Fenofibrate and rosiglitazone improve quality of lipoproteins in patients with type 2 diabetes mellitus

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Abstract

BACKROUND: Particle size distribution in both HDL and LDL is reflected in the fractional esterification rate of cholesterol by lecithin cholesterol acyltransferase (LCAT) in plasma depleted of apoB containing lipoproteins (FER_{HDL}). We studied FER_{HDL} in a group of patients with type 2 diabetes and determined the impact of two different PPAR agonists (fenofibrate and rosiglitazone) on this marker of lipoprotein particle quality.

PATIENTS AND METHODS: 66 patients with type 2 diabetes (26 women) and 32 control subjects (19 women) were included in the study. 33 patients received fenofibrate and 33 rosiglitazone as add on therapy. Average duration of treatment was 4 months. Plasma lipoprotein glucose levels were determined using an automated analyzer (COBAS Mira, Roche). LDL cholesterol concentrations were calculated by Friedewald formula. FER_{HDL} was determined by a radioassay after precipitating apo-B containing particles of plasma. The assays were performed at baseline and at the end of each treatment. SPSS base program was used for statistical evaluation.

RESULTS: Both fenofibrate and rosiglitazone resulted in a significant decrease of FER_{HDL} (24.62 ± 11.27%/h vs. 19.93 ± 10.34%/h; 20.0 ± 6.1%/h vs. 15.8 ± 5.8%/h, $p < 0.001$). Rosiglitazone was significantly more effective in FER_{HDL} lowering than fenofibrate ($p < 0,02$)

CONCLUSIONS: Both fenofibrate and rosiglitazone improve FER_{HDL} in patients with type 2 diabetes. The effect is more pronounced for rosiglitazone. Qualitative change of plasma lipoproteins reflected by FER_{HDL} can contribute to antiatherogenic action of PPAR agonists. On contrary, changes of lipoprotein composition induced by PPAR agonists cannot explain adverse cardiovascular effects observed in some large clinical trials with PPAR agonists.

ORIGINAL ARTICLE

Abbreviations :

TC	– total cholesterol
TG	– triglycerides
HDL-c	– high-density lipopoprotein cholesterol
LDL-c	– low-density lipoprotein cholesterol
FER_{HDL}	– fractional esterification rate of HDL
CVD	– cardiovascular disease
LCAT	– lecithin cholesterol acyltransferase
PPARs	– peroxisome proliferator-activated receptors

INTRODUCTION

Stimulation of peroxisome proliferator-activated receptors (PPARs) leads to a broad spectrum of effects on metabolism of glucose, lipids and lipoproteins and other intermediate metabolism pathways.

Fibrates are PPAR α agonists with major impact on plasma lipoprotein metabolism. As has been demonstrated in numerous trials, fibrates positively influence plasma lipoprotein levels raising HDL particle concentrations and decreasing triglyceride levels. Moreover, fibrates change the distribution of lipoprotein subclasess decreasing particularly small dense LDL particles (1–3). Similarly to glitazones there are also other antiatherogenic properties of fibrates that have been well documented e.g. anti-inflammatory and anti-oxidative effects, impact on free fatty acid metabolism, endothelial function etc. (4).

PPAR γ agonists (glitazones) are well known modulators of insulin sensitivity used to control glucose levels in type 2 diabetes (5,6). They also impact on plasma levels of lipids and lipoproteins, however, these effects of individual PPAR alpha agonists substantially differ. Pioglitazone significantly more reduces triglycerides (TGs) and increases HDL cholesterol levels than rosiglitazone (7,8). The adverse effect of LDL-cholesterol increase is more pronounced in rosiglitazone (9). However, detailed analyses of lipoprotein subclasses revealed greater amount of large buoyant LDL particles and decreased postprandial free fatty acid concentrations after rosiglitazone therapy (10). Furthermore, rosiglitazone exhibits additional properties such as anti-inflammatory action and other anti-atherogenic effects (11).

Interestingly, the cardioprotective effects of both glitazones and fibrates have been recently put into question after large clinical trials were published (12). There might be other, still poorly understood, mechanisms of PPAR stimulation that offset the above mentioned cardiovascular beneficial effects of these drugs.

To find out if fibrates and glitazones modify the composition and thus biological behavior of lipoprotein subspecies we have used a novel biomarker fractional esterification rate of HDL (FER_{HDL}) This biomarker tests lipoprotein quality and reflects the balance between atherogenic and anti-atherogenic lipoproteins in plasma and has had very good predictive value for positive findings on coronary angiography in clinical trials (13, 14).

