Pioglitazone and Rosiglitazone Have Different Effects on Serum Lipoprotein Particle Concentrations and Sizes in Patients With Type 2 Diabetes and Dyslipidemia

MARK A. DEEG, MD[1]
JOHN B. BUSE, MD[2]
RONALD B. GOLDBERG, MD[3]
DAVID M. KENDALL, MD[4]
ANTHONY J. ZAGAR, MS[5]
SCOTT J. JACOBER, DO[5]
MEHMOOD A. KHAN, MD[6]
ALFONZO T. PEREZ, MD[7]
MENG H. TAN, MD[5]
ON BEHALF OF THE GLAI STUDY INVESTIGATORS*

OBJECTIVE — Associated with insulin resistance in type 2 diabetes are increased serum triglycerides, decreased HDL cholesterol, and a predominance of large VLDL, small LDL, and small HDL particles. The comparative effects of thiazolidinedione insulin sensitizers on serum lipoprotein particle concentrations and sizes in type 2 diabetes are not known. We studied the effects of pioglitazone (PIO) and rosiglitazone (ROSI) treatments on serum lipoprotein particle concentrations and sizes in type 2 diabetic patients with dyslipidemia.

RESEARCH DESIGN AND METHODS — This is a prospective, randomized, double-blind, multicenter, parallel-group study. After a 4-week placebo washout period, patients randomized to PIO ($n = 369$) were treated with 30 mg q.d. for 12 weeks followed by 45 mg q.d. for another 12 weeks, while patients randomized to ROSI ($n = 366$) were treated with 4 mg q.d. followed by 4 mg b.i.d. for the same intervals. Lipoprotein subclass particle concentrations and sizes were determined by proton nuclear magnetic resonance spectroscopy at baseline and end point (PIO [$n = 333$] and ROSI [$n = 325$] patients).

RESULTS — PIO treatment increased total VLDL particle concentration less than ROSI treatment and decreased VLDL particle size more than ROSI. PIO treatment reduced total LDL particle concentration, whereas ROSI treatment increased it. Both treatments increased LDL particle size, with PIO treatment having a greater effect. Whereas PIO treatment increased total HDL particle concentration and size, ROSI treatment decreased them; both increased HDL cholesterol levels.

CONCLUSIONS — PIO and ROSI treatments have different effects on serum lipoprotein subclass particle concentrations and sizes in patients with type 2 diabetes and dyslipidemia.

Diabetes Care 30:2458–2464, 2007

From the [1]Department of Endocrinology and Metabolism, Veterans Affairs Hospital and Indiana University, Indianapolis, Indiana; the [2]Department of Endocrinology and General Medicine, University of North Carolina, Chapel Hill, North Carolina; the [3]Department of Endocrinology, Metabolism and Diabetes, University of Miami, Miami, Florida; the [4]International Diabetes Center, Park Nicollet Institute, Minneapolis, Minnesota; [5]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana; [6]Takeda Pharmaceuticals North America, Lincolnshire, Illinois; and [7]Takeda Global Research and Development, Lincolnshire, Illinois.

Address correspondence and reprint requests to Meng H. Tan, MD, Lilly Research Laboratory, Eli Lilly and Company, Indianapolis, IN 46285. E-mail: tan_meng@lilly.com.

Received for publication 12 September 2006 and accepted in revised form 18 June 2007.

Published ahead of print at http://care.diabetesjournals.org on 26 June 2007. DOI: 10.2337/dc06-1903. Clinical trial reg. no. NCT00331487, clinicaltrials.org.

*A complete list of the GLAI Study Investigators can be found in the APPENDIX.

D.M.K is currently affiliated with Amylin Pharmaceuticals, San Diego, California.

M.A.D. has received honoraria from Takeda. J.B.B. has received consulting fees from Eli Lilly, LipoScience, Lilly, and Takeda. R.B.G. has received honoraria from Lilly, Takeda, Pfizer, Merck, Merck Schering Plough, KOS, Astra Zeneca, and Abbott; has received grant/research support from Novo Nordisk, Pfizer, Merck, KOS, Astra Zeneca, and Sankyo; and has received consulting fees from Lilly and Takeda, Pfizer, Merck, Merck Schering Plough, Astra Zeneca, and Abbott.

Abbreviations: Apo, apolipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IDL, intermediate-density lipoprotein; NMR, nuclear magnetic resonance; OAM, antihyperglycemic medication; PIO, pioglitazone; ROSI, rosiglitazone; TZD, thiazolidinedione.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Two core metabolic defects contribute to the development of type 2 diabetes: insulin resistance and insulin insufficiency. Insulin resistance, present in most of these patients (1), is associated with a cluster of abnormalities that increase the risk for cardiovascular disease, including dyslipidemia (2,3) characterized by elevated triglycerides, decreased HDL cholesterol, and a predominance of small LDL particles (4–7). A major contributor to this hypertriglyceridemia is hepatic overproduction of triglyceride-rich VLDL and apolipoprotein (apo) B caused by insulin resistance and increased availability of free fatty acid substrate (8,9). Insulin suppresses large VLDL and apoB release in healthy humans (10) but not in patients with type 2 diabetes, resulting in hypertriglyceridemia (11). Decreased lipoprotein lipase activity in fat and skeletal muscle contributes to the reduced clearance of triglyceride-rich lipoproteins (10,11). The exchange of triglycerides in the VLDL particles with the cholesteryl esters in LDL and HDL particles (via the cholesteryl ester transfer protein system) leads to accumulation of small LDL and HDL particles, respectively (12). The mean particle size of VLDL increases and the LDL and HDL particle sizes decrease as insulin resistance worsens in nondiabetic subjects and type 2 diabetic patients (7).

