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***In Vivo* ^{31}P MRS Assessment of Intracellular NAD Metabolites and NAD^+/NADH Redox State in Human Brain at 4 Tesla**

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Abstract

NAD^+ and NADH play key roles in cellular respiration. Intracellular redox state defined by the NAD^+/NADH ratio (RX) reflects the cellular metabolic and physiopathological status. By taking the advantage of high/ultrahigh magnetic field strengths, we have recently established a novel *in vivo* ^{31}P MRS based NAD assay for noninvasive and quantitative measurements of intracellular NAD concentrations and redox state in animal and human brains at 16.4 T, 9.4 T and 7 T, respectively. To explore its potential for clinical application, in this study, we investigated the feasibility of assessing the NAD metabolism and redox state in human brain at a relatively lower field of 4 T by incorporating ^1H -decoupling technique with the *in vivo* ^{31}P NAD assay. The use of ^1H -decoupling significantly narrowed the linewidths of NAD and α -ATP resonances, resulting in higher sensitivity and better spectral resolution as compared to the ^1H -coupled ^{31}P spectrum. These improvements made it possible for reliably quantifying cerebral NAD concentrations and RX, which are consistent with previously reported results obtained from similar aged human subjects at 7 T. In summary, this work demonstrates the capability and utility of the ^1H -decoupled ^{31}P MRS based NAD assay at lower field strength; thus, it opens new opportunities for studying intracellular NAD metabolism and redox state in human brain at clinical setting. This conclusion is supported by the simulation results, indicating similar performance and reliability can be achieved at 3 T and 4 T with the same signal to noise ratio.

Keywords

in vivo ^{31}P MRS; intracellular redox state; NAD^+ and NADH; human brain; ^1H -decoupling

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 (1,2). Through chemical 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

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functions. In addition, NAD also involves in many biological processes (3,4) including signal transduction, calcium homeostasis (5,6), and aging (7) or cell death (2,8). The concentration ratio of NAD^+ and NADH ($\text{RX} = [\text{NAD}^+]/[\text{NADH}]$) is defined as intracellular NAD redox state, which reflects the fundamental balance of cellular oxidative-reductive reactions including glycolysis and oxidative phosphorylation (9). Moreover, accumulating evidence has also suggested a correlation between the changes of intracellular redox state and the alterations in energy metabolism during normal aging processing (10) and under many diseased conditions such as diabetes, stroke (11), cancer (12) and epilepsy (3,13). Recently, NAD^+ metabolism and NAD^+ -dependent enzymes have been considered as promising therapeutic targets for neurological diseases (14).

Although the critical roles of NAD^+ , NADH and their ratio (RX, or redox state) in cellular metabolism and regulation highlights the urgent need for *in vivo* measurement of NAD contents and its redox state noninvasively, direct assessment of NAD concentrations and NAD^+ /NADH redox state *in vivo* is still challenging. To date, there have been limited options available for the assessment of intracellular NAD^+ and NADH. Biochemical analysis uses HPLC (15), capillary electrophoresis (16), or enzymatic cycling assays (12) to measure the NAD^+ and NADH concentrations *ex vivo*. Since this method requires tissue biopsy and extraction, it is invasive and not suitable for longitudinal studies, especially when human tissues or organs are targeted, and it is certainly prohibited in human brain applications. The autofluorescence approach utilizes weak endogenous fluorescence signals to monitor the intracellular NADH level (17,18). Although less invasive, it is unable to detect the NAD^+ signal and also suffers from artifacts, limited tissue penetration, invasive surgery and potential cell injury.

By taking the advantage of high/ultrahigh magnetic field strengths, we have recently established an *in vivo* ^{31}P MRS method for noninvasive measurement of intracellular NAD concentrations and NAD^+ /NADH redox state in animal and human brains (19). By least-square fitting the partially overlapped NAD^+ , NADH and α -ATP resonances in a ^{31}P spectrum using the quantification model developed in our lab, the separation of NAD^+ and NADH signals can be achieved and the individual concentrations of NAD^+ and NADH can also be determined. This approach has been applied and validated in normal cat brains at 16.4 Tesla (T) and 9.4 T (19); in normal, ischemic (20) and hypoxic (21) rat brains at 16.4 T; and in healthy (10,22) and Parkinson's (23) human brains at 7 T. Although it prefers higher signal-to-noise ratio (SNR) and better spectral resolution offered by high/ultrahigh field (19), this *in vivo* NAD assay has the potential to be extended to lower fields and clinical scanners as long as a good spectral quality with narrow resonance linewidth and adequate SNR is achieved.

