

Pioglitazone Reduces Atherogenic Dense LDL Particles in Nondiabetic Patients With Arterial Hypertension

A double-blind, placebo-controlled study

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OBJECTIVE — The oral antidiabetic agent pioglitazone improves insulin sensitivity and glycemic control and appears to lower atherogenic dense LDL in type 2 diabetes. Insulin resistance may occur frequently in nondiabetic patients with hypertension. This study is the first to report the effect of pioglitazone on LDL subfractions in normolipidemic, nondiabetic patients with arterial hypertension.

RESEARCH DESIGN AND METHODS — We performed a monocentric, double-blind, randomized, parallel-group comparison of 45 mg pioglitazone ($n = 26$) and a placebo ($n = 28$), each given once daily for 16 weeks. Fifty-four moderately hypertensive patients (LDL cholesterol, 2.8 ± 0.8 mmol/l; HDL cholesterol, 1.1 ± 0.3 mmol/l; triglycerides, 1.4 mmol/l [median; range 0.5–7.1]) were studied at baseline and on treatment.

RESULTS — At baseline, dense LDLs were elevated (apolipoprotein [apo]B in LDL-5 plus LDL-6 >250 mg/l) in 63% of all patients. Sixteen weeks of treatment with pioglitazone did not significantly change triglycerides, total, LDL, and HDL cholesterol. However, pioglitazone reduced dense LDLs by 22% ($P = 0.024$). The mean diameter of LDL particles increased from 19.83 ± 0.30 to 20.13 ± 0.33 nm ($P < 0.001$ vs. placebo), whereas the mean LDL density decreased from 1.0384 ± 0.0024 to 1.0371 ± 0.0024 kg/l ($P = 0.005$ vs. placebo). The effect of pioglitazone on LDL size and density was independent of fasting triglycerides and HDL cholesterol at baseline and of changes in fasting triglycerides and HDL cholesterol.

CONCLUSIONS — The prevalence of atherogenic dense LDL in nondiabetic, hypertensive patients is similar to patients with type 2 diabetes. Pioglitazone significantly reduces dense LDL independent from fasting triglycerides and HDL cholesterol. The antiatherogenic potential of pioglitazone may thus be greater than that expected from its effects on triglycerides, LDL, and HDL cholesterol alone.

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Abbreviations: apo, apolipoprotein; CAD, coronary artery disease; DBP, diastolic blood pressure; PPAR, peroxisome proliferator-activated receptor; SBP, systolic blood pressure; TZD, thiazolidinedione.

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

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Insulin resistance often occurs in nondiabetic patients with arterial hypertension, and hypertension and insulin resistance are closely related (1,2). Lipid metabolism in insulin resistance is characterized by increased triglycerides, low HDL cholesterol, only slightly elevated LDL cholesterol levels (3), and a preponderance of dense LDL (4,5) that is often referred to as the atherogenic lipoprotein phenotype (6).

A link between dense LDL and increased risk of coronary artery disease (CAD) was first proposed by Austin et al. (7). Subsequent case-control and prospective studies have shown that a preponderance of dense LDL increases the risk of CAD by up to sevenfold (6,8–14). Compared with buoyant LDL, dense LDLs exhibit reduced uptake by the LDL receptor (15), are more susceptible to oxidation, and show increased binding to proteoglycans of the vessel wall (16). Dense LDL may cause endothelial dysfunction independent of LDL cholesterol, triglycerides, and HDL cholesterol (17). There is, however, evidence that large, buoyant LDL particles may increase the risk of CAD as well (18). In fact, in 837 survivors of myocardial infarction of the Cholesterol on Recurrent Events (CARE) trial, large LDL size was an independent predictor of CAD (19), and in preeclampsia, most buoyant LDL and triglyceride-rich remnant lipoproteins were associated with elevated blood pressure (20).

Pioglitazone is an antidiabetic thiazolidinedione (TZD) compound. The TZDs act as peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists. Pioglitazone and other TZDs increase the transcription of insulin-responsive genes (21) and reduce both peripheral and hepatic insulin resistance in patients with type 2 diabetes (22,23). The improved insulin sensitivity results in lowering of blood glucose and plasma insulin (22,24). Like the other TZDs, troglitazone and rosiglitazone, pioglitazone ameliorates insulin

resistance without stimulating insulin release from pancreatic β -cells, thus lowering the risk of hypoglycemia (25).