By targeting insulin resistance, the thiazolidinedione (TZD) class of oral antihyperglycemic medications (OAMs) can affect glucose and lipoprotein metabolism. Pioglitazone hydrochloride (PIO) (Actos; Takeda Pharmaceuticals North America, Lincolnshire, IL) and rosiglita-

zone maleate (ROSI) (Avandia; GlaxoSmithKline, Research Triangle Park, NC) are the currently available TZDs for the treatment of type 2 diabetes.

The many reports that suggest that PIO has different effects from ROSI on lipid parameters in type 2 diabetic patients were detailed in our recent report on the first multicenter, prospective, randomized, double-blind, parallel-group comparison of maximally effective monotherapy doses of PIO and ROSI in these patients with dyslipidemia receiving no concomitant glucose-lowering or lipid-altering therapies (13). With the advent of proton nuclear magnetic resonance (NMR) spectroscopy (14), subjects with the same LDL cholesterol levels have been found to differ in their LDL particle concentration and particle size distribution (15). We now extend our first report of the GLAI Study (13) by describing the effects of PIO and ROSI on the serum lipoprotein subclass particle concentrations and sizes in a subgroup of the parent study.

RESEARCH DESIGN AND METHODS

— The study design and methods of the GLAI Study have been published (13). Briefly, inclusion criteria included patients aged ≥35 years with a diagnosis of type 2 diabetes; fasting triglyceride levels ≥150 mg/dl and <600 mg/dl; fasting LDL cholesterol levels <130 mg/dl; fasting serum C-peptide levels ≥1 ng/ml; and A1C values ≥7%, ≤11% if naïve to previous OAM therapy; or A1C values ≥7%, ≤9.5% if previously treated with OAM monotherapy. Exclusion criteria included treatment with insulin within 60 days of screening, combination OAM therapy, any lipid-altering agent, and any weight loss agent (13). This study was conducted in accordance with the Declaration of Helsinki guidelines on good clinical practice and was approved by each investigator's institutional ethical review board. All subjects gave informed consent.

Eligible patients discontinued any current OAM therapy and received oral placebo therapy throughout a 4-week, single-blind, lead-in period. Qualified personnel provided dietary counseling on the American Heart Association weight-maintaining step I diet. Randomization occurred in a stratified fashion, with four strata corresponding to previous OAM treatment (previously treated or naïve) and sex (male or female). Patients received either 30 mg PIO q.d. or 4 mg ROSI q.d. for the initial 12 weeks. For the final 12 weeks, the doses of PIO and ROSI were increased to the maximally effective doses (for monotherapy) of 45 mg q.d. or 4 mg b.i.d., respectively. In this substudy analysis, all patients who had data for baseline and end point lipoprotein subclass particle concentrations and sizes were included. The population of subjects prespecified for the analyses was all subjects randomized. We feel it is important to use this population to be consistent with the preplanned analyses and to be consistent with the intent-to-treat paradigm.

Analytical methods

The following analyses were performed by Covance Central Laboratory Services (Indianapolis, IN): fasting blood samples (after at least 10 h of fasting) were analyzed (13) for plasma glucose, A1C, serum insulin, serum triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol. AposB and A-I (Beckman IMMAGE Immunochemistry System; Beckman Instruments, Brea, CA), Lp(a) (SPQ Antibody Reagent of Diasorin, Stillwater, MN), and apoC-III (Wako Chemicals, Richmond, VA) were determined by immunoassay. Lipoprotein subclass particle concentrations and sizes were measured using NMR spectroscopy at LipoScience (Raleigh, NC) (14–16). Ten subclass categories were measured in nanometers: large VLDL (>60), medium VLDL (35–60), small VLDL (27–35), intermediate-density lipoprotein (IDL) (23–27), large LDL (21.3–23.0), medium LDL (19.8–21.2), small LDL (18.0–19.8), large HDL (8.8–13), medium HDL (8.2–8.8), and small HDL (7.3–8.2). Homeostasis model assessment of insulin resistance (HOMA-IR) was determined as a surrogate of insulin resistance (17).The safety assessment has been previously reported (13).

Statistical methods

Baseline data are presented as means ± SD. Differences between treatment groups in demographics and baseline parameters for patients entering active drug therapy were evaluated using Fisher's exact test for categorical variables or an independent-groups *t* test for continuous variables. For the glycemic, lipid, and HOMA-IR variables, analyses of the change from baseline level were conducted on patients for whom a baseline measurement and at least one postbaseline measurement were available. The last-observation-carried-forward change from baseline level was analyzed using a fixed-effects ANOVA, with baseline level of the analyte as a covariate (ANCOVA). The ANCOVA model comprised terms for strata, geographic region in which the investigative site was located, treatment, and baseline value. The last-observation-carried-forward changes from baseline were adjusted for their baseline level using the ANCOVA model and are reported as least-squares means with 95% CIs or with the SE of the least-squares means (18,19). Treatments were compared using the least-squares means obtained from the ANCOVA model. Spearman correlation coefficients were calculated for selected variables. SAS version 8.2 (SAS Institute, Cary, NC) was used for all analyses. All tests were two sided, and results were considered statistically significant for $P < 0.05$.

RESULTS

— The patient flow through the study has been reported (13). In the PIO group 333 (of 369) patients and in the ROSI group 325 (of 366) had baseline and postbaseline lipoprotein NMR data. The distribution of patients among the various withdrawal categories was similar between treatment groups.

Baseline demographics and characteristics

As in the parent study, no statistically significant differences existed between the treatment groups with respect to demographics and baseline characteristics. Data are not repeated here, as the majority of the patients from the parent study were included in this substudy.

