The Review of DIABETIC STUDIES

ORIGINAL DATA

The Effect of PPARγ-Agonism on LDL Subclass Profile in Patients with Type 2 Diabetes and Coronary Artery Disease

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■ Abstract

Patients with type 2 diabetes (T2DM) often present a preponderance of small, dense LDL particles (small-LDL), which are associated with a high risk of myocardial infarction. Some studies suggest that PPARγ-agonists increase LDL cholesterol but have divergent effects on various LDL subclasses in T2DM patients. We studied the effect of rosiglitazone on the LDL subclass profile in T2DM patients with verified coronary artery disease (CAD). 58 patients with T2DM (HbA1c < 8.5%) and CAD were enrolled in a 16-week, randomized, double-blind and placebo-controlled trial with rosiglitazone 8mg/day (n = 29) or placebo (n = 29). The LDL subclass profile was measured with gel electrophoresis. Rosiglitazone improved insulin sensitivity and glycemic control. Total cholesterol did not change after rosiglitazone treatment (p = 0.062, ANCOVA adjusted for gender and baseline values), whereas LDL (including IDL) cholesterol increased from 2.33 ± 0.48 to 2.67 ± 0.61 mmol/l (p = 0.002 vs. baseline, p = 0.0497 vs. placebo) and large buoyant LDL (large-LDL > 250Å) increased from 1.31 ± 0.36 to 1.46 ± 0.42 mmol/l (p = 0.010 vs. baseline, p = 0.044 vs. placebo) in the rosiglitazone group. No significant changes occurred to the concentration of small-LDL (< 250Å), the average LDL particle size, or HDL or triglyceride concentrations. Whole-body insulin sensitivity was associated with the average LDL particle size after intervention in the whole population (r = 0.40, p = 0.002) and in the rosiglitazone group (r = 0.43, p = 0.020). In conclusion, in T2DM patients with CAD, rosiglitazone treatment significantly increases the concentration of large (buoyant) LDL cholesterol, but not of small dense LDL cholesterol. The long term consequences of this divergent effect of rosiglitazone on LDL subfractions require further exploration.

Keywords: type 2 diabetes · coronary artery disease · PPAR-agonism · lipoproteins

Introduction

Diabetic patients have an increased risk of myocardial infarction (MI), with a poorer outcome than in non-diabetic MI patients [1]. An elevated LDL cholesterol level is a well-established risk factor for coronary artery disease (CAD), although patients with CAD often have only slightly elevated LDL cholesterol concentration. However, these patients frequently present a preponderance of small, dense LDL particles (small-LDL), which are most atherogenic [2] and are associated with a high risk of myocardial infarction [3, 4]. Compared to large, buoyant LDL particles (large-LDL), small-LDL particles are more readily oxidized

and their uptake by LDL receptor is reduced [5, 6]. In addition, high levels of small-LDL particles are linked to endothelial dysfunction [7].

Rosiglitazone is a member of the PPARγ-agonists, which are widely used as antidiabetic agents. In addition to the effects on glucose metabolism, rosiglitazone affects lipid metabolism, inflammatory responses and cellular proliferation [8-10]. Rosiglitazone therapy has been shown to increase the levels of total and LDL cholesterol in patients with type 2 diabetes (T2DM) [11]. In previous studies, thiazolidinediones have decreased the proportion of small-LDL and increased the resistance to LDL oxidation in subjects with blunted insulin sensitivity [12-14]. In addition, it has been shown that rosiglitazone increases LDL particle size and LDL cholesterol concentration in T2DM patients without cardiovascular complications [14, 15]. To our knowledge, the effects of rosiglitazone on the lipoprotein profile of diabetic patients with existing ischemic heart disease have not been previously studied.

The purpose of this study was to determine the effects of rosiglitazone on the concentration of large-LDL and small-LDL cholesterol as well as on the average LDL particle size in patients with T2DM and CAD. LDL particle size was measured by linear gel electrophoresis at baseline and after a 16-week intervention period of rosiglitazone or placebo.

Materials and methods

Subjects

The patients were participants of the previously published trial with rosiglitazone, with the primary endpoint of the effect of rosiglitazone on the myocardial glucose uptake [16]. The effect of rosiglitazone on LDL particle size was one of the secondary endpoints. Inclusion criteria were past or present angina pectoris symptoms under stress, reversible perfusion defect in exercise-rest SPECT perfusion imaging, T2DM treated with diet or with metformin and/or sulphonylurea, and good or moderate glycemic control (HbA1c < 8.5%). Criteria for exclusion were unstable angina pectoris, symptomatic tachy- or bradyarrhythmias, a history of percutaneous transluminal coronary angioplasty during the preceding 6 months, insulin therapy, or heart failure. All patients gave their written informed consent before participating in the study. The study was conducted according to the guidelines of the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland.

