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2002

EFFECTS ON LIPID METABOLISM OF METFORMIN AND TROGLITAZONE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Biguanides and the thiazolidinediones (TZDs) have distinct anti-hyperglycemic mechanisms of action. Both classes also influence lipid metabolism but have not been adequately compared. We measured the effects on lipid parameters of metformin (M), a biguanide, and troglitazone (T), a TZD, alone and in combination (MT), in 27 type 2 diabetic subjects. Random assignment was made to either M (1000 mg BID; n=15) or T (400 mg QD; n=12) for 3 mo. Baseline characteristics were similar between groups: mean age 53.4 years, HbA1c 9.5%, and BMI 33.7 kg/m^2. After 3 mo. of M or T, subjects were placed on MT (n=23) for another 3 mo.

There was an insignificant rise in HDL-C with M or T (+6%) and an additive effect with MT (+12%, p<.01). M decreased LDL-C by 7.5% (p=.04), T insignificantly increased levels (+8.1%), and MT had a neutral effect. Both M (-15.3%) and T (-16.4%) insignificantly reduced fasting (f-) TG; MT decreased f-TG by 27.0% (p<.01). A similar pattern was seen on postprandial (pp-) TG (M, -9.8% (p=NS); T, -9.7% (p=NS); MT, -15.1% (p=.006)). M did not affect f-FFA, whereas there was a reduction with T (-30.0%, p=.03), partially offset by MT (-17.3%, p<0.01). In contrast, both agents decreased pp-FFA, with T having a greater effect (M, -32.7% (p<.01); T, -48.7% (p=.001)), and little evidence for additive effect (-53%, p<.0001).

The effects of MT on HDL-C, f-TG, and pp-TG seem to be additive, while their effects on LDL-C appear offsetting. T elicits a clear suppression of both f-FFA and pp-FFA, whereas M does not affect f-FFA and induces a less potent suppression of pp-FFA. In combination, M attenuates T's f-FFA effects, and there is only minimal additive benefit on pp-FFA. Overall, both agents have generally favorable, although differing, effects on lipid metabolism, with only some synergy demonstrated in combination.

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INTRODUCTION

Prevalence and Pathophysiology of Type 2 Diabetes Mellitus

Diabetes mellitus (DM) is a serious and common health problem. In the US alone, 16 million individuals are affected by DM; approximately 90% of these patients have type 2 DM (T2DM) (1). As the number of affected patients continues to increase, the National Health and Nutrition Examination Survey states that diabetes will become the most common chronic disease in this country (1). The national cost of treating diabetes as well as its complications (DM is the leading cause of blindness and renal failure) accounts for 1/6 of all health care expenditures (1).

Intrinsic genetic factors contribute significantly to the pathogenesis of this disease. DM is found more often within certain ethnic groups including Hispanics, African Americans, Pacific Islanders, and Native American Indians. Twin studies have also shown that monozygotic twins have a twofold or greater concordance as compared to dizygotic twins (2). Though researchers have sought to isolate individual candidate genes or clusters of genes, none have been found, suggesting that DM involves multiple defects in various genes. Acquired factors including increasing age, visceral obesity, and physical inactivity have also been correlated with the development and progression of this disease.

T2DM is defined by the presence of peripheral insulin resistance and non-autoimmune impaired insulin secretion. It begins with a compromise in insulin action. Peripheral insulin resistance occurs most notably in skeletal muscle and adipose tissue, resulting in decreased glucose disposal. Unimpaired glucagon secretion coupled with hepatic insulin resistance results in elevated basal hepatic glucose output. Adipocytes release free fatty acids (FFA), which along with glucose, are transported to the liver for triglyceride (TG) synthesis. TG becomes an alternative energy source for tissues such as muscle, thereby conserving glucose for the central nervous system. Not only is plasma glucose and FFA elevation a result of insulin resistance, but each also independently exacerbates insulin resistance ("glucotoxicity", "lipotoxicity"). FFA oxidation also further stimulates gluconeogenesis in the liver. Pancreatic β cell hyperplasia

occurs to increase insulin output. With worsening insulin resistance, however, the level of hyperinsulinemia becomes relatively insufficient, resulting in mild hyperglycemia, initially detected in the postprandial setting. β cell dysfunction soon follows. The decreased insulin response to glucose eventually results in elevated fasting blood sugar levels. The etiology of β cell dysfunction is unknown but suspected etiologies include genetically programmed failure, glucotoxicity, and/or lipotoxicity.

Diabetic Complications

Microvascular complications

Patients with T2DM are at a higher risk of developing microvascular and macrovascular complications compared to those unaffected by the disease. Up to 20% of patients with T2DM are found to have retinopathy and 7-8% are found to have neuropathy at the time of clinical diagnosis (3). The incidence of microvascular complications then rises as the duration of T2DM increases. Approximately 9% of previously unaffected patients develop neuropathy, nephropathy, and/or retinopathy within 9 years of diagnosis (4).

The Diabetes Control and Complications Trial (DCCT), a large prospective multicenter study of type 1 diabetics, was the first to demonstrate the causal role of hyperglycemia in microvascular complications and thus, establish the importance of strict glucose control in these patients (5). As expected, the Kumamoto Study (6), the Veteran Affairs Cooperative Study (7), and the United Kingdom Prospective Diabetes Study (UKPDS) (8) all went on to further demonstrate that type 2 diabetics would similarly benefit from aggressive glucose control.

Macrovascular complications

Although microvascular complications increase morbidity, macrovascular complications take the greater toll on T2DM patients. Atherosclerosis accounts for 80% of the mortality rate, with cardiovascular disease (CVD) being the number one cause of death in these patients (9).

Atherosclerosis-related morbidity, including coronary artery disease (CAD), stroke, and peripheral vascular disease (PVD) is also 2.5 times higher in diabetic men and 3.5-4.5 times higher in diabetic women than in their non-diabetic counterparts (10, 11, 12).

The main goal of current diabetes treatment is to strictly control glucose levels. Although this has been shown to benefit microvascular complications, its role on macrovascular complications has not been fully supported. A Finnish study showed that elevated levels of glycosylated hemoglobin (HbA1c) may mediate CVD in elderly diabetics through the formation of advanced glycation end products in vessel walls (13). A number of other theories have linked the genesis of atherosclerosis to endothelial dysfunction, or frequently coexisting hypertension and hyperlipidemia.

Interventional studies have yielded interesting yet inconclusive results. The Veterans Affairs Cooperative Study (7) and the UKPDS (8) both found insignificant differences in macrovascular complications between their intensively treated and conventionally treated groups. When the UKPDS subgroups were further analyzed, however, researchers found that the group intensively treated with metformin had a significant 39% reduction in the incidence of myocardial infarction (MI) compared to patients treated conventionally with diet or intensively with sulfonylureas or insulin. This finding raised the possibility that different diabetic agents may have varying effects on macroangiopathy. The cardiovascular protective effect of metformin, however, was lost for unexplained reasons when it was used in combination with sulfonylureas.

Since meticulous control of diabetic hyperglycemia has not been consistently effective in combating macroangiopathy, other etiologies have been studied. Insulin resistance and the resultant hyperinsulinemia may be key factors.

Insulin is a growth factor, which theoretically, accelerates atherogenesis through vasculature smooth muscle cell and connective tissue proliferation, as well as increased platelet adhesiveness (14). Hyperinsulinemia has been predictive for CVD in non-diabetic patients in the Helsinki Policemen Study (15), the Paris Prospective Study (16), and the Quebec Cardiovascular

Study (17). Second, insulin resistance and hyperinsulinemia are often found in conjunction with other cardiovascular risk factors, such as visceral obesity, impaired glucose tolerance or diabetes, hypertension, aging, impaired fibrinolysis with increased levels of plasminogen activator inhibitor (PAI-1), and dyslipidemia (18). When present alone, each component poses a risk factor for CVD. Since many components are often present simultaneously [known as the insulin resistance syndrome (IRS)], they may synergistically increase the risk for macrovascular complications.

Insulin resistance may, in fact, be an important causal contributor to the development of its associated features, including the CVD risk factor, diabetic dyslipidemia. Plasma FFA levels increase as adipocytes become resistant to the anti-lipolytic effects of insulin. Elevated FFA, then in turn, contribute to the decline in β cell function. Additionally, FFA coupled with high levels of glucose, as seen in T2DM patients, provide the main substrates necessary for TG-rich very low density lipoprotein (VLDL-C) production by the liver. Lipoprotein lipase (LPL), which hydrolyzes chylomicrons and VLDL-C to higher density lipoproteins, is also regulated by insulin. Insulin resistance renders this enzyme less effective. This combined decrease in VLDL-C clearance in conjunction with elevated VLDL-C production results in the most common lipid abnormality present in diabetic patients, hypertriglyceridemia (19, 20, 21). Hypertriglyceridemia, in turn, leads to the formation of smaller, more dense LDL-C cholesterol particles (22). LDL-C levels are not necessarily elevated in T2DM patients, but small, dense LDL-C particles are more easily oxidized and therefore, more atherogenic (23, 24). HDL-C levels are decreased in these patients, again, secondary to decreased production, as well as hypertriglyceridemia (17).

