Intracellular Redox State Quantification by 31P MRS measurement of NADH and NAD+ Content during Ischemia/Reperfusion in Perfused Rat Heart

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Purpose

Intracellular redox state is thought to be a key regulator in myocardial metabolism. However, non-invasive quantification of myocardial redox state is challenging. Recent studies suggest that ^{31}P MR spectroscopy has the potential to quantify intracellular NADH/NAD$^+$ (1). In this study, we aimed at evaluating the sensitivity of ^{31}P MRS to quantify alterations in NADH/NAD$^+$ ratio during ischemia/reperfusion (IR) in perfused rat hearts.

Materials and Methods

Data Acquisition: Male Sprague-Dawley rats were anesthetized. Hearts were excised, cannulated, and perfused with the Krebs-Henseleit buffer equilibrated with 95% O_2/5% CO_2 at 37˚C. The perfusion column was placed into a vertical bore 9.4T Bruker spectrometer with 20 mm volume coil. The perfusion protocol comprised of 30 min no-flow global ischemia, followed by 45 min reperfusion. ^{31}P MR spectra were acquired using the following parameters: repetition time, 6 s; spectral width, 5.7 KHz; NAV, 128; flip angle, 90˚. Spectra averages were acquired over the first 20 minute period of both ischemia and reperfusion periods.

Data Processing: In-house developed, Matlab-based code was used to perform all data processing. 5 Hz line broadening was applied to the fid before Fourier transform. The spectra were phased and corrected for baseline. Resonance peaks were quantified by fitting the peaks with Lorentzian line shapes. The αATP peak was fit and subtracted from the spectrum. Resonance peaks corresponding to NADH and NAD$^+$ were then fit using a model outlined by Lu et al. (1). A total of three parameters were fit: center frequency of NAD$^+$, total peak height for NAD$^+$, and NADH/NAD$^+$ ratio. Peak width obtained from the fitting to αATP peak was used in corresponding NAD curve fittings. Representative fitting results are shown in Fig. 1. Total NAD metabolite pool was estimated using NAD fit results to estimate total NAD metabolite pool concentrations. Relative concentrations were converted to absolute concentrations by using baseline αATP peak area as an internal reference corresponding to 16.95 μmol/g wet weight[2].

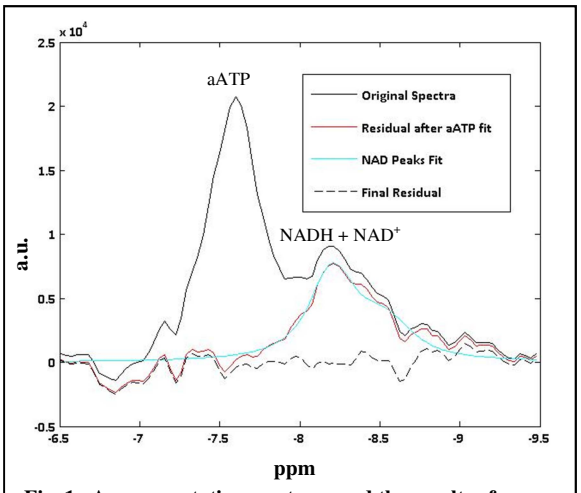

Fig. 1. A representative spectrum and the results of curve fitting.

Results

Of the 12 hearts from which baseline, ischemia, and reperfusion spectra were obtained, the fitting of 2 baseline spectra and 2 reperfusion spectra was considered unsuccessful because of the large residual errors. These spectra were excluded from data analysis.

NADH/NAD$^+$ ratio at baseline, during ischemia and reperfusion are shown in Fig. 2. NADH/NAD$^+$ ratio was 0.028±0.057 at baseline. The ratio was significantly higher during ischemia (0.361±0.301, $p<0.005$). Reperfusion caused a decrease in this ratio (0.167±0.141, $p=0.076$ compared to ischemia), however, it remained higher than that at baseline ($p<0.05$). Total NAD metabolite pool (Fig. 3) was found to remain constant between baseline, ischemia, or reperfusion periods (5.44±0.91, 5.67±0.86, and 5.05±1.86 μmol/g dry weight respectively).

Discussion & Conclusion

The NADH/NAD$^+$ ratio measured in our current study showed a similar trend as that reported in literature. However, MRS-measured NADH/NAD$^+$ ratio was consistently lower than literature reported values by a factor of ~4 (2). Total NADH/NAD$^+$ concentration was higher than HPLC-measured values by a factor of ~2. A possible reason for this disagreement might be the assumption of equal spectral line width used in curve fitting. Nevertheless, our results suggest that ^{31}P MRS can reliably track the relative changes in NADH/NAD$^+$ ratio in perfused hearts during IR.

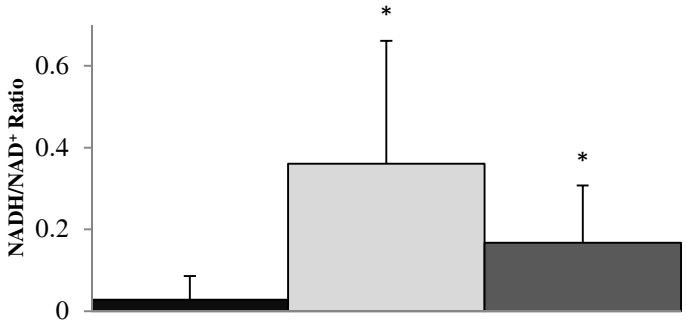

Figure 2. NADH/NAD$^+$ ratio. *p < 0.05 compared to baseline.

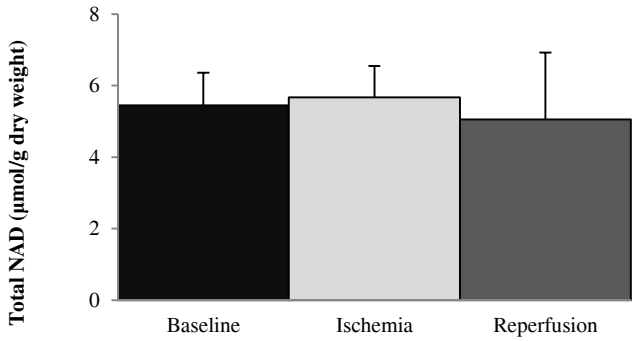

Figure 3. Estimated total NADH/NAD$^+$ concentration.

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References: 1. Lu, M. et al., Magnetic Resonance in Medicine. 2013, early view; 2. Ceconi, C. et al., Cardiovascular Research. 2012; 47:586-594