

Preliminary Study of Cerebral NAD Metabolism and Redox State in Parkinson's Patients

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Introduction Neurodegenerative diseases have become a major socioeconomic burden of modern society and Parkinson's disease (PD) is one common neurodegenerative movement disorder affecting a million peoples in the USA alone. Although energy failure stemming from mitochondrial dysfunction has been proposed as a cause of neuronal death in PD¹⁻³, the actual demonstration of energy metabolism is difficult in living human brains. Thus, the underlying mechanisms are not well understood, which has affected the development of disease modifying therapies. In this study, we applied a newly developed *in vivo* ³¹P MRS approach⁴ at high/ultrahigh field to non-invasively assess the intracellular nicotinamide adenine dinucleotide (NAD) contents and its redox ratio in the occipital lobe of PD patients and controls (CT). The capability of this methodology for providing valuable information regarding the balance of cerebral glucose and oxygen metabolism has been demonstrated in healthy human brain at 7T and in animal brains under pathophysiological conditions at ultrahigh fields⁵⁻⁶ and its utility will be further exploited in this PD patient study.

Methods Seven PD patients (Age: 54-73 years old, 4M/3F and disease duration: 7±4 years) and equal number of controls participated in this study. All MR measurements were conducted at 7 Tesla/90 cm actively shielded human scanner (Siemens). A surface coil probe consists of a quadrature ¹H coil for anatomic imaging and B₀ shimming and a single loop ³¹P coil (Dia.=5cm) for collecting ³¹P MRS from visual cortex. A small sphere with reference phosphorus compound was placed at the center of the ³¹P coil for power calibration. For each subject, ³¹P MR pulse-acquired spectra (NT=320, TR=3s and FA=84°) and 3D-MRSI data (FOV=12×12×9 cm, matrix=7×7×5, TR=1.2s, total NT=896 and FA=68°) were acquired; and a matching 3D-MRSI data was also obtained from a spherical ATP phantom (containing 10mM ATP, 10.3mM [Mg²⁺] and 45mM [Na⁺] at pH of 7.0) with the same loading and position to the subject's head. A newly developed NAD quantification method capable of simulating and fitting the spectrum of α-ATP, NAD⁺ and NADH peaks at a given magnetic field strength was applied⁴. The absolute concentrations of NADH and NAD⁺ in each subject were determined by comparing the integrals of these resonances with that of α-ATP after correcting the saturation effects based on T₁ and flip angle information. The concentration of ATP in each subject was determined via comparing the ATP signals of identical 3D-MRSI voxels within the human brain and the ATP phantom, respectively. The ratio of NAD⁺/NADH and the total NAD content ([NAD]_{total}=[NAD⁺]+[NADH]) in the brain tissue were also calculated. All results were presented as Mean±SD.

Results Figure 1A displays the original and fitting spectra of female (n=3) and male (n=4) subjects from the PD and control groups as well as the individual fitting components of α-ATP, NADH and NAD⁺. The quantification results of [ATP], [NADH], [NAD⁺], [NAD]_{total} and NAD⁺/NADH redox ratio of different groups are summarized in Table 1. Although the ATP level in the PD patients shows slightly lower trend compare to the age-matched control group, the contents of NAD, including NAD⁺, NADH and total NAD, as well as the NAD⁺/NADH redox ratio were not significantly different. However, when the PD patients were grouped according to their gender (*i.e.*, F_PD vs. M_PD), a much larger difference was observed in their [NADH], [NAD⁺] and NAD⁺/NADH ratio. Such gender-related differences were much pronounced in the PD compare to the CT brains. Interestingly, good correlations between the brain [ATP] and intracellular [NADH] or the NAD redox state were observed in PD patients (see Fig. 2) but not in age-matched control subjects.