Sixty-two patients were enrolled and 58 of them completed the study. Subject characteristics are shown in Table 1. All patients were undergoing stable medical therapy (Table 1). Ten patients had a history of previous myocardial infarction. Five patients had microalbuminuria. The mean duration of diabetes was 6.4 ± 6.2 years in the rosiglitazone group and 7.6 ± 6.7 years in the placebo group (p = n.s.).

Table 1. Characteristics of the study subjects (n = 58) at randomization

Characteristic	Placebo	Rosiglitazone
Gender (M/F)	21/8	21/8
Age (yr)	63.4 ± 7.3	64.3 ± 7.6
Body weight (kg)	89.3 ± 14.7	86.9 ± 17.9
BMI (kg/m²)	29.5 ± 3.5	30.0 ± 4.9
Systolic BP (mmHg)	148.0 ± 25.0	143 .0 ± 20.0
Diastolic BP (mmHg)	78.0 ± 8.0	76.0 ± 6.0
Medication		
β-blockers	79% (23/29)	72% (21/29)
Statins	52% (15/29)	45% (13/29)
ACE-inhibitors	41% (12/29)	41% (13/29)
Ca-blockers	21% (6/29)	24% (7/29)
ASA	76% (22/29)	86% (25/29)
Long-acting nitrates	21% (6/29)	28% (8/29)

Legend: Data are mean ± SD. M: male. F: female. BMI: body mass index. BP: blood pressure. ACE: angiotensin-converting enzyme. ASA: acetyl salicylic acid.

Study protocol

All subjects underwent screening at which SPECT perfusion imaging was performed in order to determine the exercise-induced myocardial ischemia. Patients who met the inclusion criteria and had no exclusion criteria entered a 4-week run-in period with a placebo. After the run-in period, the patients were randomized for the double-blind treatment with either rosiglitazone (8 mg/day) or a placebo for a total of 16 weeks. For biochemical measurements, blood was drawn after an overnight fast and, thereafter, a euglycemic hyperinsulinemic clamp (1 mU/kg/min) was performed for 180 minutes at baseline and after 16 weeks of treatment. Whole-body insulin sensitivity was determined from 80 to 140 minutes of the clamp, as described previously [17]. At week 16, coronary angiography was performed via the femoral artery using the Judkins technique. Angiography was performed with 5-Fr catheters (Cordis, Johnson & Johnson, Mi-

ami Lakes, FL, USA). Coronary artery diameters were analyzed with QCA software (Quantcor stenosis evaluation software, Siemens, Munich, Germany).

Biochemical analysis

The blood samples, except the samples for the analysis of lipoprotein fractions, were sent to a central laboratory (Quest Diagnostics UK Ltd, UK), where the concentration of total cholesterol, HDL cholesterol, triglycerides, HbA1c, fasting plasma glucose, fasting C-peptide, insulin and FFA were measured. Standard methods and quality control were performed. Plasma glucose concentration during the euglycemic hyperinsulinemic clamp was locally measured in duplicate using the glucose oxidase method (Analox GM7 or GM9 Analox Instruments Ltd., London, UK).

Measurement of the LDL particle size and LDL subfractions

LDL subfractions and particle size were determined in the laboratory of Turku University Hospital from frozen serum samples (-70°C) by linear gel electrophoresis with the Lipoprint LDL system (Quantimetrix, Redondo Beach, CA, USA) [18]. In addition, to investigate the stability of LDL subfractions in different sample types, twenty-four frozen (-70°C) EDTA-*plasma* samples were analyzed with the Lipoprint LDL system. Plasma samples were centrifuged and then run on the same gel with the matching serum samples. There was a strong correlation of the average LDL particle size between plasma and serum samples (Figure 1).