Glycemic, lipid, and insulin resistance variables

Baseline levels; change from baseline for fasting triglycerides; total, LDL, and HDL cholesterol; free fatty acids; A1C; fasting plasma glucose; fasting serum insulin; and HOMA-IR for each group, and the comparison of changes in these parameters between groups, are similar (Table 1) to those in the parent study (13) with one exception. At baseline, all parameters were similar except for fasting triglycerides, which was significantly higher in the PIO group than in the ROSI group in this substudy. As reported previously (13), triglycerides decreased significantly in the PIO group, while it increased (not significantly in this substudy analysis) in the ROSI group. The change in triglycerides from baseline was significantly different between the two treatments. Both treatments significantly increased total and

Table 1—*Baseline and change from baseline of lipid, glycemic, and insulin variables*

	PIO (n = 333)			ROSI (n = 325)			Least-squares mean (95% CI) between groups
	n	Baseline	Change from baseline	n	Baseline	Change from baseline	
Triglycerides (mg/dl)	331	255.3 ± 151.1	−46.7 (−62.5 to −31.0)*	324	230.8 ± 119.8†	12.3 (−3.5 to 28.1)	−59.0 (−79.4 to −38.7)‡
Total cholesterol (mg/dl)	331	193.5 ± 31.4	9.6 (5.8–13.5)*	324	192.7 ± 32.5	28.5 (24.6 to 32.3)*	−18.9 (−23.8 to −13.9)‡
LDL cholesterol (mg/dl)	332	107.2 ± 25.7	12.5 (9.3–15.7)*	324	109.3 ± 25.8	21.4 (18.1 to 24.6)*	−8.9 (−13.1 to −4.7)‡
HDL cholesterol (mg/dl)	331	38.7 ± 9.8	5.2 (4.2–6.1)*	324	39.9 ± 10.5	2.3 (1.4 to 3.3)*	2.9 (1.6–4.1)‡
Free fatty acids (mEq/dl)	331	0.64 ± 0.28	−0.11 (−0.14 to −0.08)*	324	0.62 ± 0.29	−0.12 (−0.15 to −0.09)*	0.00 (−0.04 to 0.04)
A1C (%)	330	7.6 ± 1.2	−0.75 (−0.9 to −0.6)*	320	7.5 ± 1.1	−0.64 (−0.7 to −0.5)*	−0.12 (−0.3 to 0.0)
Fasting plasma glucose (mg/dl)	332	179.6 ± 59.9	−33.5 (−38.1 to −28.9)*	324	175.7 ± 55.9	−37.2 (−41.8 to −32.6)*	3.7 (−2.2 to 9.6)
Fasting serum insulin (units/ml)	331	20.0 ± 20.0	−4.8 (−5.8 to −3.8)*	324	18.2 ± 14.9	−4.9 (−5.9 to −3.9)*	0.06 (−1.2 to 1.4)
HOMA-IR		8.3 ± 6.6	−2.9 (−3.4 to −2.5)*		7.9 ± 7.5	−3.2 (−3.6 to −2.7)*	0.22 (−0.3 to 0.8)

Data are means ± SD or least squares means (95% CI). *P value <0.001 for within-group change from baseline. †P value = 0.02 for between-group baseline comparisons. ‡P value <0.001 for between-group differences.

LDL cholesterol; however, PIO caused smaller increases. Both treatments significantly increased HDL cholesterol, with PIO resulting in a significantly greater increase. Compared with their respective baseline values, which did not differ between treatment groups, both treatments significantly decreased A1C, fasting plasma free fatty acids, fasting plasma glucose, fasting serum insulin, and HOMA-IR, resulting in similar end point values for both and no significant differences between treatments. Body weight changes from baseline were similar for PIO (2.0 ± 0.2 kg) and ROSI (1.6 ± 0.2 kg).

Particle concentration changes

Table 2 shows the baseline, change from baseline for each group, and a comparison of these changes between them for each subfraction particle concentration.

VLDL

Compared with their respective baseline values, PIO treatment significantly decreased large VLDL particle concentration but caused no change in total, medium, or small VLDL particles, whereas ROSI treatment significantly increased the total,

Table 2—*Lipoprotein subclasses particle concentration*

	PIO (n = 333)		ROSI (n = 325)		Least-squares mean between groups (95% CI)
	Baseline	Change from baseline	Baseline	Change from baseline	
VLDL particles (nmol/l)					
Total	82.8 ± 27.0	1.7 (−1.6 to 5.0)	81.6 ± 25.1	14.8 (11.4–18.1)*	−13.1 (−17.4 to −8.8)†
Large	10.6 ± 9.8	−2.7 (−3.7 to −1.8)*	9.2 ± 7.9	1.1 (0.2–2.1)‡	−3.8 (−5.0 to −2.6)†
Medium	35.3 ± 23.8	2.4 (−0.5 to 5.2)	35.5 ± 22.0	12.4 (9.6–15.3)*	−10.1 (−13.7 to −6.4)†
Small	37.0 ± 25.3	2.0 (−0.9 to 4.8)	37.0 ± 23.4	1.3 (−1.6 to 4.2)	0.7 (−3.0 to 4.3)
IDL particles (nmol/l)	36.6 ± 52.2	−6.8 (−13.3 to −0.2)‡	32.4 ± 47.8	14.70 (8.1–21.3)*	−21.50 (−30.0 to −13.0)†
LDL particles (nmol/l)					
Total	1,393.8 ± 360.7	−49.2 (−91.0 to −7.4)‡	1,368.2 ± 372.0	111.7 (69.4–154)*	−160.9 (−215 to −107)†
Large	182.6 ± 211.1	161.4 (130.7 to 192.1)*	194.7 ± 223.1	141.8 (110.9–172.8)*	19.6 (−20.2 to 59.4)
Small	1,175.1 ± 433.8	−200.9 (−256 to −146)*	1,141.6 ± 428.8	−38.8 (−93.9 to 16.4)	−162.2 (−233 to −91.4)†
HDL (μmol/l)					
Total	30.8 ± 6.1	0.8 (0.2–1.4)‡	30.5 ± 5.6	−0.4 (−1.0 to 0.2)	1.2 (0.4–1.9)§
Large	3.7 ± 2.2	0.6 (0.4–0.8)*	3.8 ± 2.3	−0.4 (−0.6 to −0.2)‡	1.0 (0.7–1.3)†
Medium	4.9 ± 3.9	1.7 (1.3–2.2)*	5.2 ± 4.3	4.0 (3.6–4.5)*	−2.3 (−2.9 to −1.7)†
Small	22.2 ± 6.1	−1.6 (−2.3 to −0.8)*	21.5 ± 5.7	−4.1 (−4.8 to −3.3)*	2.5 (1.6–3.5)†