Treating dyslipidemia is extremely beneficial in preventing the development of CVD in T2DM patients. Elevated total cholesterol, LDL-C, and TG, as well as depressed HDL-C have all been shown to be independent risk factors for CVD (25, 26). Additionally, studies have suggested that diabetics may be affected more by abnormalities in the lipid metabolism than non-diabetics. The Multiple Risk Factor Intervention Trial, for example, showed that in all patients, elevated total cholesterol was related to mortality due to coronary heart disease (27). However, in

patients with a similar degree of hypercholesterolemia, diabetic males had a fourfold greater risk for heart disease than their non-diabetic controls.

Treatments for T2DM

T2DM medications are primarily used to attain glucose control. Sulfonylureas, which stimulate the release of insulin at lower glycemic thresholds, comprised the first drug class to become available. Approximately 66% of patients respond to sulfonylureas, although 20% of the responders eventually require additional medication for adequate glucose control (28).

Sulfonylureas generally have no effect on lipid profiles. Additionally, because they increase insulin levels, there is speculation that the sulfonylureas have at best, no effect, and at worst, may potentiate macrovascular complications. They remain, however, the most widely used oral anti-diabetic agents.

Within the last decade, other agents have also become available in the US. Biguanides, which were temporarily available in the 1960s and 1970s, made a resurgence in 1995 when metformin was introduced into the US market (29). Its entry was quickly followed by other classes, such as the alpha glucosidase inhibitors, the thiazolidinediones (TZDs), and the non-sulfonylurea insulin secretagogues. The most interesting of these medications may be the biguanides and the TZDs. Both are insulin sparing agents which in addition to controlling glucose levels, may also have beneficial effects on CVD via their effects on diabetic dyslipidemia.

Metformin, a Biguanide

The anti-hyperglycemic effects of biguanides have been recognized since the 19th century. At that time, the active ingredient, guanidine, was extracted from the French lilac, *Galega officinalis*, to treat diabetic patients (30). Guanidine was later used to synthesize other biguanides. The first of these, phenformin, became commercially available in the US in the

1960s. In the 1970s, however, the drug was removed because it was found to increase the risk of lactic acidosis (29).

Metformin was used for years in Europe and Canada before it became FDA- approved in December of 1994 (31). This biguanide has proven to be much safer than phenformin. The estimated incidence of lactic acidosis is .03 per 1000 patient-years of use (31). A majority of these cases occurred in the setting of incorrect use, for example, in patients with contraindications such as renal impairment. Approximately 30% of patients experience various side effects, most notably dose-related GI symptoms such as abdominal pain, nausea, and diarrhea (31). These symptoms, however, are tolerable for most, with only 5% of patients requiring discontinuation of the medication (32).

The glucose and HbA1c lowering with metformin is equivalent to that of the sulfonylureas. Over 90% of patients initially show clinical improvement on metformin (31). With continued use, however, 5-10%/yr. become less responsive and eventually require additional medications (31). Unlike sulfonylureas, metformin does not cause hypoglycemia and has thus been categorized as an "anti-hyperglycemic."

Metformin's mechanism(s) of action have yet to be fully elucidated. A majority of studies suggest that the drug primarily decreases hepatic glucose output by decreasing glycogenolysis and/or gluconeogenesis. The drug may also have modest effects on increasing insulin sensitivity of peripheral tissues (33, 34). Some studies have shown that there are similar declines in glucose and FFA levels. Lower FFA levels could theoretically improve glucose levels through the Randle cycle (35,36). Perriello et al. showed that there were similar effects in the suppression of hepatic glucose production, plasma FFA concentration, and rates of lipid oxidation, along with the increase in rates of glucose oxidation in patients treated with metformin (35). Meanwhile, Abbasi et al. demonstrated significant decreases in fasting and postprandial glucose as well as FFA. In their study, metformin resulted in a mean glucose decrease of 17%

while FFA decreased by 19% (30). However, not all studies have shown significant reductions in plasma FFA after metformin treatment (37).

Because metformin reduces glucose levels without elevating insulin levels, there has long been speculation that the drug may also increase peripheral insulin sensitivity, and therefore, increase peripheral glucose disposal. Metformin is indeed ineffective in the absence of insulin (31). Studies have, however, yielded conflicting results as to whether improved peripheral glucose disposal is directly related to the drug or whether it is a secondary effect of improved glucose control (i.e. improved glucotoxicity) (37, 38, 39), lowering of FFA levels (i.e. improved lipotoxicity) (40), and/or changes in body weight and composition (37). Postprandial glucose lowering may also be facilitated by slow glucose absorption by the GI tract attributable to metformin (41).

Metformin has rapidly gained popularity in part because of its additional beneficial effects on various cardiovascular risk factors. Experimental models in animals have shown that metformin alters aortic lipid metabolism, providing a basis for its possible protective effects against atherosclerosis (42). In the UKPDS, overweight patients treated with metformin monotherapy experienced significantly fewer macrovascular events than the control group and the groups treated with sulfonylurea or insulin (8). This benefit may be secondary to a multitude of factors. Metformin is an insulin sparing drug, it does not cause weight gain (43, 44), and it has been shown in some studies to have lipid lowering effects (*Table1).* Overall, metformin appears to significantly lower total cholesterol, LDL-C, and TG, while modestly decreasing FFA and causing no significant change in HDL-C (62). Additionally, increased fibrinolytic activity and decreased platelet aggregation, as well as decreased blood pressure and peripheral arterial resistance have also been demonstrated (53).

TABLE 1: LITERATURE REVIEW OF THE EFFECTS ON LIPID METABOLISM OF METFORMIN

Lipid Parameter	Percent Change
Total Cholesterol	No significant change (40) or up to 17% decrease (49, 51, 55, 58, 59, 60)
VLDL-C	No significant change (40) or up to 39% decrease (46, 52, 56, 57, 58)
LDL-C	No significant change (51, 61) or up to 24% decrease (55, 59, 60)
HDL-C	No significant change (35, 61) or up to 17% increase (45, 46, 51, 55)
TG	No significant change (45, 47, 48) or up to 45% decrease (46, 49, 50, 51, 52, 53, 54, 55)
FFA	No significant change (45) or up to 17% decrease (35, 46)

Troglitazone, a TZD

TZDs comprise the other class of diabetic medications which may have beneficial CVD effects. In 1997, troglitazone became the first TZD introduced into the US market (28). Troglitazone was synthesized with an α-tocopherol moiety, similar in structure to vitamin E, in hopes of producing a TZD which would not only control hyperglycemia, but could also lower lipids via limiting lipid oxidation (63). Troglitazone was eventually removed from the US market by the FDA because of its link to rare idiosyncratic hepatocellular injury. New TZDs, such as rosiglitazone and pioglitazone, are now available with significantly safer side effect profiles.

Troglitazone has the same glucose lowering ability as sulfonylureas or metformin in diabetic patients (64). The drug is not associated with hypoglycemia, and therefore, is also referred to as an "anti-hyperglycemic". Its mechanism of action is insulin sensitization, which primarily occurs in peripheral tissues but may also affect hepatic glucose production (65). To exert its effect, the drug requires the presence of insulin (66, 67). In a study by Suter et al., 25% of patients treated with 3 months of troglitazone (400mg QD) had no clinical response (68). Interestingly, these patients also had the lowest levels of insulin secretion.

TZDs regulate insulin induced gene expression by activating various nuclear receptors of the peroxisome proliferator activated receptor (PPAR) family (69). PPAR-α, which is found in tissues such as the liver, kidney, heart, and muscle, is involved in fatty acid metabolism. The mechanism of action of the lipid lowering fibrates is via this particular receptor. TZDs primarily work via another member of the superfamily, PPAR-γ, which is found in adipose tissue. It stimulates glucose disposal by increasing transcription of glucose transporters, GLUT1 and GLUT4, necessary for glucose uptake (69). Since PPARs are also found on vascular wall cells, theoretically, TZDs could directly affect the vasculature to prevent atherogenesis (70). Glucose lowering effects have also been associated with increased β cell responsiveness. (71).

Like biguanides, TZDs also have multiple beneficial effects on CVD risk factors. They increase insulin sensitivity resulting in decreased insulin levels, perhaps even to a greater degree than metformin. The drug also appears to improve dyslipidemia (*Table 2).* Overall, review of the literature suggests that troglitazone may significantly decrease TG and FFA, while increasing total cholesterol, LDL-C, and HDL-C. The effects of troglitazone on the lipid profile appear to be dose dependent (71).

