**Introduction** The basic function of the NAD (nicotinamide adenine dinucleotide) for human is to release energy from nutrients in our diet through conversion between NADH (reduced form) and NAD⁺ (oxidized form) in various redox reactions to form adenosine triphosphate (ATP) for supporting all cellular activities and functions. The intracellular NAD⁺/NADH ratio, defined as the redox state, reflects the fundamental balance of the cellular oxidative-reductive reactions associated with the energy production [1]. In addition, NAD also involves in many important enzyme reactions, which mediates various biological processes including the metabolic signaling, calcium homeostasis, and aging or cell death [2-3]. Despite their important roles in cellular metabolism and regulation, it is unfortunate that there is no in vivo approach available for non-invasive assessment of NAD contents and redox states, in particular, in human brains. In this study, we exploited a novel $^{31}$P MRS approach recently developed in our lab to directly measure the NAD contents and the NAD⁺ and NADH resonance peaks at a given magnetic field strength was applied. The novel quantification method capable of simulating and/or fitting the spectrum of all $^{31}$P signals with very low residual, thus, the individual fitting components provided intriguing findings: i) it is feasible to robustly measure and identify the total NAD level was slowly declined in older people’s brain.

**Discussion and Conclusion** The present study, for the first time, reported following intriguing findings: i) it is feasible to robustly measure and identify the in vivo $^{31}$P MRS signals of the NAD⁺ and NADH in human brain at 7T; ii) the knowledge regarding the NAD and its redox state in human brain, which is not available in the literature, can now be readily and non-destructively obtained; and iii) the intracellular NAD concentrations and NAD⁺/NADH redox state are strongly age dependent in the healthy human brains. The age-related NAD and redox changes observed in this study are likely the indications of deteriorating cellular metabolic activities and functions occur during the aging process. Further study is needed to underline their relations with the anticipated morphological, neurochemical or metabolic changes found in aging brains [5-6]. We conclude that the newly developed in vivo $^{31}$P MRS approach provides new opportunities for studying the central roles of the NAD and its redox in human health and diseases. The same approach can be readily extended to other organs beyond the brain.

**Methods** Eleven healthy volunteers (Age: 21-64 years, 6M/5F) participated in this study. All MR measurements were conducted at 7 Tesla/90 cm bore human scanner (Siemens) with a surface coil probe placed over the visual cortex for data acquisition. This probe consists of a quadrature $^1$H coil for anatomic imaging and $^3$P shimming and a single loop $^{31}$P coil (Dia. $\approx$ 5cm) for collecting $^{31}$P MR spectroscopy data. The $^{31}$P spectra were obtained using pulse-acquire sequence, 300$\mu$s hard pulse for excitation with optimized pulse power and flip angle, 3$\sigma$ repetition time and 320 total scan number in all subjects. A novel quantification method capable of simulating and/or fitting the spectrum of all $\alpha$-ATP, NAD⁺ and NADH resonance peaks at a given magnetic field strength was applied. The absolute concentrations of $\alpha$-ATP, NADH and NAD⁺ in each subject were determined by comparing the integral of these resonances with that of $\alpha$-ATP, in which its concentration was set to 2.8$nM$ as an internal standard [4]. The ratio of NAD⁺/NADH and the total NAD content ([NAD]$_{total}$=[NAD⁺]+[NADH]) in the brain tissue were also be determined.

**Results** Figure 1 displays a typical in vivo $^{31}$P spectrum of human visual cortex obtained in a representative subject. Excellent sensitivity and spectral quality achievable at 7T ensured the reliable detection and quantification of the NAD signals in vivo. The total signals of $\alpha$-ATP, NAD⁺ and NADH determined by the model fitting matched well to the original $^{31}$P signals with very low residual, thus, the individual fitting components provided quantitative measures of the intracellular NAD contents and redox ratio with the values of [NAD⁺]=0.30$nM$, [NADH]=0.08$nM$, [NAD]$_{total}$=0.38$nM$ and NAD⁺/NADH redox state of 3.51 obtained in this subject. When the absolute concentrations of NAD⁺, NADH and total NAD in different subjects were plotted against their ages as shown in Fig. 2, strong age-dependent relations were observed in individual subject data (open symbols) as well as in grouped data (filled symbols). We found that the NADH increases and NAD⁺ declines with the rise of the subject’s age, thus, a profound reduction in the NAD⁺/NADH redox state was clearly evident in the healthy human brains with normal aging. We also found that the total NAD level was slowly declined in older people’s brain.

**Discussion and Conclusion** The present study, for the first time, reported following intriguing findings: i) it is feasible to robustly measure and identify the in vivo $^{31}$P MRS signals of the NAD⁺ and NADH in human brain at 7T; ii) the knowledge regarding the NAD and its redox state in human brain, which is not available in the literature, can now be readily and non-destructively obtained; and iii) the intracellular NAD concentrations and NAD⁺/NADH redox state are strongly age dependent in the healthy human brains. The age-related NAD and redox changes observed in this study are likely the indications of deteriorating cellular metabolic activities and functions occur during the aging process. Further study is needed to underline their relations with the anticipated morphological, neurochemical or metabolic changes found in aging brains [5-6]. We conclude that the newly developed in vivo $^{31}$P MRS approach provides new opportunities for studying the central roles of the NAD and its redox in human health and diseases. The same approach can be readily extended to other organs beyond the brain.

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