^{31}P MRS of resting muscle at 7T: differences in the alkaline pH compartment between different muscles and sedentary and elite trained athletes

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Introduction. Non-invasive determination of mitochondrial content is an important objective in clinical and sports medicine (1). ^{31}P MRS approaches to obtain information on this parameter at clinical field strengths typically require in-magnet exercise. Direct observation of the intra-mitochondrial inorganic phosphate (Pi) pool in resting muscle would constitute a much simpler alternative. In our previous research a peak 0.4 ppm downfield from the cytosolic Pi resonance (Pi_1) was found in resting skeletal muscle using the increased signal-to noise and spectral resolution that can be obtained at 7 tesla (2). This signal (which we term Pi_2), at 5.1 ppm was attributed to the Pi pool inside the mitochondrial matrix based on various considerations including the chemical shift, the locations from which the signals were recorded, and the short T_1 relaxation time. However, despite being highly suggestive, it has not been definitively proved that this signal truly is of mitochondrial origin. Seeking further clarification, the aim of this study was to measure the Pi_2 signal in soleus (SOL) and tibialis anterior (TA), and in sedentary volunteers versus highly trained athletes at 7T. If our hypothesis is correct, then differences in the Pi_2 signal should be observed between muscles with different oxidative capacities, such as the TA and SOL muscles (3) and/or subjects with severely different activity levels (1).

Methods. ^{31}P MRS was performed in three groups of subjects, each consisting of 3 healthy volunteers. One group of normal volunteers (REG), one group of reasonably active subjects (exercise between 0 and 2 times a week)(SED), and one group of endurance trained athletes (ATH) (exercise 6-9 times/week). Data were acquired as 2D CSI datasets (FOV, 160 x 160; matrix size, 8 x 8; TR = 1680 ms; hamming weighted acquisition and post processing with 32 averages at the central k-space lines (4), and an adiabatic 90° half passage pulse of 3.3 ms duration with the transmitter frequency set to 5.0 ppm downfield from the PCr peak). A custom-built double-tuned ^{1}H and ^{31}P coil setup with square coils for ^{31}P (10 cm) and ^{1}H coil (12 cm) was used in transmit/receive mode. The 2D data set was orientated perpendicular to the muscle of interest.
In the REG group two 2D CSI datasets were acquired: one with the coil placed at the posterior side of the leg for the soleus (SOL) muscle, and one with the coil at the anterior side for the tibialis anterior (TA) muscle. In the SED and ATH groups, only one CSI data set was acquired of the SOL muscle. Data were visualized using 3DCSI software (5), and voxels were identified in the SOL and TA muscles. The corresponding free induction decays were exported and fitted using the jMrui software package after filtering using Hankel Lanczos singular values decomposition (HLSVD) to remove partially-excited signals of PCr and ATP due to the limited bandwidth of the adiabatic pulse. Signals were fitted for the main Pi resonance Pi_1 and Pi_2. Differences in Pi_2/Pi_1 between SOL and TA were computed with a paired t-test in the REG group, and with an unpaired t-test for the SED and ATH groups.

Results. In the REG group, a signal from Pi_2 was reproducibly detected at ~0.4 ppm downfield of Pi_1 (Fig 1), and this signal was consistently higher in the SOL compared to the TA muscle (Fig 2a). On average, the Pi_2/Pi_1 ratio decreased from 0.05 ± 0.01 in SOL to 0.03 ± 0.01 in TA (p = 0.06). Assuming identical cytosolic Pi concentrations in resting SOL and TA, this result predicts that the mean ratio of Pi_{2_SOL}/Pi_{2_TA} is 1.6. In the comparison between the SED2 and ATH groups, the trained subjects showed a significantly increased Pi_2/Pi_1 ratio compared to sedentary individuals (0.06 ± 0.01 versus 0.03 ± 0.01) (Fig 2b). Similarly, assuming identical Pi_1 concentrations, this results in a factor-of-two difference.

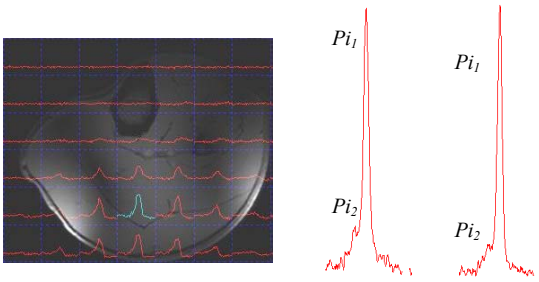

Fig1. Gradient echo image with a CSI data set overlaid (left). Example spectra of a trained (left) and an untrained volunteer (right), spectra are scaled to the Pi_1 signal.

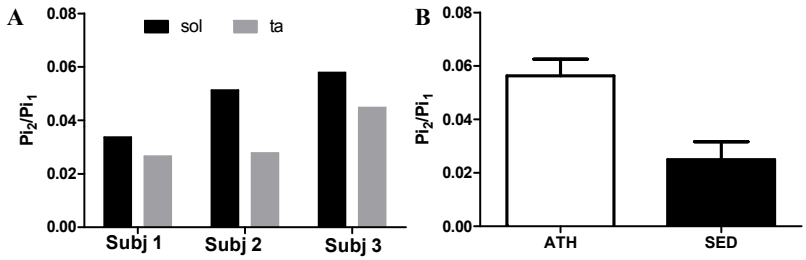

Fig 2. Histograms of the Pi_2/Pi_1 ratio in SOL and TA muscle in 3 volunteers (A) and between endurance trained and untrained subjects (B).

Conclusion. In this study we observed an increased Pi_2/Pi_1 in SOL versus TA muscle, as well as in trained versus untrained subjects. As human biopsy data have shown a similar fiber type composition of TA and SOL muscles (6) and no differences were observed in Pi_1 levels between trained and untrained subjects (7) we assume that cytosolic Pi concentrations are similar in all groups. If the amplitude of the Pi_2 resonance scales with mitochondrial density, these results would indicate that the mitochondrial density of human SOL muscle is 1.6-fold higher than for TA muscle. This number matches the finding by Forbes and coworkers of a 1.5 fold faster (22 vs 32 s) apparent time constant for PCr recovery in SOL versus TA muscle in human subjects (3). Similarly, a two-fold faster apparent time constant for PCr recovery has been observed in trained versus untrained subjects (8). The agreement between this well established MR-readout of in vivo mitochondrial capacity and the results of our CSI measurements of the Pi_2/Pi_1 ratio in the calf muscle further supports our hypothesis that resonance Pi_2 originates from the mitochondrial compartment in muscle.

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