In Vivo Assessment of Intracellular NAD^+/NADH Redox State in Human Brain at 4 Tesla

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Introduction Nicotinamide adenine dinucleotide (NAD), functioning as an important coenzyme and co-substrate, has been tightly linked to energy production and metabolic regulation in all living cells (1). Through conversion between its reduced form (NADH) and oxidized form (NAD^+), NAD participates in various redox reactions as electron donating and accepting coenzymes to facilitate the synthesis of adenosine triphosphate (ATP), which can release energy via hydrolysis for supporting all cellular activities and physiological functions. Intracellular redox state defined by the NAD^+/NADH ratio (RX) is considered reflecting the intracellular metabolic status and function, and it is sensitive to alterations in metabolism associated with aging, diabetes, stroke and cancer (2). To date, there have been only two invasive approaches available for assessment of the intracellular redox state: the biochemical analysis using tissue/cell extraction and the auto-fluorescence detection of NADH only (3). By taking the advantage of high/ultrahigh magnetic field strengths, we have recently established a novel *in vivo* ^{31}P MRS method for noninvasive measurement of intracellular NAD concentrations and RX in animal and human brains at 16.4 T, 9.4 T (4) and 7 T (5), respectively. Although it prefers higher SNR and better spectral resolution offered by high/ultrahigh field (4), as long as a good spectral quality with narrow linewidth and adequate SNR is achievable, this *in vivo* NAD assay has the potential to be extended to lower fields and clinical scanners. In this study, we applied and tested this ^{31}P MRS-based NAD assay incorporated with ^{1}H decoupling technique to assess NAD metabolites and redox state in human visual cortex at 4 T.

Method Seven healthy volunteers (Age: 23.4±4.2 years old, 4 Male/3 Female) participated in this study. All measurements were conducted on the 4 T/90 cm bore human system (Oxford) interfaced with Varian console (Agilent, CA). One passively decoupled dual-coil RF probe, consisting of a linear butterfly ^{1}H surface coil and a 5 cm-diameter single-loop ^{31}P surface coil, was tuned to 4 T frequencies (^{1}H: 170 MHz; ^{31}P: 69 MHz), respectively, and used in this study for B_0 shimming/anatomic imaging and collecting *in vivo* ^{31}P spectra from human visual cortex. A single-pulse-acquisition sequence with the option of on/off broadband ^{1}H-decoupling (WALTZ-16) during FID acquisition was applied to obtain ^{31}P spectra with a nominal 90° RF excitation pulse and following parameters: 2.5 kHz spectral width, 800 number of points for each FID, 3 s repetition time and 320 or 640 total scan number. The FID was processed by exponential filtering with a line broadening of 2 Hz to enhance apparent SNR prior to Fourier transformation. The recently developed quantification model (4) capable of simulating and/or fitting NAD signals at any given field strength was applied to 4 T spectra. Specifically, by the least-square fitting of the model outputs with those resonances within -9 to -11.5 ppm of the *in vivo* ^{31}P spectrum (using PCr resonance at -2.5 ppm as chemical shift reference), signal intensities and linewidths of the NAD^+, NADH and α-ATP resonances were determined. Based on the decomposed signals from their overlapped spectrum, individual integrals of the α-ATP, NAD^+ and NADH resonances were then calculated from the model-fitted spectrum (4). By using the α-ATP signal corresponding to 2.8 mM in normal brain tissue (4,6) as an internal standard, intracellular concentrations of [NAD^+], [NADH], total $[NAD]_{total}$ and RX can be determined.

