Detection of cerebral NAD+ in humans at 7 T

Robin A. de Graaf[1], Henk M. De Feyter[1], Peter B. Brown[1], Terence W. Nixon[1], Douglas L. Rothman[1], and Kevin L. Behar[1]
[1]*MRRC, Yale University, New Haven, CT, United States*

Introduction – Nicotinamide adenine dinucleotide (NAD^+) and its reduced form, NADH, have central roles in cellular metabolism and energy production as electron-accepting/donating coenzymes. In addition, NAD^+ is increasingly being recognized as a net substrate for a range of reactions related to gene expression, calcium mobilization, aging, cell death and the timing of metabolism via the circadian rhythm. Whereas the *in vivo* detection of NAD^+ and NADH have traditionally been limited, it was recently demonstrated that NAD^+ could be quantitatively detected by both ^1H [1] and ^{31}P [2] MR spectroscopy (MRS) on rat brain *in vivo*, whereas NADH could be detected by ^{31}P MRS [2]. Here we present an extension of the ^1H-MR-based NAD^+ detection to human brain at 7.0 T and provide validation with ^{31}P MRS.

Methods – All experiments were performed on a 7.0 T Magnex magnet interfaced to an Agilent Direct Drive spectrometer. ^1H and 31P MRS data was acquired with separate 80 mm and 90 mm diameter surface coils, respectively. ^1H MR spectra were acquired with a 1D LASER sequence (TE = 17 ms, 20 mm slice parallel to the coil) employing frequency-selective excitation (7.4 – 10.0 ppm) with an 8 ms minimum-phase SLR pulse. A repetition time of 1500 ms ensured negligible T_1 saturation due to the short ^1H NAD^+ T_1 relaxation time in the absence of water perturbation [1]. ^{31}P MR spectra were acquired with a 90° pulse – acquire sequence (TR = 5000 ms, no localization). Spectral quantification was achieved with a home-written, Matlab-based version of LCModel.

Results – Fig. 1A shows the downfield region of a ^1H MR spectrum acquired from human brain *in vivo* at 7.0 T. Besides the NAA amide-bound proton signal at 7.84 ppm and multiple, unassigned signals from purine nucleotides between 8.0 and 8.6 ppm, the downfield region displays three clear resonances at 8.8, 9.1 and 9.3 ppm originating from the NAD^+ H4, H6 and H2 nicotinamide protons. The non-overlapping NAD^+ resonances were quantified at 200 – 300 μM. Fig. 1B shows part of a ^{31}P MR spectrum acquired from human brain *in vivo* at 7.0 T. Besides the large PCr and ATP resonances, the spectrum is characterized by an upfield shoulder on the α-ATP signal that was previously described as the sum of NAD^+ and NADH [2]. All ^{31}P MR spectra had a visible contribution from uridine diphosphate glucose (UDPG) at -9.83 ppm. As the ^{31}P MR spectrum of UDPG is composed of two ^{31}P signals at -9.83 and -8.23 ppm, the upfield shoulder was decomposed as a sum of NAD^+, NADH and UDPG. NAD^+ was quantified in the 200 – 300 μM range, in good agreement with the ^1H MR data. Exclusion of UDPG from the ^{31}P spectral fit did not change the NAD^+ concentration significantly, but had a large effect on the NADH level and hence on the NAD^+/NADH redox ratio.

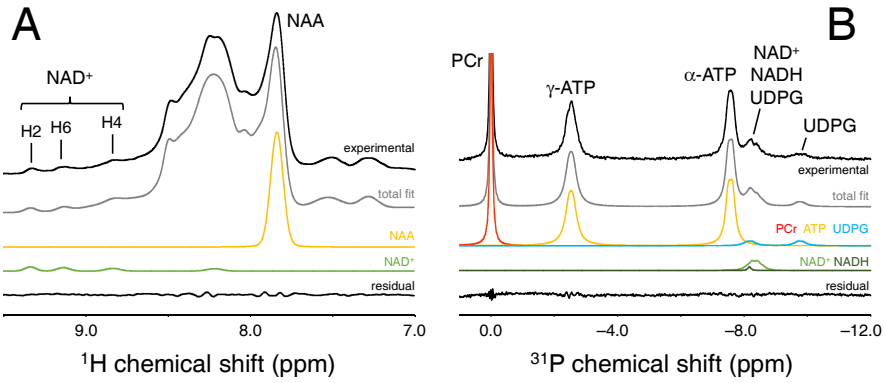

Figure 1: *In vivo* detection of NAD^+ by (A) ^1H MRS and (B) ^{31}P MRS. (A) The ^1H MR spectrum was quantified with signals for NAA, NAD^+ and 8 signals for the unassigned resonances. Using a 10 mM NAA concentration the NAD^+ concentration was calculated in the 200-300 μM range. Alternative concentration references could be water or total creatine. (B) The ^{31}P MR spectrum was quantified with signals for PCr, ATP, NAD^+, NADH and UDPG. Assuming a 2.8 mM ATP concentration, the NAD^+ concentration fell in the 200-300 μM range. The NAD^+/NADH ratio changed from 2.1 ± 0.8 to 5.1 ± 0.5 (n = 3) upon inclusion of the UDPG resonance at -8.23 ppm.

Discussion – Here we have presented the *in vivo* detection of NAD^+ on human brain by ^1H and ^{31}P MRS. The methods are in good agreement with regard to the absolute NAD^+ concentration. Preliminary inspection of the results indicates that ^1H-MR-based NAD^+ detection is more sensitive and easier to quantify due to the lack of spectral overlap. ^{31}P-MR-based NAD^+ detection is more complicated due to spectral overlap with NADH and UDPG. However, at 7.0 T the spectral resolution was sufficient to separate the three components unambiguously, thereby providing a more complete picture of the *in vivo* redox and energetic states. Unlike the ^{31}P NMR method [2], the presented ^1H NMR method cannot detect NADH due to spectral overlap [1]. However, the wide availability of proton-capable MR systems together with the ease-of-use makes ^1H NMR-based NAD^+ detection an alternative and/or complementary method for *in vivo* applications.

[1] R. A. de Graaf and K. L. Behar, NMR Biomed (2014) [2] M. Lu et al, Magn Reson Med (2013) doi: 10.1002/mrm.24859 This research was supported by NIH grants R01-MH095104 and P30-NS052519.