Mitochondrial NADH in vivo: functional test reveals a natural indicator of oxidative phosphorylation in 31P spectrum.

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Target audience: A non-invasive natural indicator of oxidative phosphorylation in vivo is of interest to investigators focused on metabolism and mitochondrial (dys)function with age and disease in muscle, heart and brain of animals and humans.

Purpose: Here we test that a co-enzyme central to mitochondrial metabolism (nicotinamide adenine dinucleotide, NADH)[2,3,4] is detectable by phosphorus spectrum (^{31}P MRS). A unique resonance downfield from the NAD+ and NADH recently reported in vivo[2] and centered at –11.05 ppm is evaluated for whether it represents NADH in mitochondria thereby providing an intrinsic probe of mitochondrial oxidation in vivo.

Methods: A 9 cm diameter surface coil tuned to the phosphorus frequency (25.9 MHz) in a General Electric 1.5 T Signa collected spectra from human vastus lateralis muscle[1]. Fully relaxed spectra were collected (16 free induction decays (FID), 16 s T_r) using a spectral width (SW) of 2500 Hz and 4096 complex points. The unfiltered PCr linewidth (full width at half-maximal height) was typically 4-8 Hz. [PCr], [ATP], [P_i] and pH during and following muscle exercise were made using a standard 1 pulse experiment with partially saturated nuclear spins (1.5 s T_r) averaging 4 FIDs for 6 s time resolution. *Chemical phantoms:* Binding constants were used to calculate the compositions for solution standards[6] with the ionic strength set at 0.175M, pH = 7.0 (36°C) and containing (in mmol L^{-1}): EGTA 15, MOPS 80, free Mg^{2+} 1, Na^+ 83 and K^+ 52. High-resolution MR spectra of individual solutions in an NMR tube containing NAD, NADH, NADP and/or UDP-glucose were taken at 4.7T (SW=10K, 16K complex, 128 FIDs, 5 s T_r). *Spectral fits:* Two fitting methods were used to cross-check values on double zero filled spectra: 1) direct integration by the MestreNova^R program and 2) fitting difference spectra (–10.4 to –11.4 ppm region) to chemical and in vivo phantoms by minimizing the sum of squares. Statistical differences at P<0.05. Means ±SE.

Results: *Unique resonance:* Figure 1 shows the fully relaxed ^{31}P MR spectrum in vivo from human vastus lateralis (VL) muscle with an expanded regions showing α-ATP and NAD(P) peak regions[1]. Three resonances present in the NAD(P) region reflect NADH (-10.6 ppm) and NAD+ (-10.75 ppm) in solution[2,3,4] (dashed lines from 4.7T) with a third unidentified peak (blue arrow at -11.05 ppm) (Fig. 1B). No overlap of the -11.05 ppm peak was found with chemical standards of other muscle metabolites (NADP+, -10.85 ppm or UDP-glucose, -10.4 & -10.65 ppm). *Quantitative analysis:* Agreement was found between integral of the -10.6 ppm, -10.75 ppm and –11.05 ppm peaks (NAD(P)/ α-ATP ATP=11.5±0.9%, *n*=40) and 11% NAD/ATP in biochemical assays of reported for human VL muscle [7]. The –11.05 ppm resonance alone is 4.4±0.4%, while the –10.75 ppm NADH peak is 0.5±0.2% of α-ATP in agreement with the NADH redox disparity between cell and mitochondria[8]. With 63% of mitochondrial NADH free in solution and detectable by MRS [4], the metabolite at –11.05 ppm represents a content of 0.41 mM, which agrees favorably with a total mitochondrial NADH of 0.55 mM estimated from the NADH content of mitochondria (3 ng/mg mitochondrial protein) and the mitochondrial volume (2.9%) for these muscles[1,5]. *Functional analysis:* Recovery from exercise activates mitochondrial NADH oxidation to NAD+. Fig. 2 shows such a decline in the –11.05 ppm peak that is matched by a reciprocal rise in NAD+ (NAD+ phantom is red dashed line) between rest and PCr recovery (elevated oxidative phosphorylation) after exercise. A quantitative agreement between the NAD+ peak area rise and –11.05 ppm decline is shown in Fig. 2C. The opposite was found with muscle exercise designed to raise mitochondrial NADH: an increased –11.05 ppm peak with a corresponding reduction in NAD+ (not shown). These results emulate the dynamics of mitochondrial NADH during stimulation and oxidative recovery revealed by optical fluorescence in classic studies[9]. *Accelerated NAD dynamics with exercise training:* A 6 month exercise training program[5] resulted in a greater decline in the –11.05 ppm peak in proportion to the rise in phosphorylation during the exercise test (Fig. 3; *n*=11-15 for each group). These results are consistent with greater mitochondrial NADH oxidation accompanying the greater phosphorylation after exercise training[5].

