Cardiac function and fat composition in STZ-induced diabetic rats

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Introduction: During the last 20 years the number of people with diabetes worldwide has risen from 30 million to 230 million, according to the International Diabetes Federation. The disease is expected to affect 380 million people worldwide by 2025. End stage diabetes is always associated with heart complications, which is the leading cause of death in diabetic patients. The usual characteristics of diabetic heart failure are left ventricular dysfunction, myocyte hypertrophy, interstitial fibrosis and fatty acid depletion (1). Streptozotocin (STZ)-induced diabetic rats have been used as a model for diabetic cardiac dysfunction (2), although the mechanisms leading to diabetic heart failure remain uncertain. In this study, we investigated cardiac function and myocardial fat content in STZ-rats over an extended period of time using High Field MRI. We used MRI cine images to calculate cardiac function and Dixon images to calculate cardiac fat volume. We hypothesized that cardiac fat composition could be a useful biomarker for diabetic heart failure in this animal model.

Methods: Nineteen age matched Wistar rats underwent cardiac examination by MRI, of which eight were controls and eleven were rendered diabetic by a single intravenous tail vein injection of STZ (Sigma; 55 mg/kg bodyweight) in isotonic saline. Blood glucose was monitored weekly via tail tip bleeding. Cardiac MR Images were acquired before STZ injection, and 8 weeks and 16 weeks after STZ injection using a Varian (Palo Alto, CA, USA) 4.7T horizontal bore magnet controlled by a Unity Inova spectrometer. Animals were prepared for MRI exams by induction of general anaesthesia with 4% isoflurane in air, placement of ECG, respiration and temperature monitoring electrodes (SAII, Stony Brook, NY) and mounting in a 72 mm ID circularly polarize bird cage coil (M2M Imaging, Cleveland, OH, USA). Core body temperature was maintained at 35-38°C throughout the study by directing a regulated warm air source over the animal. Following acquisition of scout images, a cardiac and respiration gated T_1 weighted gradient echo cine study was acquired in the cardiac short axis orientation spanning from the apex to the base of the left ventricle (TR = 2* R-R interval, ~ 280-360 msec, TE = 2.2 msec, cardiac phases = 20, flip angle = 20°, slices = 6, thickness = 2 mm, averages = 2, FOV = 60x60 mm, matrix = 128x128). A second cine study was then performed in the cardiac long axis view using the same acquisition parameters (slices = 3, and oriented to give 2-, 3- and 4-chamber views). Cardiac short axis images were then generated using the three point Dixon fat/water imaging method (3) (TR = 1000 msec, TE = 20 msec and matrix = 256x128). End diastolic volume (EDV), end systolic volume (ESV), ejection fraction (EF) and left ventricular mass (LVM) were determined from regions of interest that were manually drawn in the cine images using ImageJ. A similar method was used to estimate the cardiac fat volume in the Dixon images.

Results: Blood glucose levels in STZ-injected rats rose from < 8 mmol/L to > 25 mmol/L two days after STZ injection and stood at that level throughout the remainder of the study. Left ventricular images with high blood/myocardium contrast were generated using the cine protocol (Figure 1. a-d). Water images generated with the Dixon method had high signal to noise ratio (SNR) and clearly depicted the cardiac anatomy. However, the anatomy was not delineated in fat images which exhibited several limited regions of high intensity (Figure 1. f-k). Cardiac EF in control (SHAM) rats did not show any change in EF at weeks 0, 8 and 16. However, there is a significant decrease in EF values in the affected animals after 8 weeks (From 80% ± 1.8% to 73.6% ± 5.0%, Figure 1. e, p < 0.01, one-way ANOVA) which continues to declined at 16 weeks (65.7% ± 5.7%, 8 - 20% drop in EF values, p < 0.0001, one-way ANOVA). No significant change in LVM/body-weight ratios was observed in weeks 0, 8 and 16 rats, even though the mean value at week 16 was higher than that at week 0. The mean volume of measurable cardiac fat as determined from the Dixon images (volume of pixels above the threshold of fat intensity) was 34 ± 6.6 mm^3 in week 0 and it dropped below detectable limit in the diabetic animals after 8 weeks of diabetes (Figure 1. i-k). At week 16, measurable cardiac fat was still below the detectable limit and produced similar fat images as that of week 8.