In an observational report, the lipid effects of troglitazone, rosiglitazone, and pioglitazone were compared in a limited number of patients (26). Changes in HbA_{1c} were similar for the three agents, whereas the effects on lipoprotein metabolism were different. Pioglitazone reduced serum triglycerides and increased HDL cholesterol to a greater extent than either troglitazone or rosiglitazone. In addition, troglitazone and rosiglitazone increased LDL cholesterol, whereas pioglitazone did not (24,26). Interestingly, troglitazone was shown to decrease the proportion of dense LDL in obese subjects despite its LDL cholesterol-raising effect (27).

In an open-label multicenter study, pioglitazone reduced dense LDL in patients with type 2 diabetes (28). This study was not placebo controlled. Therefore, the present study was devised to examine the effect of pioglitazone on the LDL subfraction profile in a double-blind, randomized, parallel-group setup in hypertensive patients frequently presenting with insulin resistance and a high proportion of dense LDL.

RESEARCH DESIGN AND METHODS

Patients and study protocol

Sixty patients with moderate hypertension, as defined by the International Society of Hypertension/World Health Organization criteria (29), were enrolled in this single-center, double-blind, randomized, placebo-controlled, parallel-group, phase II study as reported previously (30). Systolic blood pressure (SBP) had to be ≥ 140 mmHg and < 180 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg and < 105 mmHg. Mean SBP of 24-h measurements > 135 mmHg and/or mean DBP > 85 mmHg (29). SBP/DBP values at baseline were $148 \pm 12/91 \pm 10$ mmHg and $144 \pm 14/88 \pm 11$ mmHg for the placebo and the pioglitazone group, respectively. Antihypertensive medication was equally distributed in both study groups. Patients were receiving mainly ACE inhibitors, diuretics, and calcium antagonists, and there were no β -blockers prescribed. Medication other than the study medication was not altered throughout the study. Patients at baseline

were insulin resistant, as assessed by the homeostasis model assessment index from fasting glucose [mg/dl] \times fasting insulin [mU/l]/22.5 (31) (in this subgroup analysis 56 ± 23.6 and 60.8 ± 64.6 for placebo and pioglitazone, respectively, and as described previously (30). Four patients did not complete laboratory assessments, and two patients presenting with type III hyperlipoproteinemia were excluded.

After a run-in period of 1 week, 19 women and 35 men between 34 and 67 years of age were randomized to receive either verum capsules containing 45 mg pioglitazone (Actos TM; Takeda, Aachen, Germany), agglomerated lactose, corn starch, and magnesium stearate ($n = 26$) or matching placebo capsules containing agglomerated lactose, corn starch, and magnesium stearate only ($n = 28$). The study medication was given once daily at breakfast in addition to the previous antihypertensive medication (30) to assess the effect on LDL subfractions.

Female patients were postmenopausal, hysterectomized, surgically sterilized, or using appropriate contraceptive methods. None of the postmenopausal women were on hormone replacement therapy. BMI had to be ≥ 20 and ≤ 35 kg/m². Exclusion criteria included known diabetes, any history of unstable angina pectoris, or any other severe heart disease.

The study protocol was approved by the Ethics Committee of the Johann Wolfgang Goethe University, Frankfurt. All patients gave written informed consent.

Laboratory assessments

At baseline and after 16 weeks of active treatment, fasting venous blood samples were drawn and immediately delivered for biochemistry. Samples for the determination of LDL subfractions were stored for up to 1 week at 4°C before lipoprotein separation. Previous experiments indicated that lipid and lipoprotein measurements were not affected by these conditions (32,33).