Data are means ± SD or least squares means (95% CI). *P value <0.001 for within-group change from baseline. †P value <0.001 for between-group differences. ‡P value <0.05 for within-group change from baseline. §P value <0.05 for between-group differences.

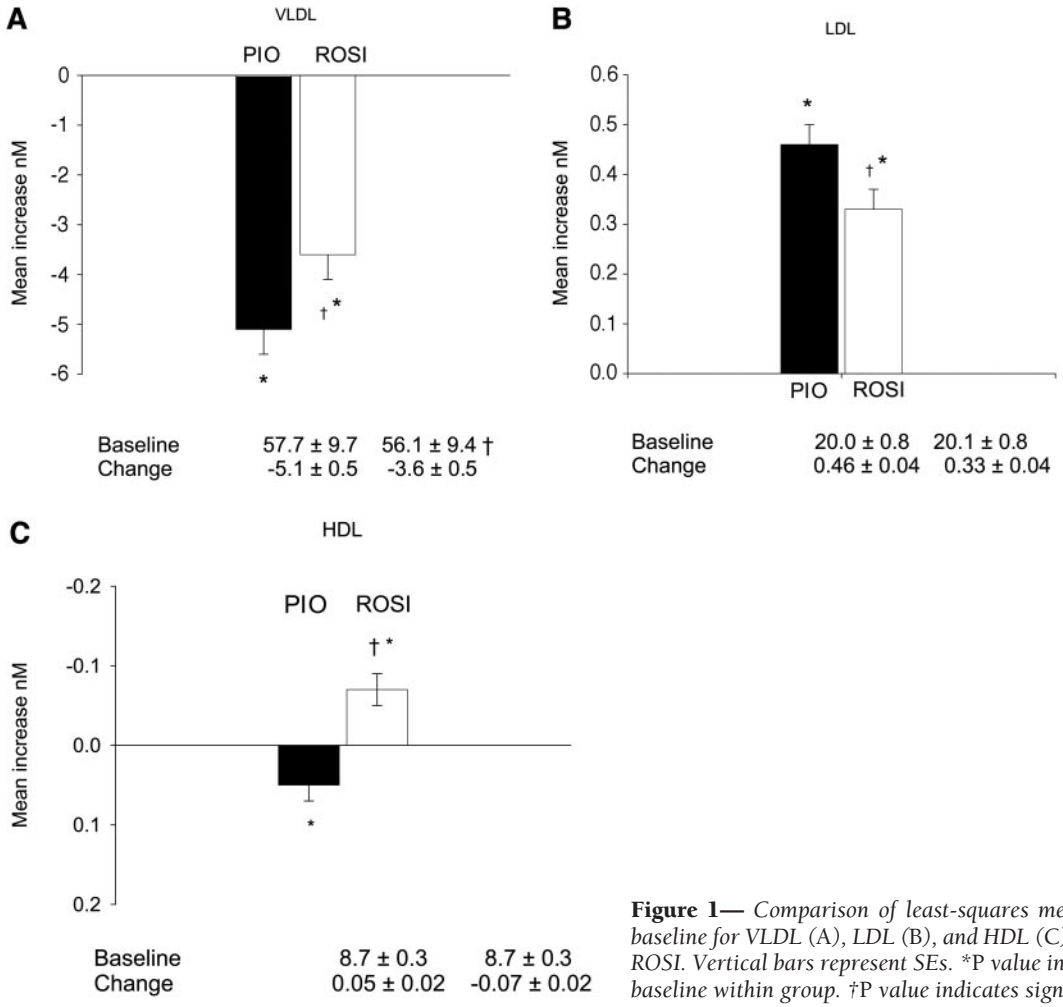

Figure 1— *Comparison of least-squares mean changes in particle size from baseline for VLDL (A), LDL (B), and HDL (C) for patients treated with PIO and ROSI. Vertical bars represent SEs. *P value indicates significant difference from baseline within group. †P value indicates significant difference between groups.*

large, and medium particle concentrations but did not change the small VLDL particles. In comparing the two treatments, the differences were significant for total, large, and medium VLDL particle concentrations but not for small VLDL particles.

IDL

PIO treatment significantly decreased IDL particle concentration from baseline, whereas ROSI treatment increased it. The between-group difference was statistically significant.

LDL

From baseline to end point, PIO treatment significantly decreased total and small, but increased large, LDL particle concentrations, whereas ROSI treatment significantly increased total and large, but caused no change in the small, LDL particle concentration. The differences between the treatments were significant for total and small LDL particles but not for large LDL particles.

HDL

Compared with their respective baseline values, PIO treatment significantly increased total, large, and medium HDL but decreased small HDL particle concentrations, whereas ROSI treatment had no effect on total HDL, significantly increased medium HDL, and decreased large and small HDL particle concentrations. The differences between the treatments were significant for total, large, medium, and small HDL particles.

