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ORIGINAL ARTICLE

Metabolic effects of muraglitazar in type 2 diabetic subjects

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Aim: To assess the effect of muraglitazar, a dual peroxisome proliferator-activated receptor (PPAR)γ-α agonist, versus placebo on metabolic parameters and body composition in subjects with type 2 diabetes mellitus (T2DM).

Methods: Twenty-seven T2DM subjects received oral glucose tolerance test (OGTT), euglycaemic insulin clamp with deuterated glucose, measurement of total body fat (DEXA), quantitation of muscle/liver (MRS) and abdominal subcutaneous and visceral (MRI) fat, and then were randomized to receive, in addition to diet, muraglitazar (MURA), 5 mg/day, or placebo (PLAC) for 4 months.

Results: $HbA1c_c$ decreased similarly (2.1%) during both MURA and PLAC treatments despite significant weight gain with MURA (+2.5 kg) and weight loss with PLAC (−0.7 kg). Plasma triglyceride, LDL cholesterol, free fatty acid (FFA), hsCRP levels all decreased with MURA while plasma adiponectin and HDL cholesterol increased ($p < 0.05$–0.001). Total body (muscle), hepatic and adipose tissue sensitivity to insulin and β cell function all improved with MURA ($p < 0.05$–0.01). Intramyocellular, hepatic and abdominal visceral fat content decreased, while total body and subcutaneous abdominal fat increased with MURA ($p < 0.05$–0.01).

Conclusions: Muraglitazar (i) improves glycaemic control by enhancing insulin sensitivity and β cell function in T2DM subjects, (ii) improves multiple cardiovascular risk factors, (iii) reduces muscle, visceral and hepatic fat content in T2DM subjects. Despite similar reduction in A1c with PLAC/diet, insulin sensitivity and β cell function did not improve significantly.

Keywords: insulin secretion, insulin sensitivity, muraglitazar, muscle/liver/adipocyte, PPARα, PPARγ, thiazolidinediones

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Introduction

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder characterized by insulin resistance in muscle and liver and impaired insulin secretion [1–4]. The insulin resistance is manifested early in the natural history of T2DM, but glucose tolerance remains normal because of a compensatory increase in insulin secretion [1–7]. With time, β cell failure ensues and overt diabetes becomes manifest [1–4]. Multiple intracellular defects, including decreased insulin signal transduction, impaired glucose transport/phosphorylation, diminished glucose oxidation and reduced glycogen synthesis, all contribute to the insulin resistance [1,2,8–10]. Type 2 diabetic individuals also manifest adipocyte resistance to the antilipolytic effect of insulin [11–13] and the resultant increase in plasma free fatty acid (FFA) concentration impairs insulin secretion [14,15] and exacerbates insulin resistance in liver and muscle [16–18]. Fat cells in T2DM also release a number of insulin-resistance-provoking and atherogenic adipocytokines and fail to secrete normal amounts of insulin-sensitizing adipocytokines, such as adiponectin [12,19]. Elevated plasma FFA levels, in combination with excessive caloric intake, lead to the intracellular accumulation of toxic lipid metabolites (FACoA, diacyglycerol, ceramides), which can exacerbate the insulin resistance by impairing insulin signal transduction [20–22]. In addition to intracellular fat accumulation in muscle [23,24] and liver [25,26], type 2 diabetic subjects are characterized by visceral adiposity, which has been linked to insulin resistance [27,28], accelerated atherosclerotic cardiovascular disease (ASCVD) and an atherogenic plasma lipid profile [29].

Thiazolidinediones are insulin-sensitizing oral agents that initiate their effects by binding to PPAR receptors [30,31]. There are three PPAR receptors: γ, α and δ. PPARγ receptors primarily are located on fat cells and vascular tissue/macrophages [32], but they are also present in muscle in low abundance and in β cells [33]. PPARγ agonists enhance insulin sensitivity in muscle and liver [34–36], improve β cell function [37,38], and sensitize the adipocyte to the antilipolytic effect of insulin [34]. PPARγ agonists also reduce liver [26,27] and intramyocellular [39] fat and cause a redistribution of abdominal fat from visceral to subcutaneous depots [25,40]. PPARα receptors primarily are located in the liver [41]. In contrast to PPARγ agonists, PPARα agonists, such as fenofibrate, when given to man, have a potent effect to reduce plasma triglycerides, but they do not lower plasma FFA, augment muscle or liver insulin sensitivity, or redistribute

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fat within the body [42]. Because of these different metabolic effects of PPARγ and PPARα agonists on insulin sensitivity, fat distribution and plasma lipid levels, new drug development has focussed on dual PPARs which possess PPARγ, as well as PPARα activity. Currently, no prior study has evaluated the effect of any dual PPAR agonist on glucose and lipid metabolism or fat distribution in man. In the present study we examined the effect of muraglitazar, a dual PPARγ/PPARα agonist, on tissue (muscle, liver, adipose) sensitivity to insulin, β cell function, fat topography and lipid metabolism in type 2 diabetic patients.

Methods

Subjects

Twenty-seven patients with T2DM and with no evidence of microvascular complications were recruited from the outpatient clinic of the Texas Diabetes Institute. The clinical, anthropometric and laboratory characteristics in the muraglitazar and placebo-treated groups prior to initiation and after 4 months of therapy are summarized in Table 1. All patients were in good general health without evidence of cardiac, hepatic, renal or other chronic diseases, as determined by medical history, physical examination, and screening blood tests. All subjects had stable body weight for at least 3 months before study, and none participated in any exercise program on a regular basis. No subject ever had taken insulin or a thiazolidinedione. Other antidiabetic medications were stable for at least 6 months and included metformin alone (n = 5), sulfonylureas alone (n = 1) and metformin plus sulfonylureas alone (n = 4); 17 diabetic subjects were drug naive. In addition, two subjects were taking a statin, three were on an angiotensin converting enzyme inhibitor and one was taking a calcium channel antagonist. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and all subjects gave their written voluntary, informed consent before participation.