Comparing with higher field strength, the major challenges of *in vivo* ^{31}P MRS application at low field are relatively poor spectral resolution and detection sensitivity (24). While optimized B_0 homogeneity with high-order shim may improve the linewidth to a certain degree, the spectral resolution still suffers from the presence of extensive J-coupling between the phosphorus (^{31}P) and hydrogen (^1H) nuclei, which results in complicated spectra with overlapping and broadening resonances and leads to further reduction in signal sensitivity. The unresolved heteronuclear coupling exists in multiple phosphorous

metabolites including phosphocreatine (PCr), ATP, phosphodiester (GPE: glycerophosphoethanolamine; GPC: glycerophosphocholine) and phosphomonoester (PE: phosphoethanolamine; PC: phosphocholine). To achieve better-resolved signals and reliable quantification, *in vivo* ^{31}P MRS applications at low field could benefit substantially from ^1H -decoupling (25). To explore the potential of the *in vivo* ^{31}P NAD assay for clinical translation, we incorporate the ^1H -decoupling technique to investigate the feasibility of assessing the cellular NAD metabolites and redox state in human brain at a relatively lower field of 4 T. Both ^1H -coupled and ^1H -decoupled spectra were acquired in the same brain to demonstrate the effect of the ^1H -decoupling.

METHODS

In Vivo ^{31}P MRS

Seven healthy volunteers (Age: 23.4 ± 4.2 years old, 4 Male/3 Female) participated in this study. The experimental procedures were approved by the Institutional Review Board of the University of Minnesota with written informed consent obtained from all subjects prior to study. All measurements were conducted on the 4 T/90 cm bore human magnet (Oxford) interfaced with Varian console (Agilent, CA). One passively decoupled dual-coil RF probe, consisting of a linear butterfly ^1H surface coil and a 5 cm-diameter single-loop ^{31}P surface coil, was tuned to 4 T operation frequencies (^1H : 170 MHz; ^{31}P : 69 MHz), and used in this study for B_0 shimming and anatomic imaging and for collecting *in vivo* ^{31}P spectra from the human visual cortex, respectively. A single-pulse-acquisition sequence with the option of on/off broadband ^1H -decoupling (WALTZ-16: 35 μs for the 90° composite pulse width, and 149 Hz B_1 decoupling strength) during the 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 (TR) and 320 (N=1) or 640 (N=6) total scan number. The radio-frequency (RF) power of broadband ^1H -decoupling for *in vivo* ^{31}P MRS acquisition was experimentally calibrated and set below the SAR limit according to the FDA guideline. The raw FID of the ^{31}P MRS data were zero-filled and processed by exponential filtering with a line broadening of 2 Hz to enhance apparent SNR prior to Fourier transformation. The SNR was calculated by dividing the peak height of PCr or α -ATP resonance by the peak-to-peak spectral noise, and multiplying it by 2.5 (24). The resonance signals of phosphorous metabolites were assigned in the *in vivo* ^{31}P MR spectrum based on their chemical shifts with PCr resonance set at -2.5 ppm as a reference. The spectrum region containing NAD^+ , NADH and α -ATP resonances (-9 to -11.5 ppm) was processed manually for spectral corrections of phase and baseline before the NAD quantification.

NAD Quantification and Model Simulation

^{31}P magnetic resonances of NAD^+ , NADH and α -ATP at 4 T follow their typical patterns as previously described at higher field strength (19). Briefly, in addition to a large α -ATP doublet centered at -10.07 ppm, NADH resonates at -10.63 ppm as a singlet while NAD^+ has four resonance peaks (quartet). The chemical shifts and relative peak intensity ratios of the NAD^+ quartet are highly dependent on the field strength, and were predicted for 4 T application based on the second-order coupling effect and high-resolution NMR results