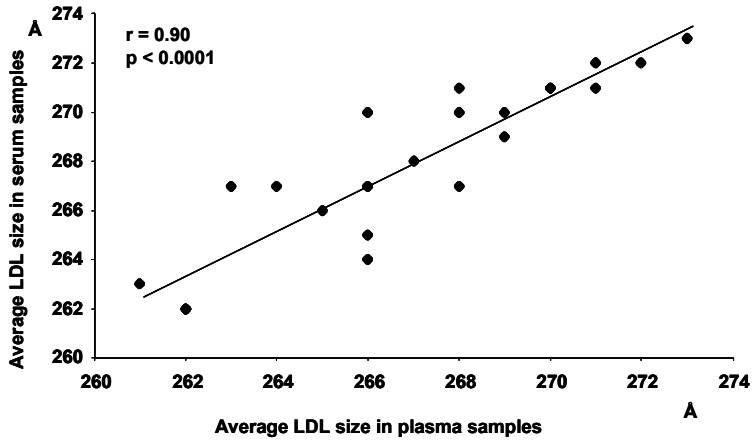

Figure 1. The average LDL particle size was well correlated between cryofrozen (-70°C) plasma and serum samples. Points (266, 267) and (270, 271) include two events each and point (262, 262) includes three events.

The LDL fraction of total cholesterol was determined as an area under the curve of IDL and LDL peaks from Lipoprint data. The detected LDL fraction was applied when the LDL concentration in total cholesterol was determined. For comparison, LDL concentration was also calculated with the Friedewald equation [19]. No difference was observed between the two different methods of analysis (Friedewald equation vs. percentage calculation from Lipoprint results). Large-LDL was determined as LDL peak 1 and LDL peak 2 (> 250 Å), and small-LDL as the sum of peaks from peak 3 to peak 7 (< 250Å) as described earlier [20].

Statistical methods

The statistical analysis was performed with 58 patients. Data are reported as mean ± SD unless otherwise stated. Student's paired *t*-test was used to compare the values between the baseline and week 16 in each group. Unpaired *t*-tests and Mann-Whitney-U tests were used to compare the rosiglitazone and the placebo groups. Because baseline total cholesterol differed between the genders and between the placebo and rosiglitazone groups, ANOVA were performed for repeated measures and ANCOVA adjusting for gender and baseline values was used to analyze the effect of rosiglitazone on total cholesterol levels. For correlation analysis, Pearson's correlation coefficients were calculated. Multiple regression analysis was performed in order to investigate the role of potential confounding factors responsible for the increase in large-LDL concentration. A p-value of < 0.05 was considered statistically significant. All statistical analyses were performed with the SAS statistical analysis system 8.2 (Cary, NC, USA).

Results

Tolerance and angiographic findings

Rosiglitazone treatment was well tolerated. The follow-up data were not obtained in four patients due to elevated liver enzyme levels during intervention, a kidney tumor, a suspected sick sinus syndrome and a consent withdrawal. Coronary angiography revealed one-vessel disease in 57% (33 of 58), two-vessel disease in 31% (18 of 58) and three-vessel disease in 12% (7 of 58) of patients. The location of the main stenosis was the LAD in 61% (35 of 58), LCX in 10% (6 of 58) and RCA in 29% (17 of 58) of pa-

tients. The median degree of stenosis was 62% (range 9-100%). Collateral circulation was found in eight patients.

Metabolic findings

Before the intervention period, the groups were well matched for fasting plasma glucose, HbA1c, C-peptide and insulin levels. After the intervention, fasting plasma glucose decreased from 7.2 ± 2.0 mmol/l to 5.9 ± 1.1 mmol/l ($p = 0.0003$ vs. baseline, $p < 0.0001$ vs. placebo) and HbA1c decreased from 7.2 ± 0.9% to 6.8 ± 0.6% ($p = 0.0007$ vs. baseline, $p < 0.0001$ vs. placebo) in the rosiglitazone group. In the placebo group, fasting plasma glucose (7.7 ± 1.7 vs. 8.1 ± 2.2 mmol/l) and HbA1c (7.1 ± 0.9 vs. 7.3 ± 0.9%) did not significantly change during the study. In the rosiglitazone group, insulin levels decreased upon fasting (from 50 ± 33 to 38 ± 22 pmol/l, $p = 0.046$ vs. baseline, $p = 0.024$ vs. placebo) and during the clamp (from 439 ± 63 to 406 ± 67 pmol/l, $p = 0.011$ vs. baseline, $p = 0.031$ vs. placebo). No change was observed in the insulin levels of the placebo group. C-peptide values were not significantly changed in either of the groups. The whole-body glucose uptake was increased from 12.1 ± 5.9 to 17.3 ± 6.9 μmol/(kg x min) ($p < 0.0001$ vs. baseline and vs. placebo) in the rosiglitazone group. There was no change in the whole-body glucose uptake in the placebo group (11.3 ± 4.1 vs. 11.9 ± 5.4 μmol/(kg x min)).

