Pilot & Feasibility Program

Intracellular P and ATP in X-linked hypophosphatemia

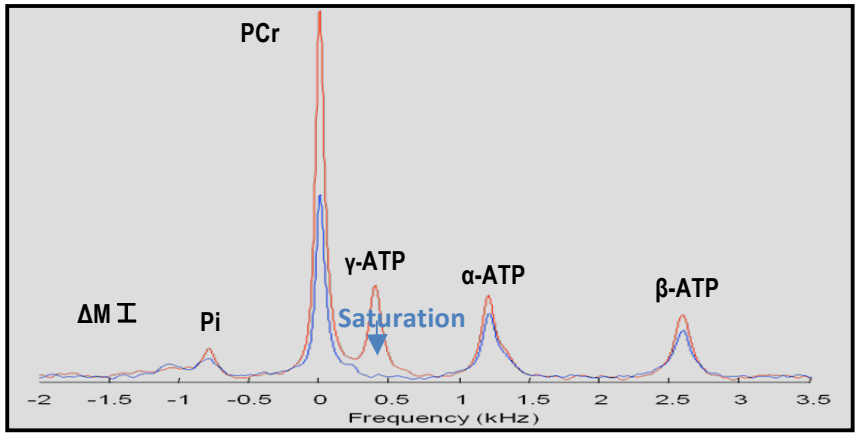
Gerald Shulman, MD, PhD, Principal Investigator

Gerald Shulman is a well-known clinical investigator in the field of carbohydrate metabolism and diabetes. His study examined the role of intracellular phosphate, as pertains to energy metabolism. Dr. Shulman notes: “In preliminary studies we have found that dietary Pi-deprivation in normal mice results in severe hypophosphatemia and a 50% reduction in skeletal muscle ATP synthesis as measured by in vivo ^{31}P-MRS saturation transfer methods. In addition, these mice exhibit reduced whole body energy expenditure, activity, and oxygen consumption. We examined intracellular phosphate metabolism, mitochondrial ATP synthesis and whole body energy metabolism in hypophosphatemic and euphosphatemic Hyp mice. These studies will provide important new insights into the role of intracellular phosphate metabolism on the regulation of mitochondrial and whole body energy metabolism under normal physiological conditions as well as provide new insights into intracellular phosphate metabolism in patients with X-Linked Hypophosphatemia.”

Findings to Date

Inorganic phosphate (Pi) is a potential regulator of mitochondrial ATP metabolism. We have shown that a ~65% decrease in plasma Pi, induced by 2 weeks of dietary Pi deprivation, promoted a ~50% reduction of the ATP synthesis flux (V_{ATP}) in skeletal muscle from 7.28±0.99 µmol/g/min to 3.73 ± 0.52 µmol/g/min ($P<0.05$). Our next step is to assess how chronic hypophosphatemia can affect *in vivo* V_{ATP} in skeletal muscle.

We have studied ATP synthesis flux noninvasively in the gastrocnemius/soleus muscle group by using a ^{31}P saturation transfer method. This technique is based on the principle that the magnetic properties of nuclei can be transferred between metabolite pools during chemical reactions. Therefore when the phosphate nucleus in the gamma position of the ATP molecule is saturated by a radio frequency pulse, becoming “NMR invisible”, a reduction in the intensity of the peak corresponding to the pool of Pi is expected due to the chemical interexchange between both pools. This difference in the intensity of the Pi resonance is therefore directly proportional to the V_{ATP} by a constant *k*. The product of *k* by the intracellular concentration of Pi equals the ATP synthesis flux. (See Figure 1 on next page.) At the end of the study, the tissues studied were collected and stored at -80°C for quantification of Pi and ATP concentrations.



Representative spectra of a ^{31}P saturation transfer performed in skeletal muscle. In each spectrum it is possible to see the resonance frequencies for inorganic phosphate (Pi), phosphocreatine (PCr) and for the three phosphate nuclei from ATP.

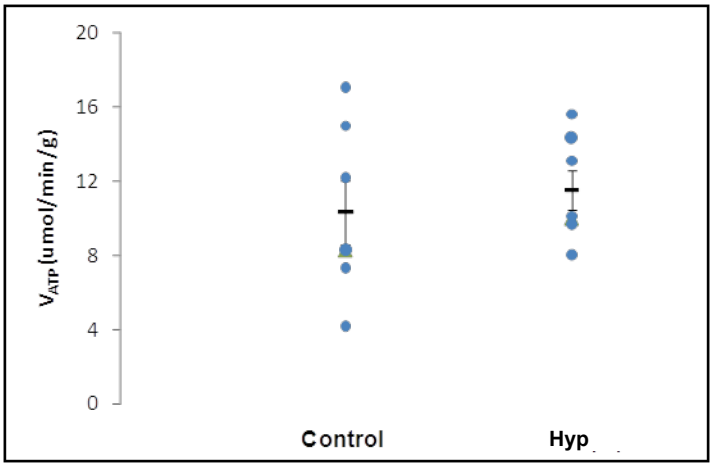
Twelve-week old control and Hyp mice (Jackson Lab, Bar Harbor, Maine) were analyzed. The results obtained so far are shown below.

Intracellular concentrations of inorganic phosphate ($[Pi]_i$), phosphocreatine ($[PCr]_i$) and ATP ($[ATP]_i$) and plasma concentrations of Pi and calcium (Ca^{2+}) for control and Hyp mice.

	Control (N = 7)	Hyp (N = 7)	P Value†
Weight (g)	25.9±1.0	15.6±0.4	0.00005
$[Pi]_i$ (µmol/g muscle)	1.3±0.0	1.4±0.2	N.S.
$[PCr]_i$ (µmol/g muscle)	18.4±0.5	17.4±1.2	N.S
$[ATP]_i$ (µmol/g muscle)	3.8±0.1	3.8±0.1	N.S.
$[Pi]_{plasma}$ (mg/dL)	9.46 ± 1.10	4.97 ± 0.53	0.008
$[Ca^{2+}]_{plasma}$ (mg/dL)	9.31 ± 0.18	9.10 ± 0.10	N.S.

† P values are the result of unpaired t tests.

Analysis of the above results shows that despite the decrease in plasma Pi concentrations, the intramyocellular concentrations of Pi, PCr and ATP remain unaltered. Correspondingly, the *in vivo* fluxes of ATP synthesis were similar between groups (10.3±1.7µmol/min/g in the control versus 11.6±1.1µmol/min/g in the Hyp mice), as shown in the figure below.



***In vivo* ATP synthesis flux (V_{ATP}) in control and Hyp**

These results show that in a setting of chronic reduction of plasma Pi levels, a regulation mechanism takes place to avoid the intracellular depletion of Pi. These results bring further insights into the regulation of phosphate homeostasis and its relevance on the regulation of ATP synthesis, and suggest that in XLH muscle Pi, as relates to energy metabolism, has adapted to reasonably normal function.