Experimental Protocol

The study design was double-blind, placebo-controlled with parallel muraglitazar and placebo arms with 3 : 1 randomization (muraglitazar : placebo). A third arm with pioglitazone was not included because the metabolic effects of this thiazolidinedione have been extensively studied. Subjects who were taking metformin or sulfonylurea had the medication discontinued 6 weeks before study, and fasting plasma lipids, glucose and HbA1c were measured every 2 weeks during the run-in period. The fasting plasma glucose (FPG) concentration differed by less than 10 mg/dl between weeks 4 and 6 in the nine subjects in whom the sulfonylurea or metformin was discontinued. During the week before randomization, subjects received: (i) 2-h 75 g oral glucose tolerance test (OGTT); (ii) quantitation of muscle [39] and liver [26,42] fat content by magnetic resonance spectroscopy (MRS) and abdominal visceral and subcutaneous fat content by MRI [25,27,42]; (iii) quantitation of total body fat mass (FM) and fat free mass (FFM) by dual energy x-ray absorption (DEXA); (iv) measurement of hepatic and peripheral tissue (muscle) insulin sensitivity with the euglycaemic insulin clamp [43] performed in combination with 6-6,^{2}H-glucose. Studies were carried out in the postabsorptive state at 08:00 h after a 10–12-h

Table 1. Baseline clinical, anthropometric, and laboratory characteristics of subjects with type 2 diabetes prior to randomization and following therapy with either muraglitazar or placebo.

	Muraglitazar		Placebo		
	Pre	Post	Pre	Post	p Value
Number	20		7		
Ethnicity (MA/C)	(17/3)		(6/1)		NS
Gender (F/M)	(13/7)		(4/3)		NS
Diabetes duration (years)	3.7 ± 2.2		3.1 ± 2.5		NS
Age (years)	50 ± 2		54 ± 3		NS
BMI (kg/m^2)	33.0 ± 0.7	34.0 ± 0.6*	29.4 ± 1.4	28.7 ± 1.3*	<0.001
Body weight (kg)	84.3 ± 2.0	86.8 ± 1.9*	75.1 ± 4.0	73.2 ± 3.6*	<0.0005
Fat mass (kg)	32.1 ± 1.5	33.8 ± 1.5*	26.4 ± 2.2	24.6 ± 2.6*	<0.0004
HbA1c (%)	8.5 ± 0.4	6.6 ± 0.3*	9.3 ± 0.7	7.2 ± 0.6*	NS
Visceral fat (kg)	0.73 ± 0.06	0.62 ± 0.04*	0.77 ± 0.11	0.71 ± 0.11	NS
Subcutaneous fat (kg)	1.79 ± 0.14	1.99 ± 0.15*	1.57 ± 0.40	1.48 ± 0.38*	<0.005
Liver fat (%)	15 ± 2	6 ± 1*	11 ± 4	9 ± 4	NS
Intramuscular fat (AU)	0.42 ± 0.07	0.21 ± 0.05*	0.32 ± 0.18	0.12 ± 0.04	NS
Triglycerides (mg/dl)	152 ± 12	119 ± 18*	157 ± 28	143 ± 16	<0.05
LDL cholesterol (mg/dl)	103 ± 5	89 ± 7*	123 ± 11	116 ± 15	NS
HDL cholesterol (mg/dl)	41 ± 3	45 ± 4*	47 ± 6	38 ± 5	<0.01
VLDL particle size (nm)	50.2 ± 1.5	52.0 ± 1.5	38.8 ± 11.4	41.5 ± 6.8	NS
LDL particle size (nm)	20.1 ± 0.2	21.2 ± 0.2*	20.5 ± 0.2	20.4 ± 0.5	<0.03
HDL particle size (nm)	8.6 ± 0.1	8.7 ± 0.1	8.5 ± 0.1	8.4 ± 0.1	NS

p < 0.05–0.01 versus baseline; p value refers to comparison of muraglitazar versus placebo; NS, non-significant.
*p < 0.05 vs. baseline.

overnight fast. Following completion of these studies, subjects participated in a 10-h ADA-approved diabetes education course and received one-on-one dietary education for 2 h with a dietitian. Subjects were instructed to consume a weight-maintaining diet containing 50% carbohydrate, 30% fat and 20% protein. Following completion of the diabetes education program, subjects were randomized to receive 16 weeks of muraglitazar, 5 mg/day (n = 20), or placebo (n = 7) at breakfast for 4 months. After the start of treatment, subjects returned to the Clinical Research Center (CRC) monthly and fasting plasma glucose (FPG), lipids, HbA1c and blood pressure were measured and body weight was recorded. Dietary advice was reinforced by a dietician on each visit. During the last week of the double-blind period, the OGTT, euglycaemic insulin clamp, MRS, MRI and DEXA measurements were repeated.

Oral Glucose Tolerance Test

Subjects were asked to refrain from eating or drinking anything except water after 20:00 h on the evening prior to study. At 07:30 h, subjects reported to the CRC and a catheter was placed in an antecubital vein for all blood withdrawal. At −30, −15 and 0 min, baseline blood samples were collected for the measurement of FPG, FFA, C-peptide, insulin, lipid, TNFα, leptin, adiponectin and hsCRP concentrations. At time zero, subjects ingested 75 g of flavoured glucose solution. Blood for plasma glucose, FFA, insulin and C-peptide determinations was obtained every 15 min after glucose ingestion for 120 min. One week later, subjects returned to the CRC for a euglycaemic hyperinsulinemic clamp study [43] which was performed with deuterated glucose infusion [44,45]. Total body fat content was determined using a DEXA whole body scanner (Hologic, Bedford, MA, USA). Hepatic, muscle, visceral and subcutaneous abdominal fat contents were determined by MRS/imaging (MRI) within 3–5 days after the insulin clamp, as previously described [25,26,39,42].