Discussion and Conclusion First, the superb quality of the ³¹P MRS data obtained at 7T made it possible for the first time to reliably quantify and distinguish low concentration NAD metabolites and to study intracellular NAD redox states in healthy human and PD patients. Second, the intracellular NAD contents including its oxidative and reduced forms were in a similar range in the two groups. This result further confirms the reliability of MRS measurement and NAD quantification method. Considering the essential role of the NAD in the cellular respiration processes and energy metabolism, the alteration in the intracellular NAD contents and redox state likely reflects the impaired cerebral energetics and a shift in the glucose/oxygen metabolism balance. Although the number of PD patients being studied so far is relatively small (4M/3F), the preliminary result reported here provides compelling *in vivo* evidence about the contribution from impaired energy metabolism in the PD pathogenesis and the potential involvement of the gender difference in the disease process. In conclusion, the present study demonstrates the new utility of high/ultrahigh field *in vivo* ³¹P MRS technique for non-invasive study of the intracellular NAD and redox state in living brains under healthy and diseased conditions, such important information is otherwise unavailable. Therefore, this method could provide valuable insights into the underlying mechanisms in PD and other neurodegenerative diseases.

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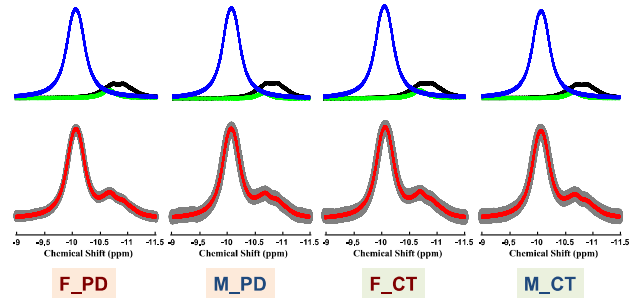


Figure 1. *In vivo* ³¹P MR spectra of human brain obtained from Parkinson's disease (PD) patients and their matched controls (CT) at 7T. The spectra of female (F, n=3) and male (M, n=4) subjects from each group are plotted separately in the bottom row where the original ³¹P signals are shown in grey and spectral fitting shown in red. The individual fitting components of α-ATP (blue), NADH (black) and NAD⁺ (green) are displayed in top row.

Table 1. Summary of cerebral ATP and NAD contents and NAD redox ratio in different groups of PD patients and control subjects.

Groups	Age (years)	[ATP] (mM)	[NADH] (mM)	[NAD ⁺] (mM)	[NAD] _{total} (mM)	[NAD ⁺]/[NADH]
PD (n=7)	60 ± 7	2.73 ± 0.10	0.09 ± 0.02	0.35 ± 0.02	0.44 ± 0.02	4.24 ± 1.50
CT (n=7)	61 ± 7	2.83 ± 0.10	0.09 ± 0.01	0.35 ± 0.02	0.45 ± 0.02	3.79 ± 0.34
F_PD (n=3)	60 ± 4	2.75 ± 0.07	0.10 ± 0.02	0.34 ± 0.02	0.45 ± 0.02	3.34 ± 0.52
M_PD (n=4)	61 ± 9	2.72 ± 0.13	0.08 ± 0.02	0.36 ± 0.02	0.44 ± 0.02	4.92 ± 1.69
F_CT (n=3)	55 ± 2	2.84 ± 0.15	0.10 ± 0.01	0.36 ± 0.02	0.46 ± 0.02	3.50 ± 0.06
M_CT (n=4)	65 ± 6	2.79 ± 0.06	0.09 ± 0.01	0.35 ± 0.02	0.43 ± 0.02	4.01 ± 0.28

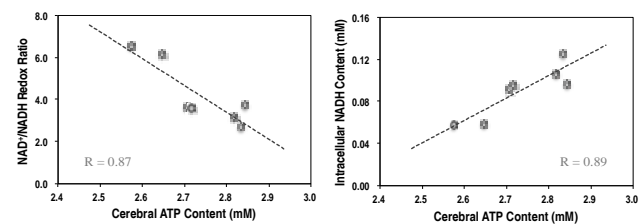


Figure 2. Correlations of intracellular NAD redox ratio (left) and NADH content (right) with the brain ATP concentration found in the PD patients. Such correlations were not observed in matched control subjects.