Lipoprotein separation

Lipoproteins were isolated by sequential preparative ultracentrifugation using the following densities: < 1.006 kg/l for VLDL, 1.006 – 1.019 kg/l for intermediate density lipoproteins, 1.019 – 1.063 kg/l for LDL, and 1.063 – 1.21 kg/l for HDL. LDL subfractions were separated according to Baumstark et al. (34): total LDLs

(1.019 – 1.063 kg/l) were fractionated into six density classes by equilibrium density gradient centrifugation. Density ranges of the subfractions were: LDL-1, < 1.031 kg/l; LDL-2, 1.031 – 1.034 kg/l; LDL-3, 1.034 – 1.037 kg/l; LDL-4, 1.037 – 1.040 kg/l; LDL-5, 1.040 – 1.044 kg/l; and LDL-6, > 1.044 kg/l. Atherogenic LDL-5 and LDL-6 are summarized as dense LDL (33,35). All centrifugation steps were carried out at 18°C using partially filled polycarbonate bottles (6 ml) in a 50-Ti rotor.

Separation at 18°C did not affect LDL composition. LDL from six healthy volunteers were isolated by ultracentrifugation at 4°C, where no enzyme-induced changes of lipoprotein composition take place, and compared with LDL isolated with the standard procedure at 18°C. Differences between separations at 18°C and 4°C of total cholesterol/apolipoprotein (apo) B, cholesterol esters/apoB, and free cholesterol/apoB ratios of LDL were 1.372 ± 1.256 , 0.351 ± 1.435 , and $4.239 \pm 1.271\%$, respectively. LDL size was calculated as described below. Separation at 18 and 4°C resulted in a difference of LDL size of $0.395 \pm 0.385\%$. Storage time at 4°C before ultracentrifugation did also not influence lipoprotein composition. Samples were analyzed in the presence or absence of 1.4 mmol/l 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman's reagent), an lecithin cholesterol acyltransferase inhibitor (36). Samples were ultracentrifuged right away and after 3 and 7 days of storage at 4°C. The ratio of free cholesterol to total cholesterol was determined in total LDL, LDL-1 through LDL-6, and HDL. Differences between blank and lecithin cholesterol acyltransferase inhibited samples were $< 5\%$ in all lipoprotein fractions, irrespective of storage time at 4°C.

Recoveries of cholesterol after centrifugation of all lipoproteins were $> 95\%$ and of LDL subfractions were $> 94\%$. The interassay coefficient of variance of the determination of apoB in each of the six LDL subfractions was $\leq 5\%$ (32).

Lipoprotein chemistry

Cholesterol and triglycerides were determined enzymatically with the cholesterol oxidase-peroxidase amino phenazone phenol (CHOD-PAP) and the glycerol-3-phosphate oxidase-peroxidase amino phenazone phenol (GPO-PAP) method (Roche Diagnostics, Mannheim, Germany), respectively. Free cholesterol and

Table 1—Summary of demographic and clinical characteristics and changes in BMI, fasting glucose, and insulin

| Variable | Pioglitazone (n = 26) | | | Placebo (n = 28) | | | P value |
|--------------------------|-----------------------|------------|----------|------------------|------------|----------|---------|
| | Baseline | Study end | % Change | Baseline | Study end | % Change | |
| Age (years) | 52.7 ± 8.3 | — | — | 54.6 ± 9.6 | — | — | — |
| Sex (female/male) | 9/17 | — | — | 10/18 | — | — | — |
| BMI (kg/m ²) | 29.1 ± 4.0 | 29.2 ± 4.0 | +0 | 27.6 ± 3.4 | 26.5 ± 5.0 | −4 | NS |
| Fasting glucose (mmol/l) | 5.4 ± 0.9 | 5.1 ± 0.5 | −6 | 5.7 ± 0.9 | 5.7 ± 0.6 | −0 | 0.049 |
| Insulin (mU/ml) | 13.0 ± 11.0 | 8.6 ± 4.6 | −34 | 12.0 ± 4.0 | 11.4 ± 6.0 | −5 | NS |

Data are means ± SD. P values were calculated by Mann-Whitney U test for differences in percent change between the pioglitazone and the placebo group. NS, not significant.

phospholipids were determined enzymatically with the cholesterol oxidase-peroxidase amino phenazone phenol (COD-PAP) method and by phospholipase D, cholineoxidase, and peroxidase, respectively, with commercially available reagents (Wako Chemicals, Osaka, Japan). The concentration of esterified cholesterol was calculated from the difference of cholesterol and free cholesterol. Concentrations of apolipoproteins were determined by turbidimetry on a Wako 30R analyzer (Wako Chemicals) using polyclonal antisera (Rolf Greiner Biochemica, Flacht, Germany) specific for the respective antigens.

