In vivo assessment of skeletal muscle ATP synthesis in Ob/Ob mice

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Introduction: Obesity is considered by the WHO, the pandemic of the XXI century and is the primary risk factor for the development of cardiovascular disease and type II diabetes. The caloric overload stresses energy homeostasis maintenance and substrate oxidation capability in organs as the heart, liver and the skeletal muscle. In the particular case of skeletal muscle this energetic imbalance is characterized by increased lipid storage and eventually inneficient ATP synthesis. Whether this encompasses a dysfunction of the mitochondrion is of intense debate [1,2]. Here we assessed mitochondrial and overall energy synthesis in the leptin deficient obese mouse (Ob/Ob) with ^{31}P MRS. For the overall ATP synthesis, the Pi→ ATP flux was determined with a ^{31}P saturation transfer (ST) experiment, whereas to infer on mitochondrial ATP synthesis we assessed phosphocreatine (PCr) recovery rate upon maximal calf-muscle contraction with gated ^{31}P MRS. Contractile force was measured simultaneously, allowing us to compare PCr levels with force production, as well as with half-relaxation time of the muscle.

Materials and methods: Male Ob/Ob mice (n=8) and WT (n=8) with 7±2 weeks of age, were fasted for 6 h and then anaesthetized with isoflurane 1-2% in gas mixture with 50%O_2/50%N_2O. All MRS experiments were performed in a 7T (Clinscan, Bruker Biospin). Mice positioned prone with the left hind-limb inside of 4-turn ^{31}P solenoid coil surrounded by an Alderman-Grant ^{1}H coil to enable shimming. A ^{31}P MR spectrum was acquired to determine Pi concentration (assuming ATP of 7.8mM) with TR=7s and 64 av. For ST experiments a low-power continuous wave train of pulses of length 100ms was used to selectively saturate the γATP resonance for 0.5,1,2,3 and 5 s. For each experiment, a saturation about Pi was performed in order to correct for RF bleeding. Unlocalized ^{31}P MR spectra were acquired with 192 av and TR of 7s.

For PCr recovery kinetics 5 Ob/Ob mice and 7 WT mice were used. Contraction of the calf muscle was achieved by electric-stimulation of the sciatic nerve as described before [3]. The optimal stimulation current was found by increasing the current through the sciatic nerve until no further increase in force was achieved. Stimulation of the skeletal muscle was achieved by a pulse train of 250ms at 150 Hz. Muscle performance was measured by a custom-made MR compatible force transducer. A dual coil setup was built consisting of a ^{31}P coil for unlocalized ^{31}P and a ^{1}H coil for reference imaging and localized shimming of the hind leg. A user interface was designed to control stimulation, measure force transduction and synchronize the measurements to the spectrometer using LabView. All ^{31}P MRS spectra were acquired with TR = 10s, number of points=2048, spectral width of 5kHz. The stimulation protocol consisted of 12-16 time series of ^{31}P MR spectra acquired before (10 scans over ~2 min), during (6 scans over 1 min) and after (50 scans over 8 min) the muscle contraction. Tetanic muscle contraction occurred every 3 seconds over a period of one minute, summing 20 contractions *per* cycle. The time series of ^{31}P spectra were added retrospectively.

All ^{31}P MR spectra were analyzed using AMARES algorithm in jMRUI with prior-knowledge. The kinetic parameters of the forward reaction Pi→ATP (or overall ATP synthesis) were estimated from the mono-exponential fitting of Msat/M0 of Pi over the different γATP saturation times and from the yielded apparent T1. Mitochondrial ATP synthesis was determined from mono-exponential fitting of PCr recovery curve. Signals derived from the force transducer were digitized to a sampling frequency of 50kHz and analyzed with Matlab, where each contraction was filtered, and peak force and half relaxation time (HRT, the time in which the force falls from half to a quarter of the maximal value) were determined. To normalize force production, muscle cross-sectional area was determined from anatomical images using Image J.