TABLE 2: LITERATURE REVIEW OF EFFECTS ON LIPID METABOLISM OF TROGLITAZONE

Lipid Parameter	Percent Change
Total Cholesterol	No significant change (71, 73, 77) or up to 5.8% decrease
LDL-C	No significant change (47, 63, 77) or up to 15.4% increase (71, 74, 75, 78)
HDL-C	No significant change (71, 75) or up to 24% increase (47, 73, 74, 76, 77)
TG	No significant change (71, 74, 75) or up to 32% decrease (47, 63, 72, 73, 76, 77)
FFA	No significant change (71) or up to 33% decrease (72, 73)

TZDs have been shown to appreciably affect TG and FFA. A study by Maggs et al. demonstrated that varying doses of troglitazone had beneficial effects on both fasting and postprandial TG while higher doses were needed to significantly decrease FFA (79). The effects of troglitazone on TG lowering are thought to be secondary to decreased hepatic production as well as increased LPL activity. The role of insulin sensitization in lowering TG levels, however, is not entirely clear. A study by Saltiel et al. found that insulin deficient mice treated with troglitazone demonstrated similar decreases in TG as their insulin resistant counterparts (80), suggesting a direct hypolipidemic effect. The increase in LDL-C found in a majority of studies is of particular interest (63). Though elevated LDL-C is an independent risk factor for macrovascular complications, in this case, it may not be as detrimental as expected. Various

studies have shown that though LDL-C levels are elevated in patients using troglitazone, the ratio of LDL-C to HDL-C (71) or the ratio of HDL-C to total cholesterol (75) remains unchanged. Additionally, larger and less dense LDL-C particles have been noted in patients treated with TZDs (77, 78, 81). These particles may not as prone to oxidation as the smaller dense LDL-C particles and may therefore, be less atherogenic.

TZDs also lower blood pressure, improve fibrinolysis, decrease platelet aggregability, and improve endothelial function (63). Further studies are necessary to evaluate whether these changes are actually associated with positive clinical outcomes.

STATEMENT OF PURPOSE AND HYPOTHESIS

Macrovascular complications are a significant cause of morbidity and mortality in T2DM patients. Strict glucose control, which has been shown to prevent and/or forestall the development of microangiopathy, has had no appreciable effect on the outcome of macroangiopathy, resulting in a search for other etiologies, as well as preventive strategies. Insulin resistance and its associated clinical features have become targets of interest.

Metformin and troglitazone are two insulin sparing diabetic agents which function via different mechanisms, but have comparable glucose lowering effects. Previous studies have also demonstrated that these two agents benefit diabetic dyslipidemia, a significant risk factor for CVD. However, their effects on lipid metabolism have not been adequately compared in a single study.

Therefore, we conducted this study with the following aims in mind:

1. To characterize the effects of metformin or troglitazone monotherapy on the lipid profiles [LDL-C, HDL-C, fasting and postprandial FFA, and fasting and postprandial TG] of T2DM patients.
2. To characterize the effects of metformin and troglitazone combination therapy on the lipid profiles [LDL-C, HDL-C, fasting and postprandial FFA, and fasting and postprandial TG] of T2DM patients.
3. To determine whether significant lipid altering effects correlate with changes in any of the following parameters: glucose, HbA1c, basal and clamp glucose production, or glucose disposal.

We hypothesized that since insulin resistance is believed to be a causal factor of IRS, that overall, troglitazone will be more effective in controlling dyslipidemia than metformin. Second, because metformin may also have minor insulin sensitizing properties, we expect that metformin

and troglitazone combination therapy will be more effective in improving lipids than metformin or troglitazone monotherapy.

METHODS

The Human Investigation Committee at Yale University School of Medicine reviewed and approved the protocol for the original study (64) as well as the use of its data for this project.

Study Subjects

The original study compared the anti-hyperglycemic potential as well as the mechanisms of action of metformin and troglitazone, when used alone and in combination. All 29 participants met the National Diabetes Data Group's criteria for T2DM, with glycosylated hemoglobin values above the upper limit of normal and plasma C-peptide concentrations ≥ 1.5ng/mL (.50 nmol/L) on diet, sulfonylurea, and/or biguanide therapy. The following patients were excluded from the study: pregnant females, patients with abnormal renal or hepatic function, and those with recent atherosclerotic events.

Initially, patients were randomly distributed between 2 treatment groups: the metformin and troglitazone monotherapy groups. Both patients and investigators were aware of their treatment. Of the 15 patients (8 females and 7 males) who were in the metformin monotherapy group, 1 patient continued on his outpatient antilipid medication and was placed on a submaximal metformin dose as a consequence of its side effects. Of the 14 patients (8 males and 6 females) who were initially enrolled in the troglitazone group, data for only 12 patients (5 males and 7 females) was available for this study. One patient continued to have glucose levels >350mg/dL and was therefore unable to complete 3 months of monotherapy. Meanwhile, a second patient did not have a substantial portion of their lipid data available. Three patients in the troglitazone monotherapy group were on anti-lipid medication, which remained stable during the study period.

After the initial 3 months of metformin or troglitazone monotherapy, patients were invited to continue an additional 3 months of metformin and troglitazone combination therapy. One patient in the metformin monotherapy group declined further participation. (The patient who was originally on the anti-lipid medication as well as a submaximal metformin dose continued

into the combination phase.) In the troglitazone monotherapy group, 1 patient declined the invitation and 2 additional patients were unable to complete the combination phase because of unrelated illness. One troglitazone patient on anti-lipid medication continued into the combination phase.

Study Design

Monotherapy and Combination Therapy

Patients were given a 2 week washout period, during which they discontinued their outpatient diabetes medication. They were then given either metformin 1000mg BID or troglitazone 400mg QD for 3 months. (One patient received a submaximal daily metformin dose of 1500mg.) At month 0 and month 3, patients were admitted for an 8-hr mixed meal-tolerance test and a hyperinsulinemic-euglycemic clamp study.

Patients who continued the second phase of the study were placed on metformin and troglitazone combination therapy for an additional 3 months. At month 6, patients were again admitted for their last 8-hr mixed meal-tolerance test and a hyperinsulinemic-euglycemic clamp study.

A diet comprised of 50% carbohydrate, 34% fat, and 16% protein was recommended for all participants to maintain baseline body weight during the course of the study.

Meal Tolerance Test

The meal tolerance test was performed after an overnight fast. At approximately 8 AM, for breakfast, and again at 12 PM, for lunch, patients were given Sustacal-HC liquid formula meals. A fasting blood sample was drawn prior to breakfast followed by eight hourly postprandial blood samples drawn from an intravenous catheter placed in the antecubital vein. Blood samples were sent to Corning Nichols Institute where glucose, HbA1c, insulin, C-peptide, HDL-C, LDL-C, FFA, and TG levels were determined.

Hyperinsulinemic-Euglycemic Clamp Study and Resulting Measurements

The hyperinsulinemic-euglycemic clamp study using [6,6-^{2}H] glucose was performed the following day after an overnight fast (64). Gas chromatography-mass spectrometry was done at the Yale Stable Isotope Core Facility. The substrate and hormone measurements were determined as follows:

1. Basal Endogenous Glucose Production = (f/BSA)x([enrichment$_{inf}$/enrichment$_{plasma}$] – 1)

f= basal [6,6-^{2}H] glucose infusate rate (mg/min)
BSA = body-surface area (m^2)
enrichment$_{inf}$ = % enrichment of [6,6-^{2}H] glucose enrichment
enrichment$_{plasma}$ = % of plasma [6,6-^{2}H] glucose enrichment

2. Glucose Disposal Rate = cEGP + GIR

cEGP = endogenous glucose production during clamping (see below for calculation)
GIR = Mean rate of exogenous glucose infusion from minutes 260 to 300 of the clamping period ($mg/m^2/min$)

3. Endogenous Glucose Production During Clamping = GIR x ([enrichment$_{inf}$/enrichment$_{plasma}$] – 1)

Data Collection and Statistical Analysis

For this project, charts collected from the original study were reviewed. Patients' lipid and glucose profiles were entered into a Microsoft Excel database. Postprandial values were calculated as the mean of the 8 existing hourly measurements.

To determine the effects of metformin and troglitazone, alone and in combination, on the lipid metabolism of patients with T2DM, statistical analysis was performed on the following 3 distinct data sets:

1. Patients who completed the initial 3 months were analyzed separately depending on the treatment group they were initially placed to directly compare the effects on the lipid metabolism of metformin and troglitazone when used alone.

2. Patients who completed six months were analyzed together regardless of their initial monotherapy group to analyze the effects on the lipid metabolism of metformin and troglitazone when used in combination.
3. Patients who completed six months were analyzed separately depending on their initial monotherapy treatment to compare the effects of adding troglitazone to metformin monotherapy versus adding metformin to troglitazone monotherapy.

