Lipid accumulation and mitochondrial function in skeletal muscle of ATGL knockout mice: a ^{31}P MRS study

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Introduction: Adipose triglyceride lipase (ATGL) is the rate-limiting enzyme of triglyceride hydrolysis and free fatty acid mobilization from lipid stores as supply for energy production. Consistent with ATGL being mainly expressed in adipocytes and myocytes, ATGL-KO mice show abnormal triglyceride accumulation in fat and muscle tissues [1]. Lipid accumulation in skeletal muscle has been associated with the development of insulin resistance and type 2 diabetes, potentially promoted by lipotoxic effects on mitochondrial function [2]. Conversely, the lack of free fatty acids for energy generation boosts whole-body glucose oxidation, resulting in an insulin sensitive phenotype of ATGL KO mice [1]. Whether this will offset a potential adverse effect of lipid accumulation on mitochondrial function is unclear. Therefore, we measured mitochondrial oxidative capacity of ATGL KO mice *in vivo,* with gated dynamic ^{31}P MRS of contracting calf muscles. Contractile force was measured simultaneously as described previously [3], allowing us to compare phosphocreatine (PCr) levels with force production, as well as with half-relaxation time of the muscle.

Materials and methods: Male ATGL KO mice (n=6) and WT (n=9) with 9 ± 2 weeks of age, were anaesthetized with isoflurane 1-2% in gas mixture with 50%O_2/50%N_2O. Contraction of the calf muscle was achieved by stimulation of the sciatic nerve. After anaesthesia, a minor incision was made in the upper leg of the mouse. To avoid contraction of the dorsal flexors, the tibial nerve was cut. Then an electrode was tunnelled through the upper leg and connected to the sciatic nerve by stitching the electrode to the surrounding tissue leaving the sciatic nerve in its natural position. A second electrode was fed through the skin near the Achilles tendon, functioning as ground reference for the stimulation pulses. Increasing the current through the sciatic nerve until no further increase in force was achieved, was used to set the optimal stimulation current. 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 (121.5 MHz) for unlocalized ^{31}P and a ^{1}H coil (300.2 MHz) for reference imaging and localized shimming of the hind leg. The complete setup was designed to enable a prone position for the mouse with the lower leg vertical into the NMR scanner for the best possible natural position while keeping both coils perpendicular to the main magnetic field. A user interface was designed to control stimulation, measure force transduction and synchronize the measurements to the spectrometer and pulse sequences using LabView. All ^{31}P MRS measurements were done in a 7T/30 cm horizontal bore magnet interfaced to a clinical console (Clinscan, Bruker Biospin) with TR = 10s, number of points=2048, spectral width of 5kHz. The stimulation protocol consisted of 8 time series of ^{31}PMRS spectra acquired before (10 scans over ~2 min), during (6 scans over 1 min) and after (30 scans over 5 min) the muscle contraction. Tetanic muscle contraction (250ms, 150Hz) occurred every 3 seconds over a period of one minute, summing 20 contractions *per* cycle. The 8 time series of ^{31}P spectra were added and analyzed using jMRUI. PCr recovery was fitted with mono- exponential function (Graphpad 4.0, CA, USA). Signals derived from the force transducer were digitized to a sampling frequency of 50kHz and analyzed with Matlab (Mathworks Inc, Natick, USA), 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.

Results & Discussion: Total body-weight of ATGL KO mice was increased compared to the WT controls (25.1 ±0.4 g *vs* 23.5±0.4 g, p=0.02), probably due to increased adipose tissue [1] and muscle triglyceride accumulation [4]. Over the 8 cycles of contractions, and under an optimal electric current of 1.92 mA, the ATGL KO mice produced a maximal force of 0.50±0.03 N compared to 1.15±0.13 N produced by the WT, p<0.001. The force decreased over the contraction block similarly in both genotypes (figure 1A). The initial values of HRT were also similar in both genotypes and rose with the contraction number, as a sign of skeletal muscle fatigue. This increase in the muscle HRT was more evident in WT than in the ATGL KO mice as shown in figure 1B. ^{31}P spectra showed that ATGL KO and WT resting muscle have comparable concentrations of [PCr] (34.6 ±3.9 *vs* 34.1±2.0 mM) and [Pi], (2.8 ±0.9 *vs* 3.8±0.9 mM) as well as ratios of PCr/γATP and PCr/Pi and pH. The electro-stimulated muscles of ATGL KO and WT, presented similar PCr depletion (~40-50%, figure 2A), as well as a decrease in pH. The fitting of PCr recovery (figure 2B) showed that ATGL KO muscle have similar PCr recovery time as the WT muscle (τPCr of 51.7 ± 7.2 s for ATGL KO *vs* 57.2 ± 6.3 s for WT).

Conclusion: Our data demonstrate that at 9 ± 2 weeks of age, ATGL KO mice have weaker muscles than the WT mice, as reflected in lower force produced under the tetanic contractions, which might be related with the impaired cardiac performance of ATGL KO [4]. On the other hand, those muscles have faster relaxations. Finally, ^{31}P MRS measurements suggest that the resting muscle of ATGL KO and WT mice have comparable phosphorus-metabolites and that the PCr recovery time does not differ between the genotypes. These findings indicate that ATGL KO mice have similar mitochondrial oxidative capacities as WT mice.

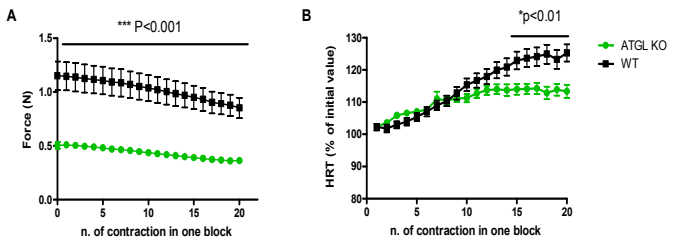

Figure 1: Absolute force production (A) and muscle half relaxation time, HRT, (% of the initial) (B) over an exercise block with 20 tetanic isometric contractions of calf muscles from ATGL KO and WT mice.

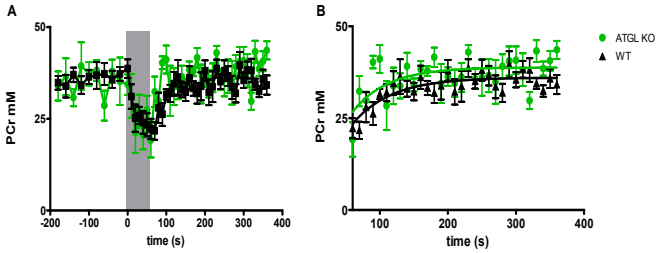

Figure 2: PCr concentration in calf muscles of ATGL KO and WT before, during and after the electric-stimulation protocol with 20 tetanic contractions (A); Mono-exponential fitting of the PCr recovery curve in ATGL KO and WT calf muscles (B).

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