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Universiteit Leiden



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Title: Ectopic fat depositions in obesity and diabetes : nutritional, exercise and pharmacological interventions

Date: 2012-10-10

Chapter 12

Pioglitazone decreases plasma cholesteryl ester transfer protein mass, associated with a decrease in hepatic triglyceride content, in patients with type 2 diabetes



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Diabetes Care 2010; 33:1625-1628

ABSTRACT

Background

Thiazolidinediones reduce hepatic steatosis and increase high-density lipoprotein (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 HDL 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.

Methods

We included 78 men with type 2 diabetes mellitus (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.

Results

Pioglitazone decreased hepatic TG content (5.9 (2.6-17.4) vs. 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 mM, $P < 0.05$). Metformin did not significantly change any of these parameters.

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 type 2 diabetes mellitus fully confirm recent findings in mice.

INTRODUCTION

Hepatic steatosis is a prevalent condition in patients with type 2 diabetes mellitus and is associated with an increased cardiovascular risk (1,2). Furthermore, many patients with type 2 diabetes mellitus display dyslipidemia characterized by high plasma levels of apoB-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 type 2 diabetes mellitus, which might contribute to the increased risk of cardiovascular disease.

To reduce this increased cardiovascular risk in type 2 diabetes, 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 statins (4), fibrates (5) or niacin (6) resulted in a reduction in plasma apoB-lipoproteins and TG levels and an increase in HDL-cholesterol. Moreover, these treatments reduced hepatic lipid content (i.e. both triglycerides 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 TG from (V)LDL to HDL. These studies thus suggest that lowering of hepatic lipid content in APOE*3-Leiden.CETP mice increased HDL-cholesterol levels by reduction of plasma CETP mass.

Since 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 pioglitazone (7). Indeed, in a previous study, we reported that both anti-diabetic drugs pioglitazone and metformin improved insulin sensitivity in men with type 2 diabetes mellitus whereas only pioglitazone reduced hepatic TG content (8). 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 type 2 diabetes mellitus, and used metformin treatment as negative control.

METHODS

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 to metformin on cardiac function and metabolism of which the results were reported previously (8). The study design will be summarized here. The study included male patients with type 2 diabetes mellitus without cardiovascular disease or diabetes related complications. Inclusion criteria were:

body mass index (BMI) 25-32 kg/m², age between 45-65 years, well-controlled diabetes with metformin, 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 prior to the study. This study was executed at two hospitals in the Netherlands (LUMC, Leiden and VUMC, 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 group 39 patients were included. In the pioglitazone group 34 patients and in the metformin group 37 patients completed the study.

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

Hepatic triglyceride content

Hepatic TG content was measured by proton magnetic resonance spectroscopy (¹H-MRS) on a 1.5 Tesla whole-body MR scanner (Gyrosan ACS/NT15; Philips, Best, The Netherlands). The technical details were described previously (8). 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 TG/ signal amplitude of water x 100%).

Plasma cholesterol, triglyceride, apoB100 and CETP mass analyses

All plasma samples were obtained in the postabsorptive state at 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 within the same blood samples. Plasma TG and cholesterol concentrations were determined using commercially available enzymatic kits (respectively no. 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 plasma by adding 10 μ L heparin (LEO Pharma, The Netherlands; 500 U/mL) and 10 μ L 0.2 M MnCl₂ (6). Plasma apoB100 was determined with the 'Human apoB ELISA kit' (no. 3715-1H; Mabtech

AB, Sweden). Plasma CETP mass was quantified using kit 'CETP ELISA Daiichi' (Daiichi Pure Chemicals Co, Ltd, Tokyo, Japan).

Statistics

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

RESULTS

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

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 total cholesterol (4.90 ± 0.16 vs. 5.19 ± 0.22 mM, $P < 0.05$), which was explained in part by an increase in HDL-cholesterol levels (1.22 ± 0.05 vs. 1.34 ± 0.05 mM, $P < 0.05$). 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).