Euglycaemic Hyperinsulinemic Clamp

Insulin sensitivity was assessed with a 3-h euglycaemic insulin clamp [11,43]. At 08:00 h (−180 min), a primed [(28 µmol/kg) − (fasting glycaemia/5 µmol/l)]-continuous (0.28 µmol/min/kg) infusion of [6,6-$^2H_2$]-glucose was started via a catheter placed into an antecubital vein and continued throughout the study. A second catheter was inserted retrogradely into a vein on the dorsum of hand, which was placed in a heated box (60°C). Baseline arterialized blood for determination of plasma [6,6-$^2H_2$]-glucose enrichment and plasma glucose/insulin/FFA concentrations was drawn at −30, −20, −10, −5 and 0 min. At time zero, a prime-continuous (60 mU/min·m^2) infusion of regular insulin (Novolin, Novo Nordisk, Princeton, NJ, USA) was started and continued for 180 min. Plasma glucose was allowed to drop to 100 mg/dl, at which level it was maintained by adjusting a 20% dextrose infusion. To minimize the changes in plasma [6,6-$^2H_2$]-glucose enrichment, 2 g of tracer were added to 500 ml of the 20% glucose solution while the constant [6,6-$^2H_2$]-glucose infusion was turned down to 20% of baseline value, i.e., to 0.056 µmol/kg min, in a stepwise fashion (by 20% every 20 min). Throughout the insulin clamp, blood was drawn every 5 min for plasma glucose determination, and every 15 min for plasma insulin and FFA concentrations and [6,6-$^2H_2$]-glucose enrichment.

Blood Analyses

Plasma glucose was measured using the glucose oxidase method (Glucose Analyzer, Beckman Instruments, Fullerton, CA, USA), plasma insulin (Diagnostic Products, Los Angeles, CA, USA) and C-peptide (Diagnostic Products) by radioimmunoassay, HbA1c by affinity chromatography (Biochemical Methodology, Drower 4350; Isolab, Akron, OH, USA) and plasma FFA by enzymatic colorimetric quantification (Wako Chemicals, Neuss, Germany). Plasma total cholesterol and triglyceride were measured enzymatically (Boehringer-Mannheim, Indianapolis, IN, USA) on Hitachi 704 autoanalyzer. Plasma HDL cholesterol was measured enzymatically on Hitachi 704 autoanalyzer after precipitation of chyomicron, and VLDL and LDL cholesterol by phosphotungstic acid. LDL cholesterol was calculated from the Friedwald equation. Plasma TNFα concentration was determined with sandwich ELISA kit (detection limit = 0.5 pg/ml; intra-assay and inter-assay coefficient of variation = 5.7 and 7.5%, respectively; Quantikine, R&D Systems, Minneapolis, MN, USA) and plasma adiponectin by radioimmunoassay (Linco Research, St Charles, MO, USA). Interleukin (IL)-6 and intracellular adhesion molecule (ICAM) were measured by ELISA (R&D Systems). High sensitive c-reactive protein (hsCRP) was measured by ELISA (ALPCO Diagnostics, Salem, NH, USA).

[6,6-$^2H_2$]-glucose enrichment was determined in plasma according to the validated gas chromatography mass spectrometry method of Wolfe [44] with modified and validated gas chromatography-mass spectrometry (GC-MS) conditions.

Body Composition

To determine overall FM and FFM, DEXA (Hologic) was performed using software version 1.3Z.

Localized proton nuclear magnetic resonance (NMR) spectra of tibialis anterior muscle [39] and liver [26,42] were acquired on a 1.9-T MRI scanner (Elscint Prestige, Elscint, Haifa, Israel) as previously described. Visceral subcutaneous adipose volumes were analysed by MRI on a 1.9-T Elscint Prestige MRI system as previously described [25,27,46].

Calculations

Tracer infusion rate (IR) during baseline was calculated by multiplying the tracer concentration (determined by the glucose oxidase method) by the pump rate and dividing by the body weight:

$$IR_{baseline}\ (\mu mol/min\ kg) = [\text{tracer conc}\ (\mu mol/ml) \times \text{pump rate}\ (ml/min)] \div \text{body weight}\ (kg). \quad (1)$$

Tracer IR during the clamp was calculated by multiplying the tracer concentration by pump rate divided by body weight

and adding the tracer infused with the exogenous glucose [calculated as % tracer in the glucose infusate (GINF) × glucose infusion rate (GIR)].

$$\begin{aligned} IR_{Clamp}\ (\mu mol/min\ kg) = {} & [\text{tracer conc } (\mu mol/ml) \times \\ & \text{pump rate } (ml/min)] \div \\ & \text{body weight } (kg) + \\ & TTR_{GINF}/(1 + TTR_{GINF}) \times \\ & GIR\ (\mu mol/min\ kg). \end{aligned} \quad (2)$$

Calculation of Glucose Production and Disposal

During the last 20 min of the basal tracer equilibration period, plasma glucose concentration and [6,6-$^2H_2$]-glucose enrichment were stable (<5%) in all subjects. Therefore, total endogenous glucose production (EGP) was calculated as the ratio of the [6,6-$^2H_2$] GIR to the plasma tracer enrichment (tracer-to-tracee ratio, $TTR_{6,6}$; mean of 3 determinations). During the baseline state, EGP = rate of glucose appearance (Ra) = rate of glucose disappearance (Rd):

$$EGP_{baseline} = \frac{IR_{baseline}}{TTR_{baseline}} \quad (3)$$

During the euglycaemic insulin clamp, the TTR was stable (<5%) and the glucose rate of appearance (Ra) was calculated using the steady-state equation:

$$RA_{Clamp} = \frac{IR_{Clamp}}{TTR_{Clamp}} \quad (4)$$

where TTR represents the mean value of samples obtained during the last 30 min of the insulin clamp and IR was calculated as described in the previous paragraph.

