A pilot study of the effects of pioglitazone and rosiglitazone on de novo lipogenesis in type 2 diabetes

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Abstract Treatment of type 2 diabetes mellitus (T2DM) patients with pioglitazone results in a more favorable lipid profile, and perhaps more favorable cardiac outcomes, than treatment with rosiglitazone. Pioglitazone treatment increases VLDL-triacylglycerol clearance, but the role of de novo lipogenesis (DNL) has not been explored, and no direct comparison has been made between the thiazolidinediones (TZDs). Twelve subjects with T2DM and hypertriacylglyceridemia were randomized to either rosiglitazone or pioglitazone treatment. Stable isotope infusion studies were performed at baseline and after 20 weeks of treatment. Both treatments reduced glucose and HbA_{1c} concentrations equally. Pioglitazone treatment resulted in a 40% reduction in hepatic DNL ($P < 0.01$) and in a 25% reduction in hepatic glucose production ($P < 0.05$), while rosiglitazone did not significantly change either parameter, although comparisons of changes between treatments were not significantly different. These pilot results indicate that pioglitazone reduces hepatic DNL while rosiglitazone does not. Larger follow-up studies are required to confirm differential effects of these agents definitively. The reduction in DNL may underlie altered assembly or atherogenicity of lipoprotein particles and may reflect PPARα or other non-PPARγ actions on the liver by pioglitazone. These differences might help explain previously reported differences in lipid profiles and cardiovascular disease outcomes for rosiglitazone and pioglitazone.—Beysen, C., E. J. Murphy, H. Nagaraja, M. Decaris, T. Riiff, A. Fong, M. K. Hellerstein, and P. J. Boyle. **A pilot study of the effects of pioglitazone and rosiglitazone on de novo lipogenesis in type 2 diabetes. *J. Lipid Res.* 2008.** 49: **2657–2663.**

Supplementary key words stable isotopes • liver metabolism • lipid metabolism • carbohydrate metabolism

Sources of triacylglycerols (TAG) assembled in the liver include plasma nonesterified fatty acids (NEFA), dietary fats, and fatty acids newly synthesized through the pathway of de novo lipogenesis (DNL). Hepatic TAG are either secreted immediately as very low density lipoprotein (VLDL)-TAG or stored in the cytosol and secreted after a delay (1). Although the majority of the fatty acids in VLDL-TAG derive from plasma NEFA, rates of DNL vary markedly in different settings, suggesting that this pathway may play important metabolic roles. DNL increases by 50–200% in the fasted state in obese (2), insulin-resistant humans (3) and in subjects with hypertriacylglyceridemia (1), with or without T2DM. DNL also contributes a significant portion (~25%) of the TAG in liver and VLDL of patients with nonalcoholic fatty liver disease (4). Perhaps most importantly, the intrahepatic metabolic milieu associated with high activity of DNL (e.g., increased availability of malonyl-CoA and glycolytic metabolites, including α-glycerol-phosphate) promotes re-esterification rather than oxidation of incoming NEFA by the liver. Accordingly, the synthesis and secretion of TAG by the liver in subjects with type 2 diabetes mellitus (T2DM) may play a role in dyslipidemia as well as in nonalcoholic fatty liver disease. Reducing hepatic DNL is therefore a potential target for reducing hepatic fat accumulation and VLDL-TAG secretion in T2DM.

The peroxisome proliferator-activated receptor (PPAR)-γ agonists, pioglitazone and rosiglitazone, are widely used for treatment of T2DM. These thiazolidinediones (TZDs) lower glucose concentrations by improving insulin sensitivity (5–7). Meta-analyses and prospective trials (8–10) have raised the possibility of different cardiovascular outcomes, however, for these two TZDs. Rosiglitazone has been suggested to cause adverse (10), or at best neutral (9), effects on cardiovascular disease outcomes, while pioglitazone has been shown to reduce adverse events in T2DM patients with a prior myocardial infarction (8).

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DNL, de novo lipogenesis; HGP, hepatic glucose production; MIDA, mass isotopomer distribution analysis; NEFA, nonesterified fatty acids; T2DM, type 2 diabetes mellitus; TAG, triacylglycerols; TZDs, thiazolidinediones; VLDL, very low density lipoprotein.