obtained at 11.7 T (see details in Fig. 1 of reference (19)). The recently developed NAD quantification model (19) capable of simulating and/or fitting NAD signals at any given field strength was then applied to 4 T spectra. For convenience, the half linewidth (HLW) of each singlet in the α -ATP doublet was employed in fitting and quantification, and the HLW values of NAD^+ and NADH were set to equal to the α -ATP HLW minus 1.5 Hz in the fitting model for achieving best fitting outcomes (19). Through regressions of the overlapped α -ATP and NAD resonances with the model functions, well-resolved signal intensities and linewidths of NAD^+ , NADH and α -ATP were determined (19). Individual integrals of NAD^+ and NADH calculated from the model-fitted spectrum were then compared with that of α -ATP, which corresponds to 2.8 mM in healthy brain tissue (19,26) and serves as an internal standard, thus, the concentrations of intracellular NAD^+ , NADH, total NAD ($[\text{NAD}]_{\text{total}} = [\text{NAD}^+] + [\text{NADH}]$) and RX can be calculated.

To examine the reliability of the NAD quantification method and its SNR dependence at lower field, eight different levels of randomly generated white Gaussian noise were added to a simulated 4 T ^{31}P spectra with predetermined half linewidth (HLW) and RX values that were similar to those of human brains measured in this study using the ^1H -decoupling method. Monte Carlo simulations of 100 trials per noise level were performed with the following parameters: $\text{RX} = 5.21$, $[\text{NAD}^+] = 0.276$ mM, $[\text{NADH}] = 0.053$ mM and $\text{HLW} = 8$ Hz. By fitting the ^{31}P spectra with eight SNR levels of α -ATP ($=5, 10, 15, 20, 40, 60, 80$ and 100), the model determined values were compared with their true values to evaluate the fitting accuracy ($=\% \text{abs}(\text{mean} - \text{real}) / \text{real}$; real: predetermined value) and error ($=\% \text{standard deviation} / \text{mean}$) of the NAD quantifications. In addition, similar simulation was also conducted for the *in vivo* ^{31}P spectra obtained with the same simulation parameters except using a broader α -ATP HLW ($=10.5$ Hz) to mimic the ^1H coupling condition.

The model simulation was also performed for 3 T application using the similar predetermined parameters as employed in 4 T simulation and 7 Hz HLW based on its field dependence (24) at 3 T with ^1H -decoupling.

All results were presented as mean $\pm$ standard deviation in this study.

RESULTS

In vivo ^{31}P MR spectra of human visual cortex acquired from a representative subject were displayed in Fig. 1. While both spectra illustrate good spectral quality and high SNR, the ^1H -decoupled spectrum (Fig. 1b) demonstrates significantly improved spectral quality when compared with the ^1H -coupled one (Fig. 1a). Specifically, narrower resonance linewidths and higher signal intensities, thus, improved SNRs due to the same spectral noise level, were observed in phosphorus metabolites including NADs, α -ATP, PCr, PE, PC, GPE, GPC and inorganic phosphate (Pi) in the spectrum acquired with ^1H -decoupling. Most importantly, as shown in the insert of Fig. 1b, ^1H -decoupling not only revealed the intrinsic split of α -ATP resonance doublets caused by ^{31}P - ^{31}P J-coupling, but also resulted in much better separation in NAD signals (i.e., three peaks were observed) than that of ^1H -coupled spectrum (insert of Fig. 1a). The excellent SNR of the ^1H -decoupled ^{31}P spectra (254 ± 60 for PCr; 71 ± 10 for α -ATP; $N=6$ with 2 Hz line broadening) and the improved peak separation in

the spectral region with overlapped NAD resonances allow reliable spectral fitting to decompose the NAD⁺ and NADH signals and consequently provide accurate NAD quantifications. The ¹H-decoupling effect significantly improved the detectability of α-ATP, NAD⁺ and NADH resonances, for instance, resulting in substantially narrowed linewidth (26±3%, p<0.001, N=6) and enhanced signal intensity or SNR gain (27±3%, p<0.001, N=6) for α-ATP with unchanged resonance integral (p=0.3) (Table 1). In contrast, such decoupling effect on γ- and β-ATP resonances was not significant (p>0.2). This is due to the J-coupling effect between the ¹H spins on the ribose C5' and the α-phosphorus spin, which is the closest one among the three phosphate groups to the adenosine; and the extensive J-coupling effect also exists for the phosphorus spins of NAD⁺ and NADH owing to their similar spin environment to α-ATP as shown in Fig. 2.