Lipid parameters which were significantly affected underwent a second phase of analysis. Regression analysis was performed to determine whether changes in lipid levels correlated significantly with changes in glucose, HbA1c, basal endogenous glucose production, endogenous glucose production during the clamp study, and/or glucose disposal rates.

RESULTS

Patient Characteristics

The metformin and troglitazone groups had similar baseline characteristics. Fifteen patients, 8 women and 7 men, were included in the analysis of the metformin monotherapy group. This group was comprised of 12 Caucasians, 2 African Americans, and 1 patient of mixed African American and Native American ancestry. Ages ranged from 32 to 74 years with a mean age of 51±13 years, while the duration of diabetes ranged from 3 months to 14 years with a mean duration of 5±4 years. Prior to the start of the study, 8 patients were taking sulfonylureas, 1 was taking metformin, 4 were taking sulfonylurea and metformin combination therapy, and 2 were diet controlled. The mean body mass index (BMI) was 33.9±6.8 kg/m^2. Twelve patients, 7 women and 5 men, were included in the analysis of the troglitazone monotherapy group. This group was comprised of 10 Caucasians, 1 African American, and 1 patient of mixed African American, Native American, and Dutch ancestry. Ages ranged from 37 to 71 years old with a mean age of 56±12 years, while the duration of diabetes ranged from 2 months to 12 years with a mean duration of 3±3 years. Prior to the start of the study, 8 patients were taking sulfonylureas, 1 was taking metformin, and 3 were diet controlled. The mean BMI was 32.7±7.4 kg/m^2 *(Table 3)*.

TABLE 3: BASELINE DEMOGRAPHICS OF PATIENTS WHO COMPLETED THE INITIAL 3 MONTHS OF MONOTHERAPY

Patient Characteristics	**Metformin Monotherapy (N=15)**	**Troglitazone Monotherapy (N=12)**
Age (yr)	51±13	56±12
Race	2 African Americans (13%) 1 African American and Native American (7%) 12 Caucasians (80%)	1 African American (8%) 1 African American, Native American, and Dutch (8%) 10 Caucasians (83%)
Gender	8 Females (53%) 7 Males (47%)	7 Females (58%) 5 Males (42%)
Anti-diabetic Medication(s) Prior to Study	8 on Sulfonylureas 1 on Metformin 4 on Combination Therapy[1] 2 Diet Controlled	8 on Sulfonylureas 1 on Metformin 3 Diet Controlled
Duration of Diabetes (yr)	5±4	3±3
Body Mass Index (kg/m^2)	33.9±6.8	32.7±7.4

**p>0.05 for above patient characteristics*
[1]Patients on combination therapy were on sulfonylureas and metformin

The patients in the metformin and troglitazone groups also had similar fasting lipid and diabetes profiles obtained at month 0 (after the washout period and prior to the start of monotherapy) *(Table 4)*.

TABLE 4: FASTING VALUES OBTAINED AT MONTH 0 AFTER THE WASHOUT PERIOD

Fasting Parameter	**Metformin Group (N=15)**	**Troglitazone Group (N=12)**
Glucose (mg/dL)	287±84	273±70
Glycosylated hemoglobin (%)	10.0±1.6	9.6±1.7
HDL-C (mg/dL)	40.7±10.1	40.6±8.0
LDL-C (mg/dL)	122.4±33.2	133.9±35.0
TG (mg/dL)	191.3±118.1	228.9±161.2
FFA (nmol/L)	.87±.27	.98±.42

**p>0.05 for above fasting parameters*

Monotherapy *(Table 5)*

After the initial 3 months, metformin and troglitazone monotherapy had similar insignificant effects on HDL-C. Metformin increased HDL-C from 40.7±10.1 to 43.2±15.1 mg/dL (+6.0%; P=.27) while troglitazone increased levels from 40.6±8.0 to 43.1±8.7 mg/dL (+6.2%; P=.14) *(Figure 1)*. In contrast, there was a significant difference between their effects on LDL-C. Troglitazone increased LDL-C from 133.9±35.0 to 144.8±37.3 mg/dL (+8.1%; P=.11), while metformin decreased levels from 122.4±33.2 to 113.2±32.5 mg/dL (-7.5%; P=.036) *(Figure 2)*. LDL-C lowering effects seen with metformin did not correlate with changes in glucose,

HbA1c, basal endogenous glucose production, endogenous glucose production during the clamp study, or glucose disposal rates.

Metformin and troglitazone monotherapy did not appreciably alter fasting *(Figure 3)* or postprandial TG levels *(Figure 4)*. Metformin decreased fasting TG by 15.3% (P=.097), from 191.3± 118.1 to 162.0± 77.9 mg/dL, and postprandial TG by 9.8% (P=.15), from 253.2±116.6 to 228.4±95.5 mg/dL. Similarly, troglitazone decreased fasting TG by 16.4% (P=.43), from 229.0±161.2 to 191.3±101.9, and postprandial TG by 9.7% (P=.18), from 290.7±147.9 to 262.6±128.0.

Metformin did not affect fasting FFA *(Figure 5)*. Levels decreased .27% (P=.96), from .873±.271 to .871±.227 nmol/L. Troglitazone significantly decreased fasting FFA by 30.0% (P=.033) from .98±.42 to .69±.24. This decrease correlated with changes in fasting glucose (R=.68; p=.016) *(Figure 6)*. Both medications decreased postprandial FFA levels (*Figure 7)*. Metformin decreased postprandial FFA levels from .39±.20 to .26±.13 nmol/L (-32.7%; P=.0091) and troglitazone decreased levels slightly more, from .37±.21 to .19±.10 (-48.7%; P=.0013). Postprandial FFA lowering did not correlate with any changes in the glucose parameters.

TABLE 5: EFFECTS OF METFORMIN OR TROGLITAZONE MONOTHERAPY ON LIPID PROFILES

Lipid Parameter	Monotherapy Group	Post-Washout Value (Month 0)	Post-Monotherapy Value (Month 3)	Percent Change	P Value
HDL(mg/dL)	*Metformin (N=15)*	40.7±10.1	43.2±15.1	+6.0%	.27
	Troglitazone (N=12)	40.6±8.0	43.1±8.7	+6.2%	.14
LDL(mg/dL)	*Metformin*	122.4±33.2	113.2±32.5	**-7.5%**	**.036**
	Troglitazone	133.9±35.0	144.8±37.3	+8.1%	.11
Fasting TG (mg/dL)	*Metformin*	191.3±118.1	162.0±77.9	-15.3%	.097
	Troglitazone	229.0±161.2	191.3±101.9	-16.4%	.43
Postprandial TG (mg/dL)	*Metformin*	253.2±116.6	228.4±95.5	-9.8%	.15
	Troglitazone	290.7±147.9	262.6±128.0	-9.7%	.18
Fasting FFA (nmol/L)	*Metformin*	.873±.271	.871±.227	-.27%	.96
	Troglitazone	.98±.42	.69±.24	**-30.0%**	**.033**
Postprandial FFA (nmol/L)	*Metformin*	.39±.20	.26±.13	**-32.7%**	**.0091**
	Troglitazone	.37±.21	.19±.10	**-48.7%**	**.0013**