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It would be helpful in evaluating the clinical outcomes data to establish whether underlying metabolic or biochemical differences exist between TZDs (i.e., whether differential biochemical actions can be identified that may explain the clinical observations). Different effects of pioglitazone and rosiglitazone have been observed on plasma lipids, with pioglitazone treatment resulting in a more favorable lipid profile than rosiglitazone treatment (5, 11). A randomized, prospective study comparing the effects of pioglitazone and rosiglitazone monotherapy in patients with T2DM, for example, showed a 12% reduction in plasma TAG concentrations with pioglitazone compared with a 15% increase with rosiglitazone in the face of identical reductions in HbA_{1c} values (5). In contrast, a meta-analysis showed no change in TAG concentrations with rosiglitazone (11). Pioglitazone as compared with rosiglitazone treatment is associated with a greater increase in HDL cholesterol (14.9% vs. 7.8%), a smaller increase in LDL cholesterol concentrations (15.7% vs. 23.3%), and less increase in apolipoprotein B (0% vs. 10%) (5). Rosiglitazone and pioglitazone have both been reported to reduce the release of fatty acids from adipocytes and decrease plasma NEFA concentrations (5, 12), thereby reducing NEFA flux to the liver. Pioglitazone has also been shown to increase the fractional clearance rate of plasma VLDL-TAG (13), although the effects of these two TZDs on intrahepatic lipid metabolism, especially as it relates to the disposition of incoming NEFA and synthesis of VLDL-TAG, have not yet been reported in humans with T2DM. Differential effects of pioglitazone and rosiglitazone on DNL, for example, might influence hepatic packaging, secretion, and the ultimate atherogenicity of VLDL particles.

The present pilot study was designed to investigate the effects of rosiglitazone and pioglitazone on hepatic lipid and glucose metabolism in T2DM, with an emphasis on hepatic DNL.

MATERIALS AND METHODS

Subjects

Twelve subjects (five males and seven females) with T2DM and hypertriacylglyceridemia were recruited and gave written informed consent. Subjects were studied at the General Clinical Research Center, at San Francisco General Hospital, University of California-San Francisco (UCSF), or at the University of New Mexico Health Science Center. The protocol was approved by the ethical committees of both institutions (Committee on Human Research at UCSF and the Human Research Review Committee of the University of New Mexico Health Sciences Center). Subjects were eligible to take part in the study if T2DM was not adequately controlled [$HbA_{1c} > 7.5\%$ or fasting glucose > 10 mmol/l (180 mg/dl)] with metformin alone or metformin in combination with a sulfonylurea, and if hypertriacylglyceridemia was present [fasting TAG concentrations between 1.7 mmol/l (150 mg/dl) and 4.5 mmol/l (400 mg/dl)]. Exclusion criteria were pregnancy, alanine aminotransferase ALT > 1.5 times the upper limit of normal, serum creatinine > 1.4 mg/dl, congestive heart failure, history of coronary artery, and pulmonary or neurologic disease. Subjects were also excluded if they had been treated with insulin or had started treatment with a statin or any fibric acid derivative within 2 months of the beginning of the study.

Study design

This was an open-label, randomized study. Subjects were randomized to pioglitazone or rosiglitazone treatments for 20 weeks. Pioglitazone was started at a dose of 15–30 mg QD for 4 weeks followed by 45 mg QD for 16 weeks. Rosiglitazone was started at 4 mg QD for 4 weeks followed 4 mg twice daily for the remaining 16 weeks of the study. Stable isotope infusion studies were performed at baseline and after completion of the 20-week intervention trial to evaluate glucose and lipid metabolism.

Stable isotope infusion protocol

Stable isotope labeled metabolites were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). The infusion protocol is shown in **Fig. 1**. On day 1 of each infusion protocol, subjects received a standardized American Diabetes Association meal at 5:00 PM. After 6:00 PM, no calories or caffeine were taken until the end of the infusion (12:00 PM on day 2). Noncaloric beverages were allowed. Sodium [1-$^{13}C_1$]acetate (10 mg/min) and [1,2,3,4-$^{13}C_4$]palmitate (0.008 mg/kg body weight/min) were infused from 12:00 AM until 12:00 PM on day 2. A bolus of [6,6-$^{2}H_2$]glucose (4.8 mg/kg body weight) was given at 8:00 AM on day 2 followed by a constant infusion (0.08 mg/kg body weight/min) until 12:00 PM the same day. Indirect calorimetry was performed using a Deltatreck unit with a ventilated hood (Sensor Medics, Yorba Linda, CA) at 8:00 AM on day 2 and was completed 1 h later. Blood samples were drawn as shown (Fig. 1). Immediately after collection, one aliquot of plasma was transferred to heptane/isopropanol (30:70, v/v) for

