Muscle-specific knockdown of Numb and Numb-like protein reduces *in situ* tetanic and twitch force production in mouse gastrocnemius muscle

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1. ABSTRACT

Numb is a protein responsible for asymmetric division, cell fate commitment and vesicular trafficking. We have demonstrated androgens upregulate Numb in denervated muscle and preliminary studies show Numb is present in the triad junction of mature muscle fibers. To gain insight into the physiologic role of Numb in adult muscle fibers, a genetic knockdown of Numb and its related protein Numb-like (NumbL) was tested for its effect on the contractile properties of the gastrocnemius. C56BL/6 mice with HSA-driven ErT2-Cre-recombinase were crossed with mice containing floxed Numb and NumbL alleles, then treated for 56 days with tamoxifen (Tam) or vehicle (Veh) [n=28: female n=15, 8 Veh and 7 Tam; male n=13, 7 Veh and 6 Tam]. At 56 days, Numb protein was reduced by 50-80% in the gastrocnemius, TA, plantaris and EDL, but unchanged in the soleus. In the Tam group, the weights of the gastrocnemius, plantaris, and EDL slightly decreased, but the soleus weight increased, consistent with compensatory hypertrophy. The gastrocnemius muscle fiber CSA was slightly reduced by ~3% in Tam-treated animals. Using *in situ* physiologic testing we discovered reduced twitch (P_t) and tetanic (P_o) force in the Tam group. Specific tension (peak P_o/gastrocnemius weight) was reduced ~25% in the Tam group. Fatigue index (FI), time-to-peak tension (TPT) and half-relaxation time (HRT) were unchanged between groups. Importantly, there were no significant reductions in Tam-treated control mice with floxed Numb/NumbL alleles lacking the HSA-Cre gene. The reduction of force following muscle-specific knockdown of Numb/NumbL without changes in FI, TPT and HRT suggests that these findings occurred independently of gross changes in cellular calcium handling. Our data indicate that expression of Numb in healthy adult muscle fibers is vital to maintain optimal contractile properties, possibly due to critical roles of Numb in either the excitation-contraction coupling process or the contractile machinery.

2. PURPOSE

The purpose of this study was to test for changes in multiple parameters of mouse *in situ* isometric and twitch muscle performance 2 months after inducing a muscle-spefic knockdown of Numb/NumbL.

3. METHODS

- C56BL/6 mice (n=28) with HSA-driven-ErT2-Cre-recombinase were crossed with mice containing floxed alleles for Numb and NumbL.
- Tamoxifen (Tam; n=8 female, 7 male) or Vehicle (Veh; n=7 female, 6 male) was administered daily for 5 days to knockdown Numb/NumbL then weekly thereafter.
- At 56 days after Numb/NumbL knockdown, muscle performance was evaluated *in situ*. Mice were anaesthetized with 3-5% isofluorane and an incision was made to expose the sciatic nerve. The gastrocnemius was then carefully isolated and the calcaneus was cut with the Achilles tendon attached. The Achilles tendon was attached to a force transducer with silk sutures and an electrode was attached to the sciatic nerve. The muscle was bathed in 37 ˚C lactated Ringer's solution.
- Physiological outcomes were isometric twitch (P_t) and tetanic (P_o) force, half-relaxation time (HRT), time-to–peak tension (TPT) and fatigue index (FI).
- Data was acquired and analyzed using an Aurora Scientific small animal physiology system and are represented as means +/- SEM.

4. RESULTS

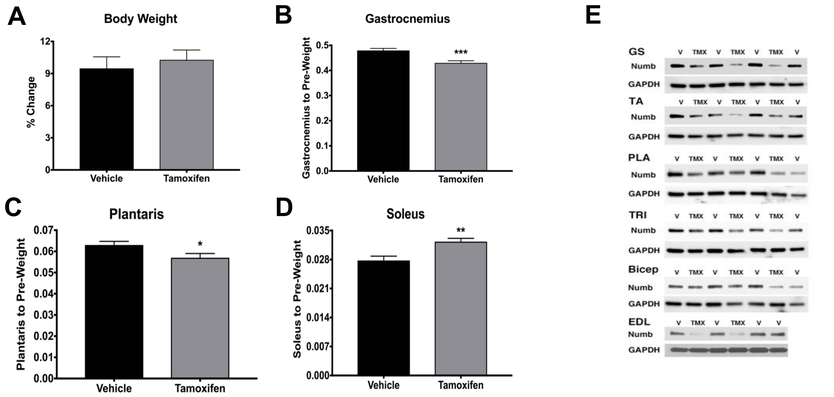

Fig. 1. Changes in body and muscle weights after 56 days of Numb/NumbL knockdown. A) There was no difference in body weight changes between the groups. However, weights of B) gastrocnemius and C) plantaris were reduced while that of D) soleus was increased in the tamoxifen animals. E) Representative blot of Numb knockdown between muscle groups at 14 days post-induction. Data are presented as mean +/- SEM. * denotes mean changes at p<0.05, ** p<0.01, *** at p<.001.