EGP during the insulin clamp was calculated by subtracting the GIR (corrected by the tracer amount) from Ra as:

$$EGP_{Clamp} = RA_{Clamp} - GIR_{Clamp} \times \left(1 - \frac{TTR_{GINF}}{1 + TTR_{GINF}}\right) \quad (5)$$

The coefficient of variation of the TTR during the last 30 min of the clamp was less than 5%, allowing the use of the steady-state equation.

Oral Glucose Tolerance Test

Areas under the curve for plasma glucose, insulin, C-peptide and FFA concentrations during the OGTT were determined using the trapezoidal rule. The mean plasma glucose, insulin, C-peptide and FFA concentrations during the OGTT were calculated by dividing the area under the curve by 120 min. The insulin secretion/insulin resistance (disposition) index during the OGTT [6] was calculated as the incremental plasma insulin response ($\Delta I_{0-120\ min}$) divided by the incremental plasma glucose response ($\Delta G_{0-120\ min}$) factored by the severity of insulin resistance, where IR = steady state plasma insulin (SSPI)/TGD during the insulin clamp and TGD = insulin-stimulated total body glucose disposal rate during the euglycaemic insulin clamp. The insulin secretory rate during the OGTT was calculated by deconvolution to the plasma C-peptide concentration [46,47].

Statistics

Statistical analyses were performed with StatView for Windows (SAS Institute, Cary, NC, USA). All values before and after treatment within each group were analysed using paired Student's *t*-test. Comparison between groups was performed using analysis of variance with Bonferroni/Dunn *post hoc* testing. Pearson's correlations between continuous variables were used as a measure of association. Stepwise multiple linear regression analysis was performed to examine multiple correlations among variables. Data are presented as mean ±s.e. p Value < 0.05 was considered statistically significant.

Results

FPG, Lipids, HbA1c and Anthropometric Measurements

All subjects tolerated the diet and medications well and there were no significant adverse events, including peripheral oedema. In the group that received muraglitazar the mean body weight increased from 84.3 ± 2.0 to 86.8 ± 1.9 kg and the body mass index (BMI) from 33.0 ± 0.7 to 34.0 ± 0.6 kg/m^2, whereas in the placebo-treated group the body weight decreased from 75.1 ± 4.0 to 73.2 ± 3.6 kg and the BMI decreased from 29.4 ± 1.4 to 28.7 ± 1.3 kg/m^2 (all $p < 0.01–0.001$ vs. baseline and vs. each other). In the muraglitazar group the FPG decreased from 183 ± 9 to 129 ± 6 mg/dl ($p < 0.001$ vs. baseline) and in the placebo group from 190 ± 15 to 160 ± 20 mg/dl ($p < 0.05$) (p = NS between groups). HbA1c decreased significantly ($p < 0.001$) in both groups from 8.5 ± 0.4 to 6.6 ± 0.3% in the muraglitazar group ($p < 0.001$) and from 9.3 ± 0.7 to 7.2 ± 0.6% in the placebo group ($p < 0.001$) (p = NS, muraglitazar vs. placebo). In the muraglitazar group, plasma triglycerides decreased from 152 ± 12 to 119 ± 18 mg/dl and HDL cholesterol increased from 41 ± 3 to 45 ± 4 mg/dl (both $p < 0.05$). LDL cholesterol decreased from 103 ± 5 to 89 ± 7 mg/dl, but this did not reach significance. LDL particle size increased mildly in the muraglitazar group (20.1 ± 0.2 to 21.2 ± 0.2, $p < 0.0001$) and did not change in the placebo group (20.5 ± 0.2 to 20.4 ± 0.5 nm, $p < 0.03$ muraglitazar vs. placebo). VLDL and HDL particle size did not change significantly in either the muraglitazar or placebo groups. After 4 months of muraglitazar treatment, there was a modest but non-significant decrease in both aspartate transaminase (AST) (31 ± 4 to 21 ± 2, $p < 0.02$) and alanine transaminase (ALT) (30 ± 3 to 18 ± 2 IU, $p < 0.002$). In the placebo groups both AST (29 ± 3 to 24 ± 3, p = NS) and ALT (32 ± 5 to 20 ± 3, $p < 0.04$) decreased or tended to decrease. Blood pressure did not change in either the muraglitazar (122 ± 4/71 ± 2 to 122 ± 4/68 ± 3 mmHg) or placebo (118 ± 3/70 ± 4 to 122 ± 3/70 ± 4 mmHg) group.

Oral Glucose Tolerance Test

During the OGTT, the mean plasma glucose decreased from 288 ± 11 to 211 ± 9 mg/dl in the muraglitazar group ($p < 0.0001$), and a similar decline from 303 ± 16 to 262 ± 21 mg/dl was observed in the placebo group (p = NS , muraglitazar vs. placebo). During the OGTT performed after 4 months, the increment in plasma glucose concentration above baseline was significantly reduced in the muraglitazar (105 ± 7 to

