Metabolic imaging in gluco-lipotoxic heart and liver disease in type 2 diabetes

Rijzewijk, L.J.

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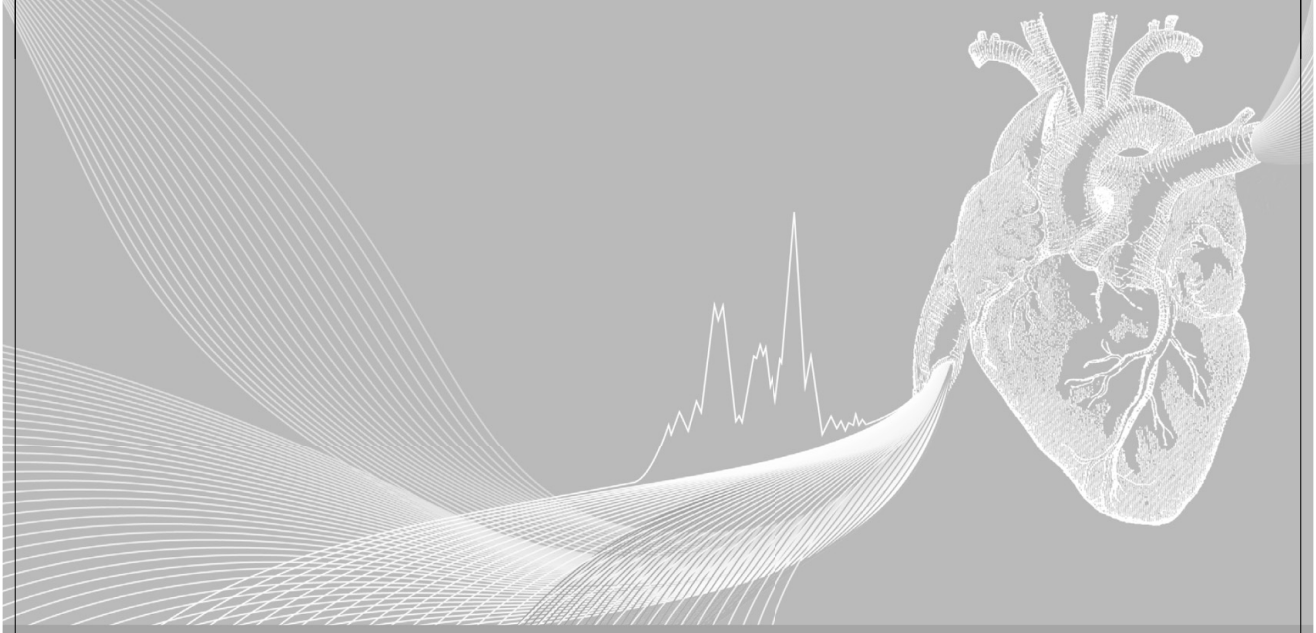
Differential effects of pioglitazone and metformin on hepatic fat content, metabolism and perfusion in diabetes

Chapter 7

Luuk J Rijzewijk
Mark Lubberink
Rutger W van der Meer
Hildo J Lamb
Johannes A Romijn
Albert de Roos
Jos W Twisk
Robert J Heine
Adriaan A Lammertsma
Johannes WA Smit
Michaela Diamant



Submitted

Abstract

CONTEXT: Hepatic steatosis is frequently present in type 2 diabetes (T2DM) and it is linked to features of the metabolic syndrome, cardiovascular and chronic liver disease. Hepatic steatosis is associated with hepatic insulin resistance, altered regulation of hepatic glucose and triglyceride metabolism, and increased triglyceride output and hepatic perfusion. Consequently, insulin sensitizing and anti-steatotic therapies may be beneficial in normalizing hepatic physiology.

OBJECTIVE: To assess in vivo the effects of pioglitazone versus metformin treatment on hepatic triglyceride content, parenchymal perfusion and metabolism in T2DM patients.

DESIGN: Randomized Clinical Trial

SETTING: General community

PATIENTS: Seventy-eight T2DM overweight males

INTERVENTION: Pioglitazone (30mg/day) or metformin (2000mg/day) and matching placebo

MAIN OUTCOME MEASURES: Positron-emission tomography: [^{15}O]H_2O, [^{11}C]palmitate and [^{18}F]-2-fluoro-2-deoxy-D-glucose and ^{1}H-MR spectroscopy: hepatic triglyceride content, before and at 24 weeks of intervention.

