing three weeks of treatment they returned to the study site and gave another blood sample. From 845 participants with data on insulin and glucose pre- and post-treatment, we excluded the following participants as measures of HOMA-IR are known to be unreliable in these groups: those who reported a diagnosis of diabetes (n = 22) or were taking a medication used to treat diabetes (n = 43). We also intended to exclude participants who had insulin concentrations ≥ 58 uU/mL or ≤ 2.88 uU/mL post-treatment; and those who had glucose concentrations ≥ 360 mg/dL or post-treatment glucose concentrations ≤ 63 mg/dL, however no subjects who met the previous criteria did not meet this criteria.

Laboratory Measurements

Details about the laboratory measurements are fully described elsewhere (37). In short, all samples were centrifuged within 20 min of collection at and stored at −70 °C to ensure they were frozen. For each analyte, specimens from each participant were assayed in the same batch to eliminate inter-assay imprecision. Fasting glucose was measured using the hexokinase-mediated reaction on a Hitachi 911 analyzer (Roche Diagnostics), while fasting insulin was measured using the human insulin specific RIA kit (Linco Research, St. Charles, MO). Triglycerides were measured using a glycerol blanked enzymatic method (Trig/GB,

Roche Diagnostics Corporation, Indianapolis, IN) and cholesterol was measured with a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics Corporation) on the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation). For HDL-cholesterol, the non-HDL-cholesterol was first precipitated with magnesium/dextran. LDL-cholesterol was measured by a homogeneous direct method (LDL Direct Liquid Select™ Cholesterol Reagent, Equal Diagnostics, Exton, PA).

Statistical Analysis

Demographic and anthropometric characteristics were calculated overall and by tertile of baseline HOMA, and differences between tertiles assessed using ANOVA analysis for continuous variables and chi-square tests for categorical variables. In previous analysis (20) the largest change in HOMA-IR among those in the GOLDN cohort occurred in those in the highest tertile of baseline HOMA-IR, therefore those with the highest baseline HOMA-IR would be expected to experience the largest changes in adiponectin concentration. Thus, changes in adiponectin were investigated by creating scatter plots of change in adiponectin by both baseline HOMA-IR and baseline adiponectin. We also investigated changes in adiponectin overall and by tertile of baseline HOMA-IR and adiponectin.

Unrelated Sub-cohort

The GOLDN study recruited participants in three generation pedigrees, thus statistical analyses of this cohort must take into account the correlation between genetic pedigrees. To

overcome the bias in correlation coefficients due to clustering within families, we created a subset of data containing only unrelated individuals (n=168) selected at random from the main GOLDN study population (see appendix I for the SAS code) and calculated the Pearson correlation coefficients between pre-/post-treatment measures of insulin resistance and pre-/post-treatment measures of adiponectin. Measures of HOMA-IR, glucose, insulin and adiponectin were log-transformed to attain normality.

Mediation Analysis

A mediator variable is one that is caused by the independent variable and causes the dependent variable (38; 39). Barron and Kenny (40) have proposed the following approach for assessing mediation: 1) Assess the relationship between the independent and dependent variables; 2) Assess the relationship between the dependent variable and the proposed mediator variable; 3) Assess the relationship between the independent variable and mediator variable; 4) Create a model that includes the independent, dependent and mediator variables. For a variable to be a mediator these models must show an association between the dependent, independent and mediator variables, and adding the mediator variable to the model relating the independent and dependent variables should attenuate the relationship. We adopted this approach to assess the relationship between fenofibrate use, changes in adiponectin and insulin resistance.

In keeping with this approach, we considered the relationship between change in HOMA-IR and fenofibrate among the entire cohort using GEE models to account for the cor-

relation between participants in the same genetic pedigree. These tests are analogous to the paired t-test, thus they were not adjusted for any covariates. This methodology was also used to examine the relationship between fenofibrate use and change in adiponectin and the relationship between change in adiponectin and change in HOMA-IR. Finally, a model was created that included fenofibrate use, change in HOMA-IR and change in adiponectin.

After conducting this analysis in the entire cohort we repeated the analysis in the unrelated sub-cohort to ensure that the results were not influenced by the correlation between members of the same genetic pedigree.

Results

Table 1 shows demographic and anthropometric characteristics of the participants overall and by baseline tertile of HOMA-IR. Participants in the highest tertile of HOMA-IR were older and had a higher BMI than those in the lower two tertiles. They also engaged in fewer hours of physical activity, as measured by the proxy variable of daily hours spent watching TV/on the computer.

Table 2 shows baseline and follow-up HOMA-IR, insulin, glucose and adiponectin concentrations. Insulin, glucose and HOMA-IR all decreased in the cohort overall, indicating that the participants were less insulin resistant following fenofibrate treatment. Adiponectin concentrations also decreased both in the overall cohort and within each tertile of baseline HOMA-IR.

Figures 1 and **2** are scatter plots of change in adiponectin by baseline adiponectin among the entire cohort and the unrelated sub-cohort, respectively**. Figure 3** shows changes in adiponectin overall and by baseline tertile of adiponectin. Adiponectin levels did not change significantly among those in the lowest tertile of baseline adiponectin ($P = 0.19$), but significant decreases were seen in the middle and highest tertile of adiponectin ($P = 0.04$, $P = 0.0001$, respectively).

Figures 4 and **5** are scatter plots of change in adiponectin by baseline HOMA-IR in the entire cohort and unrelated sub-cohort. **Figure 6**, considers these changes quantitatively which shows statistically significant ($P < 0.0001$) decreases in adiponectin after fenofibrate treatment overall. Adiponectin also decreased in each tertile of baseline HOMA-IR ($P = 0.001$, $P = 0.04$, $P < 0.0001$, respectively).