82 ± 6 mg/dl, $p < 0.03$) but not in the placebo-treated groups (113 ± 9 to 102 ± 9 mg/dl, p = NS). The fasting plasma insulin concentration fell significantly by ~4 μU/ml in the muraglitazar group and tended to decrease in the placebo group (p = NS , muraglitazar vs. placebo). The mean plasma insulin concentration (0–120 min) during the OGTT did not change significantly in the muraglitazar (41.3 ± 6.6 vs. 35.5 ± 4.5 μU/ml) or placebo (26.2 ± 8.7 vs. 34.5 ± 13.5 μU/ml) groups. The mean plasma C-peptide concentration during the OGTT increased slightly (p = NS) in the muraglitazar group and rose significantly in the placebo group (Table 2). Following muraglitazar therapy, the incremental AUC for C-peptide divided by the incremental AUC for plasma glucose concentration (figures 1, 2; Table 2) increased significantly from 3.1 ± 0.5 to 6.4 ± 0.7 pmol/l per mg/dl ($p < 0.001$); in the placebo group there was a smaller, non-statistically significant increase from 1.8 ± 0.6 to 3.5 ± 1.4. The insulin secretion (ΔISR/Δ*G*)/insulin resistance (measured with the insulin clamp) index increased significantly in the muraglitazar group (2.7 ± 0.7 to 10.1 ± 1.7, $p < 0.0001$) and tended to increase in the placebo group (1.6 ± 0.5 vs. 4.7 ± 1.6, $p < 0.07$, muraglitazar vs. placebo). In the combined muraglitazar plus placebo groups the natural log of the insulin secretion/insulin resistance index correlated strongly with the natural log of the FPG ($r = -0.74$, $p < 0.0001$) and with the natural log of the 2-h plasma glucose concentration ($r = -0.66$, $p < 0.0001$) during the OGTT. These correlations also were highly significant ($p < 0.01$) if the muraglitazar and placebo groups were analysed separately.

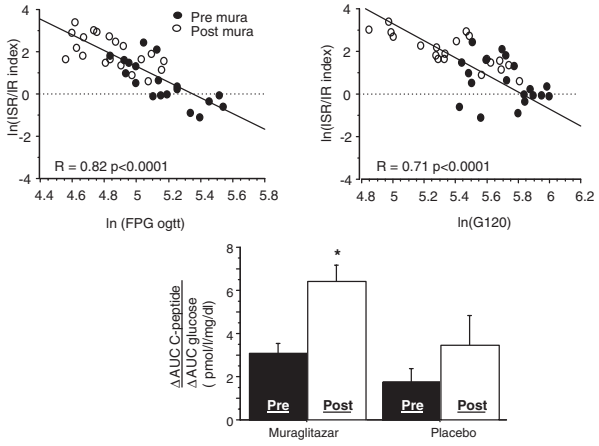

Figure 2. Relationship between the natural log of the fasting plasma glucose concentration (FPG) (left top panel) and the natural log of the 2-h plasma glucose concentration (2-h PG) (right top panel) versus the natural log of the insulin secretory rate (ISR) during the OGTT divided by insulin resistance (IR) (measured with the euglycaemic insulin clamp). Bottom panel displays the incremental C-peptide area under the curve (AUC) divided by the incremental glucose area under the curve (AUC) during the OGTT in muraglitazar- and placebo-treated subjects.

Body Composition

The percent total body fat (DEXA) increased slightly with muraglitazar therapy and decreased slightly in the placebo group (p = NS). FM increased significantly in the muraglitazar

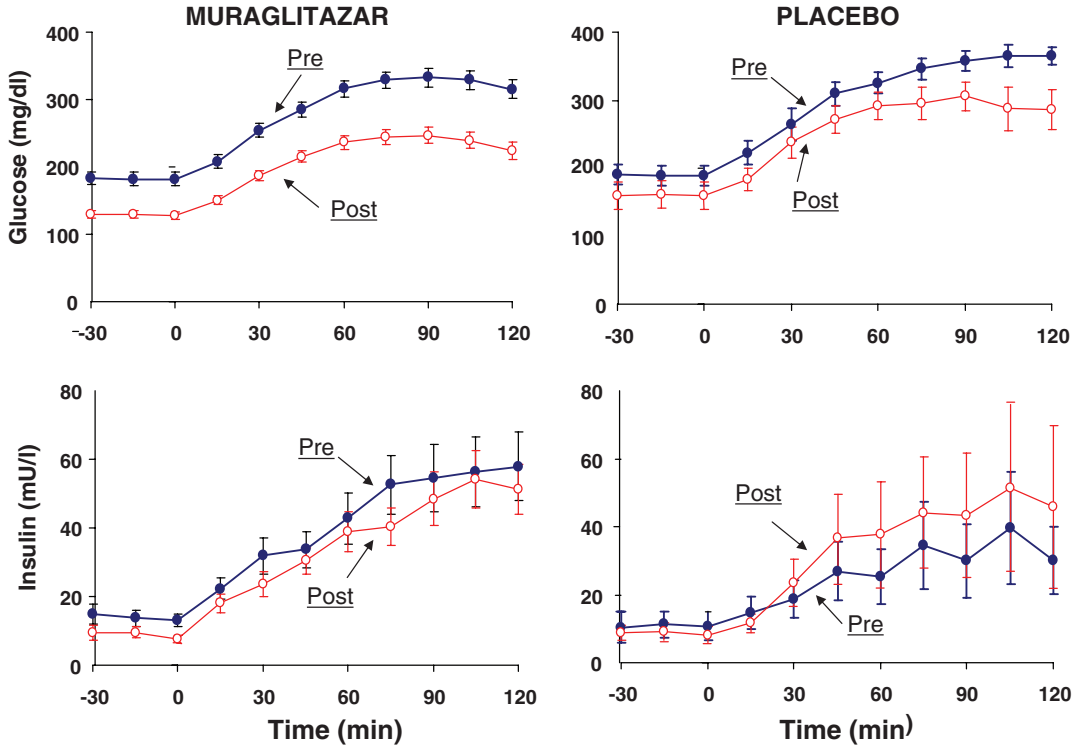

Figure 1. Effect of muraglitazar (left panel) and placebo (right panel) on the plasma glucose and insulin concentrations during the oral glucose tolerance test.

Table 2. Changes in plasma glucose and insulin concentrations and insulin sensitivity indices during the OGTT and the euglycaemic clamp in T2DM patients treated with muraglitazar versus placebo for 4 months.