In vivo measured (gray trace) and model-fitted (red trace) ¹H-decoupled ³¹P spectra obtained from another human subject at 4 T are displayed in Fig. 3a with the chemical shift range covering NAD⁺, NADH and α-ATP resonances. The selected region of spectrum was satisfactorily fitted by the linear summation of the model-decomposed signals of NAD⁺, NADH and α-ATP (top in Fig. 3b), which was evident from the small residue (within the noise level) between the original spectrum and model fittings (bottom in Fig. 3b). Figure 4 demonstrates the linewidth dependence of the NAD resonance pattern at 4 T based on model simulations with a fixed NAD⁺/NADH ratio (=4.8). When linewidths (i.e., 2×HLW) increasing from 7 Hz to 15.6 Hz, which mimics the situations from phantom solution to *in vivo* brain tissue under ¹H-decoupling condition (e.g., in the current study), the combined resonances of NAD⁺ and NADH characterized by the five sharp peaks (top in Fig. 4a) evolves to a broader spectrum with weakened separations among apparent resonance peaks (top in Fig. 4c); further linewidth broadening as in the case of ¹H-coupling diminishes such separation (see Fig. 4d). Figure 3d illustrates another example of simulated individual NAD⁺, NADH and their combined spectra using the same linewidth and RX values obtained from the *in vivo* spectrum shown in Fig. 3a. By including α-ATP doublet, Fig. 3c displays a simulated spectrum that is highly comparable to the ¹H-decoupled *in vivo* ³¹P spectrum measured in the human brain at 4 T (gray trace in Fig. 3a).

Table 2 summarizes the results of intracellular concentrations of NAD⁺ (0.31±0.02 mM, or 0.28±0.02 μmol/g after an unit conversion using a brain tissue density of 1.1 g/ml), NADH (0.058±0.004 mM, or 0.053±0.004 μmol/g), total NAD ([NAD]_{total}=0.37±0.02 mM, or 0.34±0.02 μmol/g) and NAD⁺/NADH redox ratio (RX=5.3±0.4) obtained from all seven healthy human subjects scanned at 4 T. To evaluate the reliability of the NAD quantification method, model simulation results under different SNR levels of α-ATP are summarized in Table 3a. As shown in Tables 3b and 3c, excellent fitting accuracy (less than 1%; lower value means more accurate) and small fitting error (≈5% or lower) could be achieved as long as the SNR of α-ATP reaches the level of 60 or higher. Considering the superior ³¹P spectral quality and SNR demonstrated in this study (SNR>70 for α-ATP; with 2 Hz line broadening), the reliability of the ¹H-decoupled *in vivo* NAD approach in human brain at 4 T was confirmed.

Figure 5 shows the comparison results of fitting errors between 3 T and 4 T model simulations and their dependence on SNR. Due to the unique spectral pattern of NAD⁺

quartet resonances and slightly narrow linewidth at 3 T, the fitting errors are almost the same for these two fields at a given SNR. Figure 5 also reveals that the errors for fitting the ^1H -decoupled ^{31}P spectra were approximately two times smaller than that of ^{31}P spectra without ^1H -decoupling, owing to 26% linewidth narrowing and 27% SNR gain by ^1H -decoupling (Table 1). Due to the larger fitting error, similar regression of ^1H -coupled ^{31}P spectra in most subjects failed to provide consistent NAD and RX results when the α -ATP SNR was below 40 with average linewidth or shimming quality in this study. In contrast, ^1H -decoupled ^{31}P spectra with a significantly lower α -ATP SNR of ~ 20 provided tolerable fitting error (see Fig. 5). These results suggest that both spectral resolution and SNR are critical for reliable NAD quantification, which is especially challenging for ^1H -coupled ^{31}P spectra; nevertheless, using the ^1H -decoupling method could alleviate this technical limitation.

DISCUSSION AND CONCLUSION

In this study, the feasibility of the ^{31}P MR-based *in vivo* NAD assay for assessing the cellular $[\text{NAD}^+]$, $[\text{NADH}]$ and NAD^+/NADH redox state in healthy human brains was demonstrated and validated at 4 T with ^1H -decoupling. Performing ^1H -decoupling during ^{31}P acquisition provides adequate spectral quality with improved SNR, narrow linewidth and good spectral resolution, which could be achieved on clinical scanners operating at lower field strength. The successful extension of the capability for noninvasively quantifying NAD concentrations from high/ultrahigh (7 T, 9.4 T and 16.4 T) to lower field strengths indicates a potential opportunity for studying NAD metabolism in human brains at clinical setting.

