

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/22160> holds various files of this Leiden University dissertation.

Author: Wang, Yanan

Title: Novel modulators of lipoprotein metabolism : implications for steatohepatitis and atherosclerosis

Issue Date: 2013-11-06

3

PIOGLITAZONE DECREASES PLASMA CHOLESTERYL ESTER TRANSFER PROTEIN MASS, ASSOCIATED WITH A DECREASE IN HEPATIC TRIGLYCERIDE CONTENT, IN PATIENTS WITH TYPE 2 DIABETES

Jacqueline T. Jonker, Yanan Wang, Willeke de Haan, Michaela Diamant,
Luuk J. Rijzewijk, Rutger W. van der Meer, Hildo J. Lamb,
Jouke T. Tamsma, Albert de Roos, Johannes A. Romijn,
Patrick C.N. Rensen, Johannes W.A. Smit

Diabetes Care 2010; 33: 1625-8

ABSTRACT

3

Thiazolidinediones reduce hepatic steatosis and increase HDL cholesterol levels. In mice with human-like lipoprotein metabolism (*APOE*3-Leiden.CETP* transgenic mice), a decrease in hepatic triglyceride (TG) content is associated with a decrease in plasma cholesteryl ester transfer protein (CETP) mass and an increase in high-density lipoprotein (HDL)-cholesterol levels. Therefore, the aim of the present study was to assess the effects of pioglitazone on CETP mass in patients with type 2 diabetes mellitus (T2DM). We included 78 men with T2DM (age 56.5 ± 0.6 years; HbA1c $7.1 \pm 0.1\%$) who were randomized to treatment with pioglitazone (30 mg/day) or metformin (2000 mg/day) and matching placebos, in addition to glimepiride. At baseline and after 24 weeks of treatment plasma HDL cholesterol levels and CETP mass were measured, and hepatic TG content was assessed by proton magnetic resonance spectroscopy. Pioglitazone decreased hepatic TG content (5.9 [interquartile range 2.6-17.4] versus 4.1 [1.9-12.3]%, $P < 0.05$), decreased plasma CETP mass (2.33 ± 0.10 vs. 2.06 ± 0.10 $\mu\text{g/ml}$, $P < 0.05$) and increased plasma HDL-cholesterol levels (1.22 ± 0.05 vs. 1.34 ± 0.05 mmol/l, $P < 0.05$). Metformin did not significantly change any of these parameters. In conclusion, a decrease in hepatic TG content by pioglitazone is accompanied by a decrease in plasma CETP mass and associated with an increase in HDL-cholesterol levels. These results in patients with T2DM fully confirm recent findings in mice.

INTRODUCTION

Hepatic steatosis is a prevalent condition in patients with type 2 diabetes mellitus (T2DM) and is associated with an increased cardiovascular risk^{1,2}. Furthermore, many patients with T2DM display dyslipidemia characterized by high plasma levels of apolipoprotein (apo) B-lipoproteins and triglycerides (TG) and low plasma levels of high-density lipoprotein (HDL) cholesterol. Recently, Toledo *et al.*³ showed that hepatic steatosis is associated with more severe hyperlipidemia in T2DM, which might contribute to the increased risk of cardiovascular disease.

To reduce this increased cardiovascular risk in T2DM, regular treatment algorithms include lipid lowering drugs. Our previous studies in *APOE*3-Leiden.CETP* transgenic mice, a well-established model for human-like lipoprotein metabolism, showed that treatment with either statins⁴, fibrates⁵ or niacin⁶ resulted in a reduction in plasma apoB-lipoprotein and TG levels and an increase in HDL cholesterol. Moreover, these treatments reduced hepatic lipid content (i.e., both TG and cholesterol) as well as the hepatic expression and plasma levels of cholesteryl ester transfer protein (CETP)⁴⁻⁶. CETP is a protein that mediates the heteroexchange of cholesteryl esters from HDL to (V)LDL with a simultaneous exchange of triglycerides from (V)LDL to HDL. These studies thus suggest that lowering of hepatic TG content in *APOE*3-Leiden.CETP* mice increased HDL cholesterol levels by reduction of plasma CETP mass.