	Muraglitazar		Placebo		
	Pre	Post	Pre	Post	p Value
Fasting					
Fasting glucose (mg/dl)	183 ± 9	129 ± 6*	190 ± 15	160 ± 20	NS
Fasting insulin (μU/ml)	13.9 ± 2.3	9.5 ± 1.8*	10.8 ± 4.2	8.8 ± 2.4	NS
Fasting FFA (μmol/l)	700 ± 37	523 ± 41*	720 ± 45	593 ± 60*	NS
IR adipose tissue (mmol/l × pmol/l)	50 ± 8	26 ± 4*	41 ± 15	25 ± 7	NS
Fasting EGP (μmol/kg_{ffm} min)	16.7 ± 1.5	14.9 ± 1.2*	14.1 ± 0.7	16.6 ± 3.3	<0.05
IR liver (μmol/kg_{ffm} × min/pM)	956 ± 119	576 ± 59*	780 ± 213	665 ± 318	NS
OGTT (mean 0–120 min)					
Mean plasma glucose (mg/dl)	288 ± 11	211 ± 9*	303 ± 16	262 ± 21	NS
Mean plasma insulin (μU/ml)	41.3 ± 6.6	35.5 ± 4.5	26.2 ± 8.7	34.5 ± 13.5	NS
Mean plasma c-peptide	7.0 ± 0.7	8.1 ± 0.7	5.2 ± 1.0	7.4 ± 1.1*	NS
Mean ISR	373 ± 41	451 ± 33*	299 ± 61	452 ± 69*	NS
ΔAUC-I/ΔAUC-G	25.7 ± 4.4	33.3 ± 4.1*	13.5 ± 6.8	25.1 ± 10.4*	NS
ΔAUC-C-Pep/ΔAUC-G	3.1 ± 0.5	6.4 ± 0.7*	1.8 ± 0.6	3.5 ± 1.4*	NS
(ΔAUC-ISR/ΔAUC-G)/IR	2.7 ± 0.7	10.1 ± 1.7*	1.6 ± 0.5	4.7 ± 1.6	NS
Clamp					
Clamp EGP (μmol/kg_{ffm} min)	4.2 ± 0.2	3.9 ± 0.8	3.5 ± 0.3	3.3 ± 0.4	NS
Total glucose disposal (μmol/kg_{ffm} min)	31 ± 4	54 ± 5*	30 ± 6	34 ± 6	<0.01
Total glucose disposal/SSPI (μmol/kg_{ffm} min/nM)	78 ± 15	135 ± 13*	66 ± 16	95 ± 19	0.09
Glucose clearance/SSPI (μmol/kg_{ffm} min/pM)	14.4 ± 3.0	24.6 ± 2.4*	12.0 ± 2.9	16.9 ± 3.4	NS

FPG, fasting plasma glucose; EGF, endogenous glucose production; IR, insulin resistance; FFA, free fatty acid; OGTT, oral glucose tolerance test; NS, non-significant.

*$p < 0.05$ versus baseline.

group (32 ± 1 to 34 ± 1 kg, $p < 0.001$) and decreased in the placebo group (26 ± 2 to 25 ± 3, $p < 0.0004$ vs. muraglitazar). Liver fat content decreased from 15 ± 2 to 6 ± 1 following muraglitazar therapy ($p < 0.01$) and did not change significantly in the placebo group (11 ± 4 to 9 ± 4%). Visceral abdominal total fat content decreased significantly ($p < 0.001$) in the muraglitazar group and tended to decrease ($p = NS$) in the placebo group (Table 1). The subcutaneous abdominal total fat content increased significantly in patients receiving muraglitazar ($p < 0.001$) and decreased significantly in subjects receiving placebo ($p = 0.04$) (Table 1). The intramyocellular fat content in the muraglitazar group decreased from 0.42 ± 0.07 to 0.21 ± 0.05 ($p = 0.006$), while the observed decrease in intramyocellular fat content in the placebo group was not significant (from 0.32 ± 0.18 to 0.12 ± 0.04, p = N S) (figure 3).

Inflammatory Markers

Treatment with muraglitazar caused a 2.5-fold increase in plasma adiponectin concentration from 8.4 ± 1.2 to 18.6 ± 1.9 μg/ml ($p < 0.001$), whereas plasma adiponectin did not change in the placebo group (9.2 ± 2.6 to 7.3 ± 0.9 μg/ml) ($p < 0.0005$ vs. muraglitazar). The plasma TNF-α concentration did not change significantly in either the muraglitazar (1.6 ± 0.2 to 1.6 ± 0.3 pg/ml) or placebo (1.2 ± 0.2 to 1.6 ± 0.2 pg/ml) groups. Plasma IL-6 did not change significantly in either the muraglitazar (3.2 ± 0.3 to 3.4 ± 0.6 pg/ml) or placebo (2.4 ± 0.4 to 2.9 ± 0.5 pg/ml, p = NS) groups. Plasma ICAM decreased in the muraglitazar (249 ± 15 to 231 ± 12 ng/ml, $p < 0.04$) group and did not change in the placebo (241 ± 25 to 234 ± 17, p = NS) group. The hsCRP fell significantly in the muraglitazar group (4.4 ± 0.7 to 2.5 ± 0.5 ng/ml, $p < 0.01$) but not in the placebo group (2.2 ± 0.7 to 2.1 ± 0.5, p = NS).

Muscle, Liver and Adipose Insulin Sensitivity

Total body (primarily reflects muscle) insulin-stimulated TGD and TGD/SSPI increased by 74 and 73%, respectively, following muraglitazar treatment (both $p < 0.001$) (Table 2). In the placebo group TGD and TGD/SSPI increased modestly but not significantly from baseline (Table 2). The changes in glucose clearance in both groups paralleled the changes in TGD.