RESULTS: Both therapies similarly improved glycemic control and whole-body insulin sensitivity. Pioglitazone versus metformin reduced hepatic triglyceride content ($P<0.001$). Pioglitazone, but not metformin, increased insulin-mediated hepatic glucose uptake ($P=0.025$) and liver parenchymal perfusion ($P=0.044$) from baseline. Neither treatment influenced hepatic fatty-acid influx rate constant.

CONCLUSIONS: In T2DM patients, pioglitazone, but not metformin, reduced hepatic triglyceride content in association with improvement of hepatic parenchymal perfusion and insulin-mediated hepatic glucose uptake, even though both drugs similarly improved hyperglycemia and whole body insulin sensitivity.

Introduction

The prevalence of obesity, the metabolic syndrome and type 2 diabetes (T2DM) has rapidly increased over the last decades.(1) Hepatic steatosis, a frequent finding in T2DM, is causally linked to features of the metabolic syndrome, as well as to cardiovascular and chronic liver disease.(2;3) Glucose homeostasis and lipid metabolism are under tight control of the liver with additional regulation by insulin.(4) Consequently, insulin resistance related to hepatic steatosis contributes to an increased hepatic glucose production and very-low density lipoprotein output.(5;6) In addition, alterations in splanchnic glucose and fatty-acid metabolism have been reported using both splanchnic catheterization(7-9) and positron-emission tomography (PET).(10-17)

Hepatic steatosis has been related to a change in portal vein hemodynamics.(18;19) Evidence from animal models with non-alcoholic fatty liver disease (NAFLD),(20) but also from human steatotic livers studied and immediately following donor organ retrieval using laser Doppler flowmetry, showed impaired hepatic microcirculation.(21) Moreover, an inverse relation between hepatic perfusion and liver triglyceride content was shown in T2DM patients.(17)

As hepatic steatosis and insulin resistance are considered to be closely associated with metabolic and hemodynamic alterations, anti-steatotic and insulin-sensitizing therapies may correct hepatic physiology and, consequently, systemic metabolic derangements. In addition to weight reduction and exercise, pharmacological therapies have been advocated.(3) Several studies, albeit with variable efficacy, have shown that insulin sensitizing therapy with thiazolidinediones and metformin may be beneficial as they reduce hepatic fat, improve glucose and lipid metabolism and reduce inflammation.(22-24)

In vivo mechanistic metabolic studies in human liver disease are limited, as highly invasive methodology, such as biopsies and catheterization, are required. Accurate non-invasive alternatives, however, are available, including proton-MR spectroscopy (^{1}H-MRS) for the measurement of hepatic triglyceride content,(25) and PET for the measurement of hepatic metabolism and parenchymal perfusion.(26-29) To date, no studies have reported on the effects of pioglitazone versus metformin therapy on hepatic glucose metabolism, fatty-acid uptake and parenchymal perfusion in relation to hepatic triglyceride content. The aim of the present study was to evaluate, in patients with well controlled T2DM, the effects of a 24-week treatment with pioglitazone versus metformin on hepatic glucose metabolism, fatty-acid uptake and parenchymal perfusion using PET with the tracer ^{18}F-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG), [^{11}C]palmitate and [^{15}O]H_2O, respectively and hepatic triglyceride content as measured using ^{1}H-MRS.

Methods

Subjects

Patients participated in a 24-week prospective, double-blind, double-dummy controlled intervention on the effects of pioglitazone versus metformin. This study is part of a larger project in which the effect of pioglitazone versus metformin on several aspects of hepatic and myocardial metabolism were investigated.(17;30-32) Details of the intervention have been published previously,(32) and will be recapitulated here in short. Male patients with uncomplicated T2DM, aged 45-65 years, were eligible. Inclusion criteria were glycated hemoglobin (HbA1c) level of 6.5-8.5 % at screening, BMI of 25-32 kg/m^2, and blood pressure not exceeding 150/85 mm Hg, with or without the use of antihypertensive drugs. Exclusion criteria were any clinically significant disorder, particularly any history

or complaints of liver disease and cardiovascular or diabetes-related complications, and prior use of thiazolidinediones or insulin. Written informed consent was obtained from all participants. The study was performed at two institutes in the Netherlands (VU University Medical Center, Amsterdam, and Leiden University Medical Center, Leiden). The protocol was approved by the Medical Ethics Review Committee of both centers, and it was performed in accordance with the Declaration of Helsinki.
Patients being eligible for the study entered a 10-week run-in period to allow for washout of previous blood glucose lowering agents and were all transferred to glimepiride monotherapy and titrated until a stable dose was reached 2 weeks before randomization. This procedure was followed to exclude possible confounding effects of the various glucose lowering agents on hepatic metabolism.
Patients were then randomized to pioglitazone 15 mg for 2 weeks and up-titrated to 30 mg or metformin twice daily 500 mg and up-titrated to 1000 mg twice daily in addition to glimepiride. Patients were requested to adhere to pre-study lifestyle and dietary habits throughout the study.