Mean LDL diameter

The mean diameter of LDL was calculated using the molar concentrations of free cholesterol, esterified cholesterol, phospholipids, triglycerides, and apoB-100 in the LDL fraction (1.019–1.063 kg/l) as validated by X-ray small-angle scattering

(34). The intra-assay coefficient of variance of the mean LDL diameter was ~5%.

Mean LDL density

The mean density of total LDL was calculated as the weighted (by apoB-100 content) mean of the densities of each of the LDL subfractions (28) according to the following equation: mean LDL density = (apoB in LDL-1 × 1.025 + apoB in LDL-2 × 1.0325 + apoB in LDL-3 × 1.0355 + apoB in LDL-4 × 1.0385 + apoB in LDL-5 × 1.042 + apoB in LDL-6 × 1.0535)/ (apoB in total LDL) [kg/l]. The intra-assay coefficient of variance of the mean LDL diameter was ~5%.

Statistical analysis

Changes in lipid and lipoprotein levels between baseline (week 0) and week 16 were compared between treatment groups using the nonparametric Wilcoxon signed-rank test for paired observations or the Mann-Whitney U test, as

indicated. A general linear model with sex as a cofactor was applied to test whether sex may have an influence on treatment effects. A general linear model was also used to assess whether triglycerides and HDL cholesterol influenced the effects of pioglitazone (versus placebo) on LDL diameter and LDL density. Triglycerides and HDL cholesterol (baseline values and percent change) were normalized by logarithmic transformation. Percentage changes of LDL diameter and density were used as dependent variables, treatment group was used as a fixed factor, and triglycerides and HDL cholesterol (baseline concentrations or percent changes) were used as covariates. Changes were considered statistically significant if *P* < 0.05. All calculations were performed using SPSS for Windows (version 11.0).

RESULTS — In this study, 63% of the patients with moderate hypertension had notable levels of dense LDL as defined

Table 2—Effect of pioglitazone on mean lipoprotein and apolipoprotein levels in hypertensive patients

| Variable | Pioglitazone (n = 26) | | | Placebo (n = 28) | | | P value |
|------------------------------|-----------------------|-------------|----------|------------------|-------------|----------|---------|
| | Baseline | Study end | % Change | Baseline | Study end | % Change | |
| Total cholesterol (mmol/l) | 5.2 ± 0.9 | 5.2 ± 0.9 | −0 | 5.1 ± 1.0 | 5.1 ± 1.0 | −0 | NS |
| Total triglycerides (mmol/l) | 1.09 | 1.36 | +25 | 1.42 | 1.52 | +7 | NS |
| (range) | (0.58–7.13) | (0.73–4.17) | | (0.54–5.64) | (0.43–9.53) | | |
| LDL cholesterol (mmol/l) | 2.8 ± 0.8 | 2.8 ± 0.6 | −0 | 2.8 ± 0.8 | 2.6 ± 0.8 | −7 | NS |
| HDL cholesterol (mmol/l) | 1.2 ± 0.3 | 1.3 ± 0.3 | +8 | 1.1 ± 0.3 | 1.1 ± 0.3 | −0 | NS |
| Total apoA-I (g/l) | 1.44 ± 0.22 | 1.36 ± 0.24 | −6 | 1.30 ± 0.38 | 1.34 ± 0.30 | +3 | NS |
| Total apoA-II (g/l) | 0.49 ± 0.10 | 0.51 ± 0.11 | +4 | 0.46 ± 0.09 | 0.45 ± 0.08 | −2 | 0.021 |
| Total apoB (g/l) | 1.09 ± 0.23 | 1.00 ± 0.26 | −8 | 1.03 ± 0.33 | 1.06 ± 0.25 | +3 | NS |
| Total apoC-II (mg/l) | 40 ± 17 | 47 ± 19 | +18 | 46 ± 20 | 46 ± 22 | +0 | 0.010 |
| Total apoC-III (mg/l) | 120 ± 40 | 126 ± 36 | +5 | 129 ± 46 | 125 ± 49 | −3 | NS |
| Total apoE (mg/l) | 29 ± 8 | 29 ± 7 | −0 | 32 ± 9 | 34 ± 12 | +6 | NS |
| ApoB in LDL (mg/l) | 824 ± 192 | 767 ± 171 | −7 | 803 ± 210 | 796 ± 210 | −1 | NS |
| ApoB in dLDL (mg/l)* | 305 ± 141 | 237 ± 140 | −22 | 307 ± 116 | 283 ± 132 | −8 | 0.024 |

