Functional assessment of skeletal muscle in mice lacking myostatin by multiparametric functional (mpf-)NMR *in vivo*

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INTRODUCTION: Myostatin, an endogenous TGF-[beta] growth factor, negatively regulates muscle growth [1]. Deletion of the myostatin gene results in spectacular increase in muscle mass, and has opened the path to therapeutic approaches [2]. Yet improvement in muscle strength does not necessarily match the observed increase in muscle mass [3]. If function is to be preserved in hypertrophic muscle, adequate oxygen supply and substrate utilization should also be maintained. Multiparametric functional (mpf-)NMR, initially developed for humans [4], and recently extended to mice [5], offers the possibility of exploring these aspects in vivo and non-invasively. The set-up, including a suitable repeated exercise protocol, was developed to simultaneously assess muscle perfusion, BOLD, phosphorus metabolism, and isometric force in response to electrical stimulation (ES). It was applied to investigate myostatin knock-out mice.

METHODS: Ten-week old male myostatin knockout FVB mice [6] (mstn$^{-/-}$, n=11) were compared to wild-type FVB (WT, n=10). Anesthetized animals were installed in a Bruker Biospec 4T NMR system, equipped with a 20cm diameter 200mT/m gradient and shim insert and home-built coils (actively decoupled ^{1}H whole-body transmission and surface reception, ^{31}P leg-volumetric acquisition). Muscle contractions were induced through two subcutaneous silver electrodes (popliteal fossa, internal side of the ankle), and force measurement was collected through a custom-built ergometer. Pulsed arterial spin labeled RARE images using SATIR[7,8] (inter-echoes time= 2.9s, RARE factor= 32, FOV=5x2cm, matrix= 128x32) acquired every 10 s were interleaved with 4 successive ^{31}P spectra (TR= 2.5s), using the Bruker MultiScanControl tool operating on PV3.0.2. Acquisitions were triggered to the ES, to allow perfusion measurement even during exercise. Mice were subjected to an "exercise (2 min) - recovery (10 min)" protocol, repeated 12 times, in order to compensate for intrinsically low signal-to noise ratio. By summing ^{31}P data across exercises at steady state (SS) [5], phosphocreatine resynthesis could be fitted on high temporal resolution ^{31}P data. Histology analysis was performed on gastrocnemius muscles stained for succinate dehydrogenase (SDH) activity. Immunostaining of anti-CD34 antibodies against endothelial cells was used to estimate capillary contact per fiber (CCF).

RESULTS: As previously established in controls [5], a metabolic steady state was reached after the second bout of exercise, for all variables describing force, perfusion, BOLD, and pH. The force developed at SS by mstn$^{-/-}$ (Fig. B) was not different from WT when normalized for the 36% increase in muscle cross sectional area measured on anatomical MR images (Fig. A). Depletion of PCr during exercise was identical (mstn$^{-/-}$: 58±11%, WT: 58±13%), as was end-exercise pH (mstn$^{-/-}$: 6.97±0.09, WT: 7.03±0.05, Fig.C). Maximal perfusion at exercise was also the same, but time courses of recovery of mstn$^{-/-}$ were radically different (Fig. E; p=0.05). Hyperemic perfusion was extremely prolonged, while PCr recovery was also significantly delayed (Fig. D; p<0.015). Positive BOLD contrast was also prolonged in mstn$^{-/-}$ (Fig. F). A decrease in the relative fraction of oxidative fibers (type I and IIa) was measured (WT: 34 ± 5 %, mstn$^{-/-}$: 22 ± 7 % of muscle area; p=0.02). A change in fiber distribution was also observed (Fig. G). Oxidative fibers were more scattered and interspaced with type IIb in mstn$^{-/-}$. As expected, CCF was slightly higher for small oxidative fibers as compared to large glycolytic in WT and this distribution was not different in mstn$^{-/-}$ (not shown).

CONCLUSION: This study provides a unique comprehensive series of functional parameters acquired *in vivo*, simultaneously and non-invasively in mstn$^{-/-}$ mice muscles. Since mpf-NMR can explore both vascular and energetic responses to the same exercise, it provides an integrative view of muscle adaptation to mass increase in this model. Here, muscle hypertrophy was found to be non-pathologic. Indeed, the lower mitochondrial oxidative capacities were not related to a deficit in perfusion or oxygenation level, but could most likely be attributed to a shift toward type IIb fibers. This coherent set of quantitative and functional data also highlights the immense potential of mpf-NMR for the characterization and follow-up evaluation of preclinical models of muscle gene therapy.

References: [1]McPheron, et al. Nature 1997 [2]Bogdanovitch, et al. Nature 2001 [3]Amthor, et al. PNAS 2007 [4]Carlier, et al. Magn Reson Med 2006 [5]Baligand, et al. ESMRMB 2008 [6]Grobet, et al. Genesis 2003 [7]Raynaud, et al. Mag Reson Med 2001 [8]Bertoldi, et al. Mag Reson Imaging 2008.