Exploration of Mitochondrial Respiration in Isolated Hearts: An Observation from Metabolically Produced $H_2{}^{17}O$ Using ^{17}O NMR Spectroscopy

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The heart is the most energy-demanding organ because of its non-stop contraction. There is increasing evidence that abnormal mitochondrial function and energy metabolism are characteristic of several prevalent cardiac diseases. However, our fundamental understanding of cardiac energetics in diseased hearts remains poor. To date, most of the studies on mitochondrial function have been performed in isolated mitochondria or with in vitro tissue samples. Observation of the production of metabolic $H_2{}^{17}O$ from the reduction of ^{17}O-oxygen provides the opportunity to evaluated mitochondria oxidation capacity in intact, viable tissues (1;2). However, applications of ^{17}O MRI for evaluating mitochondrial function have been limited due to the challenge of detecting metabolic $H_2{}^{17}O$ in the vast background of natural abundance $H_2{}^{17}O$. In this study, we developed a direct ^{17}O MR Spectroscopy (MRS) approach to examine the feasibility and sensitivity of detecting metabolically produced $H_2{}^{17}O$ in intact hearts at different workload.

Methods

Sensitivity optimization of perfused heart ^{17}O MRS methodology Several improvements were made to our cardiac perfusion apparatus to improve the sensitivity of $H_2{}^{17}O$ detection. First, we developed a closed-loop perfusion system with significantly reduced perfusate volume (26 ml vs. >1 L) to reduce natural abundance $H_2{}^{17}O$ signal. The recycling perfusate was also continually oxygenated by inserting a $^{17}O_2$-filled silicone tubing into the perfusion line to allow the $^{17}O_2$ gas to permeate into the perfusate. In addition, theophylline, an adenosine antagonist, was infused at 0.5 ml/min to maintain ventricular function despite adenosine accumulation.

Animal Models Isolated hearts from 2-month male Sprague-Dawley rats were perfused with oxygenated buffer containing 10 mM glucose at different $[Ca^{2+}]$ (1.5 or 2.5 mM, n=5 for each group) to induce normal/high workload. Left ventricular developed pressure (LVDP) and heart rate (HR) were measured via a water-filled balloon connected to a pressure transducer. At the start of the perfusion protocol, $^{16}O_2$-oxygenated perfusate was supplied for 15 min to ensure metabolic equilibrium. A ^{31}P spectrum was collected to verify the metabolic status of the heart. A natural abundance ^{17}O spectrum was then collected for background subtraction. Once the perfusate was switched to $^{17}O_2$-oxygenated buffer, dynamic acquisition of ^{17}O spectra began immediately for over 5 min.

Dynamic ^{17}O NMR measurements ^{17}O spectra were acquired on a Bruker 9.4T vertical-bore spectrometer with a 20 mm broadband NMR probe. Field homogeneity was adjusted by shimming on the proton signal to a linewidth of 30~50 Hz. Sequential ^{17}O spectra were acquired at 20 s temporal resolution with 64 averages. $H_2{}^{17}O$ was quantified by fitting the resonance peaks with Lorentzian curves followed by the integration of the peak area. The time course of metabolic $H_2{}^{17}O$ accumulation was obtained by normalizing signal intensity to natural abundance $H_2{}^{17}O$ concentration.

Kinetic model and analysis To quantify mitochondrial respiration rate from ^{17}O data, we developed a compartmental model that describes the transport and production of metabolic $H_2{}^{17}O$ during perfusion. Myocardial O_2 consumption rate (MVO_2) was determined from least-square fitting of the model to NMR-measured $H_2{}^{17}O$ concentration. MVO_2 was also calculated from the coronary flow and the difference in O_2 tension between the arterial supply line and the coronary effluent measured with a blood/gas analyzer.

Results

Rate pressure product (RPP=LVDP×HR) increased from 17,000±3,700 mmHg/min at normal $[Ca^{2+}]$ to 29,500±5,200 mmHg/min at high $[Ca^{2+}]$ ($p<0.05$). Both groups showed normal PCr/ATP ratio measured by ^{31}P spectra (1.5±0.2 vs. 1.43±0.2). Dynamic ^{17}O NMR spectra showed progressive increase of $H_2{}^{17}O$ resonance peak (Fig. 1a), suggesting steady production of mitochondrial $H_2{}^{17}O$. Hearts perfused with high $[Ca^{2+}]$ demonstrated accelerated increase in ^{17}O signal (Fig. 1b). Compared to normal workload, model-determined MVO_2 increased from 3.3 to 7.0 μmol/min/g under high workload. Measured MVO_2 were 3.3±0.5 and 6.2±0.2 at normal and high workload, respectively.

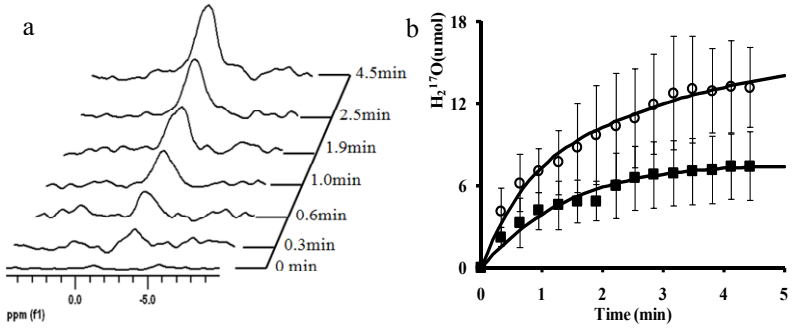

Figure 1 a. Representative dynamic ^{17}O spectra. **b.** Time course of $H_2{}^{17}O$ accumulation. Open circles: high workload; closed squares: normal workload. Solid lines were model fitted curves.

Discussion and Conclusion

In the current study, we demonstrated the feasibility and sensitivity of dynamic ^{17}O MRS in detecting altered metabolic rate associated with changes in cardiac workload. In combination with kinetic modeling, this high temporal resolution MRS method has the potential to quantify mitochondrial respiration rate within 5 min of data acquisition.

References

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