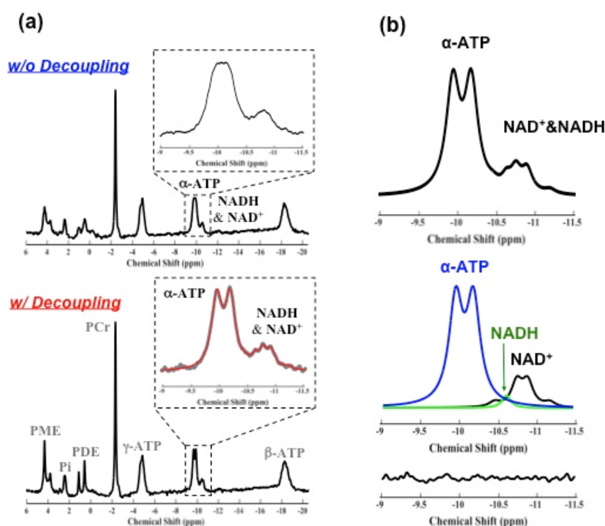

Figure 1. **(a)** ^{1}H-coupled (top) and ^{1}H-decoupled (bottom) *in vivo* ^{31}P MR spectra of human visual cortex from a representative subject. Inserts are the expanded spectra from -9 to -11.5 ppm. In the bottom insert, original signals (gray) and model fitted signals (red) are displayed. **(b)** Simulated ^{1}H-decoupled *in vivo* ^{31}P spectrum at the field strength of 4 T (top), model decomposed individual signals (middle) and fitting residue (bottom) of a representative *in vivo* ^{31}P MR spectrum from human brain.

Table 1. NAD concentrations and RX in healthy human brain at 4 T

Subjects #	1	2	3	4	5	6	7	Mean±Std
[NAD^+] (mM)	0.32	0.34	0.30	0.32	0.32	0.30	0.27	0.31±0.02
[NADH] (mM)	0.060	0.056	0.052	0.062	0.057	0.061	0.055	0.058±0.004
RX	5.3	6.0	5.7	5.2	5.6	4.9	4.9	5.3±0.4
$[NAD]_{total}$ (mM)	0.38	0.40	0.35	0.39	0.38	0.36	0.33	0.37±0.02

Result As shown in Fig. 1a, ^{1}H-decoupled ^{31}P spectrum showed narrower resonance linewidth, better-resolved signals and improved spectral quality when compared with ^{1}H-coupled spectrum. Simulated spectrum of combined NAD^+, NADH and α-ATP resonance signals (Fig. 1b, top) was highly comparable to the ^{1}H-decoupled *in vivo* ^{31}P MR spectrum of human brain at 4 T (Fig. 1a, bottom insert). All the resonances of NAD^+, NADH and α-ATP were satisfactorily fitted, as reflected by the small residue between the original spectrum and model fitting (Fig. 1b, middle & bottom). Table 1 summarizes the results of RX values (5.3±0.4) and NAD concentrations.

Discussion & Conclusion Figure 1a clearly demonstrates better-resolved resonance signals and improved spectral quality of ^{1}H-decoupled ^{31}P spectra compared to the ^{1}H-coupled one, which could result in more reliable NAD measurement. The T_1 relaxation times of NAD^+ and NADH in human brain at 4 T are unknown, but are expected to be similar to each other at the same field and to increase at lower field (4). Due to partially relaxed condition in this study, saturation effect would lead to an underestimation of the NAD concentrations, and the actual brain [NAD^+], [NADH] and $[NAD]_{total}$ should be slightly higher than those reported in this study after corrections of saturation effects once T_1 values are available. However, such correction is not necessary for RX calculation, since similar T_1 values of NAD^+ and NADH at the same field would result in the same saturation effects (4). The RX values measured at 4 T (Table 1) are in an excellent agreement with those previously reported 7 T results from similar aged human subjects (5). In summary, this work supports the feasibility and reliability of the new NAD assay based on the ^{1}H-decoupled ^{31}P MRS approach for direct assessment of intracellular NAD metabolism and redox state in human brain at 4 T. This technical advancement opens new opportunities for non-invasively investigating the critical roles of NAD in human brain functions and diseases at lower field strengths, for instance, using a clinical platform at 3 T.

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