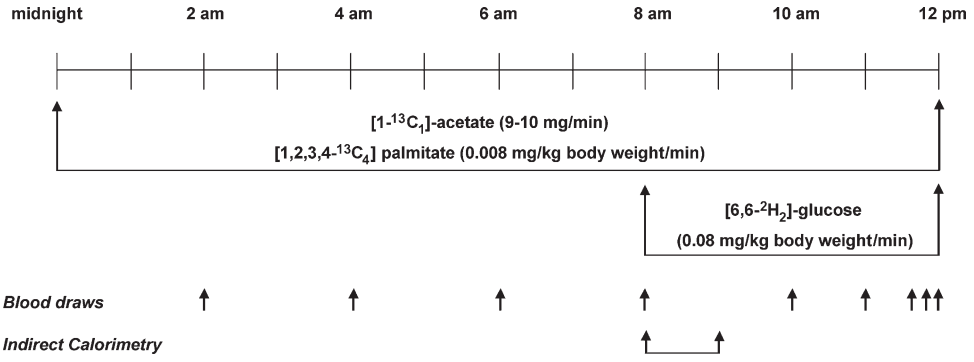

Fig. 1. Stable isotope infusion and blood drawing protocol.

plasma NEFA analysis. The remaining plasma was stored at −80°C for further analyses.

Analyses

Plasma TAG, total cholesterol, LDL-cholesterol, HDL-cholesterol, HbA1C, aspartate aminotransferase (AST), and ALT concentrations were analyzed by standard clinical laboratory techniques. Plasma insulin concentrations were determined by radioimmunoassay (Linco, St. Charles, MO). Plasma glucose concentrations were analyzed with a Yellow Springs Instrument (Yellow Springs Instruments Co., Yellow Springs, OH).

NEFA and lipoprotein isolation and analysis

VLDL particles were isolated from plasma by sequential ultracentrifugation (1). Briefly, two 30 min spins (40,000 rpm at 10°C) were performed to isolate lipoproteins with a Svedberg flotation rate (S_f) > 400 (chylomicron-like particles) followed by an 18 h spin (40,000 rpm at 10°C) to isolate VLDL particles (S_f 20–400). Total lipids from VLDL particles and plasma samples were extracted with chloroform:methanol (2:1, v/v) and were subjected to thin layer chromatography to separate VLDL-TAG and plasma NEFAs from other lipid fractions. VLDL-TAG fatty acids and plasma NEFAs were then trans-esterified to fatty acid-methyl esters for gas chromatography mass-spectrometry (GC-MS) analysis. It should be noted that separation of buoyant VLDL particles in lipoprotein remnants, to the extent that these are removed with the chylomicron-like fraction, may alter DNL values (4).

Mass spectrometry

GC-MS was performed using a model 5973 instrument (Agilent Technologies, Palo Alto, CA) with a DB-17 or VB-17 column to measure $^{13}C_1$- enrichment in VLDL-TAG-palmitate and plasma enrichment of [1,2,3,4-$^{13}C_4$]palmitate. Electron impact ionization was used, monitoring mass/charge ratio (*m/z*) 270–272 and 274, representing the parent M_0, $M_{1,}$, M_2, and M_4 isotopomers. Plasma [1,2,3,4-$^{13}C_4$]palmitate enrichments were quantified by comparisons with standard curves of [1,2,3,4-$^{13}C_4$]palmitate. The relative concentrations of fatty acids in plasma were quantified by GC with flame ionization detection, as described previously (1). Hepatic glucose production (HGP) was determined based on tracer dilution with [6,6-$^2H_2$]glucose infusion by GC-MS, as previously described (14).

Calculations

Fractional DNL: the fraction of VLDL-TAG-palmitate synthesized by the DNL pathway was calculated using mass isotopomer distribution analysis (MIDA) (15, 16). Briefly, the isotopic enrichment of the precursor for lipogenesis (hepatic cytosolic acetyl-CoA) was calculated from the ratio of excess double-labeled to excess single-labeled species (EM2/EM1) in VLDL-TAG-palmitate, based on combinatorial probabilities. Fractional DNL was determined by the standard precursor-product relationship as described previously (15). Fractional DNL at steady state was calculated as the mean of DNL values at 10 and 12 h after the start of the sodium [1-$^{13}C_1$]acetate infusion.