Based on the results of this study, to ensure accurate determination of the low concentration NAD metabolites from their overlapped ^{31}P signals, ^1H -decoupling technique is critical. As shown in the insert of Fig. 1b and Fig. 3a, the three separated peaks observed on the right shoulder of α -ATP resonance are identified to be the singlet of NADH and the two central peaks of the NAD^+ quartet, respectively. The other two smaller peaks on the edge of NAD^+ quartet are hardly visualized on the *in vivo* ^{31}P spectrum at 4 T due to their low sensitivity and/or overlapping with the dominant α -ATP doublet (Fig. 3), nevertheless, their signal contributions were accounted in the model simulation and spectral fitting.

Interestingly, at higher field strength (such as 7 T, 9.4 T and 16.4 T), the *in vivo* NAD^{31}P spectrum demonstrates two apparent peaks consisting of: 1) the NADH singlet overlapping with the left-side part of the NAD^+ resonance; and 2) the right-side part of the NAD^+ resonance (10,19,22). In contrast, the three apparent NAD peaks observed at 4 T in this study can be explained by the field dependence of the NAD^+ spectral pattern with various chemical shifts and intensity ratios between the NAD^+ quartet resonances with a fixed and field independent center chemical shift: the two central (stronger) peaks of the NAD^+ quartet move towards each other when the field strength decreases and become separated from the NADH singlet; meanwhile, the two outside (weaker) peaks depart from each other along with an increasing intensity ratio of the stronger to weaker peaks that further enhance the signals of the central quartet (19). Therefore, for *in vivo* spectra with broad resonance linewidth, mixed NADH singlet with NAD^+ quartet results in two apparent spectral peaks at

higher field strength, whereas the unique pattern of ^1H -decoupled NAD^+ spectra at lower field (e.g., at 4 T in this work) improves the separation between the NADH and NAD signals. Therefore, the three-peak-separation of NAD resonances observed in the ^1H -decoupled ^{31}P spectrum at 4 T clearly distinguishes the NADH singlet from the NAD^+ quartet in the human brain, which confirms the chemical shift assignment of NADH singlet at -10.63 ppm and subsequently conveys the reliability of the *in vivo* NAD quantifications at lower field.

To reduce the scan time, *in vivo* ^{31}P MRS experiments usually are not performed under fully relaxed condition. Although the T_1 relaxation times of NAD^+ and NADH in human brain at 4 T are unknown, they are expected to be similar to each other at the same field strength and to increase at lower field strength (19,24). Therefore, the T_1 value of NAD^+ or NADH at 4 T is expected to be longer than that of cat brain measured at 9.4 T (1.5-1.6 s) (19,27) and human brain at 7 T. Due to partially saturated acquisition condition in this study ($\text{TR}=3$ s), the saturation effect would lead to an underestimation of the NAD concentrations, i.e., the actual brain $[\text{NAD}^+]$, $[\text{NADH}]$ and $[\text{NAD}]_{\text{total}}$ should be slightly higher than those reported in this study after correcting the saturation effects once the T_1 values become available. This is evident from the comparison, showing the slightly higher values of $[\text{NAD}^+]=0.35\pm0.01$ mM, $[\text{NADH}]=0.06\pm0.01$ mM, and $[\text{NAD}]_{\text{total}}=0.41\pm0.02$ mM obtained from the age-matched young human subject group ($N=7$, age: 23.4 ± 4.2 years old) at 7 T in our previous work (10). Nevertheless, the correction of saturation effect is not necessary for RX quantification, since similar T_1 values of NAD^+ and NADH at the same field would result in the same saturation effects. The RX value (5.3 ± 0.4 , $N=7$, age: 23.4 ± 4.2 years old) measured at 4 T in this study (Table 2) is in an excellent agreement with those 7 T results (5.4 ± 0.8 , $N=7$, age: 22.7 ± 2.3) from our previous work (10), suggesting excellent reliability and accuracy of ^1H -decoupled ^{31}P MRS-based NAD assay for assessing the human brain NADs and redox state at 4 T. Moreover, the comparison of 3 T model simulation results with that of 4 T (Fig. 5) clearly indicate that similar performance is achievable for clinical scanners at 3 T with the same SNR.

