

TOWARD QUANTITATION OF PANCREATIC β CELL MASS USING A TWO-SITE EXCHANGE ANALYSIS OF MANGANESE-ENHANCED MR IMAGES

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Introduction: Type 1 diabetes is a metabolic disorder characterized by an inability to maintain normoglycemia due to autoimmune destruction of the insulin-producing β cells in pancreatic islets. In non-diabetic individuals, β cells secrete insulin into the bloodstream in response to a sensed elevation in blood glucose. Insulin secretion, stimulated by the intracellular transport of glucose, is immediately preceded by calcium ion (Ca^{2+}) influx through voltage-gated Ca^{2+} channels. Manganese (Mn^{2+}) is a paramagnetic T1-shortening contrast agent that enters specific cells (including β cells and potentially other pancreatic cells) through Ca^{2+} ion channels, leading to a compartment-dependent concentration of contrast agent, with higher intracellular concentration and lower extracellular concentration. Mn^{2+} -enhanced MRI has been used previously to probe β -cell function^{1,2}, however these MRI methods were not quantitative. We investigated using a two site exchange analysis of Mn^{2+} -enhanced MR images of the pancreas to more quantitatively estimate β -cell mass, and evaluated these methods in non-diabetic mice and in a mouse model of type 1 diabetes.

Methods: Our overall approach was to employ Look-Locker imaging after Mn^{2+} injection and apply a two site water exchange analysis to the longitudinal magnetization recovery curve of the pancreas. This approach was taken because we reasoned that the intracellular water fraction estimated from this analysis would reflect β cell mass. Type 1 diabetes was induced by a single injection of 180 mg/kg streptozotocin (STZ), a drug that is selectively toxic to pancreatic β -cells, and mice were confirmed to be diabetic by having 3 consecutive blood glucose readings above 250 mg/dl. Non-diabetic ($n = 5$) and diabetic mice ($n = 5$) were imaged on a 7T Clinscan system (Bruker, Germany). All animals received an intravenous bolus injection of 100 μ l glucose (1.5 mg/g) 2 minutes prior to an intraperitoneal injection of 50 μ l MnCl (0.1 μ mol/g). Coronal images of the pancreas were acquired using a Look-Locker inversion recovery sequence 1 hour after Mn^{2+} injection. The pulse sequence used a nonselective 180 $^\circ$ inversion pulse followed by a train of gradient echoes separated by a delay time of 20-35 msec. Other parameters were: time between inversions = 5500 msec, TE = 1.9msec, flip angle = 3 $^\circ$, slice thickness = 1mm, FOV = 35mm x 25mm, number of images = 100, and number of averages = 3. After image acquisition, a region of interest was drawn around the pancreas and the pancreatic T1 relaxation curve was used to solve a two site exchange T1 relaxation model. The two site exchange model considers two water compartments (extracellular and intracellular) with separate T1 values and water exchange between compartments. The longitudinal Bloch equations governing two site exchange are:

$$\frac{dM_{Zic}}{dt} = -[M_{Zic}(t) - M_{Zic}^{eq}] / T1_{ic} - M_{Zic}(t) \cdot k_{ic} + M_{Zec}(t) \cdot k_{ec}$$

$$\frac{dM_{Zec}}{dt} = -[M_{Zec}(t) - M_{Zec}^{eq}] / T1_{ec} - M_{Zec}(t) \cdot k_{ec} + M_{Zic}(t) \cdot k_{ic}$$

where M_{Zic}^{eq} and M_{Zec}^{eq} are the respective equilibrium longitudinal magnetizations of the intra- and extracellular water, $T1_{ic}$ and $T1_{ec}$ are the respective the intra- and extracellular relaxation times, and k_{ic} and k_{ec} are the respective exchange rates of intra- to extra- cellular water and vice versa. The intracellular fraction was calculated as $f_{ic} = M_{Zic}^{eq} / (M_{Zic}^{eq} + M_{Zec}^{eq})$. The two site exchange model was solved using a 4th order Runge-Kutta method and model parameters were determined by iteratively minimizing the error between the model and the measured relaxation curve using a nonlinear least squares algorithm with the model parameters initialized to $T1_{ic} = 500\text{msec}$, $T1_{ec} = 667\text{msec}$, $k_{ic} = 2.5\text{s}^{-1}$, $k_{ec} = 1.5\text{s}^{-1}$ and $f_{ic} = .02$.