Particle size changes

Figure 1 shows the mean baseline and change from baseline for particle size of each lipoprotein class. The baseline mean VLDL particle size was larger in the PIO treatment than in the ROSI treatment group (Fig. 1*A*). Both treatments decreased VLDL particle sizes from baseline; however, PIO treatment decreased the mean VLDL particle size more than ROSI. The mean LDL particle sizes at baseline were comparable in both groups (Fig. 1*B*). PIO and ROSI treatments significantly increased mean LDL particle size; however, PIO treatment had a greater effect. The mean HDL particle sizes were comparable in both groups at baseline (Fig. 1*C*). There were small, but statistically significant, changes in mean HDL particle size from baseline for each group, and the difference between them was significant.

Correlation between HOMA-IR and lipoprotein particle size

There was a negative correlation between HOMA-IR and LDL particle size in the PIO and ROSI groups and in the total study population (PIO: $r = -0.249$, ROSI: $r = -0.267$, and combined: $r = -0.252$; all values $P < 0.0001$). There was also a negative correlation between HOMA-IR and HDL particle size in all three groups (PIO: $r = -0.171$, $P < 0.002$; ROSI: $r = -0.181$, $P = 0.001$; and combined: $r = -0.165$, $P < 0.0001$). There was a positive correlation between HOMA-IR and VLDL particle size in all three groups (PIO: $r = 0.306$, ROSI: $r =$

0.374, and combined: $r = 0.339$; all values $P < 0.001$).

Serum apos

PIO treatment significantly decreased apoC-III (baseline 19.3 ± 7.4 mg/dl; change from baseline −1.5 mg/dl [95% CI: −2.1 to −1.0]) and ROSI increased apoC-III (baseline 18.2 ± 6.7 mg/dl; change from baseline 1.0 mg/dl [0.4–1.6]). At baseline, PIO-treated subjects had mean apoB levels of 105.0 ± 19.9 mg/dl, with a nonsignificant change of −0.05 mg/dl (−2.4 to 2.3) during treatment. Baseline apoB was 104.0 ± 19.3 mg/dl in the ROSI-treated subjects, with a significant increase of 10.5 mg/dl (8.1–12.8) during treatment. PIO treatment had no effect on apoA-I level during treatment (baseline apoA-I: 113.8 ± 23.0 mg/dl; change from baseline 1.4 mg/dl [−0.6 to 3.4]), whereas ROSI treatment significantly decreased apoA-I (baseline 113.7 ± 22.0 mg/dl; change from baseline −5.6 mg/dl [−7.6 to −3.6]). At baseline, PIO-treated subjects had mean Lp(a) levels of 18.9 ± 22.4 mg/dl, with a significant increase of 5.6 mg/dl (4.3–6.9) during treatment. Baseline Lp(a) was 19.9 ± 23.7 mg/dl in the ROSI-treated subjects, with a significant increase of 2.8 mg/dl (1.5–4.1) during treatment. Differences between treatments for apoC-III, apoB, apoA-I, and Lp(a) were all significant.

CONCLUSIONS — This study is the first to demonstrate that PIO and ROSI treatments result in significantly different effects on serum lipoprotein subclass particle concentrations and sizes despite similar effects on glycemic control and insulin resistance. These observations were made utilizing NMR spectroscopy, a novel technique that permits evaluation of these important characteristics of lipoproteins, and as such has advanced our understanding of the changes in serum lipoprotein subclass particle concentration and size under normal conditions (16,20), in specific disease states (7,21–23), and during treatment with specific drug therapies (24–26).

Garvey et al. (7) reported that all three major lipoprotein subclasses differed significantly as a function of insulin sensitivity in insulin-resistant nondiabetic subjects and type 2 diabetic patients. Mean particle size of VLDL increased and the LDL and HDL particle sizes decreased as insulin resistance worsened. The dyslipidemia in type 2 diabetic patients primarily reflected an exacerbation of the changes observed in nondiabetic subjects with insulin resistance. There was a negative correlation between insulin sensitivity and VLDL particle size and a positive correlation between insulin sensitivity and LDL and HDL particle sizes. We corroborated their findings in reporting a positive correlation between HOMA-IR and VLDL particle size and a negative correlation between HOMA-IR and LDL as well as HDL particle size. However, the correlation between insulin resistance and lipoprotein particle sizes, although significant, was modest for VLDL and small for LDL and HDL.

Many studies (13) showed that PIO treatment decreases fasting triglyceride, whereas ROSI treatment increases it. One possible explanation for the difference in action of the two treatments may be their effects on VLDL particle concentration and size. Our study demonstrated that PIO treatment decreased and ROSI treatment increased large VLDL particle concentration. PIO treatment resulted in no increase in the medium VLDL particle size and concentration, whereas ROSI treatment increased them, and PIO treatment decreased mean VLDL particle size to a greater extent than ROSI treatment. Additionally, these treatments exerted differential effects on apoC-III, an inhibitor of lipoprotein lipase–mediated lipolysis and remnant clearance (27). In our study, PIO treatment resulted in a decrease in apoC-III, whereas ROSI treatment caused an increase in apoC-III, which is associated with a delay in clearance, especially of smaller VLDL and IDL particles. The greater increase in medium-sized VLDL particle concentration and IDL in the ROSI group may be partially explained by the apoC-III changes. Nagashima et al. (28) reported that PIO treatment reduced triglyceride levels, at least in part, by increasing fractional clearance of VLDL and triglyceride from circulation. They attributed the change to increased plasma lipoprotein lipase mass and decreased levels of plasma apoC-III and also suggested that PIO treatment, by increasing hepatic insulin sensitivity, could reduce expression of the apoC-III gene. A correlation between insulin resistance and apoC-III production in humans has been reported (29). That the reductions in insulin resistance caused by the two treatments were comparable in our study suggests that other mechanisms may contribute to the differences observed in triglyceride changes between PIO and ROSI treatments. Finally, it is possible that peroxisome proliferator–activated receptor α activity in PIO can account for the differences in triglyceride effects. However, there is no clear data to indicate that PIO has peroxisome proliferator–activated receptor α effects (30).