Combination Therapy *(Table 6)*

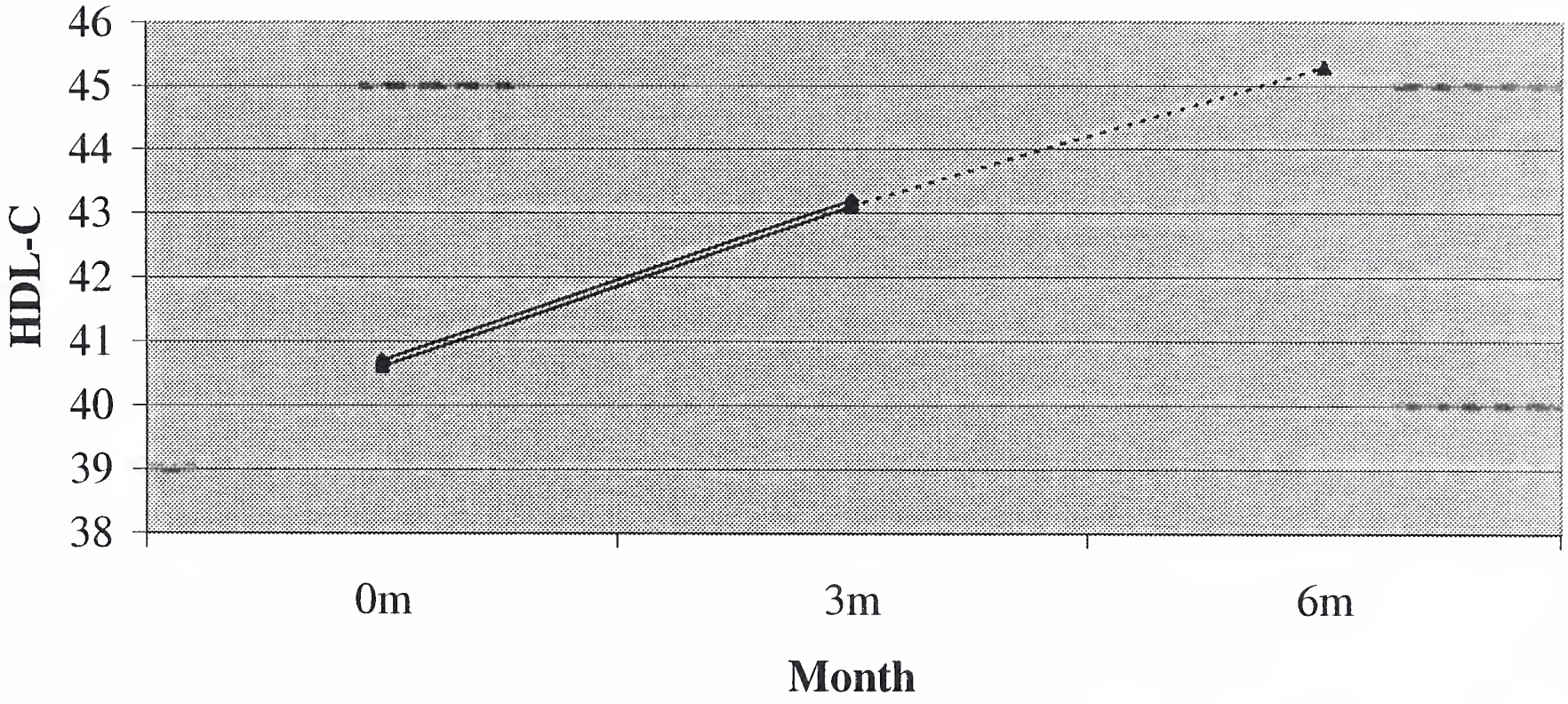
Three months of metformin and troglitazone combination therapy significantly increased HDL-C by 12% (P=.0044), from 40.4±9.1 to 45.3±12.8 mg/dL *(Figure 1)*. There was no correlation between the HDL-C increase and changes in glucose parameters. When patients who completed the entire 6 month study were analyzed according to their initial monotherapy group, adding troglitazone to metformin appreciably increased HDL-C by 7.7% (P=.012). Regarding LDL-C, the addition of metformin to troglitazone significantly decreased levels by 12.4% (P=.009). However, there was no significant overall LDL-C difference with combination therapy (-1.6%; P=.66) *(Figure 2)*.

Combination therapy decreased fasting and postprandial TG by 27.0% (P=.0083), from 219.7±145.3 to 160.5±117.6 mg/dL *(Figure 3)* and 15.1% (P=.0059), from 265.2±117.1 to 225.2±122.6 mg/dL respectively *(Figure 4)*. Fasting and postprandial FFA also appreciably decreased. Fasting FFA decreased from .94±.35 to .78±.21 nmol/L (-17.3%; P=.0017) *(Figure 5)* while postprandial FFA decreased from .38±.21 to .18±.12 (-53.0%; P=2.5E-07) *(Figure 7)*. Adding troglitazone significantly decreased fasting FFA by 15.4% (P=.035) and postprandial FFA by 38.14% (P=.0029). These significant changes did not correlate with any changes in the glucose parameters.

TABLE 6: EFFECTS OF COMBINATION THERAPY ON LIPID PROFILES (N=23)

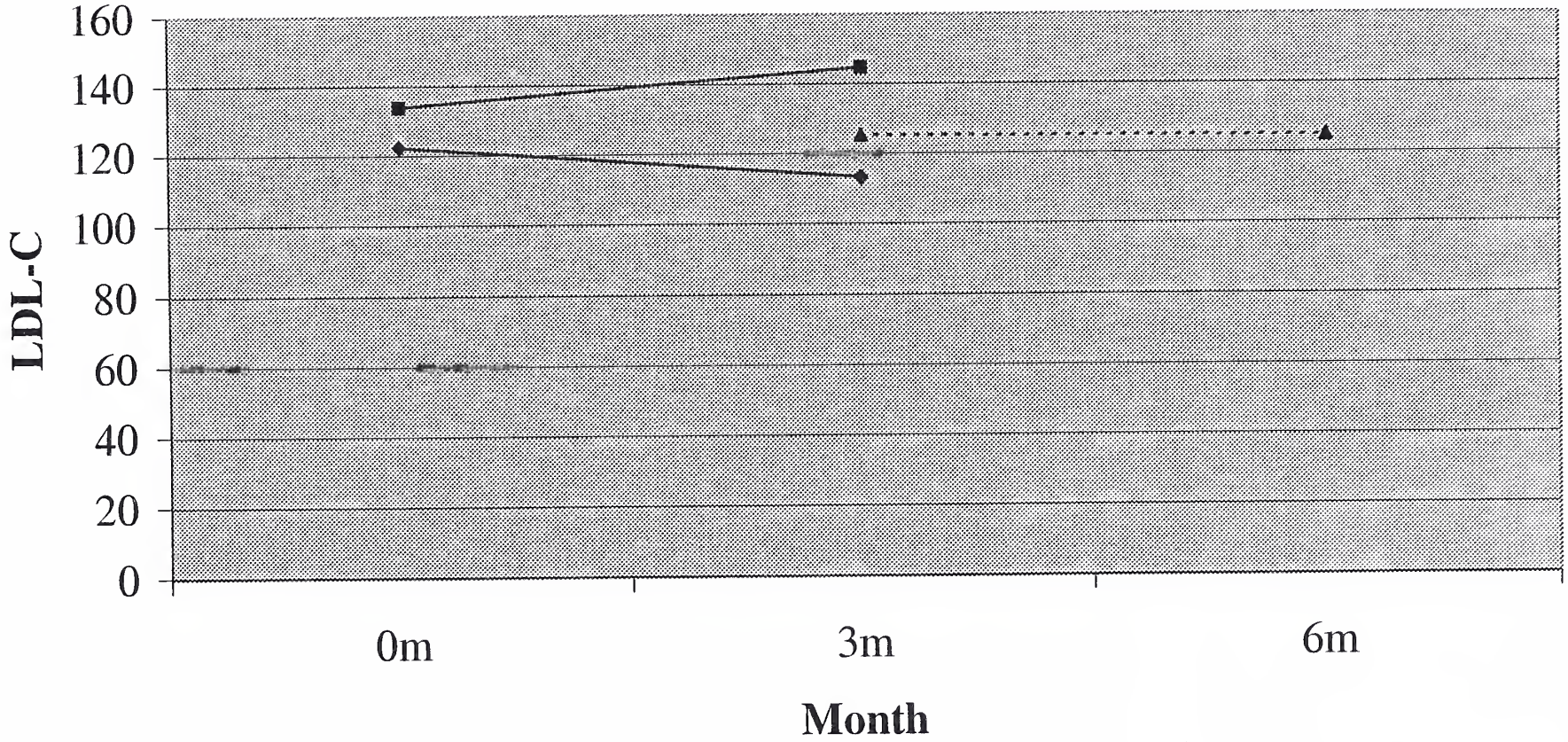
Lipid Parameter	**Post-Washout Value (Month 0)**	**Post-Combination Therapy Value (Month 6)**	**Percent Change**	**P Value**
HDL (mg/dL)	40.4±9.1	45.3±12.8	**+12.0%**	**.0044**
LDL (mg/dL)	127.0±30.0	125.0±27.7	-1.6%	.66
Fasting TG (mg/dL)	219.7±145.3	160.5±117.6	**-27.0%**	**.0083**
Postprandial TG (mg/dL)	265.2±117.1	225.2±122.6	**-15.1%**	**.0059**
Fasting FFA (nmol/L)	.94±.35	.78±.31	**-17.3%**	**.0017**
Postprandial FFA (nmol/L)	.38±.21	.18±.12	**-53.0%**	**<0.0001**