The rates of appearance (Ra) of plasma NEFA and plasma glucose were calculated by the isotope dilution technique (17). Ra (mg/kg body weight per min) = isotope infusion rate (mg/kg body weight per min)/metabolite enrichment, where metabolite enrichment is molar excess fraction. For Ra NEFA, Ra palmitate was first calculated and was then divided by the % of plasma NEFA that is palmitate. The average of the results from samples drawn at 6:00 AM, 8:00 AM, 10:00 AM, and 12:00 AM was used for the NEFA enrichment and 11:00 AM, 11:30 AM, 11:45 AM, and 12:00 PM for glucose enrichments. Under fasting conditions, Ra glucose is taken to represent HGP.

Total fatty acid oxidation rate: whole-body fat oxidation was calculated by indirect calorimetry using the following equation: whole-body fat oxidation (g/min) = {[1.67 × VO_2 (l/min)] − [1.67 × VCO_2 (l/min)]} − [1.92 × urinary N (g/min)] (18). Urinary N was assumed to be 150 mg/kg body weight/day (19). Total fatty acid oxidation was also expressed on a molar basis by converting whole-body fat oxidation to its molecular equivalent using 287 g/mol as the average molecular weight of a fatty acid.

Extra-adipose tissue re-esterification of NEFA was calculated as the difference between Ra NEFA and whole-body fatty acid oxidation

Statistical analysis

Statistical analyses were performed for three different comparisons: *1*) Baseline parameters between the two treatment groups were compared by unpaired Student's *t*-test. *2*) Within each treatment group, the pre- and post-treatment results were compared using repeated-measures ANOVA (ANOVA) with Bonferroni correction. *3*) Unpaired Student's *t*-tests were used to establish if the changes seen with pioglitazone were significantly different from changes seen with rosiglitazone.

RESULTS

Subject characteristics and metabolic parameters

Subject characteristics and metabolic parameters at baseline and post-treatment are shown in **Table 1**. There were no significant differences between the rosiglitazone and pioglitazone treatment groups at baseline with respect to age, weight, body mass index, or metabolic parameters. In the pioglitazone treatment group, two subjects were taking metformin alone and four subjects were taking metformin in combination with sulfonylurea. In the rosiglitazone group, three subjects were on metformin alone and three subjects were on metformin in combination with sulfonylurea. In all cases, the dose of metformin and/or sulfonylurea was at least 50% of the maximum recommended dose. Five subjects in the rosiglitazone treatment group and one subject in the pioglitazone group were on stable regimens of statins.

All six subjects in the pioglitazone group and five out of six subjects in the rosiglitazone group gained weight after 20 weeks of treatment, although the weight increase was statistically significant for the pioglitazone group only ($P < 0.05$). Similar results were seen for changes in body mass index after treatment [$P < 0.05$ for pioglitazone treatment but not significant (NS) for rosiglitazone treatment].

Fasting plasma glucose and HbA1C concentrations decreased significantly ($P < 0.05$) with both treatments. The reductions in mean insulin concentrations with treatment were not significant in either group.

Changes in plasma lipids with TZD treatment were not significant, although the trends were consistent with published results (5, 7, 11). Mean fasting TAG concentrations tended to increase with rosiglitazone treatment and decrease with pioglitazone treatment, mean fasting total cholesterol tended to increase with rosiglitazone treatment but not with pioglitazone treatment, and the increase in LDL cholesterol concentrations was greater for rosiglitazone

TABLE 1. Subject characteristics of the rosiglitazone and pioglitazone treatment groups