Reducing the magnetic field inhomogeneity by B_0 shimming is crucial for improving ^{31}P spectral resolution and SNR, in particular, if the ^1H -decoupling method was not used for assessment of brain NADs and RX. Nevertheless, the shimming was highly robust in this study, showing $<15\%$ variation in the measured α -ATP HLWs among inter subjects (Table 1). The averaged α -ATP HLW without ^1H -decoupling was 10.5 Hz ($N=6$). This value can be used to calculate the apparent full linewidth of α -ATP including two doublets to be 34.5 Hz ($=2\times10.5\text{Hz} + J_{\alpha\text{-ATP}} - 2\text{Hz}$ line-broadening employed in the spectral processing in this study; where $J_{\alpha\text{-ATP}}$ is the homonuclear J coupling constant of α -ATP and equals to 15.5 Hz (19)). This result is identical to the predicted value at 4T based on Eq. 2b of an independent study (24). Therefore, obtaining reasonable B_0 shimming should not be a major technical hurdle for real-time NAD and RX quantification using ^1H -decoupled ^{31}P MRS. This notion is supported by small inter-subject variations of NAD concentrations and RX shown in Table 2.

Beside the benefits of detecting α -ATP, NADs and redox state in human brain at 4 T, the use of ^1H -decoupling also significantly improves the signal intensity and SNR for other phosphorus metabolites such as PCr ($17\pm2\%$, $p<0.01$, $N=6$), PE ($66\pm15\%$, $p<0.001$), PC

($27 \pm 13\%$, $p < 0.05$), GPE ($138 \pm 45\%$, $p < 0.001$) and GPC ($81 \pm 23\%$, $p < 0.001$). Since the ^1H -decoupling does not change the signal integral as illustrated in Table 1, there is no complication for spectral quantification based on the integral analysis but with huge SNR gain for some phosphorus metabolites, for instance, $>100\%$ for GPE. In contrast, the nuclear Overhauser effect (NOE) generated by employing a low-power and continuous wave at the water proton Larmor frequency prior to the RF excitation pulse could also enhance the signals of phosphorus metabolites without linewidth change. However, the NOE induced SNR gains rely on the increase of resonance integral (28), thus, a careful calibration of the integral enhancement is required for proper quantification of metabolites concentration.

Recently, an alternative approach based on *in vivo* ^1H MRS using a frequency-selective RF pulse excitation for detecting the brain NAD^+ resonances around 9 ppm has been reported (29). This ^1H method could avoid the issue of overlapped resonances from other metabolites, however, it is incapable of detecting intracellular NADH, thus, NAD redox ratio (RX) in the brain. In addition, it also loses the useful signals of other metabolites commonly detected by conventional ^1H MRS approaches owing to a narrow band width of the selective RF pulse (29).

In conclusion, this work supports the feasibility and reliability of the new *in vivo* NAD assay based on the ^1H -decoupled ^{31}P MRS approach for direct and quantitative assessment of intracellular NAD metabolites 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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Abbreviations

ATP	Adenosine triphosphate
GPC	Glycerophosphocholine
GPE	Glycerophosphoethanolamine
NAD	Nicotinamide adenine dinucleotide
NAD^+	Oxidized NAD form
NADH	Reduced NAD form
PC	Phosphocholine
PCr	Phosphocreatine
PE	Phosphoethanolamine

Pi	Inorganic phosphate
RX	Redox state ratio
HLW	Half linewidth
SNR	Signal to noise ratio
SAR	Specific absorption rate
TR	Repetition time

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Figure 1. ${}^1\text{H}$ -coupled (a) and ${}^1\text{H}$ -decoupled (b) *in vivo* ${}^{31}\text{P}$ MR spectra of human visual cortex from a representative subject (320 scans). Inserts are the expanded spectra in the chemical shift range of -9 to -11.5 ppm. In the insert of (b), original signals (gray trace) and model fitted total signals of α -ATP and NADs (red trace) are displayed.

Figure 2. Schematic of molecular structures, showing strong heteronuclear J-coupling effects between the ^{31}P spin(s) and closest ^1H spins (red) for NADH, NAD $^{+}$ and α -ATP, respectively.

Figure 3.

