

IN VIVO QUANTIFICATION OF RENAL LIPID IN DIABETIC MICE BY MAGNETIC RESONANCE IMAGING

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Introduction Diabetic nephropathy is the leading cause of endstage renal disease worldwide and a leading cause of morbidity and mortality in diabetic patient. There is growing evidence that abnormal lipid metabolism and renal accumulation of lipids play a role in the pathogenesis of diabetic nephropathy (1-3). Kidney biopsy and histological analysis is not ideal for longitudinal follow-up studies. Proton (¹H) magnetic resonance (MR) imaging offers several non-invasive methods to obtain separate fat and water images, allowing for obtaining the fat-to-water ratio in tissues and quantifying the fat content. Sbarbat et al. applied chemical shift imaging (CSI) to quantify lipid in the brown adipose tissue *in vivo* (4). MR measurements of fat contents in the liver, skeletal muscle, pancreas and heart *in vivo* have been studied (5-8). However, to our knowledge, this non-invasive approach has not yet been applied and investigated for measuring the lipid accumulation in kidney. The purpose of this study was to study the feasibility and accuracy of *in vivo* MR measurement of lipid accumulation in kidney using the diabetes (*db/db*) mouse model.

Methods All animal experiments were approved by the institutional Committee on Animal Research. Fifteen-week-old C57BLKS/J *db/db* and wild type (WT) mice (*n*=10) with an average weight of 51.63 ± 1.89 g and 31.38 ± 1.44 g were used in this study. Phantoms were made according to the volume percentage of fat ranging from 0% to 100%. All MR experiments were carried out using a 7.0 T small animal magnetic resonance system (Bruker PharmaScan, Ettlingen, Germany). CSI were performed to measure lipid contents (LCs) in phantoms, kidneys of mice. The results were compared to known LCs in phantoms and to the reference standard from mice by kidney lipid (KL) chemical analysis. T1WI was performed to estimate adipose tissue distribution of visceral (VS) and subcutaneous (SC) of mice.

Results CSI underestimated fat concentration when FC was in range of 50% to 100%. Full details about imaging phantoms were described in our previous study (5). *In vivo*, the averaged LC_{CSI} of the renal cortex and medulla in *db/db* mice were $12.73 \pm 0.94\%$ and $4.64 \pm 1.02\%$, respectively. In comparison, the averaged LC_{CSI} of renal cortex and medulla in WT mice were $3.16 \pm 0.50\%$ and $2.67 \pm 0.70\%$. Significant differences in kidney LC in renal cortex and renal medulla between the *db/db* mice and WT mice were observed using the data from CSI ($P < 0.01$). Interestingly, the Kidney LC in renal medulla of *db/db* mice is low than that in the renal cortex ($P < 0.01$), but no significant difference between the renal cortex and medulla was found in WT mice ($P = 0.068$). The averaged KL in *db/db* mice calculated by the chemical analysis method was higher than that of WT mice with 9.22 ± 1.44 mg and 4.11 ± 0.74 mg, respectively. A strong correlation between the averaged KL measured by the chemical method and the LC calculated by the MR method ($r = 0.916$, $P = 0.000$) was observed. Visceral fat of the total amount of white adipose tissue in *db/db* mice was significantly higher than that of WT mice. In the kidney sections of *db/db* mice examined by the oil-red O staining, lipids were found accumulated in the glomeruli and proximal tubules of the renal cortex, but not in the WT mice.

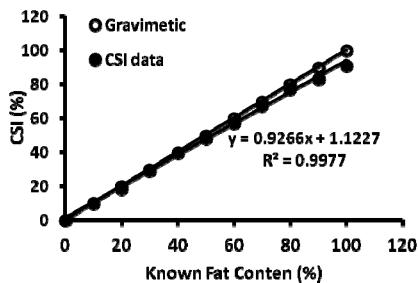


Fig.1

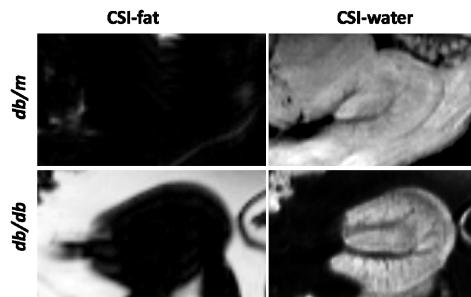


Fig.2

Figure 1. CSI underestimated fat concentration when FC was in range of 50% to 100%. CSI has a high linear correlation with the known gravimetric reference standard (CSI: $r=0.997$, $P < 0.01$).

Figure 2. *In vivo* CSI (selective water protons imaging) showed signal intensity in the cortex of diabetic kidney was lower than that of control group.

Discussion Accumulation of excess lipids in nonadipose tissues leads to cell dysfunction or cell death (9). This phenomenon, known as lipotoxicity, may play an important role in the pathogenesis of diabetes (9). Glomeruli and renal tubules (proximal tubules in particular) seem to be most susceptible to lipid accumulation. CSI includes two series, selective fat-protons imaging and selective water-protons imaging. CSI is thus able to not only accurately measure the FC, but also evaluate its distribution. But ¹H MRS of kidney parenchyma has been hampered by technical difficulties such as respiratory motion, and subsequent contamination from perirenal or hilar fat. Lipid is usually accumulated in renal cortex instead of medulla, while the least voxel of MRS is around $1.5 \times 1.5 \times 1.5$ mm, which is still much larger than the area can exclusively contain cortex, thus can lead to underestimate lipid content.

Conclusion Our results indicate that T₁WI can be used to observe fat distribution, and CSI is accurate in quantifying lipid in both phantoms and kidney in mice and evaluating its distribution. The renal lipid accumulation, lipid content in renal cortex of *db/db* mice was significantly higher than that of renal medulla. We proposed that MRI will be used to study animal models and patients with various renal diseases resulting from lipotoxicity.

Reference (1) Hsu, C. et al, *Am J Kidney Dis.* 2000; 36:272-281. (2) Wang, Z., et al, *Diabetes.* 2005; 54:2328. (3) Jiang, T., et al, *Diabetes.* 2007; 56, 2485. (4) Sbarbat, A., et al, *J Lipid Res.* 1997; 38, 343-347. (5) Peng, X.G., et al. *J Lipid Res.* 2011;52:184-1855. (6) Gaeta, M., et al. *Radiology.* 2011;259:487-494. (6) Schwenzer, N.F., et al, *Invest Radiol.* 2008;43: 330-337. (8) Goldfarb, J.W. *Radiology.* 2009;253: 65-73. (9) Weinberg, J. *Kidney Int.* 2006; 70: 1560-1566.