Lipid parameters

At randomization, total cholesterol was slightly lower in the rosiglitazone group as compared to the placebo group ($p = 0.024$), otherwise groups were well matched for lipid parameters (Table 2.). After the treatment, total cholesterol levels increased by 12% ($p = 0.001$ vs. baseline, $p = 0.015$ vs. placebo) and LDL (including IDL) cholesterol increased by 19% ($p = 0.002$ vs. baseline, $p = 0.0497$ vs. placebo) in the rosiglitazone group. In ANCOVA analyses adjusting for gender and baseline values, the change in the total cholesterol level was not significant ($p = 0.062$). Rosiglitazone increased IDL cholesterol by 23% ($p = 0.0004$ vs. baseline, $p = 0.015$ vs. placebo). From LDL subfractions, large-LDL cholesterol (> 250Å) increased by 13% ($p = 0.010$ vs. baseline, $p = 0.044$ vs. placebo) in the rosiglitazone group (Figure 2). However, no significant changes occurred in small-LDL cholesterol concentration, in average LDL particle size, or in HDL cholesterol or triglyceride concentrations. There was a significant correlation between the whole-body insulin sensitivity and the average LDL particle size after intervention in the whole population ($r = 0.40$, $p = 0.002$) as well as in the rosiglitazone group ($r = 0.43$, $p = 0.020$) (Figure 3) but not in the placebo group. No significant correlation was found at baseline between the average LDL particle size and the whole-body insulin sensitivity in either the pooled population or in

Table 2. Summary of the lipid parameters and metabolic data of the study groups

Parameter	Baseline		After 16 weeks		p
	Placebo	Rosiglitazone	Placebo	Rosiglitazone	
Total cholesterol (mmol/l)	4.61 ± 0.78	4.18 ± 0.65[2]	4.65 ± 0.86	4.66 ± 0.85	0.015[3]
HDL-cholesterol (mmol/l)	1.17 ± 0.34	1.08 ± 0.21	1.18 ± 0.34	1.15 ± 0.24	ns
Triglyceride (mmol/l)	1.88 ± 1.00	1.68 ± 0.77	1.81 ± 0.93	1.71 ± 1.14	ns
LDL-cholesterol (mmol/l)[1]	2.59 ± 0.57	2.33 ± 0.48	2.68 ± 0.57	2.67 ± 0.61	0.05[4]
IDL-cholesterol (mmol/l)	0.97 ± 0.22	0.92 ± 0.21	1.02 ± 0.23	1.12 ± 0.31	0.015
Large buoyant LDL-cholesterol (mmol/l)	1.48 ± 0.37	1.31 ± 0.36	1.50 ± 0.37	1.46 ± 0.42	0.044
Small dense LDL-cholesterol (mmol/l)	0.14 ± 0.18	0.09 ± 0.11	0.16 ± 0.23	0.08 ± 0.08	ns
Mean LDL particle size (Å)	268.50 ± 4.60	269.00 ± 4.00	268.00 ± 5.80	269.70 ± 3.60	ns
Peak LDL particle size (Å)	272.00 ± 6.40	270.50 ± 6.40	270.80 ± 8.00	271.30 ± 7.20	ns
Serum FFAs (mmol/l)	0.80 ± 0.28	0.75 ± 0.24	0.79 ± 0.25	0.68 ± 0.21	ns
Fasting plasma glucose (mmol/l)	7.70 ± 1.70	7.20 ± 2.00	8.10 ± 2.20	5.90 ± 1.10	< 0.001
HbA1c (%)	7.10 ± 0.90	7.20 ± 0.90	7.30 ± 0.90	6.80 ± 0.60	< 0.001
Whole-body glucose uptake (μmol/(kg x min))	11.30 ± 4.10	12.10 ± 5.90	11.90 ± 5.40	17.30 ± 6.90	< 0.001

Legend: Data are mean ± SD. HDL: high density lipoprotein. LDL: low density lipoprotein. IDL: intermediate density lipoprotein. ns: not significant. [1] including IDL. [2] $p = 0.024$, rosiglitazone vs. placebo at baseline. [3] $p = 0.062$ for total cholesterol in ANCOVA analysis adjusting for gender and baseline values, for the change in the rosiglitazone group vs. the placebo group. [4] $p = 0.002$ baseline vs. week 16 in the rosiglitazone group.