	Rosiglitazone			Pioglitazone		
Parameters	*Pretreatment*	*Post-treatment*	*Change*	*Pretreatment*	*Post-treatment*	*Change*
Subjects (male/female)	6 (2/4)			6 (3/3)		
Age (years)	56 ± 6			53 ± 8		
Weight (kg)	86.8 ± 26.7	89.8 ± 27.1	3.0 ± 3.4	98.5 ± 36.6	103.4 ± 40.4[a]	4.9 ± 4.2
BMI (kg/m^2)	31.3 ± 5.4	32.3 ± 5.4	1.1 ± 1.4	36.9 ± 12.5	38.7 ± 13.8[a]	1.8 ± 1.5
Fasting glucose (mmol/l)	8.9 ± 5.5	6.4 ± 3.5[a]	−2.5 ± 2.2	8.8 ± 2.5	7.3 ± 3.5[a]	−1.5 ± 1.2
Fasting insulin (pmol/l)	90 ± 26	75 ± 18	−15 ± 22	92 ± 61	83 ± 26	−9 ± 46
Fasting HbA1C (%)	8.2 ± 1.1	6.9 ± 1.1[a]	−1.3 ± 0.8	8.1 ± 0.6	7.0 ± 1.0[a]	−1.1 ± 0.6
Fasting TAG (mmol/l)	2.44 ± 0.53	2.82 ± 1.11	0.38 ± 1.13	2.53 ± 0.92	2.19 ± 1.80	−0.34 ± 0.81
Fasting NEFA (mmol/l)	0.61 ± 0.36	0.50 ± 0.15	−0.11 ± 0.26	0.75 ± 0.32	0.51 ± 0.04	−0.23 ± 0.30
Fasting total cholesterol (mmol/l)	4.24 ± 1.44	5.21 ± 1.61	0.98 ± 1.24	4.04 ± 1.04	3.99 ± 0.59	−0.05 ± 1.07
Fasting LDL-cholesterol (mmol/l)	2.27 ± 1.31	3.03 ± 1.18	0.76 ± 1.05	1.94 ± 0.91	2.13 ± 0.57	0.19 ± 1.20
Fasting HDL-cholesterol (mmol/l)	0.86 ± 0.26	0.89 ± 0.15	0.04 ± 0.19	0.94 ± 0.20	0.86 ± 0.11	−0.08 ± 0.11
Fasting AST (U/l)	27 ± 6	27 ± 9	0 ± 4	24 ± 8	22 ± 5	−2 ± 5
Fasting ALT (U/l)	36 ± 10	30 ± 10	−6 ± 6	32 ± 10	28 ± 7	−4 ± 6

AST, aspartate aminotransferase; BMI, body mass index; NEFA, nonesterified fatty acids; TAG, triacylglycerols. Data are mean ± SD.
[a] $P < 0.05$ significantly different from pretreatment by ANOVA. HbA1C data from one subject was not measured in the pioglitazone group.

treatment compared with pioglitazone treatment. HDL cholesterol concentrations did not change with either treatment. Changes with treatment were not significantly different between groups for any of these parameters.

Hepatic glucose production

At baseline, mean hepatic glucose production (HGP) was 2.75 ± 0.65 and 2.46 ± 0.42 mg/kg body weight per min for the rosiglitazone and the pioglitazone groups, respectively (NS). Pioglitazone treatment resulted in a significant reduction in HGP (1.91 ± 0.43 mg/kg body weight per min, $P < 0.05$), while rosiglitazone treatment did not (2.32 ± 0.57 mg/kg body weight per min, $P = 0.2$). The change with treatment was not significantly different between the two groups by unpaired *t*-test (−0.57 ± 0.42 mg/kg body weight per min for pioglitazone vs. −0.43 ± 0.60 mg/kg body weight per min for rosiglitazone, NS).

DNL contribution to VLDL-TAG-palmitate

The time course of hepatic DNL during a constant infusion of [1-$^{13}C_1$]acetate is shown in **Fig. 2A, B**. Fractional DNL (%) represents the fraction of plasma VLDL-TAG-palmitate derived from the DNL pathway (20). Prior to treatment, fractional DNL was not significantly different between groups. Pioglitazone treatment significantly reduced fractional DNL at steady-state, from 6.6 ± 0.7% at baseline to 4.2 ± 0.6% ($P < 0.01$), while rosiglitazone treatment did not significantly alter fractional DNL, from baseline 6.9 ± 1.4%, to post-treatment 5.5 ± 1.4% ($P = 0.5$). The change with treatment was not significantly different between the two groups by unpaired *t*-test (−2.4 ± 1.2% for pioglitazone vs. −1.4 ± 4.5% for rosiglitazone, NS).

Ra NEFA, whole-body fat oxidation, and extra-adipose tissue re-esterification of plasma NEFA

Neither rosiglitazone nor pioglitazone treatment resulted in statistically significant changes of Ra NEFA, whole-body fat oxidation or extra-adipose tissue re-esterification rates (**Table 2**).