proton-MR-spectroscopy and PET

All patients underwent (^{1}H)-MRS on a 1.5-T whole-body MR scanner (Gyroscan ACS/NT15, Philips, Best, the Netherlands) before randomization and at 24 weeks of intervention following an overnight fast to determine hepatic triglyceride content. The technical procedures were described previously in detail elsewhere.(32) In addition, an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA) was used for PET studies on hepatic perfusion, glucose and fatty-acid uptake, which were performed within two days from MRS assessments. The technical procedures were described previously in detail elsewhere.(17;32) In short, hepatic parenchymal perfusion was quantified using [^{15}O]H_2O (1100 MBq), and the hepatic fatty acid uptake rate and hepatic glucose uptake (HGU) using [^{11}C]palmitate (185 MBq) and [^{18}F]FDG (170 MBq) respectively. Parenchymal perfusion and fatty-acid uptake were assessed in the fasting state, whereas HGU was performed under hyperinsulinemic euglycemic conditions to achieve an isometabolic steady state with a plasma glucose level of 5 mmol/l. Moreover whole body insulin sensitivity (M/I value), insulin clearance rate (ICR) and post-hepatic insulin delivery rate (IR) were calculated as described in detail previously.(17) The rate constant parameter K_i of [^{11}C]palmitate was not multiplied by fasting plasma fatty-acid levels as was done for glucose, as plasma fatty-acid levels may not accurately reflect portal vein concentrations. Hence only [^{11}C]palmitate K_i values will be reported.

Statistics

Reported values are means ± standard error (SE) or median (interquartile range, IQR) when non-normally distributed. Linear regression analyses with an adjustment for baseline values were performed to analyze differences between groups. To additionally test within-group changes from baseline, independent-paired t-tests or Wilcoxon signed-ranks tests (where appropriate) were performed. All statistical tests were two-sided, significance was considered at the level of 0.05. Analyses were performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). This trial is registered with Current Controlled Trials, number ISRCTN53177482.

Results

Baseline characteristics were previously described in detail elsewhere.(32) In short, both the pioglitazone and metformin group consisted of 39 males. They were well matched with respect to age (pioglitazone; 56.8±1.0 vs metformin; 56.4±0.9), BMI (pioglitazone; 28.2±0.5 vs metformin; 29.3±0.6), waist (pioglitazone; 104±2 vs metformin 105±2) and concomitant medication. Glycemic control improved similarly in both groups from baseline (Table 1). Pioglitazone and metformin differentially altered the lipid profile, γ-GT and ALT (Table 1). Pioglitazone and metformin had similar effects on the M/I value, insulin clearance rate and the post-hepatic insulin delivery rate (Table 1).

Hepatic ^{1}H-MRS measurements were obtained from all 78 patients and. PET data were performed in the first 60 patients. Pioglitazone, but not metformin, significantly decreased liver triglyceride content (6.9 (2.6-17.4) to 4.1 (1.9-12.1) %, P=0.001, versus 7.7 (3.7-23.9) to 10.7 (5.1-22.0) %, P=0.157; between-group P<0.001). The pioglitazone related decrease in liver triglyceride content was paralleled by an increase from baseline in HGU (20.4±1.4 to 24.7±1.6 mmol·gr^{-1}·min^{-1}, P=0.025, versus 24.3±2.5 to 22.9±3.0 mmol·gr^{-1}·min^{-1}, P=0.386; between group P=0.211), hepatic parenchymal perfusion 0.733±0.042 to 0.858±0.057 mL·cm^{-3}·min^{-1}, P=0.044, versus 0.696±0.039 to 0.745±0.042 mL·cm^{-3}·min^{-1}, P=0.386 , between group P=0.174), whereas the hepatic fatty-acid influx rate constant remained unaltered in both groups (0.203±0.025 to 0.220±0.011 mL·cm^{-3}·min^{-1}, P=0.139, versus 0.196±0.009 to 0.198±0.010 mL·cm^{-3}·min^{-1}, P=0.726; between group P=0.197). During scan time no [^{18}F]FDG loss from the liver was detected before and after intervention. Details on detection strategy is described elsewhere.(17)