Because the correlation between hepatic TG content and plasma CETP mass has not been studied in humans, the aim of this study was to evaluate whether the relationship between lowering of hepatic TG content and decreased plasma CETP mass also exists in humans. Hepatic TG content can be lowered by thiazolidinediones, including pioglitazone⁷. Indeed, in a previous study, we reported that both antidiabetic drugs pioglitazone and metformin improved insulin sensitivity in men with T2DM, whereas only pioglitazone reduced hepatic TG content⁸. Therefore, we used pioglitazone treatment as a model to study the effects of a change in hepatic TG content on CETP mass in patients with T2DM and used metformin treatment as a negative control.

MATERIALS AND METHODS

Study design

This study used the data from the Pioglitazone Influence on tRiglyceride Accumulation in the Myocardium in Diabetes (PIRAMID) study. This was a prospective, randomized, double-blind, intervention study, which compared the effects of pioglitazone and

metformin on cardiac function and metabolism. The results were reported previously⁸. The study design will be summarized here. The study included male patients with T2DM without cardiovascular disease or diabetes related complications. Inclusion criteria were body mass index (BMI) 25-32 kg/m², age between 45-65 years, and diabetes well controlled with metformin, a sulfonylurea or both (HbA1c 6.5-8.5%). Patients were excluded on the following criteria: uncontrolled hypertension (blood pressure > 150/85 mmHg), medical history of diabetes-related complications, liver disease, cardiovascular disease, or use of thiazolidinediones or insulin before the study. This study was executed at two hospitals in the Netherlands (Leiden University Medical Center, Leiden and VU University Medical Center, Amsterdam), and both local ethics committees gave their approval. All participants signed informed consent.

If patients met the inclusion criteria, their glucose-lowering medication was switched to glimepiride monotherapy, until a stable dose was reached 2 weeks before the start of the intervention. At baseline, patients were randomized to metformin (500 mg twice daily, titrated to 1000 mg twice daily) or pioglitazone (15 mg once daily, titrated to 30 mg once daily after 2 weeks) in addition to glimepiride. In both the metformin and pioglitazone groups, 39 patients were included. In the pioglitazone group 34 and in the metformin group 37 patients completed the study.

Patients were studied at baseline and after 24 weeks treatment. Blood sampling and magnetic resonance spectroscopy were performed after an overnight fast.

Hepatic triglyceride content

Hepatic TG content was measured by proton (¹H) magnetic resonance spectroscopy on a 1.5 Tesla whole-body magnetic resonance scanner (Gyrosan ACS/NT15; Philips, Best, the Netherlands). The technical details were described previously⁸. The voxel was placed in the hepatic parenchyma, carefully preventing placement of the voxel in vascular structures. Java Magnetic Resonance User interface software (jMRUI version 2.2, Leuven, Belgium) was used for fitting of the spectra. Technical details of spectra acquisition and spectra quantification were formerly described^{8,9}. Spectra with and without water suppression were acquired to calculate hepatic TG content relative to water (signal amplitude of triglyceride/ signal amplitude water x 100%).

Plasma cholesterol and CETP mass analysis

All plasma samples were obtained in the postabsorptive state on the day of randomization and at 24 weeks and analyzed in one laboratory (Leiden, the Netherlands). To ascertain that we could make adequate correlations, these measurements were performed in the same blood samples. Plasma triglyceride and cholesterol concentrations were

determined using a commercially available enzymatic kit (1488872 and 236691; Roche Molecular Biochemicals, Indianapolis, IN, USA). This cholesterol assay was also used for determination of plasma HDL cholesterol after precipitation of apoB-lipoproteins from 20 μ l of plasma by adding 10 μ l of heparin (500 unites/ml; LEO Pharma, The Netherlands) and 10 μ l of 0.2 mol/l $MnCl_2$ ⁶. Plasma apoB100 was determined with a Human ApoB ELISA kit (3715-1H; Mabtech, Nacka Strand, Sweden). The plasma CETP mass was quantified using a CETP ELISA Daiichi kit (Daiichi Pure Chemicals, Tokyo, Japan).

