Novel Biomarkers of Mitochondrial Function: the Mitochondrial Index and the Crossing Point of Glucose and Oxygen Consumption Curves obtained <in vivo> by Dynamic Deuterium Magnetic Resonance

Gheorghe D Mateescu[1], Chris A Flask[2,3], Allen Ye[4], Bernadette Erokwu[5], Michael Twieg[6], Karishma Gupta[5], and Mark Griswold[3,5]

[1]Chemistry, Case Western Reserve University, Cleveland, OH, United States, [2]Biomedical Engineering, Case Western Reserve University, Cleveland, OH, United States, [3]Case Western Reserve University, OH, United States, [4]Bioengineering, University of Indiana at Chicago, IN, United States, [5]Radiology, Case Western Reserve University, OH, United States, [6]Biomedical Engineering, Case Western Reserve University, OH, United States

Target audience Scientists and clinicians interested in finding new avenues for the diagnostic of bio-energetic (metabolism) deficiencies and the improvement of functional MR imaging interpretation.

Purpose To establish a non-invasive method for *simultaneous in vivo* determination of glucose and oxygen consumption in the whole-body and discrete organs and tissues, and to define a *new biomarker* of mitochondrial function.

Methods Athymic mice are prepared for two different kinds of experiments – one with *intravenous* (tail vein) catheter, the other with *intraperitoneal* catheter – for infusion of D-Glucose-6,6-d_2 ($G66d_2$). The animal is placed in a container allowing for proper anesthesia, breathing and temperature monitoring (MR-compatible Monitoring System, SA Instruments, Inc); it is then introduced in a preclinical MRI scanner (9.4 T Bruker Biospec), and five baseline (natural abundance) ^{2}H-spectra are taken (shown on the left of Fig 2). Subsequently, 200 µL sterile saline $G66d_2$ solution (250 mg/ml) is infused *via* intra-peritoneal (*ip*, Fig. 2) or intravenous (*iv*, Fig. 3) injection and *Dynamic Deuterium MR (DDMR)* spectra are taken in 16 s blocks. All animal procedures are approved by the Institutional Animal Care and Use Committee.

Results and Discussion The sketch in Fig 1 illustrates the origin of the *nascent mitochondrial water, HDO*. Fig 2 shows a complete *DDMR* experiment. An intrinsic benefit is the *simultaneous* measurement of *both* the deuteriated glucose (right, decreasing intensity peak) and of the nascent *HDO* (left, increasing intensity peak). Fig 3 demonstrates the feasibility of quantitative determinations.

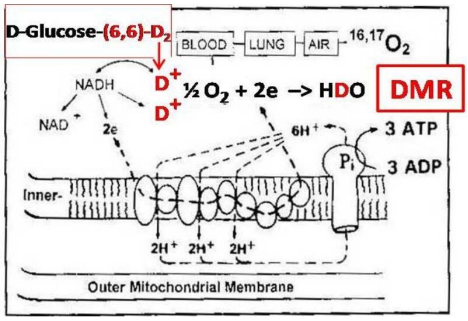

Fig 1. Infused deuteriated glucose reaches mitochondria, where it is metabolized into CO_2 and water. Proton – deuteron exchange results in *HDO* formation.

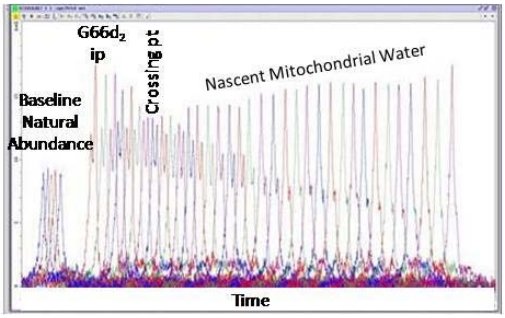

Fig 2. Raw DDMR data (screen shot) before (first 5 peaks) and after *ip* injection (35 spectra with *HDO* peak on the left, and $G66d_2$ on the right). 10 mm slice centered on the mouse head. PW = 100 us; AQ = 0.5 s; NS = 32. Experiment time 10800 s.

Preliminary results show that the *crossing point* of the glucose and oxygen consumption curves occurs approximately 6.5 times faster for *iv* infusion (at ~ 6 minutes), when compared with *ip* infusion (at ~ 39 minutes).

Conclusions The results shown above demonstrate that it is possible to *simultaneously* measure, *in vivo*, by *DDMR,* the rates of oxygen and glucose consumption in a live organism, following administration of deuteriated glucose. It is thus possible to define a *biomarker* of mitochondrial function, namely the *crossing point* (the occurrence time of the intersection of the glucose and oxygen consumption curves). We also propose the establishment of a *mitochondrial index*, the ratio of the rates of glucose consumption and *nascent mitochondrial water* (MW_n) formation (both expressed in µMole/g·min): $M_{ind} = MRGlc / MRMW_n$, where the denominator is indicative of the *oxygen consumption*. M_{ind} can be correlated with the state of health of organs and tissues. Applications of interest to us are in fatty liver and cancer detection and staging. Corroboration of fMRI, PET and M_{ind} results could bring considerable improvement in the interpretation of fMRI and a better understanding of the variability of $CMRO_2$ in normal human brain.[1] Currently, the optimal voxel size is ~ 0.5 mL. Considering the loss of sensitivity in moving to clinical (lower field) MRI scanners, it would be possible to make the measurement in more than 1,000 voxels in a human brain. Similar estimates may be made for other organs and tissues. Work in progress includes attempts to indirectly detect deuterium *via* proton MR. Preliminary measurements are being made on a home-built ^{1}H-{^{2}H} coil. Employing Magnetic Resonance Fingerprinting[2] is also being explored.

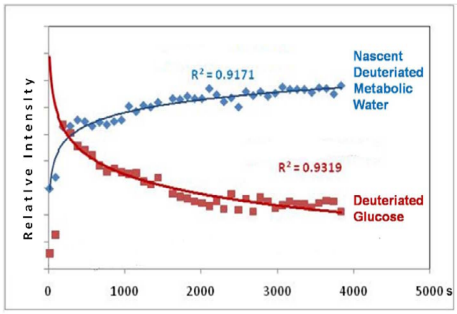

Fig 3. *MRGlc* and $MRMW_n$ after *iv* $G66d_2$ infusion

References

1. Gjedde A, Anerud J, Peterson E,Ashkanian M, Iversen P, Vafaee M, Møller A, & Borghammer P. Variable ATP Yields and Uncoupling of Oxygen Consumption in Human Brain. Adv Exp Med Biol 2009;701:243-248, and references cited therein.
2. Ma D, Gulani V, Seiberlich N, Liu K, Sunshine JL, Duerk JL & Griswold MA. Magnetic resonance fingerprinting. Nature 2013;495: 187-193.