Table 3 compares the entire cohort and the unrelated sub-cohort. The unrelated sub-cohort had a slightly smaller percentage of female participants and a greater percentage of current smokers and drinkers. The sub-cohort also had higher HOMA-IR and concentrations of insulin and glucose at baseline, and slightly lower adiponectin concentrations. These differences, especially for the main variables (HOMA-IR, glucose and insulin), were quite small.

Tables 4 and **5** present the correlation coefficients among the unrelated sub-cohort for measures of insulin sensitivity pre-/post-treatment and change in insulin sensitivity, including HOMA-IR, fasting plasma glucose and fasting plasma insulin, and adiponectin concentration pre-/post-treatment and change in adiponectin. There is a significant correlation between

post-treatment adiponectin and all of the measures of insulin resistance, however none of the measures of change in insulin resistance are significantly correlated with change in adiponectin.

Mediation analysis results in the entire cohort

Previous analyses undertaken in this cohort (20) found a significant association between fenofibrate use and change in HOMA-IR, with fenofibrate use resulting in a 0.24 unit decrease in HOMA-IR (95% CI: -0.32, -0.14). We began by assessing the relationship between change in HOMA-IR and change in adiponectin, which was not significant ($P = 0.55$). This relationship remained insignificant after stratifying by baseline tertile of HOMA-IR (lowest tertile $P = 0.44$, middle tertile $P = 0.82$, highest tertile $P = 0.14$).

A statistically significant relationship between fenofibrate use and change in adiponectin was observed; fenofibrate use resulted in a 365 ng/mL decrease in adiponectin ($P < 0.0001$). This relationship remained significant when stratifying by baseline tertile of HOMA-IR (lowest tertile -495 ng/mL; $P < 0.0001$, middle tertile -370 ng/mL; $P< 0.0001$, highest tertile -235 ng/mL; $P = 0.001$).

The final model assessed the relationship between fenofibrate use and change in HOMA-IR and included change in adiponectin as a covariate. When change in adiponectin was not included in the model, fenofibrate use resulted in a 0.24 unit decrease in HOMA-IR ($P < .0001$). When adiponectin is included in the model, however, we found a 1.63 unit decrease in HOMA-IR, although this result was not significant ($P = 0.18$). We also considered

this relationship within each tertile of baseline HOMA-IR and found that, in all cases, adding adiponectin to the model attenuated the association between fenofibrate use and change in HOMA-IR.

Mediation analysis results in the unrelated sub-cohort

In the unrelated sub-cohort, fenofibrate use resulted in a 4.35 unit decrease in HOMA-IR, however this result was not statistically significant ($P = 0.07$). Similarly to the results found in the entire cohort, change in HOMA-IR was not associated with change in adiponectin ($P = 0.39$). Fenofibrate use was associated with a 313 ng/mL decrease in adiponectin which was statistically significant ($P < 0.01$).

The final model assessed the relationship between fenofibrate use and change in HOMA-IR with change in adiponectin included as a covariate. In the model without change in adiponectin as a covariate, fenofibrate use resulted in a 4.35 unit decrease in HOMA-IR ($P = 0.07$). When change in adiponectin was included in the model fenofibrate use resulted in a 0.34 unit decrease in HOMA-IR that was statistically significant ($P = 0.001$).

Discussion

We did not find evidence to support the notion that adiponectin is a mediator between fenofibrate use and change in HOMA-IR in the GOLDN cohort. While fenofibrate use is associated with statistically significant decreases in HOMA-IR, the relationship between

change in HOMA-IR and change in adiponectin is not statistically significant. This lack of a relationship between change in HOMA-IR and adiponectin suggests that change in adiponectin may not be a mediator between fenofibrate use and change in insulin resistance as measured by HOMA-IR, assuming no increase in adiponectin production and uptake consequent to up-regulation of adiponectin receptors following treatment with fenofibrate.

We also examined these relationships in a randomly selected sub-cohort of unrelated participants. In the unrelated sub-cohort fenofibrate use was not associated with statistically significant changes in HOMA-IR at the $\alpha = 0.05$ level, likely due to the smaller sample size ($n = 168$) since we observed this association in the analyses that included the whole study population. We found no statistically significant relationship between change in adiponectin and change in HOMA-IR ($P = 0.39$), indicating that change in adiponectin may not be a mediator in the relationship between fenofibrate use and change in HOMA-IR. This was further confirmed when adding change in adiponectin to the model relating fenofibrate use to change in HOMA-IR strengthened the relationship between fenofibrate use and change in HOMA-IR; were adiponectin a mediator of this relationship adding it to the model would have attenuated this relationship.

These results stand in contrast to many of the studies that have previously considered this relationship. The majority of studies (25; 27-32; 41; 42) have found an increase in adiponectin concentrations after treatment with fenofibrate. Thus, it is unusual that we observed the opposite effect, with fenofibrate use resulting in statistically significant decreases in adiponectin. Adiponectin is negatively correlated with BMI, so we investigated changes in BMI in this cohort to determine if this may have had an impact on our findings. The cohort did

undergo an increase in BMI of 0.16 kg/m^2 ($P < 0.0001$), however this change is small, making it unlikely that the change in adiponectin we observed is attributable to changes in BMI.

One potential reason we did not observe a relationship between change in adiponectin and change in BMI is the measure of adiponectin we used. Studies (43-45) have suggested that the ratio of high-molecular-weight adiponectin to total adiponectin is more closely correlated to glucose intolerance than total adiponectin is, although this has not been seen in all studies (46; 47). In the GOLDN study only total adiponectin was measured, making it impossible to model the relationship between the ratio of high-molecular-weight adiponectin to total adiponectin.

It is also possible that fenofibrate use resulted in increased expression of adiponectin receptors and thus more efficient use of adiponectin. Expression of these receptors has been shown to be positively correlated with insulin sensitivity (48), and thus negatively correlated with insulin resistance, although not all studies have observed this relationship (49). In addition, fenofibrate has been found to increase expression of adiponectin receptors (7), although studies assessing this have been contradictory (50).