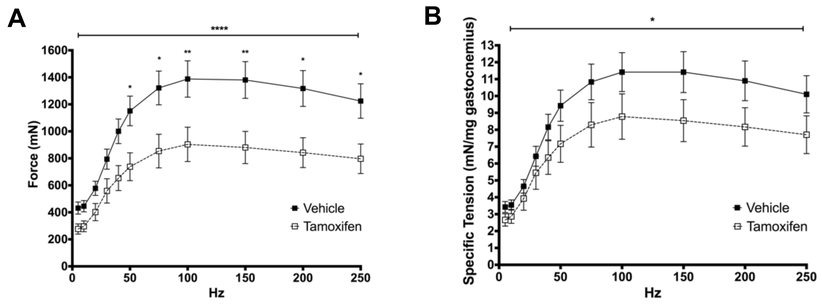

Fig. 2. Force-frequency curves of supramaximally stimulated gastrocnemius muscle. A) There is a strong Treatment x Frequency interaction effect which results in reduced force at all frequencies in the tamoxifen animals. B) Decreases in force production are independent of the reduced gastrocnemius size. Data are presented as mean +/- SEM. * over long brackets denotes interaction effect at p<0.05 and **** at p<.0001. * over individual data points denotes simple effect differences at p<0.05 and ** at p<0.01.

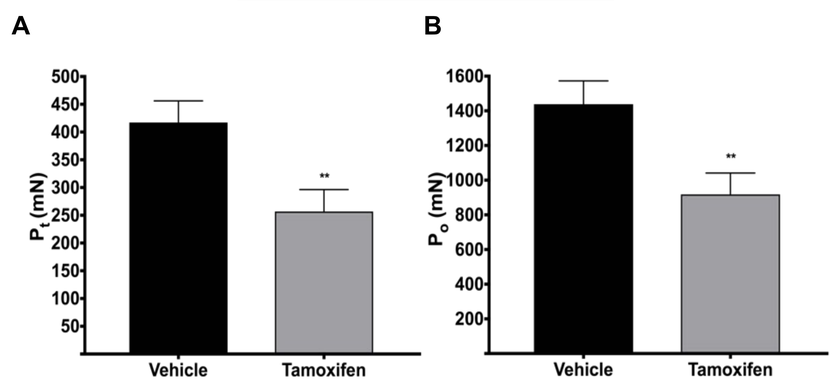

Fig. 3. Maximal force production is reduced in animals administered tamoxifen compared to animals treated with vehicle. A) Maximal twitch force (P_t) is reduced by approximately 40% and B) maximal tetanic force (P_o) is reduced by a similar magnitude. Data are presented as mean +/- SEM. ** denotes mean changes at ** p<0.01.

5. RESULTS CONT.

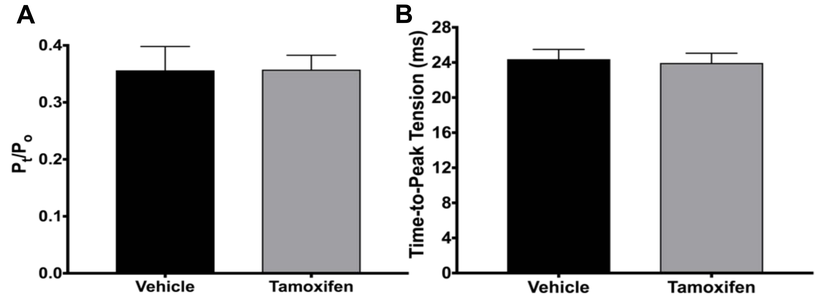

Fig. 4. Parameters of muscular force and force generation are unchanged between vehicle-treated and tamoxifen-treated animals. A) The ratio of maximal twitch to tetanic force production and B) time-to-peak tension is not affected by tamoxifen administration. Data are presented as mean +/- SEM.

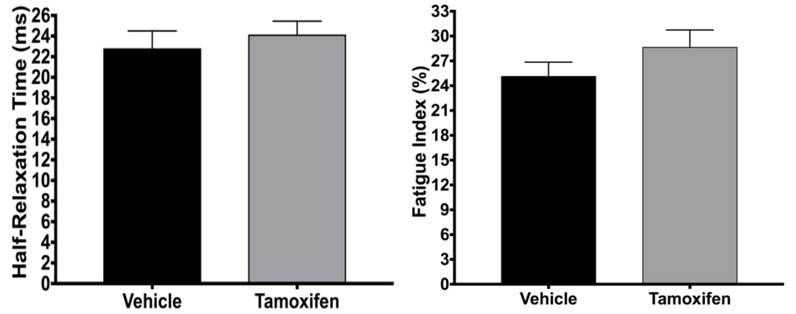

Fig. 5. Markers of muscle recovery following isometric twitch contractions are similar between vehicle and tamoxifen groups. A) There was no mean change in half-relaxation time after tamoxifen treatment. B) The fatigue index, measured as the force of the last contraction of a series of 120 consecutive contractions divided by the maximal force produced during the protocol, is not significantly different between groups. Data are presented as mean +/- SEM.

6. CONCLUSIONS

- Animals with tamoxifen-induced, muscle-specific knockdown of Numb and its related protein, NumbL, have preserved body mass over 56 days.
- The gastrocnemius, plantaris and extensor digitorum longus had reduced mass after tamoxifen treatment when normalized to controls.
- Soleus weight was elevated after tamoxifen, suggesting compensatory hypertrophy.
- Tamoxifen animals demonstrated large reductions in isometric gastrocnemius tetanic force production after correction for the loss in muscle mass.
- Maximal isometric twitch and tetanic force were reduced, while their ratio remained unchanged between groups.
- Time-to-peak tension, half-relaxation time and fatigue index were unchanged between groups, meaning gross changes in calcium handling are most likely not effected by reduced levels of Numb and NumbL.

7. ACKNOWLEDGMENTS

Research support was provided by a VA RR&D Service Merit to CPC (B7756R) and a VA RR&D Service Center Award to WAB (B9212C).