In vivo and simulated ^{31}P spectra of human brain at 4 T: (a) Expanded *in vivo* ^1H -decoupled ^{31}P MR spectrum (from -9 to -11.5 ppm) of human visual cortex from another representative subject (640 scans) (gray trace) with its model fittings (red trace). (b) Model decomposed individual component of $\alpha\text{-ATP}$ (blue), NAD^+ (black) and NADH (green) with small fitting residue shown in the bottom. (c)&(d) Model simulated spectra of NAD^+ quartet, NADH singlet, total NAD and combined $\alpha\text{-ATP}$ with NAD signals using the

linewidth of 14.3 Hz and NAD^+/NADH redox (RX) ratio of 4.8, which are the same as those measured from the spectrum displayed in (a).

Figure 4.

Linewidth dependence of the NAD resonance pattern at 4 T. Model simulations of NAD^+ quartet (bottom), NADH singlet (middle) and combined NAD resonance (top) with predetermined NAD^+/NADH redox ratio (RX) of 4.8 are displayed as the α -ATP HLW increasing from 3.5 Hz (a), 6 Hz (b), 7.8 Hz (c) for mimicking *in vivo* ^1H -decoupled ^{31}P spectrum, to 10.5 Hz (d) for mimicking *in vivo* ^1H -coupled ^{31}P spectrum.

Figure 5.

Comparison results of fitting error between 4 T and 3 T, and between ^1H -decoupled and coupled *in vivo* ^{31}P spectra, and their dependence on α -ATP SNR for (a) RX, (b) NAD^+ and (c) NADH.

Table 1

Signal intensity and linewidth improvements of α -ATP resulting from ^1H -decoupling

Subjects #	1		2		3		4		5		7		Mean±Std
	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	
Peak Intensity (×10 ⁻³ a.u.)	3.2	4.1	3.2	4.0	3.2	4.0	2.7	3.4	3.2	4.2	3.4	4.2	+(27.0±2.8) %
	+28.5%		+26.9%		+24.7%		+25.3%		+31.8%		+24.8%		
	10.0	7.3	10.4	7.9	10.5	8.2	10.0	7.8	10.5	7.4	11.4	8.3	
HLW (Hz)	-26.8%		-24.7%		-22.2%		-22.2%		-29.5%		-27.4%		-(25.5±3.0) %
Integrals (×10 ⁻³ a.u.)	2.7	2.6	2.8	2.7	2.8	2.8	2.3	2.2	2.8	2.7	3.1	3.0	-

Subject #6 is not included in this Table for comparison because the ^1H -coupled spectrum was not available in this subject.

Table 2

Results of NAD concentrations and NAD⁺/NADH redox state (RX) measured in healthy human brain at 4 T using *in vivo* ³¹P MRS with ¹H decoupling

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

Table 3

Model simulation results at 4 T (100 Trials per SNR level)

(a) Fitting Results

SNR (α -ATP)		20	40	60	80	100	W/O Noise
4T Fitted Values	HLW	8.01±0.09	8.00±0.04	7.99±0.03	8.00±0.02	8.00±0.02	8.00
	RX	5.22±0.77	5.27±0.44	5.23±0.28	5.20±0.19	5.21±0.14	5.21
	[NAD*]	0.275±0.009	0.277±0.005	0.276±0.003	0.276±0.002	0.276±0.002	0.276
	[NADH]	0.054±0.006	0.053±0.004	0.053±0.002	0.053±0.002	0.053±0.001	0.053
	[NAD]_{total}	0.329±0.007	0.329±0.004	0.329±0.002	0.329±0.002	0.329±0.001	0.329

(b) Fitting Accuracy (%)

SNR (α -ATP)		20	40	60	80	100	W/O Noise
4T Fitted Values	HLW	0.13	0	0.13	0	0	-
	RX	0.19	1.15	0.38	0.19	0	-
	[NAD*]	0.36	0.36	0	0	0	-
	[NADH]	1.89	0	0	0	0	-
	[NAD]_{total}	0	0	0	0	0	-

(c) Fitting Error (%)

SNR (α -ATP)		20	40	60	80	100	W/O Noise
4T Fitted Values	HLW	1.12	0.50	0.38	0.25	0.25	-
	RX	14.75	8.35	5.35	3.65	2.69	-
	[NAD*]	3.27	1.81	1.09	0.72	0.72	-
	[NADH]	11.11	7.55	3.77	3.77	1.89	-
	[NAD]_{total}	2.13	1.22	0.61	0.61	0.30	-