Results & Discussion: Anatomical images showed that despite greater body-weight of the ob/ob mice (48.7±1.7g vs. 23.4±0.5g, p<0.0001) the CSA of the hindlimb, for the same tibial bone area, was significantly smaller (28.2±1.1 mm^2 vs. 38.8±0.8 mm^2, p<0.0001) compared to the WT mice. At rest, and assuming an ATP concentration of 7.8 mM, the levels of PCr in Ob/Ob mice were similar to those of WT (21.3±0.8 vs. 22.7±0.5 mM). PCr/ATP ratio (2.7±0.1 vs. 2.9±0.1) and intracellular pH (7.08±0.01 vs. 7.12±0.02) were also equivalent between the two groups of mice. Pi concentration was found increased in Ob/Ob mice compared to WT mice (2.3±0.4 mM vs. 1.8±0.5mM, p=0.02). The overall muscular ATP synthesis flux ($V_{Pi\rightarrow ATP}$), assessed by ST was comparable between the Ob/Ob mice and WT mice (0.35±0.13 mM/s vs. 0.29±0.07 mM/s, p=0.7, see Fig. 1).

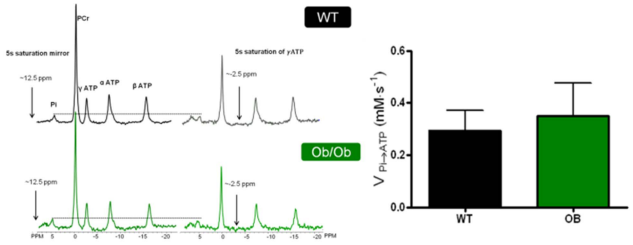

Fig. 1 *^{31}P MR saturation transfer in skeletal muscle of Ob/Ob and WT mice. (Right) Example of spectra acquired with 5s saturation at γATP and mirrored frequency about Pi. (Left) Estimates of overall ATP synthesis flux in skeletal muscle of Ob/Ob (green) and WT mice(black).*

Concurrent with a lower muscle mass, when stimulated at optimal current, the force produced by Ob/Ob muscles was significantly lower than that of WT, even when normalized for CSA (average of 0.010±0.0001 N/mm^2 vs. 0.026±0.0004 N/mm^2, p<0.0001). Muscle HRT of Ob/Ob mice was only significantly increased at the onset of contraction cycle (16.9±0.7 s vs. 13.7±0.3s, p<0.05) after which it was comparable to that of WT mice.

Dynamic ^{31}P MR spectra acquired during electro-stimulation showed that in WT mice, PCr was depleted by ~35% (Fig. 2) whereas in Ob/Ob mice PCr was depleted ~37%. The mono-exponential fitting of PCr recovery curve informs on muscle mitochondrial oxidative capacity and for the WT mice it yield a recovery time τPCr of 60.9±12s whereas in Ob/Ob mice, the preliminary experiments, show a τPCr of 25.3±9s.

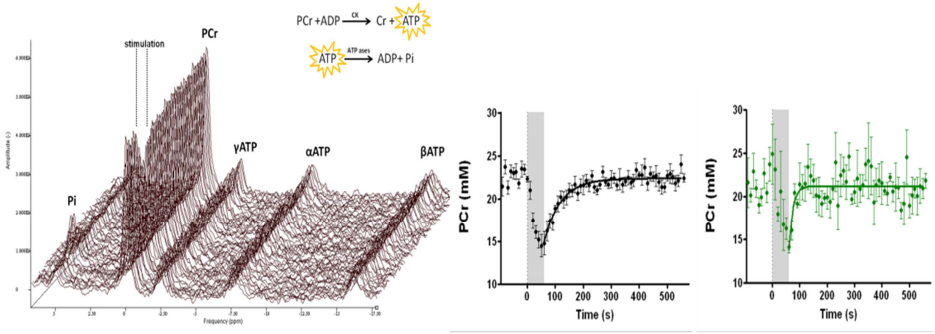

Fig. 2 *Dynamic ^{31}P MR spectra acquired from a WT mouse, before, during and after electro-stimulation. PCr dynamics in electro-stimulated calf muscles of WT mice (black), and Ob/Ob mice (green).*

Conclusion: Our data shows that the Ob/Ob mice present a lower muscle mass compared to WT mice, despite greater body-weight. The overall ATP synthesis assessed in the resting muscles of Ob/Ob mice showed no significant differences compared to WT mice.

References[1] Goodpaster BH, Diabetes 62, 2013. [2] Holloszy JO, Diabetes 62, 2013. [3] Kan HE *et al.*, NMR in Biomed 22, 2009.

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