Previous studies, using methods other than NMR spectroscopy, have reported that PIO treatment (31–33) and ROSI treatment (34) increase LDL particle size. Our study, using NMR spectroscopy, is the first to directly compare the effects of these two treatments on LDL particle size and show that they had different effects not only on LDL particle size but also on concentration. The mechanism by which this differential effect on LDL particle size occurred is probably unrelated to the reduction of insulin resistance and glycemic improvement, as both drugs decreased insulin resistance and improved A1C similarly. These independent effects were demonstrated in a study in which metformin and PIO treatment resulted in a reduction in the small LDL subfraction, whereas there was no change with a sulfonylurea despite similar improvement in glycemia (33). Florez et al. (35) reported that apoC-III correlated inversely with LDL particle size, probably through effects on triglyceride-rich lipoprotein metabolism. The increase in total plasma and LDL apoB by ROSI treatment (34) suggested an increase in LDL particle concentration. We confirmed this with the NMR spectroscopy technique and extended this previous finding by showing that PIO treatment decreased LDL particle concentration, whereas ROSI treatment increased it. This may explain the smaller increase in LDL cholesterol occurring with PIO treatment than with ROSI treatment. Cromwell and Otvos (36) have reported that LDL particle concentration, as measured by NMR spectroscopy, was a strong, independent predictor of coronary heart disease and was more strongly associated with coronary heart disease risk than LDL particle size. Finally, the two treatments have divergent effects on IDL and remnants, both independent predictors of cardiovascular disease (37).

Freed et al. (34) reported that ROSI treatment increased HDL cholesterol predominantly by increasing the HDL_2 subclass, with minimal change in apoA-I level. We extend these findings by showing that *1*) PIO treatment raised HDL cholesterol more than ROSI treatment; *2*) PIO treatment had no effect on apoA-I, whereas ROSI treatment decreased it; *3*)

PIO treatment increased HDL particle size, whereas ROSI treatment decreased it; and *4*) there were significant differences in their treatment effects on HDL particle concentrations. The reason for these differences in the effect between the two treatments on HDL particles, in the presence of similar reductions of insulin resistance, remains to be elucidated. It is possible that PIO treatment, in causing a decrease in triglyceride and large VLDL, reduced the cholesteryl ester transfer protein–mediated exchange of VLDL, triglycerides, and HDL cholesteryl ester, resulting in significant increases in large- and medium-sized HDL particles and a reduction in small particles, leading to a net increase in size. By contrast, the trend toward an increase in triglyceride levels among ROSI-treated patients may explain why there was a decrease in large HDL particles. The increase in HDL cholesterol in the ROSI treatment group was apparently due to a reduction of smaller-sized and an increase in medium-sized HDL particles by ROSI treatment, and a qualitatively similar change was also noted for the PIO treatment group. These changes could reflect the effect of TZDs on ATP-binding cassette-1 activity, which might be expected to produce these size changes (38). Finally, there are TZD effects that do not appear to result from an improvement of insulin sensitivity. The difference between PIO and ROSI on lipids and lipoproteins may be one of these. Whether these differences are clinically relevant is not known, since evidence for cardiovascular benefit exists for interventions that may increase larger HDL_2 particles with nicotinic acid (39) as well as those that appear to increase small HDL particles with fibrates (40).

In summary, our study demonstrates that PIO treatment and ROSI treatment differ significantly in their effects not only on serum lipids but also on lipoprotein subclass particle concentrations and particle sizes. These differences were observed despite similar improvements in many nonlipid cardiovascular disease risk factors associated with insulin resistance and type 2 diabetes. Associations of lipoprotein subclass particle concentration and size have been reported. Whether these differences in lipoprotein subclass particle concentrations and sizes translate into differences for the risk of cardiovascular disease remain to be established. Further clinical trials are needed to determine whether these differences in lipoprotein particle size results in clinically meaningful differences.

Acknowledgments— This study was jointly funded by Eli Lilly and Company and Takeda Pharmaceuticals North America.

We acknowledge the efforts of the investigators of the GLAI Study (listed below); Lisa Martin; Sandra Althouse; Melvin Prince, MD; and Janet Tobian, MD; without whom this project could not have been completed.