Statistical analysis

Data are expressed as means \pm standard error of the mean (SEM) or as median (interquartile range) because hepatic TG content and plasma TG were not normal distributed. Paired *t* tests or Wilcoxon signed-ranks tests were used for within-group differences. We used ANOVA to assess between-group differences. For correlation analysis, Spearman correlation analyses were used. We used SPSS software (version 16.0, SPSS, Chicago, Illinois, USA) for statistical analyses. *P* < 0.05 was considered statistically significant.

RESULTS

At baseline, patients in both treatment groups were well-matched for age (pioglitazone 56.8 ± 1.0 years and metformin 56.4 ± 0.9 years, between group *P* > 0.05), duration of diabetes (pioglitazone 4 [3-6] years and metformin 3 [1-5] years, *P* > 0.05), and body mass index (pioglitazone 28.2 ± 0.5 kg/m² and metformin 29.3 ± 0.6 kg/m², between group *P* > 0.05) ⁸.

Table 1. Hepatic triglyceride content, plasma CETP mass, cholesterol, triglycerides, and apoB100 at baseline and after 24 weeks of treatment

	Pioglitazone		Metformin		<i>P</i> value between groups
	Baseline	24weeks	Baseline	24weeks	
Hepatic TG content (%) #	5.9 (2.6-17.4)	4.1 (1.90-12.3)*	7.7 (3.7-23.9)	10.7 (5.1-22.0)	< 0.01
CETP mass (μ g/ml)	2.33 ± 0.10	$2.06 \pm 0.10^*$	2.44 ± 0.08	2.24 ± 0.12	NS
HDL cholesterol (mmol/l)	1.22 ± 0.05	$1.34 \pm 0.05^*$	1.22 ± 0.06	1.23 ± 0.06	0.02
Total cholesterol (mmol/l)	4.90 ± 0.16	$5.19 \pm 0.22^*$	5.15 ± 0.18	4.99 ± 0.18	< 0.01
Triglycerides (mmol/l)	1.74(0.97-3.09)	1.37(0.88-1.80)	2.08(1.08-2.74)	1.23(0.68-1.92)	NS
ApoB100 (mg/dl)	84.88 ± 5.08	$75.41 \pm 4.03^*$	89.72 ± 5.01	$74.58 \pm 4.22^*$	NS

Data are means \pm SEM or median (interquartile range). # Data from van der Meer et al ⁸.

*Within group *P* < 0.05; NS = not significant

Treatment effect

The effects of treatment on hepatic TG content and plasma lipid profiles are presented in Table 1. As we showed before ⁸, treatment with pioglitazone for 24 weeks decreased hepatic TG content [5.9 (2.6-17.4) vs. 4.1 (1.9-12.3)%, $P < 0.05$]. This was accompanied by a decrease in plasma CETP mass (2.33 ± 0.10 vs. 2.06 ± 0.10 $\mu\text{g/ml}$, $P < 0.05$) and an increase in plasma HDL cholesterol levels (1.22 ± 0.05 vs. 1.34 ± 0.05 mmol/l , $P < 0.05$). Treatment with metformin did not significantly affect either of these parameters. Treatment with pioglitazone and metformin both decreased plasma TG and apoB100 significantly; however, there was no difference in the reduction between the groups (Table 1).

Correlations

Changes in plasma CETP mass after 24 weeks in the pioglitazone-treated patients correlated with changes in hepatic TG content ($r = 0.34$, $P < 0.05$), although this association was not present in the metformin group.