This study has a number of strengths that add to the credibility of its findings. First, the sample size was large enough to afford ample statistical power to detect differences before and after treatment. In addition, most of the participants were compliant with their treatment, and this was ascertained by pill counts, an objective measure. The blood samples were taken in accordance with one established protocol and the laboratory work done at one site, thus minimizing the impact of variability between laboratories.

There are two key limitations in this study: lack of a control group that was not taking fenofibrate and lack of a differentiation between the various molecular weights of adiponectin. Because we cannot compare the change in adiponectin seen in our cohort to those seen in a control group it is not possible to determine if the decreases in adiponectin seen in this cohort are the result of a secular trend. We also cannot determine if fenofibrate use changed the ratio of high-molecular weight adiponectin to total adiponectin. One other limitation is lack of data on expression of adiponectin receptors 1 and 2. Increased expression of adiponectin receptors in response to fenofibrate could increase uptake of adiponectin by tissues and thus manifest as a net decrease in circulating concentrations despite an increase in adiponectin production.

Since the results from our study are different from those obtained from a few previous studies on this topic, the question of whether adiponectin is a mediator of the relation between fenofibrate use and insulin resistance or not remains unanswered. More studies with more detailed assays for different forms of adiponectin, expression of adiponectin receptors, changes in patient weight are needed to determine if change in adiponectin mediates the relationship between fenofibrate use and HOMA-IR. These studies should include a placebo-controlled group and also collect data on other factors that can impact adiponectin levels both at baseline and after fenofibrate treatment. Such analyses will be necessary to definitively determine what, if any, role adiponectin plays in the relationship between fenofibrate use and changes in insulin sensitivity as measured by HOMA-IR.

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Table 1. Demographic and anthropometric characteristics of included participants

		Tertile of HOMA-IR		
	All participants (n=780)	**1 (n=259)**	**2 (n= 261)**	**3 (n= 260)**
Age, y	47.4 (15.9)	45.4 (15.0)	46.3 (16.6)	50.64 (15.43)
BMI, kg/m^2	28.2 (5.32)	24.85 (3.60)	28.02 (4.48)	31.65 (5.37)
Average timespent on TV/Computer, hr/week	2.66 (1.91)	2.33 (1.92)	2.54 (1.79)	3.11 (1.94)
Female, %	49.5	57.9	47.89	57.3
Current smokers, %	7.82	10.04	8.43	5.00
Current alcohol users, %	50.5	57.9	51.0	42.69

Numbers are presented as mean (s.d.) or percent

Except for smoking, all variables showed statistically significant ($P<0.05$) differences in their distribution across tertiles of baseline HOMA-IR.

Table 2. Change in HOMA-IR, insulin, glucose and adiponectin

	Treatment or *P**	All participants (n = 780)	Tertile of Baseline HOMA-IR		
			1 (Lowest) (n = 259)	2 (n =261)	3 (Highest) (n =260)
HOMA-IR	Pre-treatment	3.43 (2.32)	1.80 (0.37)	2.87 (.33)	5.61 (2.87)
	Post-treatment	3.19 (2.13)	1.94 (.69)	2.77 (.82)	4.84 (2.82)
	P	< .0001	0.001	0.04	< .0001
Insulin	Pre-treatment	13.65 (7.59)	7.88 (1.60)	11.86 (1.61)	21.19 (8.63)
	Post-treatment	12.99 (6.92)	8.49 (2.70)	11.67 (3.21)	18.78 (8.41)
	P	< .0001	0.0002	0.17	< .0001
Glucose	Pre-treatment	99.20 (13.86)	92.78 (7.39)	98.46 (8.27)	106.32 (19.03)
	Post-treatment	96.86 (13.52)	91.72 (7.32)	96.16 (8.29)	102.67 (19.15)
	P	< .0001	0.0004	< 0.0001	< 0.0001
Adiponectin	Pre-treatment	8147.61 (4741,10237)	9857 (5998,12717)	8403 (5115,10465)	5953 (3,674,7879)
	Post-treatment	7781.0 (4564,9823)	9362 (5730,11836)	8033 (5108,9927)	5949 (3265,7879)
	P	< .0001	0.001	0.04	< 0 .0001

Table 3. Comparison of total cohort and unrelated sub-cohort

	Unrelated sub-cohort (n = 168)	Total cohort (n = 780)
Female	47.02	49.49
Current Smokers	8.93	7.82
Current Drinkers	57.14	50.51
Age	50.30 (15.38)*	47.44 (15.87)
BMI	28.84 (4.74)	28.18 (5.32)
Baseline HOMA-IR	3.49 (2.24)	3.43 (2.32)
Post-treatment HOMA-IR	3.17 (1.90)	3.19 (2.13)
Baseline insulin	13.93 (8.08)	13.65 (7.59)
Post-treatment insulin	12.98 (7.17)	12.99 (6.92)
Baseline glucose	99.50 (9.24)	99.20 (13.86)
Post-treatment glucose	97.11 (10.09)	96.86 (13.52)
Baseline adiponectin	7,901 (4,639)	8,147 (4,682)
Post-treatment adiponectin	7,588 (4,345)	7,781 (4,360)

All numbers are presented as mean (s.d.) or percent
* Significantly different from the total cohort at $P < 0.05$