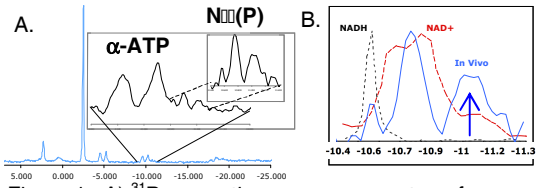

Figure 1: A) ^{31}P magnetic resonance spectrum from human vastus lateralis at 1.5T with expansion of α-ATP and NAD(P). B) Chemical phantoms for NADH and NAD+ (dashed lines) vs. in vivo spectrum (blue) reveal an unidentified peak (arrow, -11.05 ppm). [1]

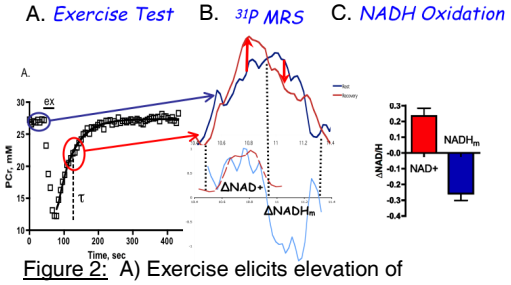

Figure 2: A) Exercise elicits elevation of oxidative phosphorylation (PCr resynthesis). B) Subtraction of NAD region in resting (blue) and recovering (red) muscle reveals rise in NAD+ [phantom in red dashed line] and drop in -11.05 ppm ($NADH_m$) peak. C) Reciprocal changes in NAD+ vs. -11.05 ppm ($NADH_m$) peak areas [recovery minus resting muscle].

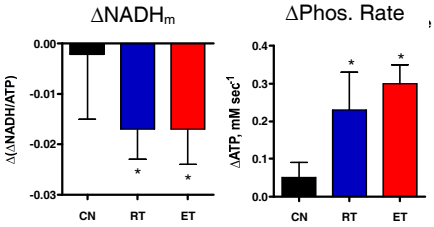

Figure 3: Decreased –11.05 ppm peak (ΔNADHm) with increased oxidative phophorylation during exercise test after resistance (RT) and endurance (ET) training vs. controls (CN) in VL muscle. [5]

Discussion: Quantitative and functional evidence identifies the –11.05 ppm in the ^{31}P spectrum in vivo as reflecting mitochondrial NADH. This –11.05 ppm peak is downfield from an NADH peak identified by a chemical phantom. A change in NADH physical properties with compartmentation is well known from the unique optical fluorescence of NADH in mitochondria[4]. A similar downfield chemical shift with compartmentation is found in the ^{31}P spectrum of phosphoryl compounds located inside erythocytes vs. plasma[10]. An elevation in hydrogen bonding due to the high protein concentration in the erythrocyte is the explanation for the downfield chemical shift in red blood cells [11]. Thus the chemical shift between the chemically identified NADH peak (-10.6 ppm) and the -11.05 ppm resonance, as well as the ratio of their contents[8], are consistent with compartmentation of NADH between cell and mitochondria.

Conclusions: A unique resonance in the NAD(P) region of the ^{31}P MR spectrum in vivo has properties consistent with NADH located in mitochondria. This natural indicator of mitochondrial NADH reflects the improvements in mitochondrial function with treatment and, in combination with the chemically defined NAD+ and NADH in vivo[2], reveals the redox disparity of mitochondria vs. cell in vivo.

Acknowledgements: R01 AGAR10853, RC2 AG036606, R01 AR41928. **References:** [1]*J Physiol* **526**, 203 (2000),[2]*Magn Reson Med* **71**, 1959 (2014), [3]*FEBS Lett* **585**, 1657 (2011).[4]*Biochem* **47**, 9636 (2008).[5]*J Appl Physiol* **90**, 1663 (2001).[6]*J Physiol* **540**, 485 (2002).[7] *J Physiol* **380**, 441(1986).[8]*Biochem J* **103**, 514 (1967),[9]*J Gen Physiol* **50**, 1009 (1967).[10] *Biochem* **27**, 8803-8810 (1988).[11] *Biochem* **27**, 8795-8802 (1988).