APPENDIX — The following is a complete list of the GLAI Study Investigators: United States: Dr. Andrew J. Ahmann, Dr. Jeanine Albu, Dr. David M. Kendall, Dr. John Bernard Buse, Dr. Edward James Busick Jr, Dr. Peter J. Campbell, Dr. John V. Capotorto, Dr. John J. Cavanaugh, Dr. RoseMarie Pasmantier, Dr. A. Jay Cohen, Dr. Samuel E. Crockett, Dr. Michael H. Davidson, Dr. Mark A. Deeg, Dr. Diana G. Dills, Dr. Steven V. Edelman, Dr. Victor A. Elinoff, Dr. Keith C. Ferdinand, Dr. David Fitz-Patrick, Dr. Thomas M. Flood, Dr. Vivian Andrew Fonseca, Dr. Zachary Roy Freedman, Dr. Ronald B. Goldberg, Dr. Lisa Harris, Dr. Willa A. Hsueh, Dr. Rajeev Kumar Jain, Dr. Lois Jovanovic, Dr. Roy A. Kaplan, Dr. Robert Lang, Dr. German G. Lasala, Dr. Anne Lucia Leddy, Dr. Michael A. Lerner, Dr. Philip A. Levin, Dr. George Liu, Dr. Michael J. McCartney, Dr. Rafal Chojnacki, Dr. Edward J. Meyer, Dr. James David Mumper, Dr. Kwame Osei, Dr. David A. Price, Dr. Philip Raskin, Dr. Harvey Resnick, Dr. Wendy F. Bone, Dr. Nathaniel G. Clark, Dr. Anthony M. Roselli, Dr. Timothy R. Smith, Dr. Ralph Joe Teague, Dr. Louis H. Philipson, Dr. Joseph Ralph Tucci, Dr. Timothy Owen Wahl, Dr. Mark Lowe Warren, Dr. Jack D. Wahlen, Dr. Gary D. Yeoman, Dr. Joseph D. Krantzler, Dr. Frank N. Cole, Dr. Julio C. Pita Jr, Dr. Ayman A. Zayed, Dr. Julio Rosenstock, Dr. Victor L. Roberts, Dr. Mark S. Kipnes, Dr. Larry D. Stonesifer, Dr. Lee R.Ralph, Dr. K. Jean Lucas, Dr. Charles B. Herring, Dr. Sriranga V. Prasad, Dr. Miguel A. Franco Jr, Dr. Clifford James Houser, Dr. Arvid Lade, Dr. Richard E. Mills, Dr. Meenakshi Patel, Dr. Marina Raikhel, Dr. Rodney D. Michaels, Dr. Russell K. Havlicek, Dr. James P. Capo Jr, Dr. William Johnson Georgitis, Dr. Gregory V. Collins, Dr. John Eric Liljenquist, Dr. Kenneth G. Davis, Dr. Robert C. Hood. Puerto Rico: Dr. Elizabeth A. Barranco Santana, Dr. Vilma M. Rabell, Dr. Pedro J. Colon Ortiz, Dr. Yolanda Sierra Quinones, Dr. Miguel Sosa Padilla, Dr. Efrain Rodriguez Vigil, Dr. Shirley W. Pagan Ocasio, Dr. Jose A. Tavarez Valle, Dr. Myriam Portilla Oliva, Dr. Jorge Aguilera-Montalvo, Dr. Francisco Abreu Feshold, Dr. Luis Rodriguez Carrasquillo, Dr. Maria de Lourdes Miranda. Mexico: Dr. Maria Guadalupe Fabian San Miguel, Dr. Melchor Alpizar Salazar, Dr. Guillermo Gonzalez Galvez, Dr. Fernando Flores Lozano. Colombia: Dr. Patricio Lopez-Jaramillo, Dr. Carlos Alberto Cure, Dr. Luis Alejandro Orozco Linares, Dr. Luz Angela Casas, Dr. Monica Alexandra Terront, Dr. Diego Luis Saaibi Solano, Dr. Adriana Santamaria Cogollos.

References

1. Haffner SM, D'Agostino R Jr, Mykkanen L, Tracy R, Howard B, Rewers M Selby J, Savage PJ, Saad MF: Insulin sensitivity in subjects with type 2 diabetes: relationship to cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 22:562–568, 1999
2. Nesto RW: Correlation between cardiovascular disease and diabetes mellitus: current concepts. *Am J Med* 116 (Suppl. 5A):11S–22S, 2004
3. Hsueh WA, Lyon CJ, Quinones MJ: Insulin resistance and the endothelium. *Am J Med* 117:109–117, 2004
4. Siegel RD, Cupples A, Schaefer EJ, Wilson PW: Lipoproteins, apolipoproteins, and low-density lipoprotein size among diabetics in the Framingham Offspring Study. *Metabolism* 45:1267–1272, 1996
5. Ginsberg HN: Insulin resistance and cardiovascular disease. *J Clin Invest* 106:453–458, 2000
6. Krauss RM: Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care* 27:1496–1504, 2004
7. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, Pugh K, Jenkins AJ, Klein RL, Liao Y: Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes* 52:453–462, 2003
8. Taskinen MR, Beltz WF, Harper I, Fields RM, Schonfeld G, Grundy SM, Howard BV: Effects of NIDDM on very-low-density lipoprotein triglyceride and apolipoprotein B metabolism: studies before and after sulfonylurea therapy. *Diabetes* 35:1268–1277, 1986
9. Cummings MH, Watts GF, Umpleby AM, Hennesy TR, Naoumova R, Slavin BM, Thompson GR, Sönksen PH: Increase hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in NIDDM. *Diabetologia* 38:959–967, 1995
10. Sparks CE, Sparks JD, Bolognino M, Salhanick A, Strump PS, Amatruda JM: Insulin effects on apolipoprotein B lipoprotein