DISCUSSION

The central questions addressed in this pilot study were whether TZDs reduce hepatic DNL in T2DM patients and whether pioglitazone and rosiglitazone have different

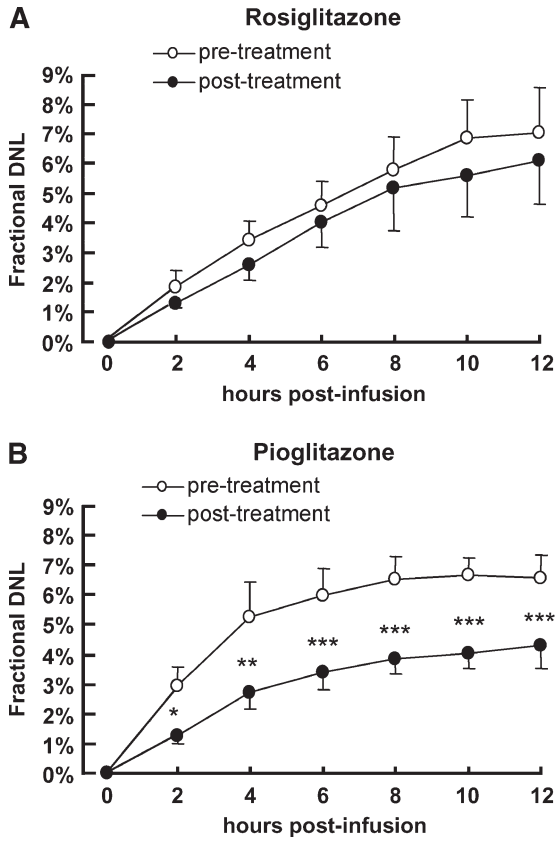

Fig. 2. The effect of 20 weeks of rosiglitazone and pioglitazone treatment on hepatic de novo lipogenesis (DNL). The time course of fractional DNL is shown during a constant infusion of [1-$^{13}C_1$] acetate before and after rosiglitazone treatment (A) and pioglitazone treatment (B). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant from pretreatment by ANOVA.

TABLE 2. Effect of rosiglitazone and pioglitazone treatment on nonesterified fatty acid (NEFA) fluxes

	Rosiglitazone			Pioglitazone		
Parameters	*Pretreatment*	*Post-treatment*	*n*	*Pretreatment*	*Post-treatment*	*n*
Ra NEFA	6.3 ± 2.7	6.1 ± 2.4	6	7.7 ± 3.4	6.9 ± 3.0	6
Whole-body fatty acid oxidation	3.7 ± 1.8	3.5 ± 1.7	4	3.3 ± 1.2	2.7 ± 1.4	4
Extra-adipose tissue re-esterification of NEFA	2.5 ± 2.7	1.9 ± 1.6	4	3.7 ± 3.0	3.4 ± 1.6	4

All data are expressed as μmol/kg body weight per minute. Values are mean ± SD.

metabolic effects, particularly on the liver, in addition to the previously established differences on lipoprotein profiles (5, 7, 11). Different effects on the regulation of hepatic metabolic pathways may be relevant to the differences in cardiovascular disease outcomes that have been reported for these two TZDs (8–10). Using sensitive in vivo stable isotope-mass spectrometric techniques, we identified differences in effects on hepatic lipid and glucose intermediary metabolism for pioglitazone and rosiglitazone. Treatment with these TZDs improved plasma glucose and HbA1C concentrations equally, as shown previously (5, 7, 11), but the hepatic metabolic response to the two TZDs was not the same. The most interesting and novel finding here is that hepatic DNL was significantly reduced (by 40%) by pioglitazone, whereas no significant change was seen with rosiglitazone treatment. The change in fractional DNL before and after pioglitazone treatment was not significantly different from the change in fractional DNL with rosiglitazone treatment, however, perhaps because of the small group sizes in this pilot study. Power calculations show that, with the same means, standard deviations and effects as were observed here, one would need at least 41 subjects per group to detect a statistically significant difference between pioglitazone and rosiglitazone treatment. The DNL values reported in the present study represent newly synthesized fatty acids in VLDL-TAG and not newly synthesized fatty acids retained in the liver. Longer labeling protocols might include a contribution from the cytosolic delay pool and might result in greater % DNL values than seen in this study.