Results: Figure 1 shows 5 images during T1 relaxation from a typical Look-Locker mouse imaging experiment. An example pancreatic longitudinal relaxation curve in figure 2 compares the fit resulting from the two site exchange model (blue) to a monoexponential fit (red), demonstrating a better fit to the measured data by incorporating two water compartments. For the groups of non-diabetic and STZ-induced diabetic mice, intra- and extracellular T1 values and the intracellular fractions are shown in figure 3. In non-diabetic mice, the intracellular fraction was 0.040 ± 0.001 , whereas the intracellular fraction decreased to 0.025 ± 0.001 in diabetic mice ($p < 0.001$), consistent with loss of β cell mass. T-tests revealed significant differences between normal and diabetic mice for all model parameters.

Discussion: Since Mn^{2+} is compartmentalized, with higher intracellular concentration and lower extracellular concentration, we hypothesized that a two site water exchange analysis of measured T1-relaxation curves could estimate the intracellular fraction, and that the intracellular fraction would reflect β cell mass. Our results in mice showed that two pools of water (extra- and intracellular) in exchange and relaxing at different rates are visible, and fit the measured data better than a monoexponential fit. In addition, significant differences in the model parameters were observable in STZ-induced diabetic mice relative to non-diabetic mice, including a decreased intracellular fraction. The residual intracellular fraction observed in diabetic mice may in part represent another cell population, such as neurons, that takes up Mn^{2+} ions and is not affected by STZ. Future work will be aimed at further elucidating the relationship between the intracellular fraction and β cell mass.

References: 1). Gimi, B. et al. *Cell Transplant.* 2006; 15 (2): 195:203 2). Antkowiak et al. *Proc 16th ISMRM*, 2008.

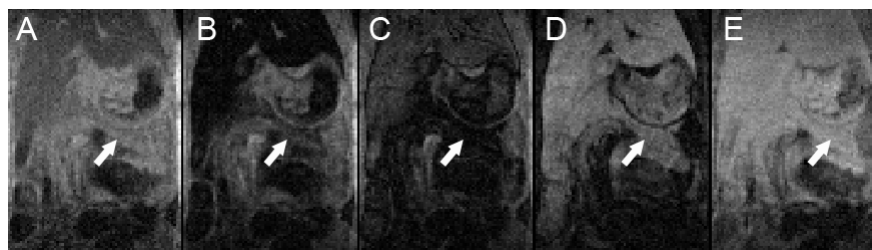


Fig 1 – Look – Locker images of pancreatic T1 relaxation after injection of Mn^{2+} . A). Image immediately after inversion. B-C). Image near (B) and at (C) null time of pancreas. D). Image after null time. E). Image near thermal equilibrium. Pancreas shows enhancement relative to other tissues due to intracellular Mn^{2+} . White arrows denote pancreas.

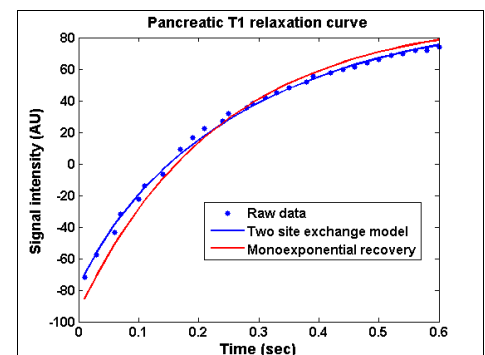


Fig 2 – Pancreatic relaxation curve 1 hour post Mn^{2+} injection. Raw data shown with two site exchange model solution (blue) and monoexponential recovery fit (red). Data is more accurately represented by a two site exchange model.

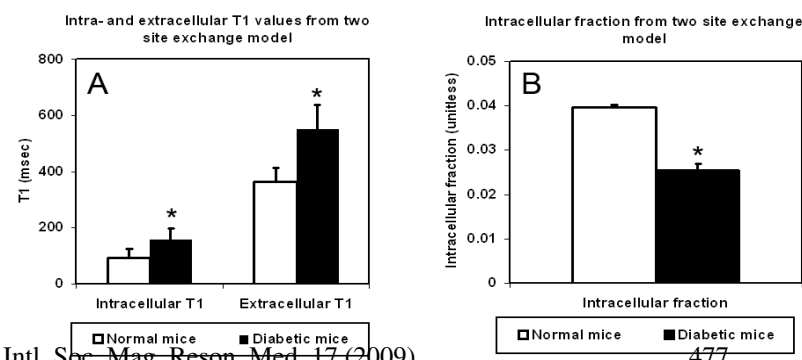


Fig 3 (at left) – Comparison of two site exchange model parameters between normal and diabetic mice at 1 hour after Mn^{2+} injection. A). Intra- and extracellular compartment T1 values. Diabetic mice had significantly greater intra- and extracellular T1 values compared to normal mice ($p < .05$). B). Intracellular fraction. Normal mice had significantly greater intracellular fraction than diabetic mice ($p < .001$).