Basal endogenous (primarily reflects liver) glucose production declined significantly in the muraglitazar group and rose slightly in the placebo group ($p < 0.05$ vs. muraglitazar) (Table 2). The hepatic insulin sensitivity index decreased by 40% in the muraglitazar group ($p < 0.01$) and fell slightly but not significantly in the placebo group (Table 2).

Mean baseline plasma FFA concentration decreased from 700 ± 37 to 523 ± 41 μmol/l ($p < 0.0003$) after muraglitazar treatment, and decreased modestly in the placebo group (720 ± 45 to 593 ± 60 μmol/l, $p = 0.03$). The basal (fasting) adipocyte insulin resistance index (fasting plasma FFA × FPI) decreased significantly in the muraglitazar group from 50 ± 8 to 26 ± 4 μmol/l × pmol/l ($p < 0.01$) and decreased modestly in the placebo group (41 ± 15 to 25 ± 7 μmol/l × pmol/l, p = NS). Suppression of the plasma FFA concentration during the insulin clamp performed prior to the start of therapy was similar in both groups and decreased significantly after 4 months of muraglitazar treatment (from 123 ± 10 to 57 ±

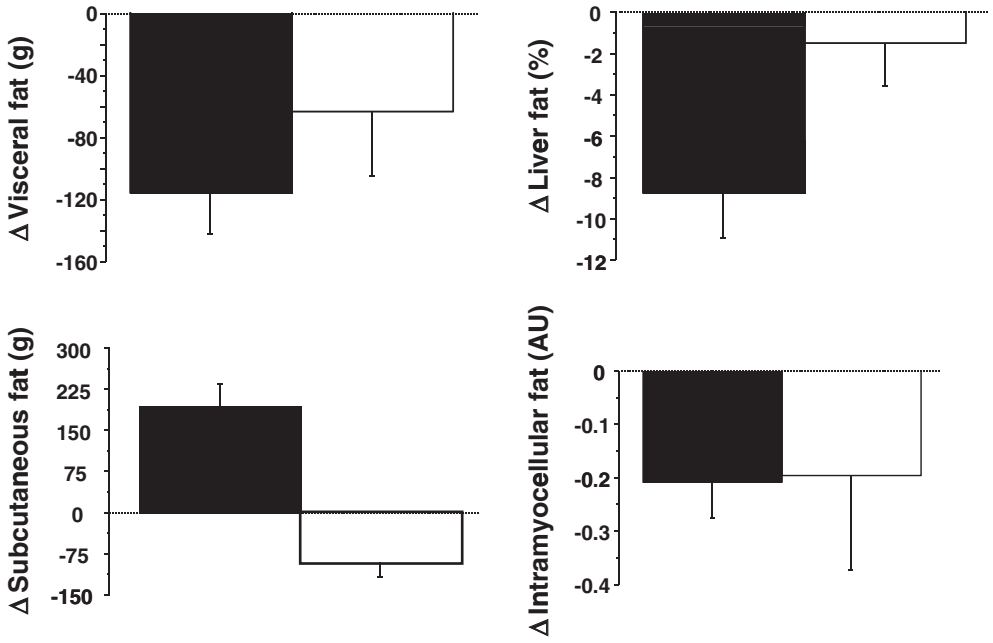

Figure 3. Changes in visceral (upper left) and subcutaneous (lower left) fat contents, percent liver fat (upper right) and percent intramyocellular fat (lower right) before and after muraglitazar (black bars) and placebo (white bars) treatments.

6 μmol/l, $p = 0.008$) but not in the placebo group (from 132 ± 22 to 117 ± 33 μmol/l, p = NS).

Discussion

Type 2 diabetic individuals are at increased risk for both microvascular and macrovascular complications [48–50]. Although the microvascular complications clearly are related to both the duration and severity of hyperglycemia [48,49,51], the macrovascular complications are more related to the classic risk factors for ASCVD including dyslipidemia, hypertension and obesity [29,52]. Collectively these have been referred to as the metabolic or insulin resistance syndrome [13,53,54]. A characteristic feature of the metabolic syndrome is the underlying insulin resistance [13,53,54], and many studies have shown that insulin resistance predicts the subsequent development of ASCVD [55–60]. Therefore, there has been great interest in the development of oral antidiabetic medications which effectively reduce the elevated plasma glucose levels in T2DM, simultaneously correct the underlying insulin resistance, a core defect in diabetic patients, and reverse known cardiovascular risk factors. PPARγ agonists are potent insulin sensitizers in liver, muscle and adipocytes [25,26,36,39,42] and cause a redistribution of fat from visceral to abdominal areas [25,40,42], while PPARα agonists correct the underlying diabetic dyslipidemia [42]. Muraglitazar is a dual PPAR agonist that possesses both PPARα and PPARγ activities [61]. In the present study, we examined the effect of muraglitazar on the multiple components of the insulin resistance (metabolic) syndrome and compared the results to placebo-treated T2DM patients who received intensive dietary intervention. Although both groups were instructed to maintain their caloric intake and body weight constant, the placebo-treated group lost a mean of 1.9 kg over 4 months, while the muraglitazar group gained 2.5 kg ($p < 0.001$). Body FM declined by a mean of 1.8 kg in the placebo group and increased by 1.7 kg in the muraglitazar group ($p < 0.001$). These results indicate that only 30% of the weight gain in the muraglitazar group could be accounted for by fluid retention and is consistent with the absence of edema in any patient in this group. Both the murglitazar- and placebo-treated groups experienced similar declines in the HbA1C (2.1%), despite the divergent changes in body weight and body fat content. Despite the similar decline in HbA1c in both groups, it is noteworthy that muraglitazar-treated T2DM subjects had a nearly twofold increase in insulin-stimulated glucose disposal compared to the placebo group (Table 2). In contrast, placebo-treated T2DM subjects had no improvement in insulin sensitivity despite the modest reduction in body weight and body fat content. During the euglycaemic insulin clamp, the majority of glucose uptake occurs in muscle [62]. Of note, intramyocellular fat content decreased mildly in the muraglitazar-treated group. Although MRS measures the amount of muscle triglyceride, which is metabolically inert, the decreased muscle triglyceride content is paralleled by a decrease in muscle long-chain fatty acyl-CoA esters (FACoAs) [39] which have been implicated in the development of insulin resistance [2,8,20–24,39,53,63]. It should be noted that increased muscle triglyceride content also can be observed in insulin-sensitive states, that is well-trained athletes [64]. But, in this case, the triglyceride is contained in discrete lipid droplets surrounding the mitochondria, is not associated with elevated FACoA levels, and is used efficiently as an energy source. The improvement in insulin sensitivity with muraglitazar also was closely correlated with the decrease in plasma FFA concentration ($r = 0.42$, $p < 0.03$),

