Impact of Viral Mediated Overexpression of IGF-I on Skeletal Muscle

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Introduction
Insulin-like growth factor I (IGF-I) is a potent myogenic factor that has been shown to play a critical role in muscle myogenesis, hypertrophy, and regeneration. Magnetic resonance (MR) imaging, due to its noninvasive nature and short acquisition periods, allows longitudinal repeated measures of muscle morphology. The objective of this study was to quantify the changes in muscle morphology of the lower hindlimb muscles in mice with IGF-I overexpression.

Experimental
35 C57BL6 mice, 3 weeks of age, were injected with self-complimentary adeno-associated virus vector for IGF-I (rAAV2/8 sc.CB rat IGF-I 5X10^10 particles diluted in 80µl of PBS) into the posterior compartment of one of their lower legs, targeting the soleus (SOL) muscle. The contralateral control leg received an injection of the same volume of PBS solution. Lower posterior hindlimb muscle size was quantified based on 3D volumetric MRI at 4, 8, 12, 16 weeks after injection. All imaging procedures were performed in a horizontal, 4.7T magnet equipped with a Bruker Avance spectrometer running Paravision 3.1.1 software (Bruker Medical, Ettlingen, Germany).

Results and Discussion
In a pilot study, MRI of the mouse hindlimbs was optimized to obtain high-resolution, 3D GE T₁-weighted images at 4.7T (TR=100ms, TE=7.5ms, FOV=21.5x42.9x375 mm³; ~20 min acquisition). These images are used to calculate muscle volume of the dorsiflexor muscles (DF = tibialis anterior, extensor digitorum longus) and the plantar flexor muscles (TS = triceps surae). Figure 1A shows a transaxial image of the mouse hindlimb with the DF and PF muscles outlined. Muscles are outlined on multiple contiguous slices from origin to insertion, and the CSA of all slices is summed to obtain total muscle volume. High correlations were observed between MR assessed muscle volumes and wet weights of the anterior compartment muscles (DF) and posterior compartment muscles (TS) obtained upon tissue harvesting in 21-23 week old mdx mice (Fig 1B).

We found that MRI is sensitive to changes in muscle size following targeted muscle expression of IGF-1 by AAV delivery in mice. Muscle hypertrophy could be easily detected in young murine hindlimb muscles following the injection of AAV-IGF-1 (Fig 2), compared to the contralateral (control) limb which was injected with an equal volume of PBS. The muscle growth during development as well as the enhancement in muscle size could easily be detected by MRI (P<0.001; n=6; Fig 2). This was confirmed ex vivo, in which there was a 15% and 11% increase in the wet weight of soleus and gastroc muscles of the injected limb 8 wks post injection.

Conclusions
Overexpression of IGF-I induced muscle hypertrophy in mice can be monitored non-invasively and longitudinally using MR imaging.

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References