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

	Pioglitazone		Metformin		P between groups
	Baseline	24 weeks	Baseline	24 weeks	
Hepatic TG content (%)*	5.9 (2.6 -17.4)	4.1 (1.9 - 12.3) [†]	7.7 (3.7-23.9)	10.7 (5.1 - 22.0)	<0.01
CETP mass (µg/mL)	2.33 ± 0.10	2.06 ± 0.10 [†]	2.44 ± 0.08	2.24 ± 0.12	NS
HDL cholesterol (mmol/L)	1.22 ± 0.05	1.34 ± 0.05 [†]	1.22 ± 0.06	1.23 ± 0.06	0.02
Total cholesterol (mmol/L)	4.90 ± 0.16	5.19 ± 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 ± 4.03 [†]	89.72 ± 5.01	74.58 ± 4.22 [†]	NS

Data are mean ± SEM or median (interquartile range). [†] Within group P<0.05. TG, triglyceride; CETP, cholesteryl ester transfer protein; NS, not significant; HDL, high-density lipoprotein. * Adapted from van der Meer et al. *Circulation* 2009; 119: 2069-2077

Correlations

Changes in plasma CETP mass after 24 weeks in the pioglitazone treated patients correlated with changes in hepatic TG content ($r=0.34$, $P<0.05$), whilst 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 non-statin 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 mM, $P<0.05$) and decreased CETP mass (2.64 ± 0.14 vs. 2.16 ± 0.12 µg/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 only significantly decreased 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		P between groups
	Baseline	24 weeks	Baseline	24 weeks	
Hepatic TG content (%)	6.4 (2.5-18.9)	4.9 (1.9-14.7)*	8.0 (2.8 - 16.5)	3.7 (1.9 - 10.9)*	NS
CETP mass (µg/mL)	2.64 ± 0.14	2.16 ± 0.12*	1.98 ± 0.09	1.96 ± 0.15	0.03
HDL cholesterol (mmol/L)	1.14 ± 0.06	1.32 ± 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 ± 5.34*	NS

Data are mean ± SEM or median (interquartile range). * Within group P < 0.05.

TG, triglyceride; NS, not significant; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein

DISCUSSION

This study assessed the associations between changes in hepatic TG content, plasma CETP mass and lipid profiles in patients with type 2 diabetes mellitus. 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 a decreased plasma CETP mass (4-6).

In this study plasma TG and plasma apoB100 equivalently decreased in both the pioglitazone and metformin group. Accordingly other studies have shown that pioglitazone and metformin both decrease plasma TG (10-13). Given the generally observed inverse relationship between plasma triglycerides and HDL-cholesterol found in epidemiological studies, a decrease in plasma triglycerides only might induce an increase in HDL-cholesterol. However in the present study the decrease in plasma triglycerides was not different between the groups and plasma HDL-cholesterol only increased in the pioglitazone group, indicating 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 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 at this moment not possible 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 (PPAR γ), which is most dominantly expressed in adipose tissue (15). 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 might 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, might in fact be larger.

To our knowledge only one other study evaluated the effect of thiazolidinediones on CETP expression, but they did not study hepatic TG content. Chappuis et al. (17) performed a cross-over study comparing pioglitazone versus rosiglitazone in 17 patients with type 2 diabetes mellitus. 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 the results on the effects of pioglitazone with our study might be due to the shorter treatment duration of 12 weeks and/or the smaller sample size in the previous study.

Lastly, we found that pioglitazone did not further decrease CETP mass in patients who already used a statin. Experimental studies in APOE*3-Leiden.CETP mice have demonstrated that statins decrease plasma CETP mass by decreasing hepatic mRNA expression, again related to decreased hepatic cholesterol content (4). Apparently the effect of statins is dominant to 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. As Kinoshita et al. (18) showed that men have lower CETP activity than women, these data may not be readily extrapolated to women with type 2 diabetes mellitus.

In conclusion, this study shows that in male patients with type 2 diabetes mellitus 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.

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