Data are mean ± SD or median (range). *LDL-5 plus LDL-6. P values were calculated by Mann-Whitney U test for differences in percent change between the pioglitazone and the placebo group.

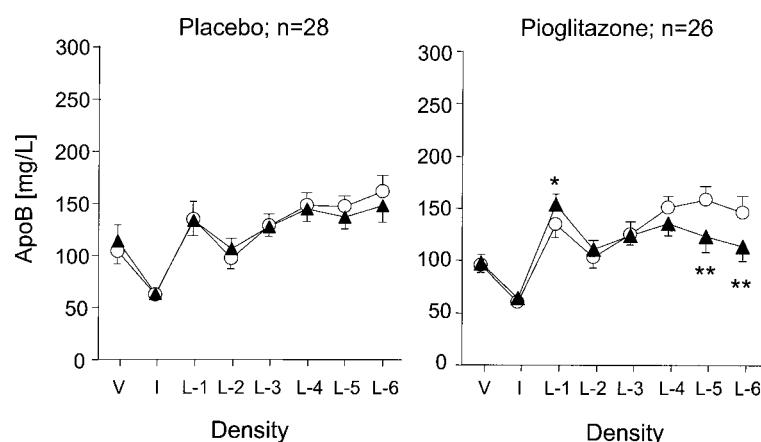


Figure 1—Mean change in subfractions of LDL following 16 weeks of treatment with 45 mg pioglitazone daily. ○, baseline values; ▲, values after 16 weeks of treatment. * $P < 0.05$ and ** $P < 0.01$ for changes between baseline and end of 16 weeks of treatment (Wilcoxon signed-rank test). Error bars represent SEM.

previously (35). All patients had similar clinical characteristics and lipid and lipoprotein concentrations at baseline (Tables 1 and 2).

The mean age of all patients was 53.7 years, and BMI was 28.3 kg/m^2 .

Fasting glucose was lowered by 6% during pioglitazone treatment and remained within the reference range in both groups during the study, and insulin levels decreased by 34%, although changes in insulin concentrations were not significant versus placebo (Table 1).

Total cholesterol, triglycerides, LDL and HDL cholesterol, and apoB in LDL did not change significantly. The concentrations of apoA-II and apoC-II increased, and the concentration of apoB in LDL decreased, although the reduction of LDL apoB was not significant between the treatment groups (Table 2).

Each apoB-containing lipoprotein particle (VLDL, intermediate density lipoproteins, and LDL) possesses one molecule of apoB. Therefore, the concentration of apoB in each density fraction is a measure of the number of lipoprotein particles present in that fraction. Pioglitazone significantly reduced apoB in LDL-5 and LDL-6 (dense LDL) and slightly increased apoB in LDL-1 (Fig. 1). Absolute concentrations of apoB in dense LDL (LDL-5 plus LDL-6) were reduced by 22% ($P = 0.024$, compared with placebo) (Table 2). This resulted in a decrease of mean LDL density from 1.0384 ± 0.0024 to $1.0371 \pm 0.0024 \text{ kg/l}$ (placebo, 1.0389 ± 0.0030 to $1.0385 \pm 0.0030 \text{ kg/l}$; $P = 0.005$ vs. placebo); the mean diameter of LDL particles

in the pioglitazone group increased from 19.83 ± 0.30 to $20.13 \pm 0.33 \text{ nm}$ (placebo, 19.83 ± 0.37 to $19.79 \pm 0.47 \text{ nm}$; $P < 0.001$ vs. placebo).