Table 4. Correlations between metabolic parameters in the unrelated sub-cohort

	Pre-treatment adiponectin	Post-treatment adiponectin	Change in adiponectin	Pre-treatment HOMA-IR	Post-treatment HOMA-IR	Change in HOMA-IR
Pre-treatment adiponectin		0.97*	-0.28**	-0.45*	-0.43*	0.21+
Post-treatment adiponectin	0.97*		-0.05	-0.44*	-0.42*	0.20++
Change in adiponectin	-0.28**	-0.05		0.14	0.1	-0.07
Pre-treatment HOMA-IR	-0.45*	-0.44*	0.14		0.82*	-0.44*
Post-treatment HOMA-IR	-0.43*	-0.42*	-0.1	0.82*		0.08
Change in HOMA-IR	0.21+	0.20++	-0.07	-0.44*	0.08	
Pre-treatment insulin	-0.43*	-0.42*	0.14	0.97*	0.80*	-0.44*
Post-treatment insulin	-0.41*	-0.40*	0.11	0.81*	0.98*	0.08
Change in insulin	0.17++	0.16++	-0.05	-0.41*	0.11	0.98*
Pre-treatment glucose	-0.34*	-0.33*	0.06	0.53*	.47*	-0.21
Post-treatment glucose	-0.28**	-0.29*	-0.03	0.44*	0.57*	0.03
Change in glucose	0.06	0.02	-0.14	-0.07	0.23++	0.37

*Significant at P < 0.0001, ** significant at p < 0.001, + significant at P < 0.01, ++ significant at P < 0.05

Measures of pre-/post-treatment HOMA-IR, insulin, glucose and adiponectin were log-transformed to attain normality

Table 5. Correlations between metabolic parameters in the unrelated sub-cohort, continued

	Pre-treatment insulin	Post-treatment insulin	Change in insulin	Pre-treatment glucose	Post-treatment glucose	Change in glucose
Pre-treatment adiponectin	-0.43*	-0.41*	0.17	-0.34*	-0.28**	0.06
Post-treatment adiponectin	-0.42*	-0.40*	0.16^{++}	-0.33*	-0.29*	0.02
Change in adiponectin	0.14	0.11	-0.05	0.06	-0.03	-0.14
Pre-treatment HOMA-IR	0.99*	0.81*	-0.41*	0.53*	0.44*	-0.07
Post-treatment HOMA-IR	0.80*	0.98*	0.11	0.47	0.57*	0.23^{++}
Change in HOMA-IR	-0.44*	0.08	0.98*	-0.21^{+}	0.03	0.34*
Pre-treatment insulin		0.81*	-0.41*	0.39*	0.32*	-0.04
Post-treatment insulin	0.81*		0.12	0.34*	0.41*	0.16^{++}
Change in insulin	-0.41*	0.12		-0.16^{++}	-0.01	0.21
Pre-treatment glucose	0.39*	0.34*	-0.16^{++}		0.82*	-0.17^{++}
Post-treatment glucose	0.32*	0.41*	-0.01	0.82*		0.42*
Change in glucose	-0.04	0.16^{++}	0.21^{+}	-0.17^{++}	0.42*	

*Significant at P < 0.0001, ** significant at p < 0.001, + significant at P < 0.01, ++ significant at P < 0.05

Measures of pre-/post-treatment HOMA-IR, insulin, glucose and adiponectin were log-transformed to attain normality

Figure 1. Change in adiponectin by baseline adiponectin in the entire cohort

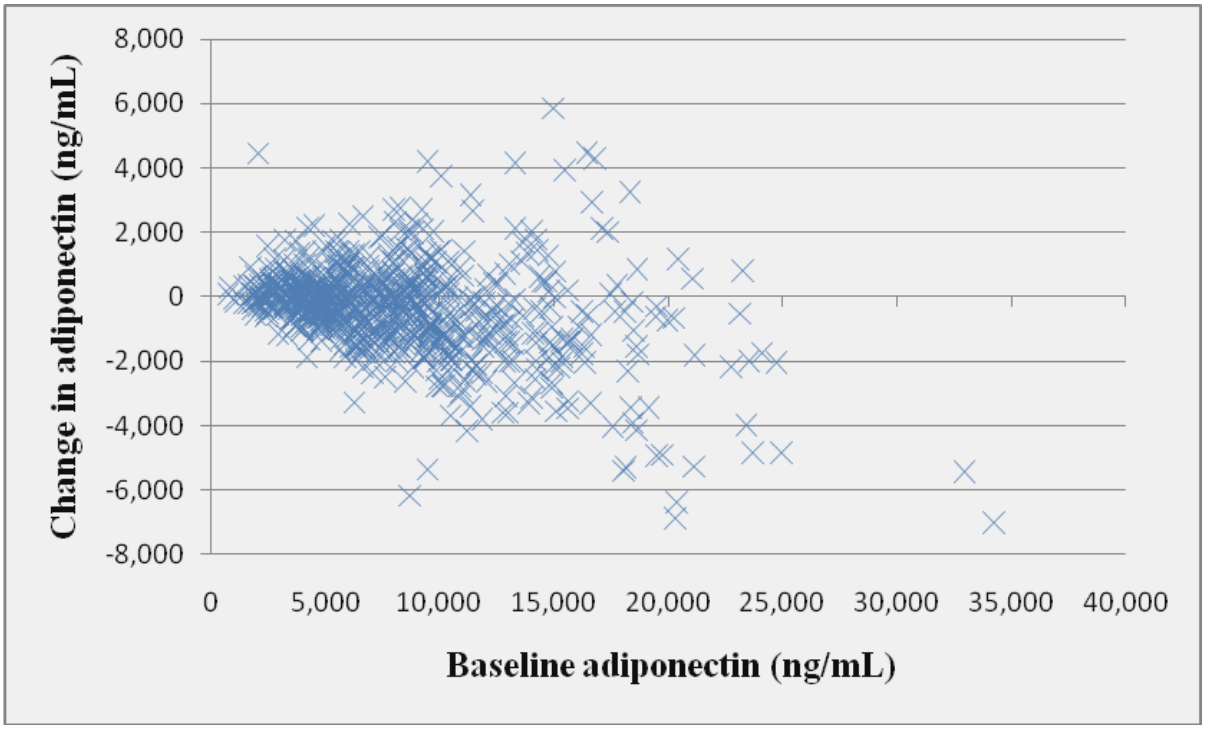


Figure 2. Change in adiponectin by baseline adiponectin in the unrelated sub-cohort

