Novel approach for the assessment of the bioavailability of exogenous phosphate by *in vivo* dynamic ^{17}O and ^{31}P MRS and MRI

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Introduction Inorganic phosphate (P_i) bioavailability affects a multitude of structural and functional attributes of live organisms, from mitochondrial bioenergetics to bone biochemistry. According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) phosphate homeostasis comprises more than 2700 chemical reactions. A large number of publications exists on the ^{31}P magnetic resonance evaluation of the *endogenous* P_i. However, to the best of our knowledge, *in vivo* MR metabolism measurements of *exogenous* P_i have not been reported. We present here preliminary results with ^{17}O enriched P_i that demonstrate the feasibility of tracking exogenous P_i *in vivo*, within the pool of natural abundance phosphates.

Materials and methods Oxygen-17 labeled monopotassiumphosphate ($KH_2P^{16,17}O_4$) was synthesized from phosphorus pentoxide and a 45% ^{17}O-enriched water solution of potassium hydroxide. After purification by crystallization the substance was used to prepare a MR phantom consisting of a 5 mm NMR tube filled with $KH_2P^{16,17}O_4$ solution (~1 mM). A melting point capillary filled with $H_2{}^{16,17}O$ was placed in the center of the tube. O-17 MRS and MRI (9.4 Tesla microimager; TE 3.8 ms, TR 32 ms, ET 2 to 12 min, 16x16 matrix) are shown in Fig 1. Image distortions are due to low resolution, exceedingly high brightness, and air bubbles. The ^{31}P spectrum of $KH_2P^{16,17}O_4$ in $D_2{}^{16}O$ is shown in Fig 2. A sterilized, buffered solution was injected *ip* (~400 mg $KH_2P^{16,17}O_4$/Kg) in a 27 g mouse (C57/B1). *In vivo* localized ^{17}O spectra (10 mm brain-centered slice) taken at 5, 10, and 30 min after injection are shown in Figure 3.

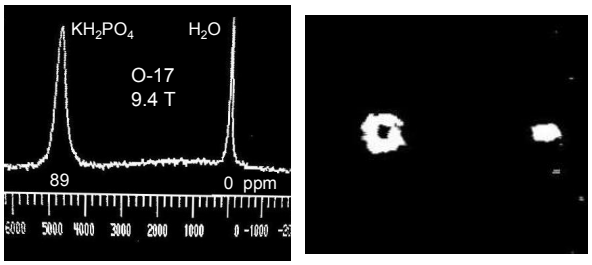

Figure 1. The 54 MHz ^{17}O MRS (left) and the MRI of the phantom (see text).

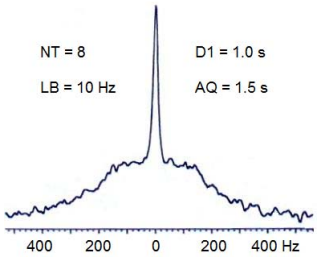

Figure 2. The 243 MHz ^{31}P MRS of $KH_2P^{16,17}O_4$ in $D_2{}^{16}O$.

Results and Discussion In spite of the fact that the localized ^{17}O spectra shown in Fig 3 display a relatively low S/N, they could yield satisfactory quantitative results. The fact that the phosphate peak is 89 ppm downfield from water greatly facilitates its quantitative determination. On the other hand, ^{31}P spectroscopy performed with ^{17}O decoupling would be an excellent means to corroborate the ^{17}O results. In Fig 2, the very large band width (FWHM ~420 Hz) is due to $^{31}P - {}^{17}O$ J - and quadrupolar - coupling, while the much narrower center line corresponds to the unlabeled (natural abundance) $KH_2P^{16}O_4$. Decoupling the ^{17}O nucleus (work in progress) will result in a much sharper, high intensity peak that would readily identify the exogenous phosphate.

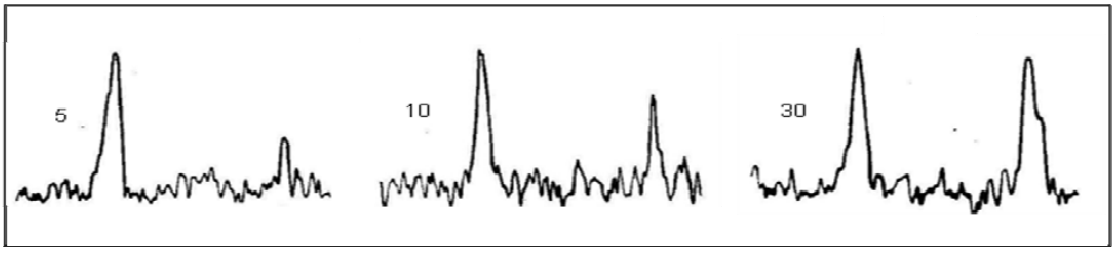

Figure 3. *In vivo* dynamic ^{17}O MR spectra obtained from a 10 mm slice that includes the brain of the mouse. The spectra are normalized to the posphate peak in order to emphasize **the transfer of the phosphate ^{17}O label to water** *via* hydrolytic reactions. Numbers are minutes after *ip* injection of $KH_2P^{16,17}O_4$.

Conclusion Preliminary results demonstrate that it is possible to track the utilization of exogenous (dietary or pharmaceutical) inorganic phosphate in living organisms employing ^{17}O-labeled phosphate. This new approach may become important in relating phosphate homeostasis defects to metabolic or other diseases.

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