synthesis and secretion by primary cultures of rat hepatocytes. *Metabolism* 35: 1128–1136, 1986
11. Malmstrom R, Packard CJ, Caslake M, Bedford D, Stewart P, Yki-Jarvinen H, Shepherd J, Taskinen MR: Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia* 40:454–462, 1997
12. Ginsberg HN: Hypertriglyceridemia: new insights and new approaches to pharmacologic therapy. *Am J Cardiol* 87:1174–1180, 2001
13. Goldberg RB, Kendall DM, Deeg MA, Buse JB, Zagar AJ, Pinaire JA, Tan MH, Khan MA, Perez AT, Jacober SJ, the GLAI Study Investigators: A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes mellitus and dyslipidemia. *Diabetes Care* 28:1547–1554, 2005
14. Otvos JD: Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. *Clin Lab* 48:171–180, 2002
15. Jeyarajah EJ, Cromwell WC, Otvos JD: Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clinics in Lab Med* 26:847–870, 2006
16. Freedman DS, Otvos JD, Jeyerajah EJ, Shalaurova I, Cupples LA, Parise H, D'Agostino RB, Wilson PWF, Schaefer EJ: Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem* 50:1189–1200, 2004
17. Matthews DR, Hosker JB, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
18. Searle SR: *Linear Models for Unbalanced Data.* John Wiley & Sons, New York, 1987
19. Gallo PP: Practical issues in linear models analyses in multi-center clinical trials. *Biopharma Rep* 6:2–9, 1998
20. Yu HH, Ginsburg GS, O'Toole ML, Otvos JD, Douglas PS, Rifai N: Acute changes in serum lipids and lipoprotein subclasses in triathletes as assessed by proton nuclear magnetic resonance spectroscopy. *Arterioscler Thromb Vasc Biol* 19:1945–1949, 1999
21. Freedman DS, Otvos JD, Jeyarajah EJ, Barboriak JJ, Anderson AJ, Walker JA: Relation of lipoprotein subclasses as measured by proton nuclear magnetic resonance spectroscopy to coronary artery disease. *Arterioscler Thromb Vasc Biol* 18: 1046–1053, 1998
22. Goff DC, D'Agostino RB Jr, Haffner SM, Otvos JD: Insulin resistance and adiposity influence lipoprotein size and subclass concentrations: results from the Insulin Resistance Atherosclerosis Study. *Metabolism* 54:264–270, 2005
23. Festa A, Williams K, Hanley AJG, Otvos JD, Goff DC, Wagenknecht LE, Haffner SM: Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 111:3465–3472, 2005
24. Suter PM, Marmier G, Veya-Linder C, Hanseler E, Lentz J, Vetter W, Otvos J: Effect of orlistate on postprandial lipemia, NMR lipoprotein subclass profiles and particle size. *Atherosclerosis* 180:127–135, 2005
25. Ikewaki K, Tohyama J, Nakata Y, Wakikawa T, Kido T, Mochizuki S: Fenofibrate effectively reduces remnants, and small dense LDL, and increases HDL particle number in hypertriglyceridemic men: a nuclear magnetic resonance study. *J Atheroscler Thromb* 11:278–285, 2004
26. McKenney JM, McCormick LS, Schaefer EJ, Black DM, Watkins ML: The effect of niacin and atorvastatin on lipoprotein subclasses in patients with atherogenic dyslipidemia. *Am J Cardiol* 88:270–274, 2001
27. Shachter NS: Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. *Curr Opin Lipidol* 12:297–230, 2001
28. Nagashima K, Lopez C, Donovan D, Ngai C, Fontanez N, Bensadoun A, Fruchart-Najib J, Holleren S, Cohn JS, Ramakrishnan R, Ginsberg HN: Effects of the PPARγ agonist pioglitazone on lipoprotein metabolism in patients with type 2 diabetes mellitus. *J Clin Invest* 115:1323–1332, 2005
29. Cohn JS, Patterson BW, Uffelman KD, Davignon J, Steiner G: Rate of production of plasma and very-low-density ipoproteins (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. *J Clin Endocrinol Metab* 89:3949–3955, 2004
30. Hirakata M, Tozawa A, Imura Y, Sugiyama Y: Erratum to "Comprison of the effects of pioglitazone and rosiglitazone on macrophage foam cell formation" [*Biochem Biophys Res Commun* 323:782–788, 2004]. *Biochem Biophys Res Commun* 327: 967–968, 2005
31. Winkler K, Konrad T, Fullert S, Friedrich I, Destani R, Baumstark MW, Krebs K, Wieland H, Marz W: Pioglitazone reduces atherogenic dense LDL particles in nondiabetic patients with arterial hypertension: a double-blind, placebo-controlled study. *Diabetes Care* 26:2588–2594, 2003
32. Winkler K, Friedrich I, Baumstark MW, Wieland H, Marz W: Pioglitazone reduces atherogenic dense low density lipoprotein (LDL) particles in patients with type 2 diabetes mellitus. *Br J Diabetes Vasc Dis* 2:143–148, 2002
33. Lawrence JM, Reid J, Taylor GJ, Stirling C, Reckless JPD: Favorable effects of pioglitazone and metformin compared with gliclazide on lipoprotein subfractions in overweight patients with early type 2 diabetes. *Diabetes Care* 27:41–46, 2004
34. Freed MI, Ratner R, Marcovina SM, Kreider MM, Biswas N, Cohen BR, Brunzell JD, the Rosiglitazone Study 108 Investigators: Effects of rosiglitazone alone and in combination with atorvastatin on the metabolic abnormalities in type 2 diabetes mellitus. *Am J Cardiol* 90:947–952, 2002
35. Florez H, Mendez A, Casanova-Romero P, Larreal-Urdaneta C, Castillo-Florez S, Lee D, Goldberg RB: Increased apolipoprotein C-III levels associated with insulin resistance contribute to dyslipidemia in normoglycemic and diabetic subjects from a triethnic population. *Atherosclerosis* 188: 134–141, 2006
36. Cromwell WC, Otvos JD: Low-density lipoprotein particle number and risk for cardiovascular disease. *Curr Atheroscler Rep* 6:381–387, 2004
37. Twickler T, Dallinga-Thie GM, Chapman MJ, Cohn JS: Remnant lipoproteins and atherosclerosis. *Curr Atheroscler Rep* 7: 140–147, 2005
38. Zhang L, Chawla A: Role of PPAR gamma in macrophage biology and atherosclerosis. *Trends Endocrinol Metab* 15:500–505, 2004
39. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ: Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 345:1583–1592, 2001
40. Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ: Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation* 113:1556–1563, 2006