If only those patients with triglycerides $<150 \text{ mg/dl}$ ($n = 18$ in both groups) were considered, LDL diameter in the placebo group was $19.93 \pm 0.33 \text{ nm}$ at baseline and $19.94 \pm 0.34 \text{ nm}$ at study end, respectively. LDL diameter in the pioglitazone group was $19.90 \pm 0.30 \text{ nm}$ and increased to $20.19 \pm 0.33 \text{ nm}$ ($P = 0.014$ vs. placebo). To assess whether triglycerides and HDL cholesterol influenced the effects of pioglitazone (versus placebo) on LDL diameter and LDL density, a general

linear model was used. The effect of pioglitazone compared with placebo on LDL size and LDL density was independent of triglycerides and HDL cholesterol at baseline and of changes in triglycerides and HDL cholesterol (Table 3).

We also applied a general linear model to assess whether sex may have had an influence on treatment effects. Differences between pioglitazone and placebo were still significant if sex was included as cofactor. The sex-corrected P values for comparisons between treatment groups of percent changes of LDL diameter and LDL density were <0.001 and 0.019 , respectively. Therefore, sex did not affect changes in LDL diameter and LDL density.

None of the patients in the study experienced any serious drug-related adverse event. γ -Glutamyl transferase, hepatic aspartate aminotransferase, hepatic alanine aminotransferase, and plasma creatine phosphokinase activities remained $<3\times$ the upper limit of normal range in all patients on all occasions during the study. The activities of liver enzymes actually dropped; the decreases in the activities of γ -glutamyl transferase ($P < 0.001$) and the liver-specific enzyme alanine aminotransferase ($P = 0.027$) were significant versus placebo (data not shown).

CONCLUSIONS — The current study provides two major findings. First, nondiabetic, normolipidemic patients with hypertension have a prevalence of dense

Table 3—Influence of triglycerides and HDL cholesterol (baseline or percent change) on changes (percent) of LDL diameter and LDL density (pioglitazone versus placebo)

| | Difference between baseline and study end (EMM \pm SEM) | | P value |
|--|---|----------------------|----------|
| | Pioglitazone (n = 26) | Placebo (n = 28) | |
| Without adjustment | | | |
| LDL diameter (percent change) | 1.498 ± 0.280 | -0.227 ± 0.270 | <0.001 |
| LDL density (percent change) | -0.134 ± 0.029 | -0.0332 ± 0.028 | 0.018 |
| Adjusted for baseline HDL and triglycerides | | | |
| LDL diameter (percent change) | 1.498 ± 0.279 | -0.227 ± 0.268 | <0.001 |
| LDL density (percent change) | -0.130 ± 0.29 | -0.0366 ± 0.028 | 0.025 |
| Adjusted for percent change of HDL and triglycerides | | | |
| LDL diameter (percent change) | 1.523 ± 0.289 | -0.251 ± 0.278 | <0.001 |
| LDL density (percent change) | -0.123 ± 0.026 | -0.04339 ± 0.025 | 0.036 |

Triglycerides and HDL cholesterol (baseline concentrations or percent change) were normalized and used as covariates in a univariate general linear model to correct for their influence on changes of LDL diameter and LDL density (dependent variables) in the treatment groups (fixed factor). EMM, estimated marginal mean.

LDL similar to newly diagnosed patients with type 2 diabetes (35). Second, pioglitazone substantially decreases dense LDL in this type of patient (Fig. 1) independently from fasting triglycerides and HDL cholesterol (Table 3). As a consequence, pioglitazone treatment resulted in larger and less dense LDL with an increase in calculated LDL size by $\sim 1.5\%$. This may well be relevant in terms of CAD risk, because in a prospective nested case-control study the incidence of fatal or nonfatal CAD was associated with LDL that was by 1.9% smaller than in control subjects (11). Thus, our research extends previous observations of an open-labeled trial with pioglitazone in patients with type 2 diabetes (28).

