

***In vivo* assessment of the effects of pioglitazone on myocardial triglyceride content and cardiac function in diabetic mice using ¹H MRS and MRI**

D. Abdurrachim¹, K. Nicolay¹, and J. J. Prompers¹

¹Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands

Introduction

Myocardial disease in diabetes patients which cannot be ascribed to coronary artery disease or hypertension is termed diabetic cardiomyopathy and affects 60% of well-controlled type 2 diabetes patients [1]. The diabetic heart is characterized by increased fatty acid oxidation, which is however not sufficient to cope with the excessive fatty acid supply. This leads to myocardial triglyceride (TG) accumulation, which is potentially toxic to the heart. Some studies have indeed shown that myocardial TG accumulation is associated with cardiomyopathy [2, 3]. Pioglitazone is one of the commonly prescribed anti-diabetic drugs; however, its effects on cardiac function and metabolism are not yet clear [4, 5]. In this study, the effects of pioglitazone on myocardial TG content and cardiac function were investigated *in vivo* in a diabetic mouse model using ¹H MRS and MRI, respectively.

Methods

Animals: Nine female C57BL/KsOlaHsd diabetic (db/db) and eight non-diabetic (db/+) mice (Harlan, UK) were used in this study. The animals were given access to water and food *ad libitum* and underwent MR measurements at 7 and 13 weeks of age. After the measurements at 7 weeks, four db/db mice were treated with pioglitazone (37.4±3.1 mg/kg/day) admixed in chow for 6 weeks. Prior to MR measurements, fasting blood glucose levels were measured.

MR protocol: The animals were anesthetized with 1.5–2% isoflurane in medical air. ECG signal and respiration were measured and used for cardiac triggering and respiratory gating. MRI and ¹H MRS were performed on a 9.4T horizontal bore MR scanner (Bruker) using a 35-mm quadrature birdcage coil (Bruker) or a 53.8-mm quadrature birdcage coil (Rapid Biomedical) for both RF transmission and signal reception. Cardiac movies were acquired in 4–5 contiguous short axis and 2 long axis slices (thickness: 1 mm) using prospective triggered cinematic FLASH MRI (TR/TE: 7/1.8 ms, α: 15°, matrix: 192x192, FOV: 30x30 mm², frames: 15–18, NSA: 6). To assess diastolic function, FLASH MRI with higher time resolution (TR/TE: 4.85/1.76 ms, α: 30°, matrix: 128x128, FOV: 30x30 mm², frames: 24–28, NSA: 6) was performed on the mid-ventricular slice. Localized ¹H MR spectra were acquired in diastole in the interventricular septum using a cardiac triggered and respiratory gated PRESS sequence (TR/TE: ~2s/9.1 ms, voxel: 1x2x2 mm³, NSA: 256) with CHESS water suppression, as described previously [6].

Data analysis: Left ventricular (LV) endocardium was semi-automatically segmented using CAAS MRV 2.0 (Pie Medical) to obtain ejection fraction (EF) as a measure for systolic function. For diastolic function, LV peak filling rates (PFR) were determined from the dV/dt of the endocardial volume normalized to the end diastolic volume (EDV). Spectral processing and fitting were performed using AMARES in jMRUI [7]. Myocardial TG levels were then calculated from the TG-CH₂ signal relative to the unsuppressed water peak. All data are presented as means ± SD. Statistical analysis was performed with SPSS 17.0 using ANOVA for repeated measures with age (7 and 13 weeks) as the within-subject factor, and group (db/+, non-treated db/db and pioglitazone-treated db/db) as the between-subject factor.

