Cardiac Alterations in Mice with Altered GLUT4 Expression

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Introduction: Cardiac hypertrophy, as an adaptive response to hypertension, has been observed in experimental animals and patients, and is often associated with depressed ventricular performance. Often hypertrophied hearts have an increased susceptibility to damage from myocardial ischemia. Cardiovascular complications in hypertrophied hearts often coincide with metabolic abnormalities.

Genetic ablation of GLUT4, the major insulin sensitive transporter, results in insulin resistance without overt diabetes. GLUT4 Null mice exhibit significantly decreased adipose tissue and severe cardiac hypertrophy (2x Control) while maintaining normal whole body glycemia. The hypertrophy seen in Null animals is not due to pressure overload. Histological examination revealed enlarged myocytes and the presence of ischemic damage in Null cardiac tissue. GLUT4 Null hearts also suffer from metabolic abnormalities, which could potentiate the hypertrophy. MRI and Langendorff perfusion studies of the Null and Control hearts were performed to determine the degree of hypertrophy and hemodynamic performance under normal and ischemic conditions using substrates containing glucose.

Methods: The MRI experiments were performed using a GE OMEGA 400WB spectrometer equipped with a cardiac gating box and a microimaging accessory with 40 mm imaging coils and 50 mm shielding gradients. Mice were anesthetized with 60mg/kg sodium pentobarbital. A set of standard human ECG leads ending in silver wires were attached to the limbs of the mouse and the ECG signal was fed to a Gould ECG coupler associated with a Gould 2200S recorder and Ponemah physiology system. The anesthetized mouse was wrapped in a small blanket and was placed in a plastic animal holder designed to position the mouse within the 40 mm imaging coil. Heart rate and ECG were monitored continuously using the Ponemah system. The rising phase of the QRS complex was used to trigger a standard 5 volt square wave gating signal to initiate acquisition. Just prior to each acquisition the heart rate was determined from the ECG record and the gating delay was set to acquire data at a time after the QRS complex equal to 75% of the R-R interval for diastolic images, or 15-25% of the R-R interval for systolic images.

Images were acquired using the ECG-gated spin warp acquisition sequence with an echo time of 18 ms and a repetition time of approximately 500 ms (varying with heart rate). A 40 mm field of view with a 256 x 256 pixel image matrix was typically used. After locating the heart, a series of transverse images separated by 1 mm were acquired from the base to the apex of the heart.

Typically, 4-5 slices of 1 mm each were required to define the heart. Dimensions were recorded from on screen coordinates.

Once significant hypertrophy was established by the MRI measurements, hearts were excised for perfusion studies. Hearts were perfused in the Langendorff mode at 60 mmHg with a Krebs-Henseleit buffer containing 5mM glucose and 0.1 ng/mL insulin. Excised hearts equilibrated for 20 min. before undergoing a 20 min. ischemic period. After the ischemia, hearts were reperfused with the initial buffer. Left ventricular function was constantly monitored using a Millar catheter associated with the Ponemah physiology system.

Results: Cardiac gated diastolic images were acquired for Control and Null mice. Figure 1 shows short axis images of Control and Null hearts.

![Figure 1: Top 2 images show Null and Control hearts in diastole. Bottom image shows Control heart in systole. Cardiac wall thickness measurements of left free wall (L), septal (S), anterior (A) and posterior (P) were determined in these 8 month old male mice.](image)

<table>
<thead>
<tr>
<th>Thickness in mm</th>
<th>L</th>
<th>S</th>
<th>A</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>1.69</td>
<td>2.59</td>
<td>1.55</td>
<td>2.49</td>
</tr>
<tr>
<td>Control</td>
<td>1.34</td>
<td>1.55</td>
<td>1.51</td>
<td>1.82</td>
</tr>
</tbody>
</table>

Both Control and Null hearts were then used in perfusion studies. Contrary to the compromised function exhibited by the Controls, Null hearts were able to retain their performance after an ischemic episode (% Decrease in dP/dt in Control after ischemia was 63% ± 10%; % Increase in dP/dt in Null after ischemia was 5% ± 10%). This recovery is dependent on adequate energy to maintain ion channel integrity and contractile function. This suggests the Null heart may use glucose more efficiently in pre- and post-ischemic conditions than Control.

Conclusions: Non-invasive cardiac gated MRI was successfully used to monitor the progression of myocardial hypertrophy in GLUT4 Null mice. Preliminary perfusion studies provided evidence of an association between metabolic alterations and hypertrophy. More extensive studies, including investigation of the MAP kinase hypertrophic pathways are currently in progress.