In general, dense LDL are positively correlated with triglycerides. Drugs capable of lowering triglycerides are hence attributed the potential to lower dense LDL as well. Like other TZDs, pioglitazone reduces insulin resistance in patients with type 2 diabetes (22), which in turn may lead to a reduction of triglycerides (28). Interestingly, this was not observed in this study. To the contrary, there was rather a nonsignificant increase in triglycerides upon pioglitazone treatment, which appears to be paralleled by the increase of apoC-II. This unexpected finding may be explained in part by the fact that triglyceride levels at baseline were already within the reference range. If only patients with triglycerides <150 mg/dl were considered, there was still a significant increase of LDL diameter in the pioglitazone group compared with placebo. Further, the effect of pioglitazone was not related to fasting triglycerides or HDL cholesterol, thus metabolically decoupling dense LDL from the other components of the atherogenic lipoprotein phenotype. Pioglitazone, therefore, appears to modify the distribution LDL subfractions by a mechanism independent of fasting triglycerides.

This raises the interesting question of how dense LDL can be generated in the presence of normal fasting triglyceride levels. On the other hand, dense LDL may, on a lower prevalence level, also be present in nondiabetic, nonhypertensive, and even normolipidemic adults—especially in middle-aged and older men. A putative mechanism may involve postprandial hypertriglyceridemia that escapes sampling at fasting conditions or lipolytic enzyme activities like lipoprotein

lipase or hepatic lipase (37). However, this study did not investigate putative mechanisms and, therefore, lipase activities were not determined.

The effects of pioglitazone on dense LDL may have tremendous practical implications. Austin et al. (38) showed in a prospective study that subjects with small LDL had a more than twofold increased risk for the future development of type 2 diabetes. This association was independent of HDL cholesterol but was not independent of fasting triglycerides. This raises the question whether discrete alterations in lipoprotein metabolism like the dominance of dense LDL in moderately hypertensive patients with otherwise normal lipoprotein parameters may precede the development of overt type 2 diabetes and whether administration of pioglitazone might help to prevent atherogenic dyslipidemia or type 2 diabetes in this patient population.

HDL cholesterol and apoA-I, the major apolipoprotein of HDL, were not increased. However, in line with our previous observations in patients with type 2 diabetes (28), we saw a significant increase of apoA-II. This typically occurs during treatment with PPAR α agonists such as fibrates (39). In general, TZDs are selective for PPAR γ (40). However, KRP-297, a TZD agonist active toward both PPAR α and PPAR γ , has recently been discovered (41) and pioglitazone was shown to have PPAR α activity in pharmacological concentrations (42). Our findings thus support that pioglitazone may have some PPAR α -activating properties, although pioglitazone does not increase apoA-I levels, a feature also described for PPAR α activation (43). Whatsoever, the increase in apoA-II may entail additional clinical benefit. In the Veterans Affairs HDL Intervention Trial, a prospective end point secondary prevention study with gemfibrozil, increases in the concentration of HDL $_3$ cholesterol, indicative of smaller HDL particles containing apoA-II, led to significantly fewer coronary events (44).

A daily 45-mg dose of pioglitazone was well tolerated. However, because a previous TZD was withdrawn from the market by the Federal Drug Administration because of idiosyncratic liver dysfunction and toxicity (45), pretherapy and bimonthly liver function monitoring has been recommended during the first year of pioglitazone therapy and periodically thereafter (46). During this study,

the activities of liver enzymes actually dropped and decreases in the activities of γ -glutamyl transferase and the liver-specific enzyme alanine aminotransferase were significant (data not shown).

In this subgroup analysis fasting glucose dropped slightly but remained within the reference range throughout the study and, although not significant, insulin levels decreased (Table 1). Thus, pioglitazone improved insulin sensitivity (30) without stimulating insulin release, which avoided hypoglycemia (25).

In conclusion, this study shows for the first time that normolipidemic patients with moderate hypertension have a prevalence of dense LDL similar to patients with type 2 diabetes and that pioglitazone is capable of lowering dense LDL independently of fasting triglycerides and HDL cholesterol. As dense LDL may predispose to the development of type 2 diabetes (38) and may cause endothelial dysfunction independent of triglycerides and HDL (17), treatment with pioglitazone may not only improve glyemic control but may also prevent type 2 diabetes and may reduce cardiovascular risk independent from fasting triglycerides and HDL cholesterol.

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