

# DELETION OF NEBULIN ALTERS THE LENGTH-TENSION PROPERTIES OF NEONATAL SKELETAL MUSCLE

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## INTRODUCTION

The magnitude of isometric stress production by skeletal muscle is predicted by the sliding filament theory and is described as the length-tension curve (LTC). The LTC is sensitive to actin thin filament length. As thin filament length increases, the shallow portion of the ascending limb of the LTC widens due to an increased domain of thin filament double-overlap. This results in an overall widening and rightward shift of the LTC (Granzier et al., 1991).

Nebulin is a large, rod-like protein (500-900 kDa) believed to act as a thin filament “ruler” that specifies thin filament length during sarcomere assembly (McElhinny et al., 2003). Nebulin-knockout (NKO) mice have thin filaments up to 25% shorter than wild-type (WT) mice, exhibit rapid postnatal myofibrillar degeneration, and die after about 1-2 postnatal weeks (Bang et al., 2006). Sliding filament theory predicts an altered LTC in NKO muscle. Therefore, the goal of this project was to test this prediction and better understand the role of nebulin *in vivo* by subjecting neonatal NKO and WT muscles to a series of functional physiological assays.

## METHODS

Bilateral bone-tendon-muscle-tendon units associated with the gastrocnemius (GN) muscle from WT and NKO mice were used at postnatal days 1 (P1) and 7 (P7). Fatigue responses and LTCs were measured only at

P1 to minimize the confounding effect of postnatal degeneration in NKO muscle.

**Isometric contractions.** GN muscles were transferred to a custom muscle-testing chamber filled with Ringer solution. Tendons were secured with silk suture. Slack muscle length ( $L_m$ ) was measured using an eyepiece crosshair reticule. Fiber length ( $L_f$ ) was computed as  $0.46L_m$ , where 0.46 is the characteristic  $L_f:L_m$  ratio for the mouse GN. The GN 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, GN muscles were weighed. Isometric stress was estimated by normalizing maximum isometric force to GN physiological cross-sectional area.

**Fatigue responses.** A series of 10 isometric contractions (Iso1-Iso10) was imposed at slack length. Fatigue was defined as a reduction in isometric stress production across the isometric exercise bout.

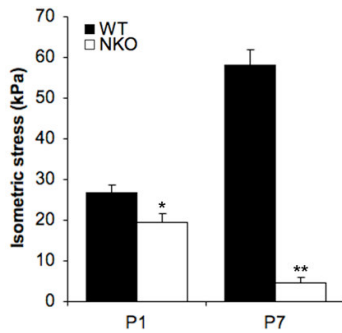
**LTCs.** Isometric contractions were imposed at slack length and then at a series of muscle lengths  $\pm 0.2L_f$ ,  $\pm 0.4L_f$ ,  $\pm 0.6L_f$ ,  $\pm 0.8L_f$ , and  $\pm L_f$  from slack length. Isometric stress was normalized to maximum isometric stress (to compute the fraction of peak tension), and muscle length was normalized to slack length (to compute relative muscle length). The fraction of peak tension was then plotted as a function of relative muscle length, and parabolic regression was applied. An applet

written in MATLAB computed the optimum relative muscle length ( $L_{opt}$ ) and full-width at half-maximum (FWHM) of each parabola.

**Statistics.** Comparisons were made using the unpaired Student's t-test. Data are shown as mean±SEM, and significance was  $P<0.05$ .

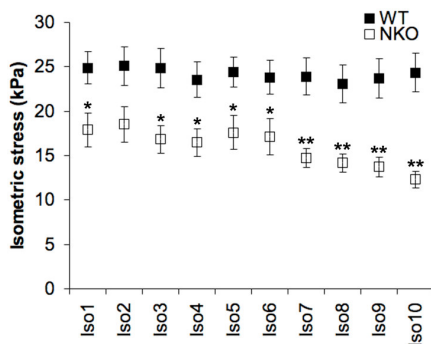
## RESULTS

The isometric stress produced by the WT GN increased ~2-fold from P1 to P7, whereas the stress produced by the NKO GN deteriorated by ~75% from P1 to P7, consistent with severe myopathy in NKO mice (Fig. 1).



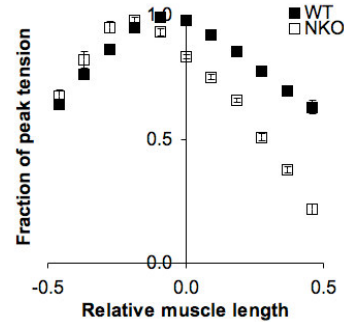
**Figure 1.** Isometric stress production in WT and NKO GN at P1 and P7. \*,  $P<0.05$ ; \*\*,  $P<10^{-7}$  compared to WT.

During 10 cyclic isometric contractions, the NKO GN consistently generated less stress than WT (Fig. 2). The WT GN exhibited no decline in stress production, whereas the NKO GN exhibited a ~20% decline ( $-0.5\pm 0.9$  kPa vs.  $-5.6\pm 1.7$  kPa,  $P<0.05$ ).



**Figure 2.** Fatigue responses of WT and NKO GN. \*,  $P<0.05$ ; \*\*,  $P<0.01$  compared to WT.

The LTC of the NKO GN was narrower than the WT GN and shifted leftward, consistent with shorter thin filaments (Fig. 3). Parabolic regression analysis of the LTCs verified these observations quantitatively, with reduced  $L_{opt}$  and FWHM in the NKO GN (Table 1).



**Figure 3.** LTCs of WT and NKO GN at P1.

Parameter	WT	NKO
$L_{opt}$	$-0.025\pm 0.008$	$-0.160\pm 0.014^{**}$
FWHM	$1.10\pm 0.02$	$1.02\pm 0.02^*$

**Table 1.** Parabolic regression analysis of LTCs. \*,  $P<0.01$ ; \*\*,  $P<10^{-5}$  compared to WT.

## DISCUSSION

These data confirm the severe functional consequences of nebulin deletion in skeletal muscle. The LTC of the NKO GN was consistent with reduced thin filament length. Future work will use site-specific mutagenesis to tease apart the particular *in vivo* functions of individual nebulin protein domains.

## REFERENCES

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## ACKNOWLEDGEMENTS

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