suggesting an important role for reversal of lipotoxicity in the insulin-sensitizing effect of muraglitazar. Muraglitazar also improved the basal hepatic insulin sensitivity index (Table 2) and reduced hepatic fat content (figure 1), consistent with previous studies performed with rosiglitazone and pioglitazone [26,35,36,65,66]. The decrease in hepatic fat content was correlated with the increase in hepatic insulin sensitivity index ($r = -0.53, p < 0.02$). The decrease in liver fat content with muraglitazar also was associated with a reduction in liver function tests (AST and ALT).

We previously have shown that thiazolidinediones are associated with an improvement in β cell function [37,38]. Consistent with these prior results, both insulin secretion/insulin resistance indices ($\Delta I/\Delta G \div$ IR and $\Delta ISR/\Delta G \div$ IR) improved after muraglitazar treatment, whereas no change was observed in the placebo-treated group. The improvement in β cell function following muraglitazar treatment was associated closely with the decline in plasma FFA concentration ($r = -0.47$, $p < 0.04$), suggesting a potential role for reduced lipotoxicity [67]. As PPARγ receptors are present on the β cell [33], a direct effect on the β cannot be excluded. The decrease in basal plasma FFA concentration and FFA turnover is consistent with previously published studies with PPARγ agonists [31].

Muraglitazar also exerted a beneficial effect on diabetic dyslipidemia, and a number of other cardiovascular risk factors including adiponectin, TNFα, hsCRP, and abdominal fat distribution. Reduced adiponectin levels and increased TNFα concentration have been associated with insulin resistance and accelerated atherosclerosis (reviewed in Refs. [12] and [19]). Muraglitazar treatment was associated with an increase in the ratio of subcutaneous to visceral fat, a change that is also consistent with a less atherogenic risk profile [28,29]. Muraglitazar-treated T2DM patients experienced significant reductions in plasma triglycerides ($\Delta = 33$ mg/dl) and LDL cholesterol (14 mg/dl) and a significant rise in plasma HDL cholesterol (4 mg/dl) (all $p < 0.01$ vs. baseline) (Table 1). LDL partial size increased, indicating less atherogenic LDL particles. Diastolic and systolic blood pressures were normal in T2DM subjects prior to the start of therapy, and did not change significantly after 4 months of muraglitazar therapy. The hsCRP, a well-established risk factor for ASCVD [67], also decreased significantly after 4 months of muraglitazar treatment.

Lastly, some comments about the decline in A1c in the placebo group are indicated because the decrease was more than expected based upon the amount of total body (1.9 kg)/fat (1.8 kg) weight loss and cannot be explained by improved muscle or hepatic insulin sensitivity. As the decline in FPG was somewhat (although not significantly) less in the placebo-treated group, this cannot account for the similar reduction in A1c in the muraglitazar- and placebo-treated groups. During the OGTT the decline in glucose was also similar in the two groups. By exclusion, these results suggest that the postprandial rise in glucose in response to mixed meals during everyday life must have been greater in the placebo group. With regard to this, the mean insulin secretory rate during the OGTT was lower in the placebo group and increased more (although not significantly more) in the placebo group (Table 2). It is possible that the weight loss in the placebo-treated group resulted in a greater insulin secretory response to mixed meals and a greater reduction in postprandial glucose levels while subjects were consuming more typical meals at home. The baseline A1c also was slightly (although not significantly) higher in the placebo-treated group and, the higher the baseline A1c, the greater is the decline in A1c with any intervention.

In summary, the dual PPAR agonist muraglitazar augments insulin sensitivity, improves β cell function, mobilizes fat out of liver and muscle, increases the ratio of subcutaneous to visceral fat content, improves diabetic dyslipidemia, and improves hsCRP and a number of other insulin-resistance-provoking and atherogenic adipocytokines. With the exception of the decrease in LDL cholesterol, these effects of muraglitazar are very similar to those observed with pioglitazone.

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Conflict of Interest

C.T. serves as a speaker for Amylin, Eli Lilly and Novartis. E.C. serves as a speaker for Takeda, Amylin and Eli Lilly. E.F. serves as a speaker for Bristol-Myers Squibb. R.D. has received investigational grants and consulting honoraria from Takeda Pharmaceuticals North America, BMS, Amylin, Eli Lilly, Novartis, Pfizer, Takeda, Roche and Merck, and serves as a speaker for Amylin, Eli Lilly and Takeda. N. M. received research support from National Institutes of Health (grants AG030979, DK80157, DK089229).

E. F., R. D. and A. G. designed the study. M. F., C. T., A. C. and P. T. conducted the study and collected the data. M. F., R. P, J. H., N. M., A. G., E. C., E. F. and R. D. analysed the data. M. F., N. M., A. G., E. C., E. F. and R. D. wrote the manuscript. M. F., A. G., J. H., N. M., A. C., P. T. and R. P. have no conflict of interest regarding this manuscript.

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