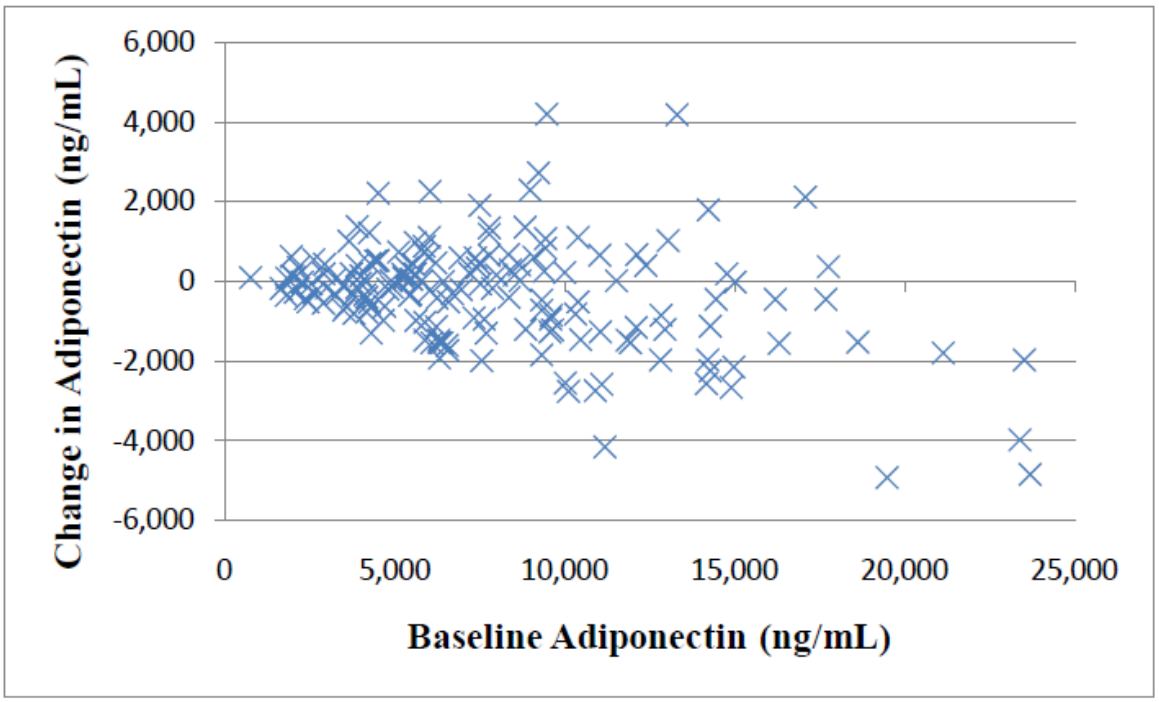


Figure 3. Change in adiponectin overall and by baseline tertile of adiponectin

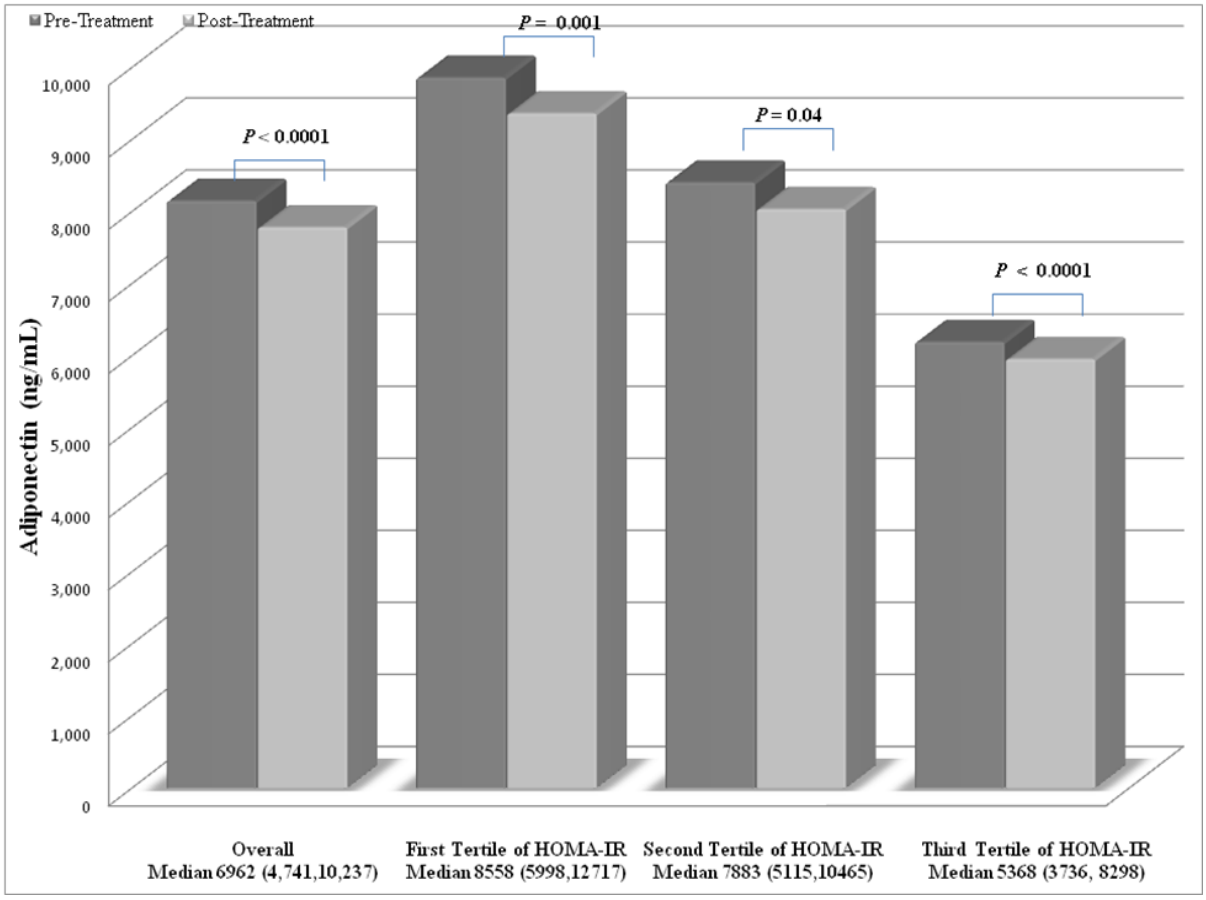


Figure 4. Change in adiponectin by baseline HOMA-IR in the entire cohort

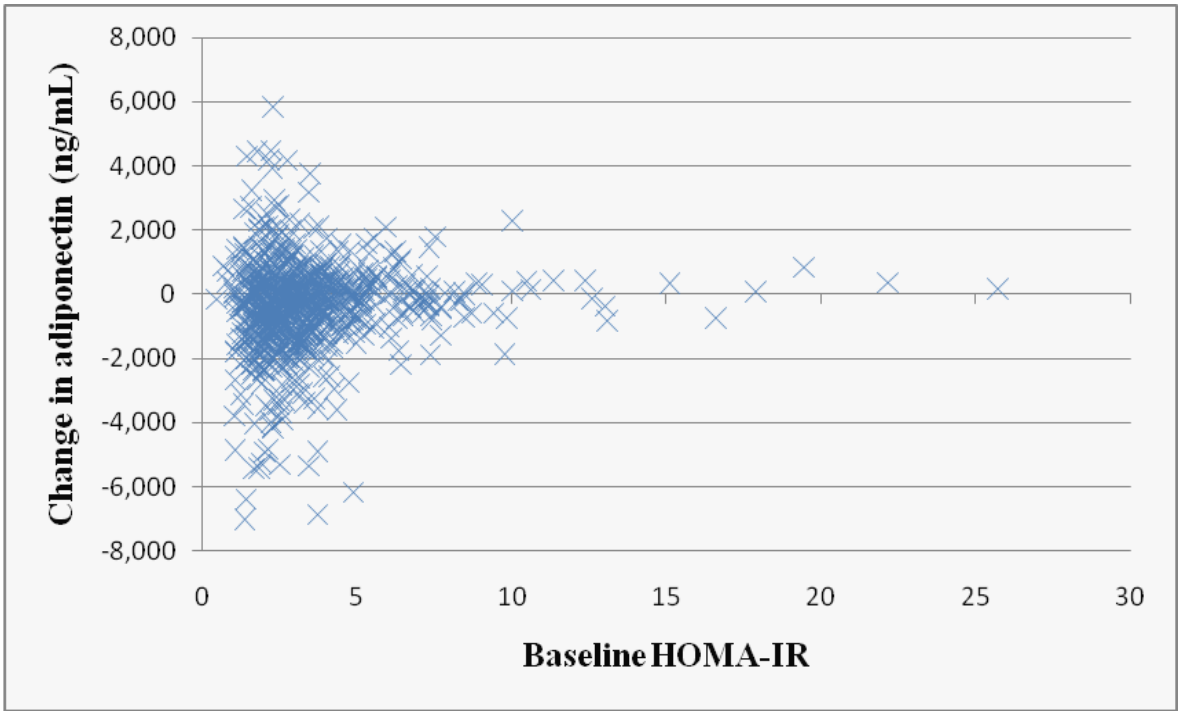


Figure 5. Change in adiponectin by baseline HOMA-IR in the unrelated sub-cohort

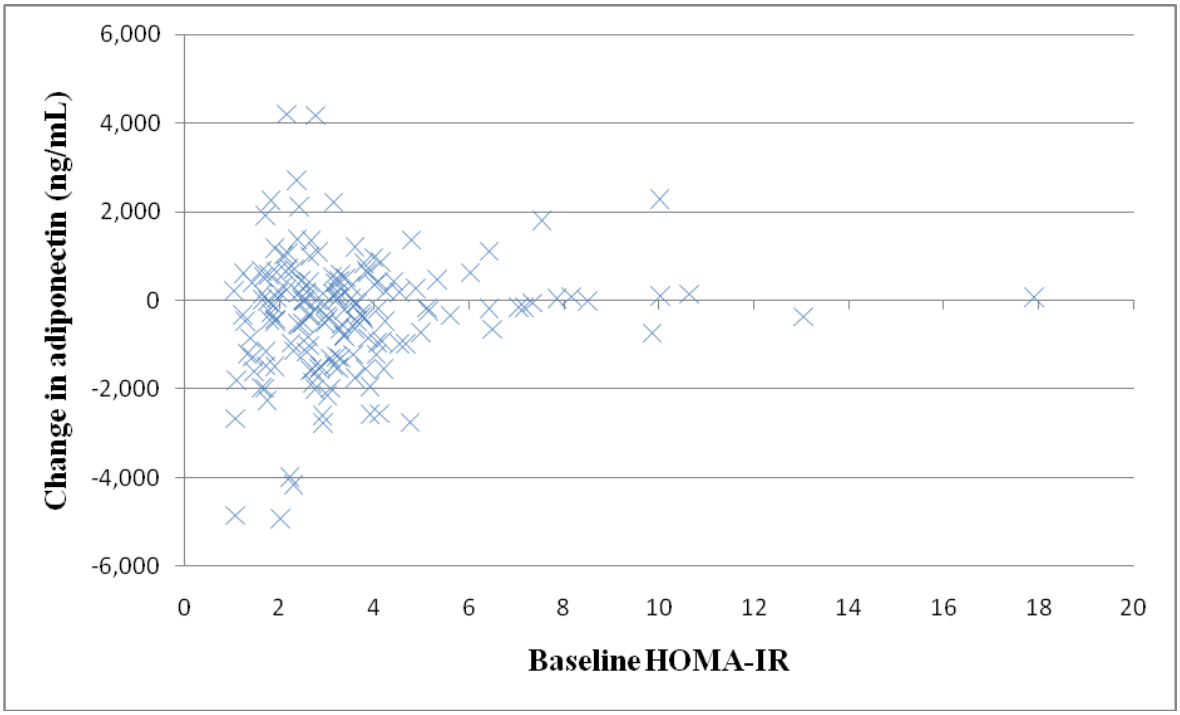
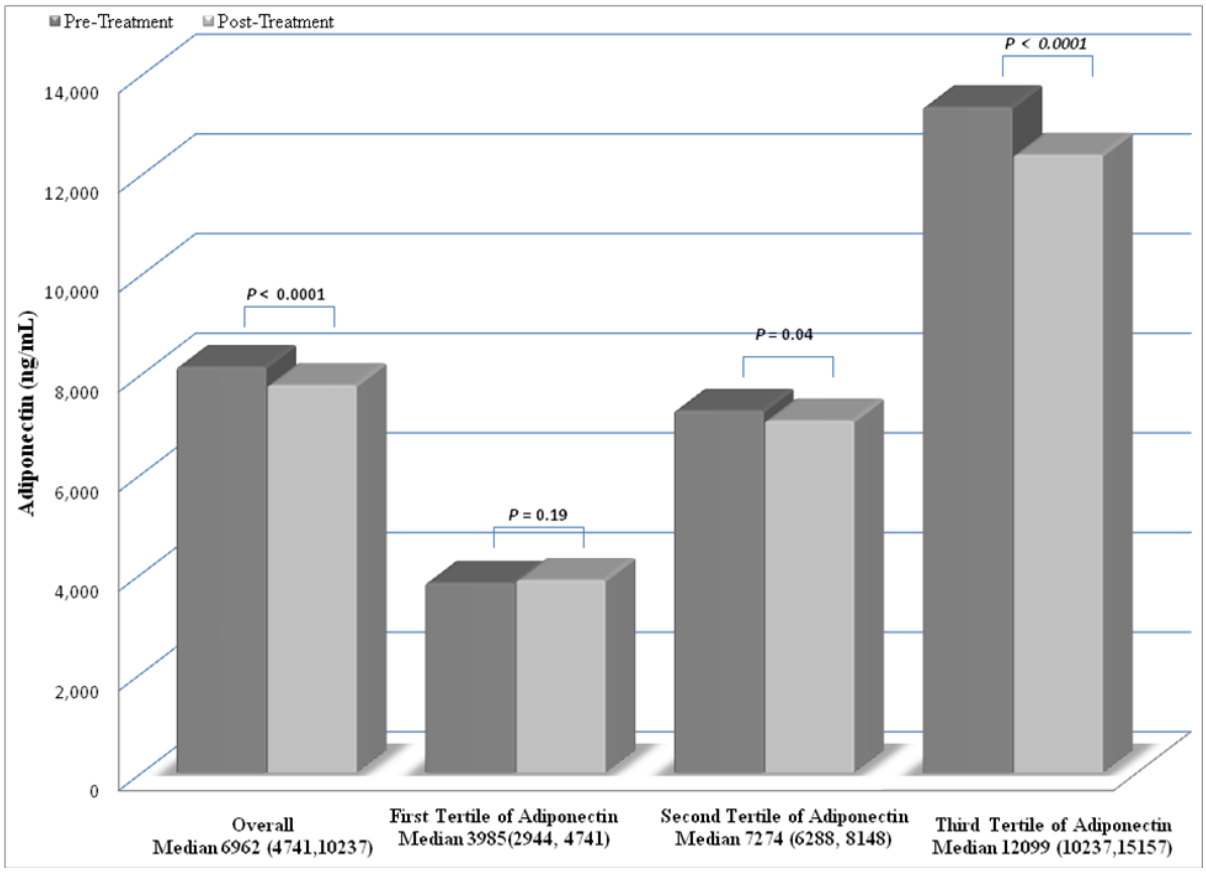


Figure 6. Change in adiponectin overall and by baseline tertile of HOMA-IR



CONCLUSION

In the GOLDN cohort, fenofibrate use was found to result in statistically significant decreases in insulin resistance as measured by HOMA-IR, insulin and glucose concentrations. The meta-analysis also found that fenofibrate use decreases HOMA-IR, insulin concentrations