

# GROWTH-DEPENDENT ENHANCEMENT OF MOUSE NEONATAL MUSCLE MORPHOLOGY AND CONTRACTILE FUNCTION

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## INTRODUCTION

Postnatal skeletal muscle growth is attributable to hypertrophy of existing muscle fibers and action of myogenic stem cells, but the precise sequence of biochemical and biological events that results in muscle growth is poorly defined. A number of transcriptional regulatory molecules have been identified as mediators of skeletal muscle growth (Knapp et al., 2006). However, these studies typically consider “growth” primarily in terms of muscle mass and morphology independent of muscle quality or contractile function. As such, they do not provide insight into the nature of the development of mechanical function. Other work has focused on developmental changes in myosin heavy-chain isoform distribution (Agbulut et al., 2003), but these experiments also failed to consider contractile function.

Therefore, the objective of this study was to measure the time-course of neonatal skeletal muscle contractility and correlate these changes in muscle function with changes in tissue morphology and fiber architecture. These data are also necessary to provide a reference for studying certain muscle-specific proteins, such as nebulin (Bang et al., 2006), whose knockout is neonatal-lethal in mouse models. In such models, severe postnatal myofibrillar disorganization and muscle degeneration render invalid any comparison to mature controls. Rather, comparison to immature wild-type controls from neonatal time-points is preferred.

## METHODS

Bilateral mouse hindlimbs were used at postnatal days 1, 7, 14, 21, and 28 (P1-P28; n=8/time point).

**Morphology.** Frozen transverse sections (thickness: 10  $\mu\text{m}$ ) of the tibialis anterior (TA) were generated. On one set of sections, Alexa Fluor 488-conjugated phalloidin staining was used to localize sarcomeric actin. Image analysis was performed to compute the cross-sectional area fraction of contractile material (a metric of myofibrillar concentration within muscle fibers). On another set, immunohistochemistry was used to localize pericellular laminin. Image analysis was performed to compute pixel area enclosed by laminin and thus quantify muscle fiber cross-sectional area.

**Mechanics.** Mouse hindlimbs were transferred to a custom muscle-testing chamber filled with Ringer solution. Hindlimbs were secured proximal to the TA with a steel pin through the femur and distal to the TA with silk suture tied around the foot and ankle, passing through the tibiotarsal joint space. Muscle length ( $L_m$ ) was measured using an eyepiece crosshair reticule. Muscle fiber length ( $L_f$ ) was computed as  $0.6L_m$ , where 0.6 is the characteristic  $L_f/L_m$  ratio for the mouse TA as determined by a pilot study. Plantarflexors were then released at the Achilles tendon and carefully resected. Dorsiflexors underwent maximal isometric stimulation using a 400-ms train of 0.3-ms

pulses delivered at 100 Hz. Isometric force was measured 3 times with 2 min between contractions. After testing, TA muscles were weighed. Maximum isometric stress was estimated by normalizing maximum isometric force to TA physiological cross-sectional area (PCSA).

**Statistics.** Data are presented as mean±SEM. The effect of postnatal day was assessed using one-way ANOVA with *post hoc* Fisher's PLSD analysis to locate where differences existed ( $\alpha=0.05$ ).

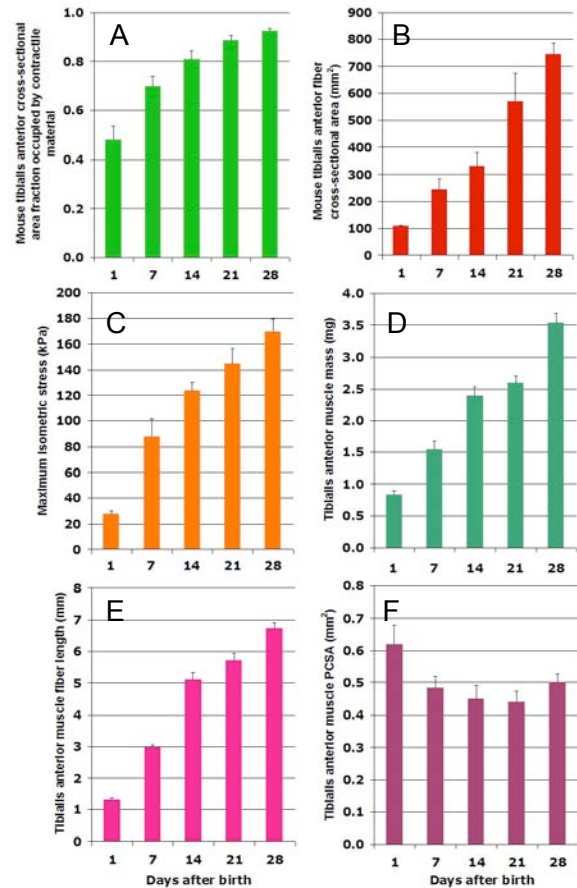
## RESULTS AND DISCUSSION

Muscle morphology exhibited rapid postnatal maturation. The cross-sectional area fraction of contractile material increased from 0.48 at P1 to 0.92 at P28, indicating a near-total filling of the intracellular space by myofibrillar contractile material by P28 (Fig. 1A). Simultaneously, fiber cross-sectional area increased ~7-fold (Fig. 1B). Morphological maturation occurred synchronously with functional enhancement, as evidenced by a ~6-fold increase in maximum isometric stress from P1 to P28 (Fig. 1C). Certain architectural parameters followed similar growth time-courses, namely muscle mass, which increased ~4-fold (Fig. 1D), and  $L_f$ , which increased ~5-fold (Fig. 1E). Interestingly, muscle PCSA decreased by 29% from P1 to P21 but began to recover slightly by P28 (Fig. 1F). This decrease in PCSA was driven primarily by  $L_f$  increasing more rapidly than muscle mass.

## SUMMARY/CONCLUSIONS

These data offer the first direct evidence for the improvement of functional quality during postnatal development of skeletal muscle. The increase in myofibrillar contractile material at least partially

accounts for the functional enhancement. Future work will attempt to develop a multivariate model of isometric muscle stress production.



**Figure 1.** Muscle morphology, maximum isometric stress, mass, and muscle architecture as functions of postnatal day.

## REFERENCES

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