SUBJECTS AND METHODS

Study subjects

Sixty-six subjects (36 males) with type 2 diabetes mellitus and 32 age and sex matched healthy controls were included into the study. The group of patients with type 2 diabetes mellitus had been treated with oral antidiabetic drugs (metformine) in monotherapy for at least 6 months before the beginning of the study. Body weight of the subjects remained stable for at least three months before enrolment in the study. Written informed consent was provided by all participants before being enrolled in the study. The study was approved by the Human Ethical Review Committee, 1st Faculty of Medicíne and General University Hospital, Prague, Czech Republic.

Thirty-three patients (18 males) were assigned to fenofibrate at a dose of 267mg and thirty-three subjects (18 males) received receive rosiglitazone at a dose of 4mg daily daily as add-on medications for four months.

Laboratory assays

Measurements of clinical and laboratory parameters were performed before the beginning and at the end of the 4th month of treatment. Control subjects underwent only one physical examination and blood drawing and received no medication. All subjects were measured and weighted.. Blood samples were collected between 7 and 8 AM after an overnight fast. Aliquots of plasma were frozen at _70 °C until analyses. Plasma total cholesterol (TC), TGs, total HDL-C, and were measured enzymatically. LDL-C was calculated using the Friedewald equation, The radioassay for FER_{HDL} had been described previously (15). Briefly, apoB containing lipoproteins are precipitated from EDTA plasma by phosphotungstic acid and MgCl2. To the supernatant, which contains plasma with HDL only, is added a filter-paper disk containing a trace of [3H]cholesterol. After an overnight incubation at 4 °C, the disk is removed and the plasma with labeled HDL is heated to 37 °C and incubated for 30 min. After the incubation, lipids are extracted by ethanol and separated by thin-layer chromatography. FER_{HDL} (%/h) is calculated from the ratio of radioactive unesterified to radioactive esterified cholesterol. Statistical analysis used paired samples test to evaluate pre- a posttreatment differences in both patients groups. To evaluate the differences between patients and controls we used independent samples T-Test. using SPSS base 15.0 software.

RESULTS

Comparison of the baseline characteristics of the patients and controls showed the rosiglitazone patients were significantly older than the fenofibrate group patients and controls. Compared to controls the patients of both groups had significantly higher body mass index

Table 1 . Fenofibtrate and rosiglitazone patients´ groups; comparison with controls and pre- to posttreatment values.

	Fenofibrate (n=33)		Rosiglitazone (n=33)		Controls (n=32)
	Baseline	After treatment	Baseline	After treatment	
Age (years)	57 ± 11.2		63.7 ± 10.1^y		56.38 ± 8.57
BMI (kg/m2)	30.4 ± 3.0^z	30.1 ± 2.9	29.1 ± 3.16^z	29.1 ± 3.2	25.7 ± 3.1
Waist (cm)	100 ± 9^z	100 ± 8	101 ± 8^z	99 ± 8^a	85 ± 11
sBP (mmHg)	136 ± 11^z	133 ± 15	135 ± 16^z	132 ± 17	120 ± 17
dBP (mmHg)	82 ± 6	80 ± 10	81 ± 12	76 ± 7	76 ± 11
Glucose (mmol/L)	9.7 ± 3.0^z	9.5 ± 2.73	9.18 ± 2.00^z	8.03 ± 1.93^b	5.06 ± 0.47
HgbA1c (%)	5.6 ± 0.88^z	5.71 ± 0.77	5.57 ± 0.98^z	5.69 ± 0.10	3.79 ± 0.37

Values expressed as means ± SD. Statistical significance between baseline values of patiens compared to controls ^{x} p<0.05, ^{y}p<0.001, ^{z}p<0.0001 and between post treatment values compared to baseline values ^{a}p<0.05, ^{b}p<0.001, ^{c}p<0.0001
BMI – body mass index; **sBP** – systolic blood pressure; **dBP** - diastolic blood pressure;
HgbA1c – glycated hemoglobin according to IFCC standards

Table 2. Lipid levels in fenofibtrate and rosiglitazone patients´ groups; comparison with controls and pre- to posttreatment values.