and glucose concentrations. Thus, we conclude that fenofibrate use results in small but statistically significant decreases in insulin resistance. This is consistent with the majority of the literature that has examined this relationship (49-54)

Clinical Significance of Findings

While the changes in insulin resistance are statistically significant, it is difficult to determine the clinical significance of these changes. This is particularly true for HOMA-IR, which has not been widely studied as a risk factor and if often measured in quantiles and not as a continuous variable (110-114) This categorical modeling, while useful in determining whether increased HOMA-IR is associated with a particular outcome, is not as helpful in determining what a clinically significant change in HOMA-IR would be.

One exception to this is a study conducted by Bonora and colleagues (115) which assessed whether HOMA-IR is a risk factor for cardiovascular disease among subjects with type 2 diabetes and found that a 1 unit increase in log(HOMA-IR) resulted in an odds ratio for incident cardiovascular disease of 1.56 (95% CI: 1.14, 2.12). The change in HOMA-IR we observed among fenofibrate users in the GOLDN cohort was -0.24 units, which corres-

ponds to a log (change HOMA) of -0.62. While this change is less than the 1 unit change assessed in Bonora's study, this does suggest that a change of -0.24 does have clinically significance with regard to cardiovascular disease. More studies that look at a variety of outcomes will be needed to further determine what change in HOMA-IR is clinically meaningful.