Discussion

The present study indicates that pioglitazone, but not metformin, increases hepatic parenchymal perfusion and insulin mediated hepatic glucose uptake in patients with T2DM. These changes were accompanied by previously reported effects of pioglitazone, including a decrease in hepatic triglyceride content, improved whole body insulin sensitivity and plasma lipids profiles. The major asset of the present study is the combined use of highly advanced ^{1}H-MRS and PET technology to further assess differential effects of pioglitazone and metformin on hepatic pathophysiology in T2DM patients.

The present study confirms several reports showing a decrease in hepatic triglyceride content by thiazolidinediones (TZDs), as measured by ^{1}H-MRS and markers of hepatocyte injury.(22;33;34) Metformin did not decrease hepatic triglyceride content, which is in line with other reports using ^{1}H-MRS.(22;34) Studies in non-diabetic subjects with NAFLD using metformin have shown contrasting effects on histology and liver enzymes.(35;36)

Various animal studies have shown that diet induced hepatic steatosis impairs hepatic hemodynamics as a result of decreased and impaired sinusoidal perfusion, proportional to the degree of steatosis.(20;37;38) In humans, portal vein hemodynamics was decreased in human NAFLD.(18;19) Furthermore, human steatotic livers, studied immediately following organ retrieval using laser Doppler flowmetry, showed impaired hepatic microcirculation.(21) We recently showed a direct relation between liver triglyceride content and hepatic perfusion.(17) To our knowledge, only one non-controlled pilot intervention study has reported a beneficial effect of a combined lifestyle and pharmacological intervention on portal blood flow,(24) but no study has evaluated the effect on parenchymal perfusion. In

11 biopsy proven NAFLD patients, a 6 months intervention with diet, increased physical activity and 1500 mg metformin significantly improved portal blood flow.(24) It is likely that this beneficial effect was not related to the use of metformin, as no effect of metformin per se was found in the present study. Moreover, dietary interventions and exercise can both independently improve steatosis.(39;40). Results of the present study show, for the first time, that thiazolidinedione therapy increases hepatic parenchymal perfusion in T2DM patients, which is associated with a decrease in hepatic triglyceride content. This improvement may be related to alterations in sinusoidal architecture and functionality, which has been shown to be perturbed in hepatic steatosis.(37)

Insulin mediated hepatic glucose uptake was increased by pioglitazone, but not by metformin. Moreover, both treatments improved whole body insulin sensitivity and insulin clearance rate, but not the post-hepatic insulin delivery rate. Previously Bajaj et al(33) evaluated the effects of a 16 week treatment with pioglitazone (45 mg/day) on splanchnic glucose uptake and whole body insulin sensitivity in 14 T2DM patients, using the euglycemic hyperinsulinemic clamp in combination with a 75 g oral glucose load. In addition, they performed ^{1}H-MRS and found pioglitazone to improve insulin mediated suppression of endogenous glucose production, augment splanchnic and peripheral tissue glucose uptake, and reduce hepatic fat content. Those latter findings concur with the present study. Iozzo et al(41) randomized a total of 30 T2DM male and female patients to rosiglitazone (8 mg/day), metformin (2000 mg/day) or placebo for 26 weeks and measured hepatic and whole body glucose uptake using the combined ^{18}F-FDG PET and clamp technique, but ^{1}H-MRS measurements of hepatic triglyceride content were not included. Rosiglitazone and metformin increased hepatic glucose uptake, albeit the latter effect was only borderline significant. Both treatments enhanced whole body insulin sensitivity. The present results are comparable with those of Iozzo et al, except for an effect of metformin on hepatic glucose uptake, in spite of similar metformin dosing. Furthermore, in 20 T2DM patients, Tiikkainen et al,(22) evaluated the effects of 16 week rosiglitazone (8 mg/day) versus metformin (2000 mg/day) therapy on hepatic triglyceride content using ^{1}H-MRS. Rosiglitazone relative to metformin decreased hepatic triglyceride content. This finding is in line with the observed decrease in liver triglyceride content in the present pioglitazone group.