	Fenofibrate		Rosiglitazone		Controls
	Baseline	After treatment	Baseline	After treatment	
TC (mmol/L)	5.1 ± 0.93^y	4.7 ± 0.74^a	4.40 ± 0.83^y	4.92 ± 0.93	5.03 ± 0.79
TG (mmol/L)	2.1 ± 1.96^{y,b}	1.71 ± 1.66^a	1.52 ± 0.57^y	1.79 ± 1.20	1.15 ± 0.45
HDL-C (mmol/L)	1.15 ± 0.61^z	1.39 ± 0.44^b	1.22 ± 0.22^z	1.42 ± 0.29^c	1.64 ± 0.35
LDL-C (mmol/L)	2.67 ± 0.44	2.41 ± 0.35^b	2.50 ± 0.68^x	2.75 ± 0.83^b	2.87 ± 0.64
FER$_{HDL}$ (%/hour)	24.62±11.27^z	19.93±10.34^c	20.02±6.09^z	15.76±5.34^c	13.43±4.92

Values expressed as means ± SD. Statistical significance between baseline values of patiens compared to controls ^{x} p<0.05, ^{y}p<0.001, ^{z}p<0.0001 and between post treatment values compared to baseline values ^{a}p<0.05, ^{b}p<0.001, ^{c}p<0.0001
TC – total cholesterol; **TG** – triglycerides; **HDL-c** – high-density lipopoprotein cholesterol;
LDL-c – low-density lipoprotein cholesterol; **FER$_{HDL}$** – fractional esterification rate of HDL
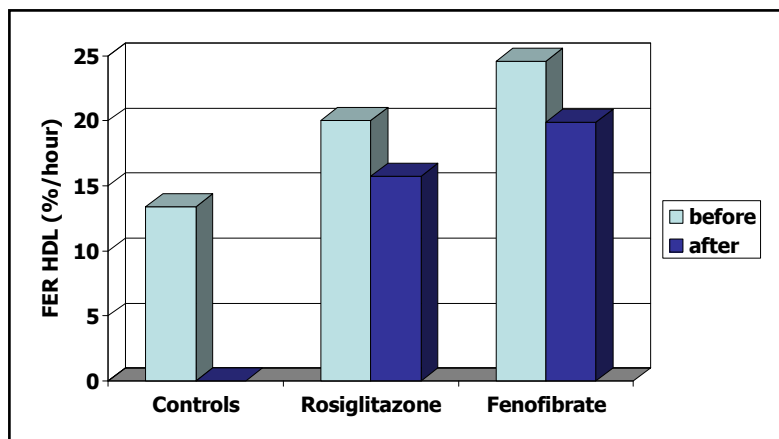

Figure 1. Comparison of FER$_{HDL}$ in controls and rosiglitazon and fenofibrate groups before and after treatment

and waist circumference, blood pressure and, of course, fasting blood glucose and hemoglobin A1C levels. Except for age there was no significant difference between the group of patients assigned fenofibrate and those on rosiglitazone (Table 1).

Total cholesterol levels at baseline were significantly higher in the fenofibrate group and in controls than in the rosiglitazone patients (5.1 ± 0.93 and 5.03 ± 0.79 vs. 4.40 ± 0.83mmol/L, $p < 0,001$). Both fenofibrate and rosiglitazone patients had significantly greater triglyc-

eride levels levels (2.1 ± 1.96 and 1.52 ± 0.57 vs. 1.15 ± 0.45 mmol/l, $p < 0,001$) while HDL-cholesterol concentrations in patients were significantly lower (1.15 ± 0.61 and 1.22 ± 0.22 vs. 1.64 ± 0.35mmol/L, $p < 0,0001$). LDL-cholesterol levels were significantly lower in rosiglitazone patients than in the fenofibrate group and controls (2.50 ± 0.68 vs. 2.67 ± 0.44 and 2.87 ± 0.64, $p < 0.05$).

At baseline, FER$_{HDL}$ was significantly greater in the fenofibrate group compared to both the rosiglitazone

patients and controls (24.62 ± 11.27 vs. 20.02 ± 6.09 and 13.43 ± 4.92% / hour, $p < 0.0001$).

Fenofibrate treatment resulted in significant decrease of total and LDL-cholesterol levels as well as triglyceride concentrations. HDL-cholesterol rose significantly. FER_{HDL} fell significantly from 24.62 ± 11.27%/hour to 19.93 ± 10.34%/hour, $p < 0.001$. Rosiglitazone administration produced significant increase of HDL and LDL-cholesterol levels and lowered FER_{HDL} from 20.02 ± 6.09 to 15.76 ± 5.34, $p < 0.0001$. Contrary to fenofibrate rosiglitazone treatment significantly lowered glucose concentrations (9.18 ± 2.00 vs. 8.03 ± 1.93, $p < 0.001$) and was accompanied by a significant decrease of waist circumference (101 ± 8 vs. 99 ± 8, $p < 0.05$). All results are summarized in Table 2, FER_{HDL} changes are shown in Figure 1.