Fenofibrate in Comparison to Statins

Fenofibrate's effects on insulin resistance are especially notable when they are considered in light of statins' impact on insulin resistance. In a recent meta-analysis statins were found to increase risk of diabetes (116), and in another study (117) simvastatin in particular was found to increase risk of diabetes. This suggests that prescribing both fenofibrate and a statin for a patient with dyslipidemia may be an effective way of managing their risk for diabetes. The ACCORD study, which randomized participants taking a statin to fenofibrate or placebo, did not report any insulin resistance outcomes. While this study did not find any beneficial effects of fenofibrate use on cardiovascular outcomes (118) the findings of the FIELD study with regard to decreases in amputations (42) and the need for laser surgery for retinopathy (41) in subjects treated with fenofibrate suggest that fenofibrate use may have benefits outside of preventing cardiovascular outcomes.

Adiponectin as a Mediator Between Fenofibrate Use and Insulin Resistance

This analysis does not support the hypothesis that changes in adiponectin mediate the relationship between fenofibrate use and change in insulin resistance. This finding is surprising as the existing literature strongly supports both an association between fenofibrate use and increased adiponectin concentrations (76) and an association between adiponectin concentrations and insulin resistance (105-108). One potential explanation for this is that some studies (89; 90; 93; 94) have found that the ratio of high molecular weight adiponectin to total adiponectin correlates more strongly with insulin resistance and changes in insulin resistance than total adiponectin does. In one study (88) the correlation between the ratio of high-molecular weight adiponectin and change in insulin resistance was significant (r^2 =0.95, P = 0.001) while the correlation between total adiponectin and change in insulin resistance was not (r^2 = 0.107, P > 0.05). The GOLDN study did not measure adiponectin in its constituent molecular forms – high, medium and low molecular weight – and thus we could not model the ratio of high-molecular-weight adiponectin to total adiponectin.

It is also possible that fenofibrate did not result in increases in adiponectin and thus decreases in insulin resistance but rather that fenofibrate use resulted in increased expression of adiponectin receptors and thus more efficient use of adiponectin. Expression of these receptors has been shown to be positively correlated with insulin sensitivity (119), and thus negatively correlated with insulin resistance, in some studies while other studies have found no relationship (120). Fenofibrate has been found to increase expression of adiponectin receptors (121), although studies have been contradictory (122). While it is possible that this explains the decrease in insulin resistance without an increase in adiponectin, there is currently not enough evidence to evaluate this hypothesis.

Strengths and Limitations

The three papers in this dissertation have several key strengths and limitations. The GOLDN cohort is large (n=780), thus while other studies assessing insulin resistance outcomes may have lacked sufficient statistical power these analyses do not. In addition, venipuncture for the GOLDN study took place following a well-described protocol and all laboratory assays were conducted in the same laboratory, eliminating the possibility of inter-laboratory variation. Compliance with fenofibrate treatment was objectively verified using pill counts, and the overwhelming majority of the subjects were compliant with treatment.

The GOLDN study is limited, however, by its lack of a control group that was not treated with fenofibrate. Without such a control group it is impossible to determine whether the effects that were seen are true treatment effects or represent secular trends and/or regression to the mean. In the cases of the analysis considering whether changes in adiponectin mediate the relationship between fenofibrate and insulin resistance the analysis is limited by the lack of measures of high-molecular-weight adiponectin, which may be more predictive of insulin resistance.

The meta-analysis also has strengths and limitations. The use of a pre/post design results in effect sizes being biased towards the null, an important limitation, although it also eliminates the impact of residual confounding, an important strength. The meta-analysis may also be impacted by publication bias; however, every effort was made to minimize such bias,

including directly contacting authors who stated they had gathered measures of insulin resistance but had not reported them.

One strength of this analysis overall is the consistency of the findings between the meta-analysis and the analysis in the GOLDN cohort. The concordance of the two analyses with and without including the GOLDN data suggests that it was not chance that resulted in this positive finding but rather that it is a true finding that can be confirmed by other analyses. Thus, we conclude that fenofibrate use does indeed result in small but statistically significant decreases in insulin resistance and this relationship is not mediated by changes in adiponectin concentrations

Areas for Future Research

Future studies in this area should focus on replicating the findings related to fenofibrate use and insulin resistance, ideally through studies that include a control group not treated with fenofibrate. They should also focus on the ratio of high-molecular-weight adiponectin to total adiponectin and whether it is a mediator in the relationship between fenofibrate use and decreased insulin resistance. More importantly, clinical studies to determine if taking a statin and fenofibrate simultaneously results in decreased risk of diabetes or harmful sequelae related to diabetes are also important to understanding the full impact of these findings.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on October 26, 2010. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56 and ICH GCP Guidelines.

Principal Investigator:	PAEGLOW, CORRIE E
Co-Investigator(s):	
Protocol Number:	**E100630003**
Protocol Title:	*Fenofibrate in the Management of Insulin Resistance*

The above project was reviewed on 8/18/10. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This project qualifies as an exemption as defined in 45CF46.101, paragraph 4.

This project received EXEMPT review.

IRB Approval Date: 8/18/10

Date IRB Approval Issued: 8/18/10

Sheila Moore, CIP
Director, Office of the Institutional
Review Board for Human Use (IRB)

Investigators please note:

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

470 Administration Building
701 20th Street South
205.934.3789
Fax 205.934.1301
irb@uab.edu

The University of
Alabama at Birmingham
Mailing Address:
AB 470
1530 3RD AVE S
BIRMINGHAM AL 35294-0104

APPENDIX B

SAS CODE TO CREATE UNRELATED SUB-COHORT

```
proc surveyselect data=goldn.fenotrial
method=srs n=1
seed=1953 out=Goldn.unrelated;
strata gpedid;
run;
```