Effect of statin use in the pioglitazone-treated group

In the pioglitazone group, 19 of the 39 patients used a statin at start of the study. Table 2 compares the changes in lipid levels and hepatic TG content between statin users versus non-statin users in the patients treated with pioglitazone. In nonstatin users, pioglitazone decreased liver TG content [6.4 (2.5-18.9) vs. 4.9 (1.9-14.7)%, $P < 0.05$], increased HDL cholesterol levels (1.14 ± 0.06 vs. 1.32 ± 0.06 mmol/l , $P < 0.05$), and decreased CETP mass (2.64 ± 0.14 vs. 2.16 ± 0.12 $\mu\text{g/ml}$, $P < 0.05$). However, remarkably, in statin users, pioglitazone also reduced liver TG content [8.0 (2.8-16.5) vs. 3.7 (1.9-10.9)%, $P < 0.05$], but did not affect either HDL cholesterol levels or CETP mass. ApoB100 decreased significantly only in the statin users (83.07 ± 8.29 vs. 68.77 ± 5.34 mg/dl , $P < 0.05$)

Table 2. Hepatic triglyceride content, plasma CETP mass, cholesterol, triglycerides, and apoB100 in patients treated with pioglitazone, selected on the use of statins at baseline

	No statin use		Statin use		<i>P</i> value between groups
	Baseline	24weeks	Baseline	24weeks	
Hepatic TG content (%)	6.4 (2.5-18.9)	4.9 (1.90-14.7)*	8.0 (2.8-16.5)	3.7 (1.9-10.9)*	NS
CETP mass ($\mu\text{g/ml}$)	2.64 ± 0.14	$2.16 \pm 0.12^*$	1.98 ± 0.09	1.96 ± 0.15	0.03
HDL cholesterol (mmol/l)	1.14 ± 0.06	$1.32 \pm 0.06^*$	1.30 ± 0.06	1.36 ± 0.08	NS
Total cholesterol (mmol/l)	5.32 ± 0.22	5.87 ± 0.35	4.44 ± 0.19	4.55 ± 0.19	NS
Triglycerides (mmol/l)	1.74(0.82-3.11)	1.32(0.80-3.02)	1.61(1.02-2.41)	1.46(0.99-1.69)	NS
ApoB100 (mg/dl)	86.59 ± 6.22	82.06 ± 5.77	83.07 ± 8.29	$68.77 \pm 5.34^*$	NS

Data are means \pm SEM or median (interquartile range).

*Within group $P < 0.05$; NS = not significant

Discussion

In this study, we assessed the associations among changes in hepatic TG content, plasma CETP mass, and lipid profiles in patients with T2DM. The results show that pioglitazone decreased hepatic TG content, associated with decreased plasma CETP mass and increased HDL cholesterol levels. These findings are in full concordance with our recent studies in *APOE*3-Leiden.CETP* mice, which showed that classical lipid-lowering drugs concurrently lowered hepatic lipid content and decreased hepatic CETP mRNA expression, resulting in decreased plasma CETP mass⁴⁻⁶.

In this study, plasma TG and plasma apoB100 decreased equivalently in both the pioglitazone and metformin group. Accordingly, other studies have shown that pioglitazone and metformin both decrease plasma TG¹⁰⁻¹³. Given the generally observed inverse relationship between plasma TG and HDL cholesterol found in epidemiological studies, a decrease in plasma TG may induce an increase in HDL cholesterol only. However, in the present study the decrease in plasma TG was not different between groups and plasma HDL cholesterol only increased in the pioglitazone group, indicating that another mechanism may be responsible for eliciting this difference, i.e., the reduction in CETP.

It is intriguing to speculate on the mechanism underlying the correlation between hepatic lipid content and plasma CETP mass. It is known that cholesterol derivatives are agonists for the nuclear receptor liver X receptor α (LXR α). Activation of LXR α results in increased transcription of CETP and sterol regulatory element-binding proteins-1c (SREBP-1c)¹⁴. Thus, our studies in *APOE*3-Leiden.CETP* mice suggest that a decrease in the hepatic cholesterol content, associated with a decrease in cholesterol derivatives, such as oxysterols, reduces LXR α activation, thereby further downregulating CETP mRNA transcription. It is therefore likely that in our study pioglitazone decreased hepatic cholesterol content in addition to TG content, thereby downregulating LXR α -activity. Although it is not possible at this time to noninvasively measure hepatic cholesterol content in humans, studies in *APOE*3-Leiden.CETP* mice with a human-like protein profile generally demonstrate a strong relation between hepatic cholesterol and hepatic TG content.