DISCUSSION

PPAR agonists are widely used drugs in the treatment of dyslipidemia (PPARα and type 2 diabetes (PPARγ). Both these drug classes have favorable impact on plasma levels of anti-atherogenic HDL particles, an effect assumed to be at least as important as LDL lowering in the prevention of cardiovascular events particularly in patients with metabolic syndrome and type 2 diabetes (16). However, recent clinical trials with fenofibrate (the FIELD study, 17) and metaanylses of cardiovascular effects of rosiglitazone (18) have changed our views of the value of these medications in the prevention of vascular events. Also other trials of HDL levels modifying therapies failed to prove clinical benefits (e.g. CETP inhibitor torcetrapib, 19). One of the explanations could be the medications promote production of large amounts of ineffective HDL particles unable to play role in reverse cholesterol transport and other anti-atherogenic functions of HDL particles. Therefore, we tested effects of PPARα agonists (fibrates) and PPAR γ agonists (glitazones) on HDL using a novel biomarker of its function –fractional esterification rate of HDL particles in apoB depleted plasma.

Fractional esterification rate of cholesterol in LDL/VLDL depleted plasma has been shown to be the strongest predictor test of positive findings on coronary angiography (13) and one of best indicators of changes in the progression of coronary artery disease (CAD) after treatment with statins and antioxidants (14). Its predictive potential bears upon the interaction lecithin-cholesterol bilayers of differently sized HDL subclasses with lecithin cholesterol acyltransferase (LCAT). The size of lipoprotein particles is crucial for cholesteryl esters (CE) production and destination (20). The destination of newly produced CE appears to be more essential for the origin of CAD than their total production. Differently sized lipoprotein particles play a protective (buoyant HDL and LDL particles) or an atherogenic role (small HDL and LDL particles) in CAD (21). Thus FER_{HDL} as a marker of lipoprotein particle size (22)

serves as a functional test of lipoprotein quality. It has been reported earlier that FER_{HDL} correlated with HDL particle size (23) and also with LDL particle size (24).

In our study both fenofibrate and rosiglitazone caused a significant decrease of FER_{HDL}. This result implies the overall impact of PPAR agonists on HDL metabolism and the size distribution of lipoprotein particles is beneficial. However, the FER_{HDL} levels after treatment remained significantly higher in patients than in controls suggesting the lipoprotein associated atherosclerotic risk was not reduced to the healthy population level. Previous studies demonstrated positive effects of fibrates (e.g. ciprofibrate) on FER_{HDL}, however lipid levels changes induced by ciprofibrate were significantly greater than those we observed in our study with fenofibrate (25). This difference is most likely due to more severe dyslipidemia in the latter study which was more modifiable by fibrate treatment. Significant decrease of FER_{HDL} after fenofibrate treatment observed in our study suggests the treatment is associated with higher concentrations of small HDL particles, very active in the reverse cholesterol transport. Moreover, lower FER_{HDL} stands for lower concentration of small dense LDL particles as there exists linear relationship between FER_{HDL} and LDL particle size (23). Therefore our results support the notion of beneficial changes of lipoprotein quality induced by fenofibrate in patients with type 2 diabetes.

There are numerous studies documenting neutral or rather negative impact of rosiglitazone on plasma lipids in humans (26, 27). Our findings are basically in line with the previous works- rosiglitazone increased total cholesterol and triglyceride levels (insignificantly) and LDL-cholesterol concentrations significantly. Nevertheless, LDL raising effect could be in part counteracted by shift in LDL subclasses distribution towards large r(and thus less atherogenic) LDL particles. This argument is supported by our finding of a significant decrease of FER_{HDL} after rosiglitazone, which is in indirect relationship with LDL particle size. Therefore, even slight rosiglitazone increase of LDL cholesterol levels doesn't have to be proatherogenic if it is accompanied by a qualitative change in LDL subclasses.

As there is a direct relationship between HDL particle size and FER_{HDL}, rosiglitazone associated increase of HDL concentrations seems to be accompanied by positive changes of subfractions representation also in this lipoprotein class (23, 24). Taken together, rosiglitazone, despite negative quantitative changes of plasma lipids, induces rather beneficial qualitative changes of plasma lipoprotein particles.

We conclude both fenofibrate and rosiglitazone induce positive qualitative changes of lipoproteins in patients with type 2 diabetes and thus decrease lipoprotein associated cardiovascular risk in these patients. Qualitative changes of plasma lipoproteins after PPAR agonists treatment cannot explain the adverse cardio-

vascular effects of these drugs observed in large clinical trials. This finding suggests other than lipid related mechanisms of possible adverse cardiovascular effects of PPAR agonists.

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