FIGURE 1: EFFECTS OF METFORMIN AND TROGLITAZONE, ALONE AND IN COMBINATION, ON HDL-C



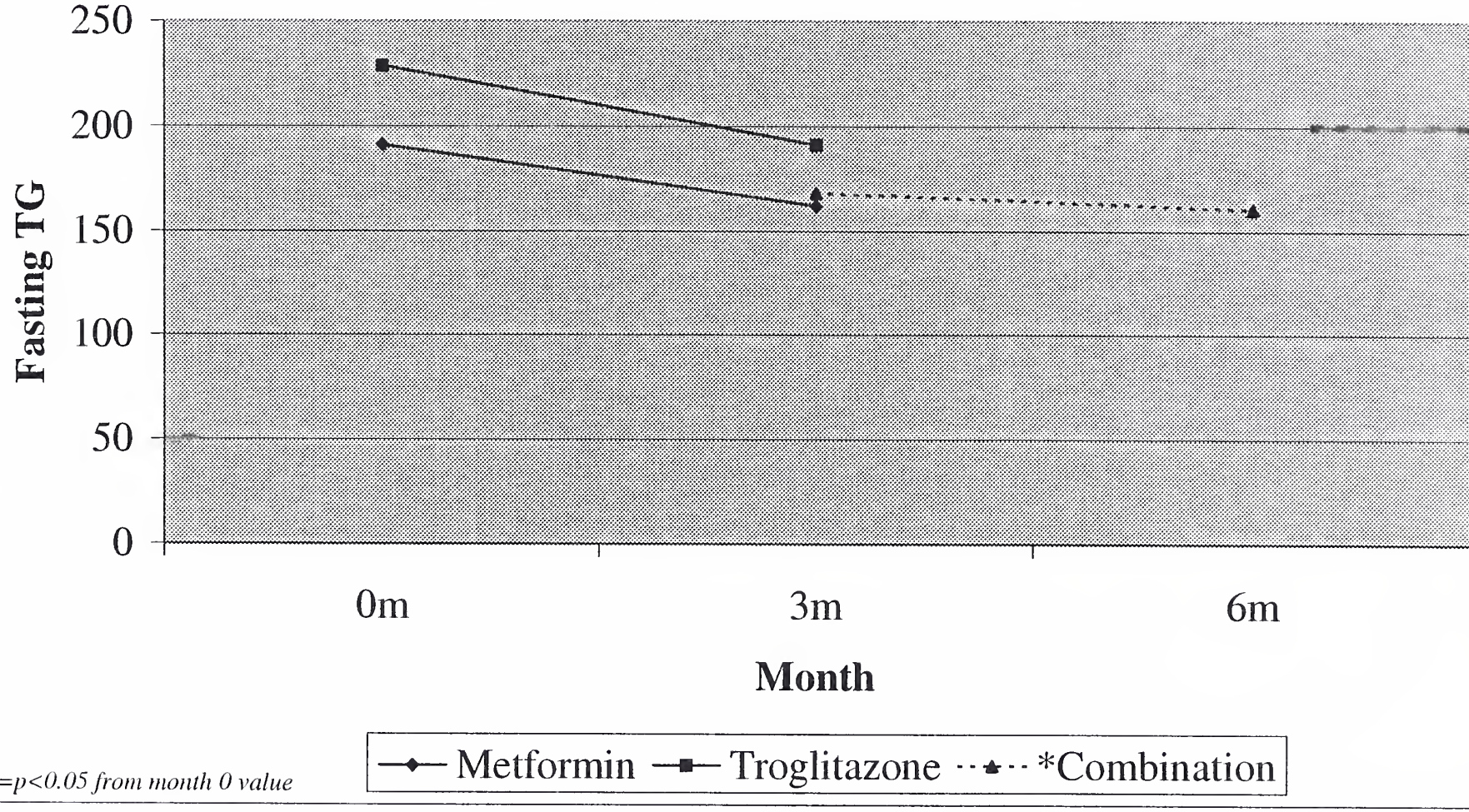
*=p<0.05 from month 0 value

FIGURE 2: EFFECTS OF METFORMIN AND TROLGITAZONE, ALONE AND IN COMBINATION, ON LDL-C



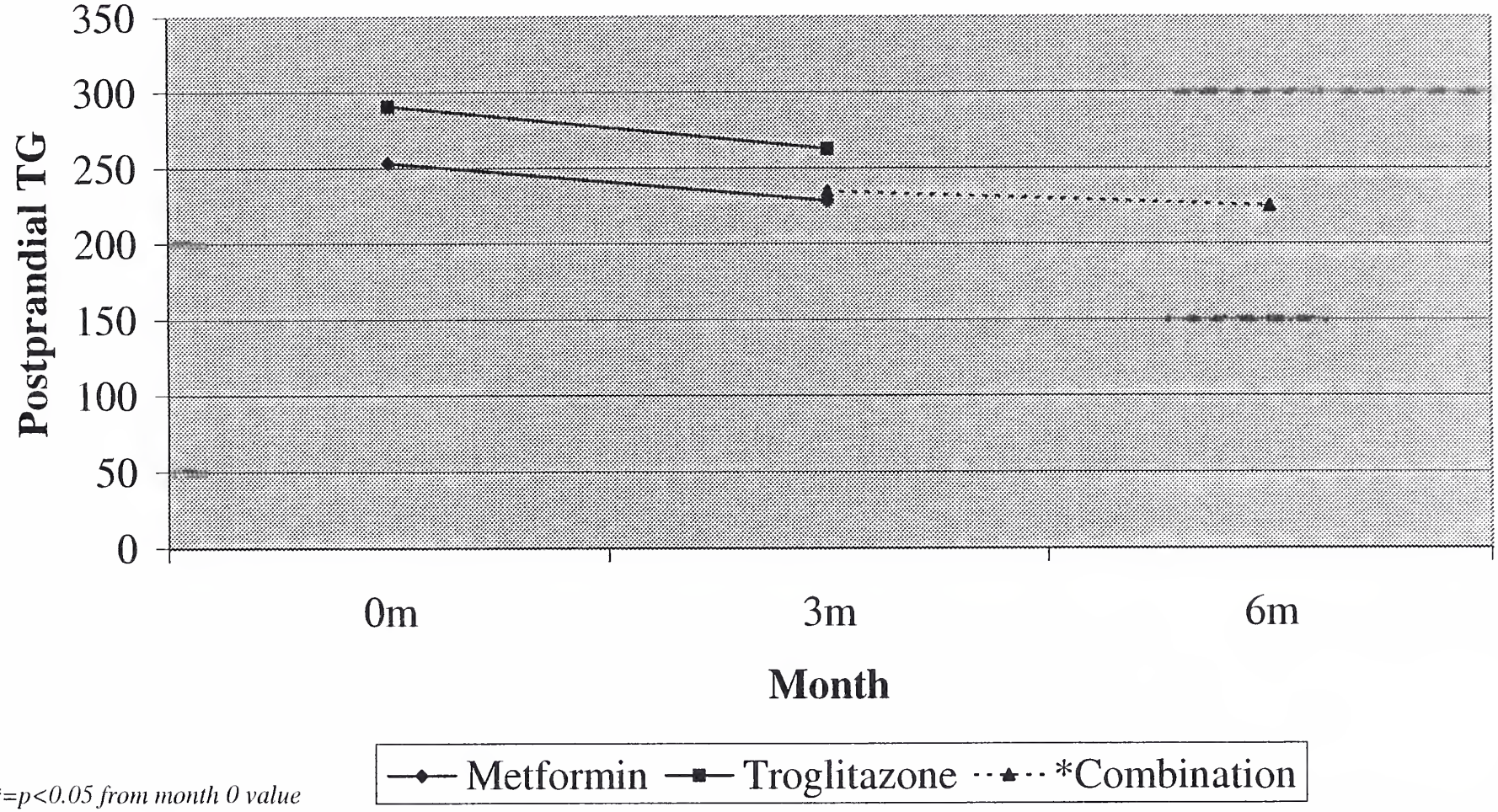
*=p<0.05 from month 0 value

FIGURE 3: EFFECTS OF METFORMIN AND TROGLITAZONE, ALONE AND IN COMBINATION, ON FASTING TG



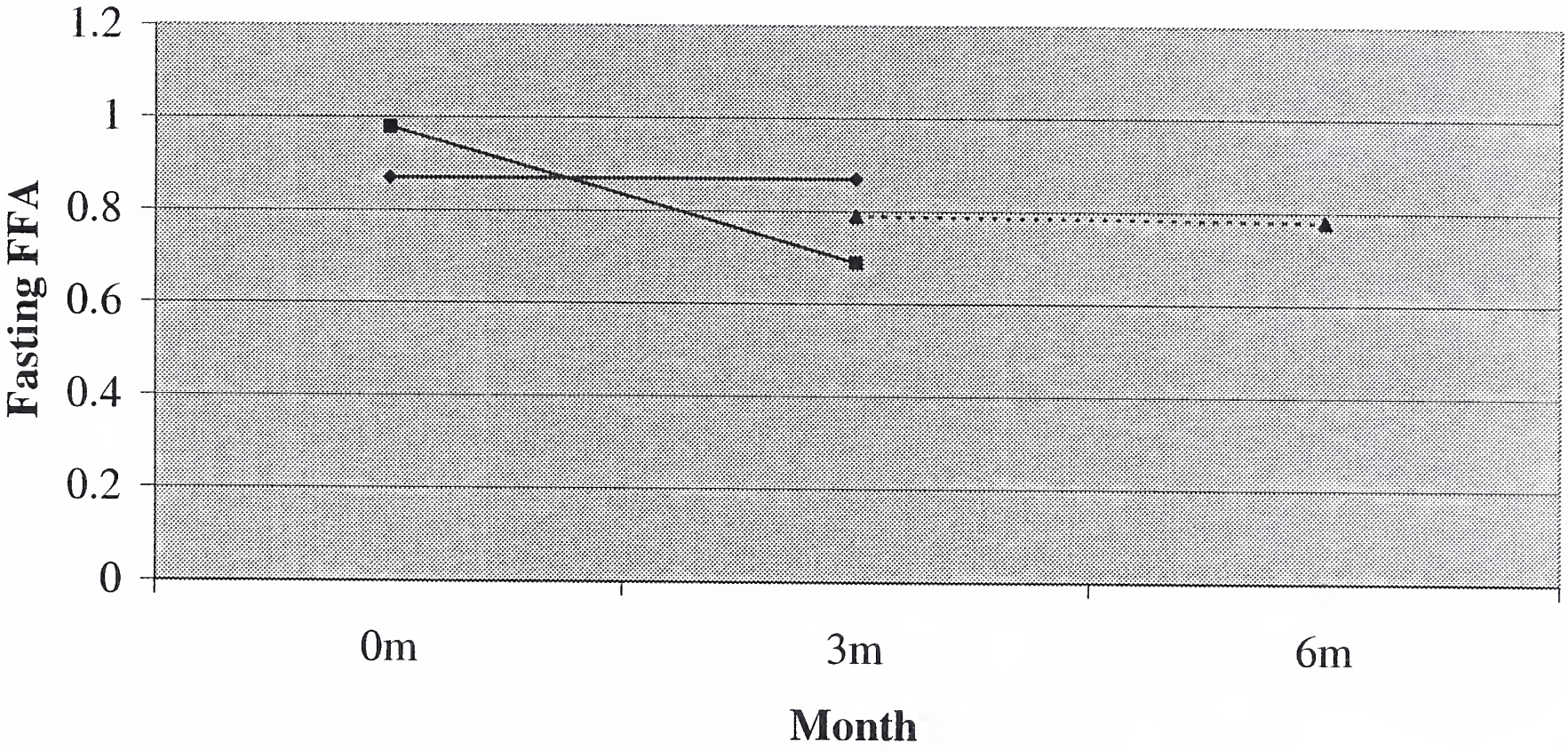
*=p<0.05 from month 0 value

FIGURE 4: EFFECTS OF METFORMIN AND TROGLITAZONE, ALONE AND IN COMBINATION, ON POSTPRANDIAL TG



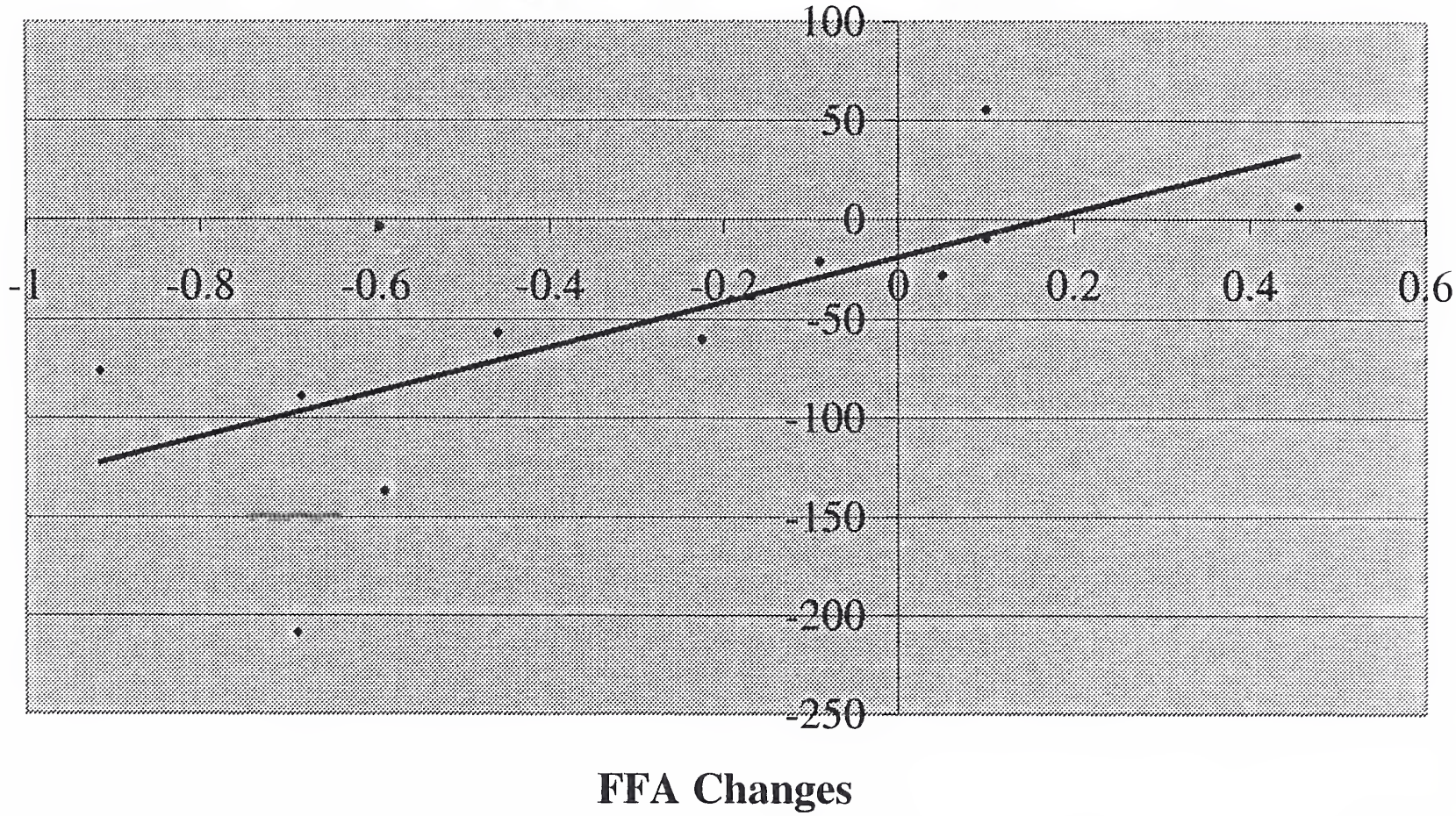
*=p<0.05 from month 0 value

FIGURE 5: EFFECTS OF METFORMIN AND TROGLITAZONE, ALONE AND IN COMBINATION, ON FASTING FFA



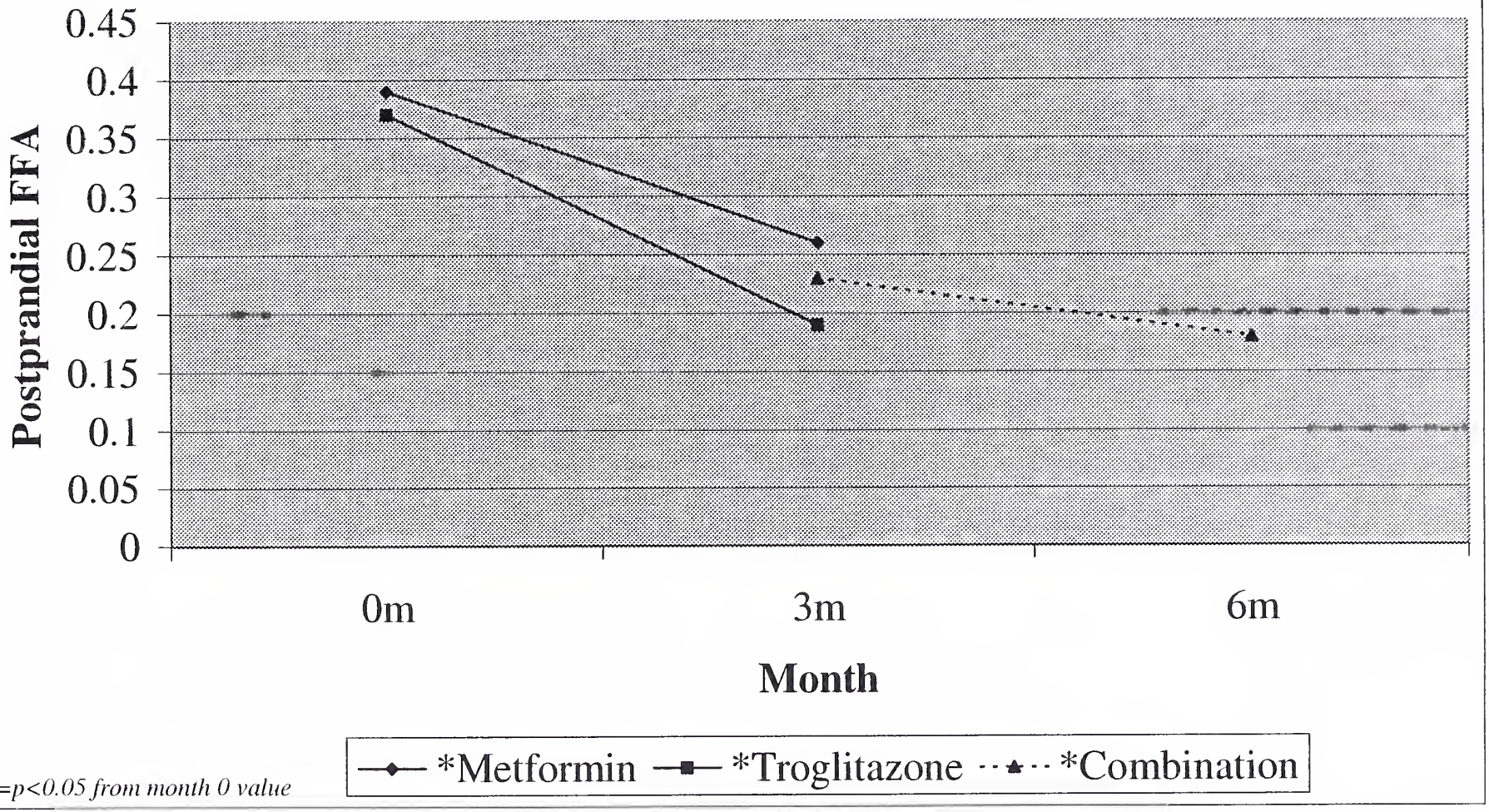
*=p<0.05 from month 0 value

FIGURE 6: CORRELATION PLOT OF GLUCOSE CHANGES VS. FASTING FFA CHANGES IN THE TROGLITAZONE MONOTHERAPY GROUP



R=.68; p=.016

FIGURE 7: EFFECTS OF METFORMIN AND TROGLITAZONE, ALONE AND IN COMBINATION, ON POSTPRANDIAL FFA



*=p<0.05 from month 0 value

DISCUSSION

Our study demonstrates that metformin and troglitazone had similar insignificant effects on HDL-C. The marginal effect on HDL-C seen with metformin is consistent with the literature. However, past studies have demonstrated that troglitazone can appreciably increase HDL-C levels in patients with T2DM (45, 46, 51, 55). The reason for the discrepancy between our findings and the existing literature is unclear. A plausible explanation may be an insufficient sample size of the troglitazone monotherapy group. In fact, not only did HDL-C concentrations rise significantly with combination therapy, but also, the addition of troglitazone to the metformin monotherapy group appreciably increased HDL-C levels. This suggests that the greater sample size found in the combination group may have allowed the effect of troglitazone on HDL-C elevation to become statistically evident. The significant effect seen with combination therapy also raises the possibility that simultaneous metformin and troglitazone administration may have some additive beneficial effects on CVD risk.

The divergent effects of metformin (55, 59, 60) and troglitazone (71, 74, 75, 78) on LDL-C are consistent with past studies. Metformin demonstrated significant LDL-C lowering effect. Its effects were significantly better than troglitazone, which marginally elevated LDL-C instead. Additionally, metformin and troglitazone administered simultaneously overall had a neutral effect on LDL-C. Although LDL-C lowering seen with metformin are noteworthy and may play a beneficial role in cardiovascular risk reduction as was implied by the UKPDS (8), the implications of LDL-C elevation with troglitazone are less clear. The LDL-C increase may be secondary to LDL-C particle size. As was previously mentioned, patients treated with troglitazone are noted to have larger, more buoyant LDL-C particles, which may be less susceptible to oxidation, and therefore, less atherogenic (77, 78, 81). Because calculated LDL-C is affected by both the number and size of the particles, it is difficult to ascertain in this study, which component was actually responsible for the elevation in the troglitazone treated group. Future studies which measure apoprotein B levels in conjunction with LDL-C levels or directly