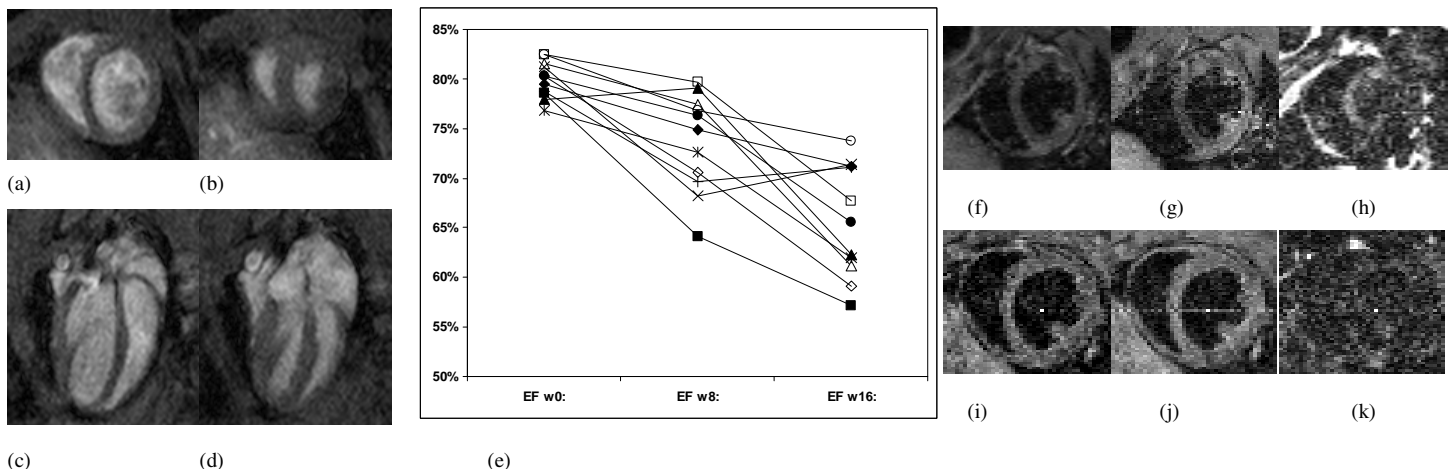(a) (b) (c) (d) (e) (f) (g) (h) (i) (j) (k)

Figure 1. (a-d) Cardiac cine images of a rat heart at diastole (a. short axis and c. long axis) and systole (b. short axis and d. long axis). (e) The change in EF during the 16 week experiment period. (f-k) Cardiac Dixon short axis images at diastole of a rat heart before STZ injection (f. water & fat signals combined, g. water signal only, h. fat signal only); and 8 weeks after STZ injection (i. water & fat signals combined, j. water signal only, k. fat signal only).

Discussion: A significant decrease in ejection fraction was detected using cine MRI in rats with STZ-induced diabetes at 8 weeks and 16 weeks. This is consistent with our previous studies on isolated perfused hearts of STZ rats, which showed that cardiac output was significantly attenuated (4). Histological studies in the same report also showed significant structural damage in LV of STZ rats. Diabetes in humans is always accompanied by LV hypertrophy and LV dysfunction under glycemia (5). Our results showed that STZ-diabetic rats can mimic the cardiac impairment observed in human disease. The observation suggests that STZ-injected rats can be used as an animal model for diabetic heart failure. Furthermore, diabetes caused a noticeable fat depletion in rat heart within 8 weeks. Fat depletion was always associated with heart failure (1). Fat composition in diabetic human heart has never been thoroughly investigated. The present study suggests that cardiac fat composition could be a useful biomarker for diabetic heart failure, and detecting fat content in heart using MRI could be a useful technique to determine this biomarker.

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