The DNL pathway contributes a minority of the nonessential fatty acids present in TAG secreted from the liver (20, 21) but represents an integrated signal of hepatic carbohydrate availability and a key regulatory pathway in the liver. Intermediary metabolites generated from the metabolism of glucose or glycogen, such as pyruvate, oxaloacetate, acetyl-CoA, and citrate, increase the generation of malonyl-CoA by acetyl-CoA carboxylase, the first metabolic step in DNL, and stimulate flux through the DNL pathway. Malonyl-CoA is also the primary regulator of fatty acid transport into mitochondria for β-oxidation, by modulating the activity of carnitine palmitoyl transferase 1 (22). Accordingly, states of increased fatty acid synthesis in a tissue are typically associated with reduced fatty acid oxidation, and, in the liver, a milieu of activated DNL results in increased cytosolic re-esterification of NEFA, rather than mitochondrial oxidation (22). The result is partitioning of NEFA into TAG synthesis, which must either be secreted as VLDL-TAG or accumulate as hepatic fat droplets. Moreover, newly synthesized fatty acids exert intrahepatic signaling actions on the assembly and secretion of VLDL particles, and may have specific actions to regulate the assembly and atherogenicity of plasma lipoprotein particles (23). Agents that reduce hepatic DNL are therefore expected to alter the partitioning of incoming NEFA toward oxidation rather than TAG synthesis and VLDL assembly, and are being evaluated as a therapeutic target in dyslipidemia and insulin resistance (24).

The effect of treatment with specific TZDs on hepatic DNL in humans with T2DM were difficult to predict a priori. Although TZDs are classically recognized to stimulate DNL in adipocytes, their effects on hepatic lipogenesis have been uncertain. Activation of PPARγ by TZDs in animal models is associated with reduced hepatic expression of DNL enzymes (25) and increased expression of fatty acid oxidation enzymes (26). On the other hand, PPARγ gene expression is upregulated in liver in models of obesity and diabetes (27, 28), and induction of hepatic PPARγ2 gene expression by high-fat feeding or other nutritional interventions has been linked to stimulation of hepatic DNL and development of hepatic steatosis (29–31). Moreover, the effects of TZDs are further complicated by the possibility of cross-reactivity with PPARα (32), in that PPARα agonists are well established to increase hepatic fatty acid oxidation pathways and reduce hepatic TAG synthesis (33). Pioglitazone may directly bind to PPARα (32); moreover, activation of genes related to PPARα appears to occur with pioglitazone binding to PPARγ, so that increased α-like effects may be driven through this indirect mechanism (34).

In this context, the preliminary finding that pioglitazone but not rosiglitazone reduces hepatic DNL in T2DM patients becomes potentially important. Even though we observed no differences in plasma NEFA flux or delivery of NEFA to the liver, a reduction in DNL could alter partitioning of incoming NEFA and influence the assembly and secretion of apoB-containing particles, in addition to directly contributing fewer new fatty acids to hepatic TAG (1). Pioglitazone has also been shown to alter the clearance of VLDL-TAG in people with T2DM (13), in addition to these effects on hepatic fatty acid metabolism.

Moreover, although fasting blood glucose concentrations and HbA1C levels were not different in the two TZD treatment arms, fasting glucose production by the liver was significantly reduced by pioglitazone, but not by rosiglitazone. Previous studies have reported a decrease in fasting HGP with pioglitazone (35) and rosiglitazone (12, 36) treatment in T2DM. In the present study, the findings

for HGP complement the findings for hepatic lipid synthesis and might suggest greater activity of pioglitazone on liver metabolism in humans. Again, these metabolic actions may reflect greater functional PPARα cross-reactivity for pioglitazone, as has been reported previously (32), compared with rosiglitazone, at least in the liver of people with T2DM.

In summary, our pilot results indicate that pioglitazone reduces hepatic DNL while rosiglitazone does not in T2DM patients in the absence of differences in glycemic control. The metabolic implications of reduced hepatic DNL and glucose production are not certain but could, in principle, contribute to differences in plasma lipoprotein profiles or atherogenicity. At a minimum, these results support the notion that pioglitazone and rosiglitazone, though both in the TZD class of PPARγ activators, cannot be considered the same drug. These findings may be germane to the recent debate concerning cardiovascular outcomes of rosiglitazone and pioglitazone treatment in T2DM (8, 10, 37).

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