measure LDL particle size (through ultracentrifugation techniques) will be helpful for clarification.

Metformin and troglitazone had minimal effects on fasting and postprandial TG when used alone, but significantly decreased these parameters when used in combination. The monotherapy results contradicted existing literature. Most studies demonstrate that both anti-hyperglycemics lower overall TG levels, with troglitazone faring somewhat better (46, 47, 49, 50, 51, 52, 53, 54, 55, 63, 72, 73, 76, 77). TG reduction seen with metformin treatment is attributed to decreased hepatic VLDL-C synthesis. Troglitazone, however, is believed to decrease the precursors for TG production through insulin sensitization and/or direct anti-lipolytic effects on adipocytes. The discrepancy between our monotherapy results and the existing literature may again, be attributable to the sample size. The ability of combination therapy to significantly reduce fasting and to a lesser magnitude, postprandial TG, is possibly secondary to the larger sample size or may again be due to an additive effect achieved with combination therapy.

Last, this study demonstrated the effects of metformin and troglitazone on fasting and postprandial FFA. Troglitazone had significant effects on fasting FFA. Not only did it decrease FFA to a significantly greater extent than metformin, but also, the addition of troglitazone to metformin monotherapy significantly lowered fasting FFA in this subgroup of patients. Combination therapy seemed to offset the degree of reduction seen with troglitazone alone. Meanwhile, both metformin and troglitazone significantly decreased postprandial FFA when used alone and in combination, postprandial FFA were decreased to a slightly greater extent. These results are similar to existing studies. Metformin has yielded inconsistent FFA results (35, 45, 46) whereas troglitazone has been noted to consistently decrease overall FFA (72, 73). Troglitazone works through the nuclear receptor, PPAR, which is predominantly found in adipose tissue (69, 70). Therefore, its insulin sensitizing properties may work directly through fat cells by decreasing lipolysis, and, therefore, FFA release. Indeed, there was a greater FFA percentage change found in the postprandial state, when insulin is more abundant, than in the fasting state.