In the present study no effect of either pioglitazone or metformin on hepatic fatty-acid uptake was observed. Iozzo et al,(42) showed similar hepatic fatty-acid uptake and esterification rates in lean and obese humans, however fatty-acid oxidation was increased in obese subjects. As hepatic fatty-acid esterification and oxidation were not quantified in the present study, the effect of pioglitazone and metformin remains to be established on these parameters. Several investigators, assessing the effects of diet and/or physical activity,(43-45) found a reduction in fatty-acid uptake related to a reduction in hepatic fat. In spite of decreased hepatic triglyceride content and increased hepatic insulin sensitivity, differences in energy balance and subsequent changes in substrate metabolism may explain the observed differences. It may, however, be speculated that the combination of pioglitazone with the life-style measures described above may have an additional beneficial effect on hepatic steatosis and metabolism with subsequent favorable changes in systemic metabolism in T2DM.

Table 1*. Biochemical data and metabolic characteristics at baseline and at 24 weeks

	Pioglitazone			Metformin			
	Baseline	**24 weeks**		**Baseline**	**24 weeks**	**P value**	**P value (between groups)**
Fasting							
HbA_{1c}, %	7.1±0.2	6.5±0.1	< 0.001	7.0±0.1	6.3±0.1	< 0.001	0.146
Glucose, mmol/L	9.0±0.4	8.1±0.4	0.007	8.3±0.4	6.8±0.3	0.002	0.052
Insulin, pmol/L	62 (40-83)	50 (35-71)	0.439	64 (30-93)	47 (28-88)	0.316	0.708
Total cholesterol, mmol/L	4.5±0.1	4.6±0.2	0.374	4.9±0.2	4.5±0.2	0.001	0.042
LDL cholesterol, mmol/L	2.5±0.1	2.5±0.1	0.380	2.9±0.1	2.6±0.2	0.001	0.107
HDL cholesterol, mmol/L	1.07 (0.94-1.28)	1.23 (0.99-1.46)	0.003	1.13 (0.90-1.42)	1.02 (0.86-1.26)	0.133	0.009
ALT, U/L	31 (21-50)	26 (20-39)	0.002	33 (26-41)	33 (23-41)	0.313	0.409
γ-GT, U/L	36 (25-47)	28 (21-39)	< 0.001	35 (20-46)	29 (21-39)	0.001	0.206
During hyperinsulinemia							
M/I value, (mg/kg·min)/(pmol/L)	0.46 (0.28-0.73)	0.54 (0.43-0.97)	0.001	0.45 (0.19-0.80)	0.58 (0.35-1.00)	0.033	0.501
Insulin, pmol/L	575 (503-620)	522 (467-599)	0.022	614 (535-722)	523 (461-608)	0.002	0.943
ICR, ml/min	1017 (964-1094)	1111 (951-1345)	0.005	9.33 (857-1066)	1100 (960-1240)	0.004	0.242
IDR, pmol/min	57 (39-95)	54 (43-85)	0.443	68 (33-87)	56 (31-100)	0.620	0.949

Data are mean ± SE or median (IQR). HbA_{1c} = glycated hemoglobin. LDL = low-density lipoprotein. HDL = high-density lipoprotein. ALT = alanine aminotransferase. γ-GT = γ-glutamyl trasnferase. M/I value = whole body insulin sensitivity adjusted during the steady state. ICR = insulin clearance rate. IDR = post-hepatic insulin delivery rate.
* Adapted from reference 32.

Conclusion

In T2DM patients, pioglitazone and metformin similarly improved glycemic control and whole body insulin sensitivity. However, only pioglitazone reduced hepatic triglyceride content. Pioglitazone and metformin exerted differential effects on hepatic parenchymal perfusion and insulin mediated HGU, which both increased by pioglitazone treatment. These data add to the beneficial pleiotropic effects of thiazolidinediones in T2DM.

Limitations

There are several limitations which should be mentioned. Firstly, PET measurements were made under different conditions (fasting and clamp), precluding a direct comparison between hepatic glucose and fatty-acid metabolism. Secondly, hepatic triglyceride content was quantified using ^{1}H-MRS. To that purpose, only three MR slides of the liver were made for voxel localization. Therefore data on total liver volume is not available and the study's conclusions are limited to liver tissue studied within the volume of the voxel. Thirdly, only ^{11}C-palmitate uptake was determined, hence the present study precludes a statement about the effects of pioglitazone and metformin on fatty-acid esterification and oxidation. Finally, the present study was designed to investigate the effects of pioglitazone versus metformin on liver triglyceride content in patients with T2DM rather than in NAFLD. Hence, all patients included had T2DM, but with a variable amount of hepatic triglyceride content.

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