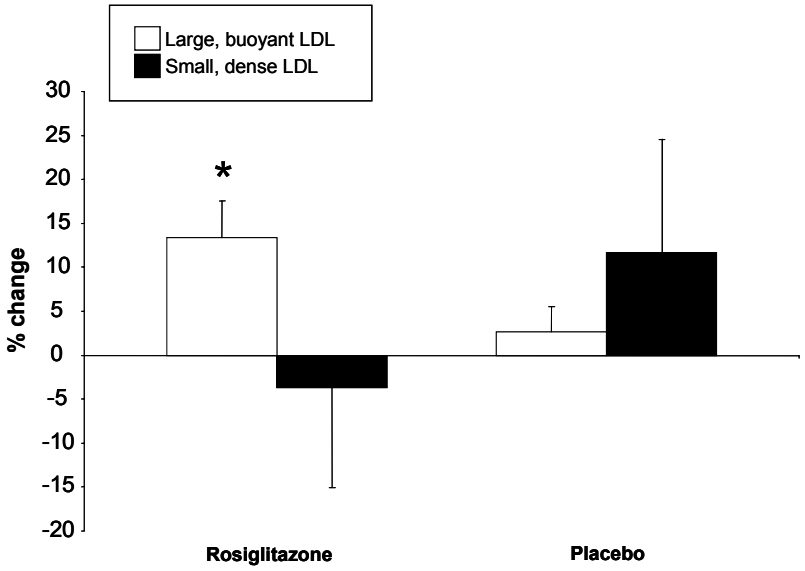

Figure 2. Large, buoyant LDL was increased by 13% in the rosiglitazone group. Values are reported as mean ± SEM. * p = 0.044 as compared to the placebo group.

the separate groups. In the multiple regression analysis adjusted for gender, baseline total cholesterol, baseline LDL cholesterol, the change in HbA1c level and the change in whole-body insulin sensitivity, the change in the large-LDL in the rosiglitazone group was no more significant (Table 3), suggesting that rather the improvement in the glycemic control than the specific drug effect is a key player in the improvement in the LDL lipid profile.

Discussion

In the current study, rosiglitazone treatment increased the level of large-LDL cholesterol but not the concentration of small-LDL cholesterol. The average LDL particle size was associated with the whole-body glucose uptake in the rosiglitazone group, suggesting that, in these patients, rosiglitazone treatment may affect cardiovascular risk factors beyond the decrement in insulin resistance.

A link between total cholesterol concentration and mortality from CAD is well established. However, the measurement of total cholesterol includes both atherogenic and anti-atherogenic lipoprotein particles; thus, the risk is more accurately determined from the LDL cholesterol concentration [21]. The elevated LDL cholesterol concentrations lead to an increased risk of cardiovascular events [22] and especially small-LDL particles are associated with a high risk of myocardial infarction [3, 4]. A direct relationship has been proposed between small-LDL and endothelial dysfunction, a prominent feature of subclinical and clinical atherosclerosis [7, 23]. Poor glycemic control has been linked to the increased amount of small-LDL particles and a shift to the preponderance of large-LDL particles has been observed with improved glycemic control [24]. In contrast, Rivellese and associates have shown that changes in the lipoprotein profile are independent of changes in glycemic control achieved by different antidiabetic medications [25]. We found that rosiglitazone therapy modestly, but significantly, improved glycemic control with the simultaneous changes in the LDL subclass profile. In addition, the effect of rosiglitazone on the LDL profile was suppressed when several confounding factors were taken into account. Thus, the data presented here do not allow the discrimination of a specific drug effect, but support a significant role of indirect factors including, but not limited to, improved glycemic control.

Here we found that rosiglitazone therapy increased total and LDL cholesterol concentration, which is in agreement with previous studies with PPARγ-agonists [12, 26]. Considering the high risk profile of cardiovascular death in T2DM patients, the increment in the LDL cholesterol level has raised some concerns about the safety of these therapies. Previous studies in non-CAD patients with blunted insulin sensitivity have shown that the most atherogenic subfraction of LDL, small-LDL, decreases and the LDL subclass characteristics move towards increased LDL particle size with PPARγ-agonists [12-15]. The current study further ex-

Table 3. Multiple regression analysis on factors explaining the change in the large-LDL cholesterol concentration

Dependent variable	Independent variable	p
The change in large-LDL cholesterol	Intervention	0.25
	Gender	0.18
	Total cholesterol at baseline	0.56
	LDL cholesterol at baseline	0.55
	The change in HbA1c	0.009
	The change in whole-body insulin sensitivity	0.042