The decrease of fasting FFA in the troglitazone group was the sole parameter found to correlate with glucose lowering. The reason is unclear. Perhaps a decrease in glucotoxicity facilitated improved FFA metabolism or vice versa. Theoretically, a relationship does exist between FFA and glucose through the Randle cycle. There is speculation that medications which decrease FFA improve insulin sensitivity, which then facilitates glucose disposal and oxidation in muscle and fat, and decreases hepatic glucose production in liver. There was, however, no significant correlations found between fasting FFA and glucose production and/or glucose disposal.

This study, which directly compared the effects on lipid metabolism of metformin and troglitazone, when used alone and in combination, had limitations. Data was derived from an original prospective study that was not designed to analyze changes in lipid metabolism, but rather, changes in glucose and HbA1c. Therefore, the small sample size may not be powered to identify all of the significant changes in the lipid parameters. In addition, the lipid profiles of patients may have been affected by factors other than the oral anti-hyperglycemic medications. Several patients were on anti-lipid medications. However, since these patients had been taking anti-lipid medications prior to the study and then continued on a fixed constant dosage throughout the study, it was not expected to be a significant confounder. Second, a diet comprised of 50% carbohydrate, 34% fat, and 16% protein was recommended to all participants to maintain baseline body weight. Although diet does beneficially affect lipid metabolism, all participants were encouraged to follow the same diet. In addition, all values were obtained at least 2 weeks after diet recommendations were made. This 2 week washout period should have provided sufficient time for changes attributable to diet alone to become evident.

Although a small pool of patients was analyzed, there were several significant findings. This study demonstrated that metformin and troglitazone may have comparable anti-hyperglycemic effects (64), but have differing effects on lipid metabolism. Metformin, which primarily decreases hepatic glucose production, was found to significantly alter LDL-C and

postprandial FFA, whereas troglitazone, an insulin sensitizer with effects primarily on adipose tissue, appreciably decreased fasting and postprandial FFA. When used in combination, metformin and troglitazone demonstrated additive effects, most notably on HDL-C and fasting and postprandial TG, and to a much lesser degree, on postprandial FFA.

The beneficial effects on diabetic dyslipidemia demonstrated by metformin and troglitazone, both alone and in combination, are intriguing. These effects may indeed play a crucial role in reducing the risk for CVD, which is the major cause of mortality in T2DM patients. Additionally, the beneficial effects of troglitazone and possibly other TZDs on FFA (ie. improved lipotoxicity) may not only reduce macrovascular complications, but also improve β cell function. Further larger scale prospective studies are needed to better understand the long-term clinical implications of these effects.

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