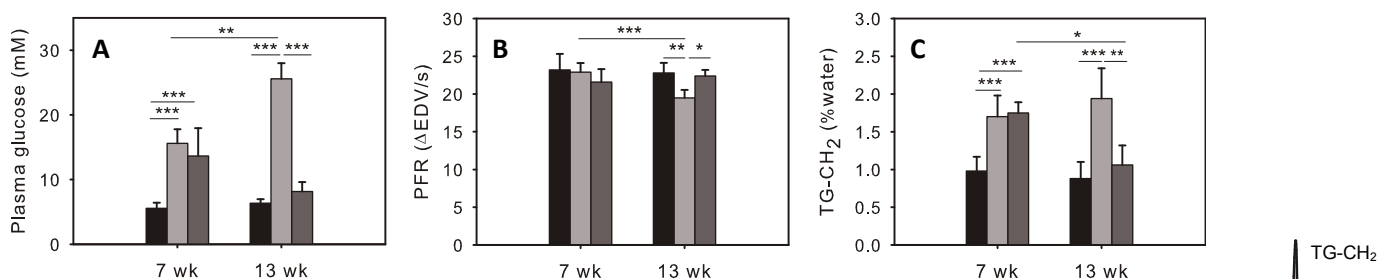


Figure 1. (A) Plasma glucose levels, (B) LV peak filling rate and (C) myocardial TG levels at 7 and 13 weeks (left bar: db/+, middle: non-treated db/db, right: pioglitazone-treated db/db; *P<0.05, **P<0.01, ***P<0.001)

Results

Plasma glucose levels: At 7 weeks, db/db mice were already hyperglycemic compared to db/+ mice (Fig. 1A). At 13 weeks, plasma glucose levels of non-treated db/db mice were further increased, while those of pioglitazone-treated db/db mice were normalized to the levels of db/+ mice (Fig. 1A).

Cine MRI: LV ejection fraction was similar in all animal groups and at both 7 and 13 weeks (data not shown). At 7 weeks, LV peak filling rate (PFR) was identical in all groups. However, at 13 weeks, LV PFR was significantly reduced in non-treated db/db mice, while in pioglitazone-treated db/db mice LV PFR remained the same as in db/+ control mice (Fig. 1B).

¹H MRS: Typical cardiac ¹H MRS spectra are shown in Fig. 2. At 7 weeks, db/db mice showed significantly higher myocardial TG content compared to db/+ mice (Fig. 1C). This was also the case at 13 weeks for non-treated db/db mice, while for pioglitazone-treated db/db mice the myocardial TG level was normalized to the value in db/+ mice (Fig. 1C).

Discussion

In this study, we confirmed the association between myocardial TG accumulation and cardiac dysfunction as measured by ¹H MRS and MRI, respectively. The myocardial exposure to a high level of TG in non-treated db/db mice was paralleled by the development of diastolic dysfunction. In pioglitazone-treated db/db mice plasma glucose and myocardial TG levels were normalized and cardiac function was preserved. Our findings show that pioglitazone treatment in an early stage of diabetes prevents the development of diabetic cardiomyopathy, possibly by reducing lipotoxic effects by diverting fatty acids to adipose tissue.

References: [1] Redfield, M.M, 2003, *JAMA*, 289:194. [2] Christoffersen, C, 2003, *Endocrinology*, 144:3483. [3] Rijzewijk, L.J, 2008, *J Am Coll Cardiol*, 52: 1793. [4] Van der Meer, R.W, 2009, *Circulation*, 119:2069. [5] Zhou, Y.T, 2000, *Proc Natl Acad Sci USA*, 97:1784. [6] Bakermans, A.J, 2010, *Proc ISMRM*, 18:1320. [7] Vanhamme, L, 1997, *J Magn Reson*, 129:35

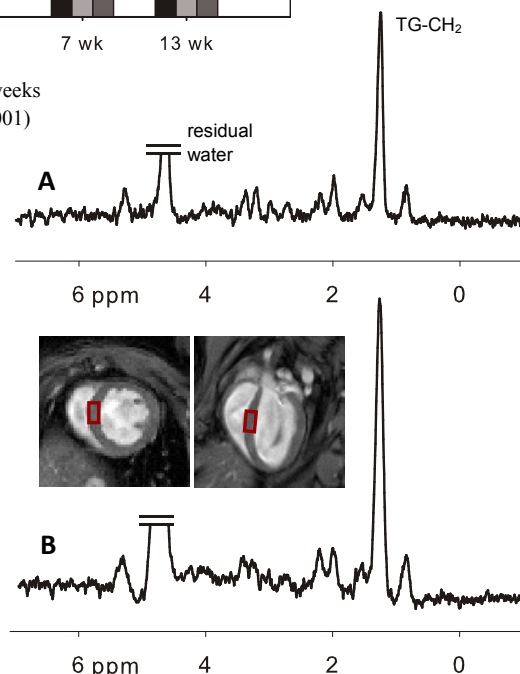


Figure 2. Typical cardiac ¹H spectra from the septum (red voxel) of 7-week old (A) db/+ and (B) db/db mice