Notably, thiazolidinediones such as pioglitazone, can also upregulate LXR α expression via activation of peroxisome proliferator-activated receptor- γ , which is most dominantly expressed in adipose tissue¹⁵. Moreover, CETP expression is prominent in adipose tissue. Radeau *et al.*¹⁶ have shown that CETP mRNA expression in adipose tissue correlates with plasma CETP concentrations. The increased subcutaneous fat mass as induced by pioglitazone treatment may contribute to increased plasma CETP

levels, thereby potentially counteracting the decrease in plasma CETP levels due to the decrease in hepatic lipid content by pioglitazone. Therefore, the observed decrease in plasma CETP mass due to the decrease in hepatic TG content may, in fact, be larger.

To our knowledge only one other study evaluated the effect of thiazolidinediones on CETP expression, but the hepatic TG content was not studied. Chappuis *et al.*¹⁷ performed a cross-over study comparing pioglitazone versus rosiglitazone in 17 patients with T2DM. They found that rosiglitazone increased total cholesterol levels and decreased plasma CETP activity in accordance with our study. However, pioglitazone did not have any effect on these parameters. The differences in their results on the effects of pioglitazone compared with our study may be due to the shorter treatment duration of 12 weeks and/or to the smaller sample size in the previous study.

Finally, we found that pioglitazone did not further decrease CETP mass in patients who already used a statin. Experimental studies in *APOE*3-Leiden* mice have demonstrated that statins decrease plasma CETP mass by decreasing hepatic mRNA expression, again related to decreased hepatic cholesterol content⁴. Apparently the effect of statins is dominant over the effect of pioglitazone. We hypothesize that statins specifically decrease hepatic cholesterol content and downregulate CETP mRNA expression. Therefore, additional lowering of hepatic TG content will not result in an additional decrease in CETP expression. However, the current study was not designed to assess the effects of statins on these parameters.

A limitation of this study is that only men were included. Kinoshita *et al.*¹⁸ showed that men have lower CETP activity than women; thus, these data may not be readily extrapolated to women with T2DM.

In summary, this study shows that in male patients with T2DM a decrease in hepatic TG content by pioglitazone is associated with a decrease in CETP mass and an increase in HDL cholesterol levels. Furthermore, we confirmed that use of statins is associated with a decreased plasma CETP mass. Both findings are in full agreement with our recent findings in *APOE*3-Leiden.CETP* mice and support the validity of these mice as a model for human-like lipoprotein metabolism.

ACKNOWLEDGEMENTS

This research was performed within the framework of the Center for Translational Molecular Medicine (CTMM; www.ctmm.nl), project PREDICt (grant 01C-104), and supported by the Netherlands Heart Foundation (NHS2003B136 and NHS2007B81 to P.C.N. Rensen), Dutch Diabetes Research Foundation and Dutch Kidney Foundation and Eli Lilly, the Netherlands. P.C.N.R. is an Established Investigator of the Netherlands Heart Foundation (2009T038).