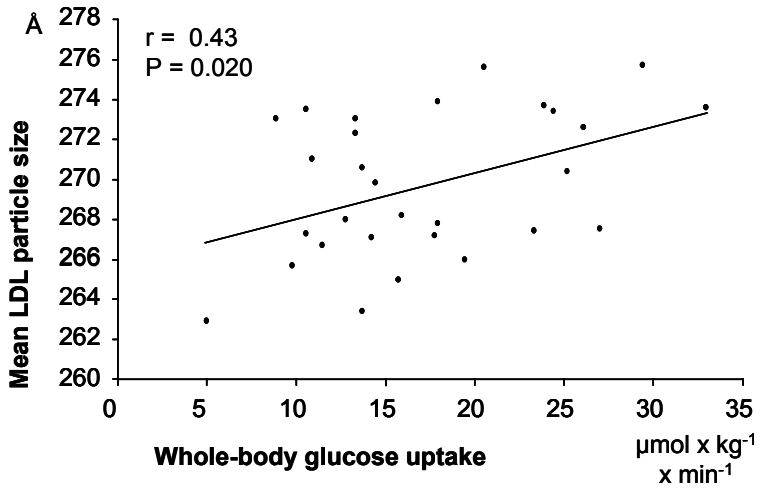

Figure 3. Whole-body insulin sensitivity was significantly correlated with the mean LDL particle size after intervention in the rosiglitazone group.

tends our knowledge of rosiglitazone action by showing that, in T2DM patients with confirmed ischemic coronary disease, rosiglitazone significantly increases the concentration of the large-LDL, but does not affect the small-LDL level. In addition, IDL cholesterol concentration increased during intervention with rosiglitazone. To our knowledge, the effect of rosiglitazone on IDL cholesterol has not been previously reported. Patients with high IDL cholesterol concentrations may have an increased risk of coronary artery disease [27], and IDL cholesterol has been linked to the progression of coronary artery lesions [28].

Insulin resistance is characterized by increased triglyceride and decreased HDL cholesterol concentrations [29]. Usually small, dense LDL goes along with those findings, forming the atherogenic triad [30]. In previous studies, LDL particle size has been linked to whole-body insulin sensitivity [31, 32]. However, with PPARγ-agonists a significant improvement in insulin sensitivity has been found but generally no or relatively small changes in mean triglyceride concentrations, and an improvement in HDL cholesterol concentration has been seen only in a few studies [13, 26, 33]. In addition, recently the effect of rosiglitazone on serum lipids and lipoproteins were compared to that of another PPARγ-agonist, pioglitazone, in patients essentially free from CAD [14]. Pioglitazone decreased triglycerides and increased HDL-cholesterol slightly more than rosiglitazone. However, the observed differences between the two drugs may well be explained by the fact that pioglitazone also acts as a PPAR-α agonist; thus, our findings of the effects of rosiglitazone are more specific with respect to PPARγ-agonism. Furthermore, the previous studies are in agreement with the current study, in which the whole-body glucose uptake was significantly correlated with average LDL particle size in the rosiglitazone group and, since no change was observed in triglyceride or HDL concentrations, no significant decrement was found in small-LDL concentration either.

In the current study, the total cholesterol concentration was significantly lower in the rosiglitazone group as compared to the placebo group at baseline. After intervention, the groups had similar total cholesterol concentrations. Whether the effect of intervention on the cholesterol level would be similar in a case of comparable baseline values remains open. Additionally, at baseline the average LDL particle size was rather high in these patients. Lately, a number of studies have used a relatively new method of linear gel electrophoresis for the analysis of lipoprotein profiles [34-36]. With this method, the results of the average LDL particle size are consistently higher than the results obtained with the conventional gradient gel electrophoresis; thus, the results are not interchangeable with one another. In addition, the division of the lipoprotein profiles to the phenotypes of A and B [37] is not applicable with the Lipoprint system due to these methodological discrepancies. However, despite the high absolute values of LDL particle size, the effect of rosiglitazone on the lipid profile is unique and the methodological differences between gel electrophoresis techniques do not hamper the interpretation of the main findings of the present study.

In summary, the present study extends our knowledge of rosiglitazone action in patients with T2DM and established ischemic coronary artery disease by showing that the concentration of the most atherogenic small-LDL concentration remains unchanged, whereas the concentration of the large-LDL increases. The long-term consequences of this divergent effect of rosiglitazone on LDL subfractions require further exploration.

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