REFERENCES

- 1 Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288:E462-E468.
- 2 Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. *Atherosclerosis* 2007;191:235-40.
- 3 Toledo FG, Sniderman AD, Kelley DE. Influence of hepatic steatosis (fatty liver) on severity and composition of dyslipidemia in type 2 diabetes. *Diabetes Care* 2006;29:1845-50.
- 4 de Haan W, van der Hoogt CC, Westerterp M, Hoekstra M, Dallinga-Thie GM, Princen HM, Romijn JA, Jukema JW, Havekes LM, Rensen PC. Atorvastatin increases HDL cholesterol by reducing CETP expression in cholesterol-fed APOE*3-Leiden.CETP mice. *Atherosclerosis* 2008;197:57-63.
- 5 van der Hoogt CC, de Haan W, Westerterp M, Hoekstra M, Dallinga-Thie GM, Romijn JA, Princen HM, Jukema JW, Havekes LM, Rensen PC. Fenofibrate increases HDL-cholesterol by reducing cholesteryl ester transfer protein expression. *J Lipid Res* 2007;48:1763-71.
- 6 van der Hoorn JW, de Haan W, Berbee JF, Havekes LM, Jukema JW, Rensen PC, Princen HM. Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester transfer protein in APOE*3Leiden.CETP mice. *Arterioscler Thromb Vasc Biol* 2008;28:2016-22.
- 7 Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med* 2004;11:1106-18.
- 8 van der Meer RW, Rijzewijk LJ, de Jong HW, Lamb HJ, Lubberink M, Romijn JA, Bax JJ, de Roos A, Kamp O, Paulus WJ, Heine RJ, Lammertsma AA, Smit JW, Diamant M. Pioglitazone improves cardiac function and alters myocardial substrate metabolism without affecting cardiac triglyceride accumulation and high-energy phosphate metabolism in patients with well-controlled type 2 diabetes mellitus. *Circulation* 2009;119:2069-77.
- 9 van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007;56:2849-53.
- 10 Betteridge DJ, Verges B. Long-term effect on lipids and lipoproteins of pioglitazone versus gliclazide addition to metformin and pioglitazone versus metformin addition to sulphonylurea in the treatment of type 2 diabetes. *Diabetologia* 2005;12:2477-81.
- 11 Derosa G, Cicero AF, D'Angelo A, Gaddi A, Ciccarelli L, Piccinni MN, Salvadeo SA, Pricolo F, Ferrari I, Gravina A, Ragonesi PD. Effects of 1 year of treatment with pioglitazone or rosiglitazone added to glimepiride on lipoprotein(a) and homocysteine concentrations in patients with type 2 diabetic mellitus and metabolic syndrome: a multicenter, randomized, double-blind, controlled clinical trial. *Clin The* 2006;5:679-88.
- 12 Goldberg RB, Kendall DM, Deeg MA, Buse JB, Zagar AJ, pinaire JA, Tan MH, Khan MA, Pere AT, Jacober SJ. Comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 2005;7:1547-54.
- 13 Jeppesen J, Zhou MY, Chen YD, Reaven GM. Effect of metformin on postprandial lipemia in patients with fairly to poorly controlled NIDDM. *Diabetes Care* 1994;10:1093-99.
- 14 Luo Y, Tall AR. Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element. *J Clin Invest* 2000;105:513-20.
- 15 Juvet LK, Andresen SM, Schuster GU, Dalen KT, Tobin KA, Hollung K, Haugen F, Jacinto S, Ulven SM, Bamberg K, Gustafsson JA, Nebb HI. On the role of liver X receptors in lipid accumulation in adipocytes. *Mol Endocrinol* 2003;17:172-82.

- 16 Radeau T, Robb M, Lau P, Borthwick J, McPherson R. Relationship of adipose tissue cholesteryl ester transfer protein (CETP) mRNA to plasma concentrations of CETP in man. *Atherosclerosis* 1998;139:369-76.
- 17 Chappuis B, Braun M, Stettler C, Allemann S, Diem P, Lumb PJ, Wierzbicki AS, James R, Christ ER. Differential effect of pioglitazone (PGZ) and rosiglitazone (RGZ) on postprandial glucose and lipid metabolism in patients with type 2 diabetes mellitus: a prospective, randomized crossover study. *Diabetes Metab Res Rev* 2007;23:392-9.
- 18 Kinoshita M, Teramoto T, Shimazu N, Kaneko K, Ohta M, Koike T, Hosogaya S, Ozaki Y, Kume S, Yamanaka M. CETP is a determinant of serum LDL-cholesterol but not HDL-cholesterol in healthy Japanese. *Atherosclerosis* 1996;120:75-82.