# Effects of early-life exposure to exercise on phenotypic properties of mouse skeletal muscle

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## Abstract

The impact of 10 weeks of voluntary early-life exercise on the phenotypic properties of mouse skeletal muscle was evaluated in this preliminary study. Three control (C) mice were housed in individual cages with a locked running wheel. Two mice were kept in identical cages, but the running wheel was free and accessible to the mice (low resistance, LR). Three mice were housed in identical cages with free running wheels that included a 15% resistive load (high resistance, HR). Wheel revolutions were quantified. Mice were then euthanized and hindlimb muscles were dissected, frozen, and stored at -80ºC for protein analyses. The muscles were analyzed for slow muscle fiber protein content (slow/cardiac troponin isoforms) to detect the amount of fast to slow muscle adaptation, as typically occurs with endurance training. On average, LR mice ran 79428, HR mice 50930, and C mice 0 revolutions/week. HR mice showed increases in troponin I slow protein content (1.76 Relative Units, RU) compared to LR (1.05 RU), and C (1.00 RU). Interestingly, both HR (1.93 RU) and LR (1.60 RU) mice showed increases in troponin C cardiac protein content compared to C (1.00 RU). The elevations in troponin I slow and troponin C cardiac protein in HR and LR mice are indicative of a fast to slow muscle phenotype transition with exercise. Despite running less, the HR runners displayed greater adaptations in slow phenotypic protein content, suggesting that resistive load is a critical factor in determining the extent of adaptation.

## Introduction & Methods

Mammalian skeletal muscle contains distinct muscle fiber types generally classified as slow-twitch and fast-twitch phenotypes based on their speed of contraction (force generation) (for review, Talmadge et al., 1993). The protein known as troponin regulates the initiation of contraction at the thin filament of the myofibrillar apparatus (aka, sarcomere). Troponin has three subunits: troponin-I (an inhibitory subunit); troponin-T (a tropomyosin-binding subunit); and troponin-C (a calcium-binding subunit) that function together as a multimeric protein. Each subunit (C, I, and T) has multiple fast vs. slow isoforms (i.e., versions of the same protein). For instance, troponin C has a fast isoform that is present in fast-twitch fibers and a slow isoform (also known as cardiac) that is present in slow-twitch fibers. Troponin I also has a fast and slow isoform found in fast-twitch and slow-twitch fibers, respectively. In humans, endurance exercise frequently involves some form of running activity, and in response to endurance exercise, muscle fibers undergo a fast to slow muscle fiber type transition, which would involve an increased expression of the slow isoforms of troponin (Härtner & Pette, 1990).

However, few studies have assessed the impact of endurance training on young mice as a model for assessing the impact on young humans. In mice, wheel running is a common model of exercise used to assess muscle adaptation to exercise. Therefore, in this preliminary investigation we assessed the impact of early-life endurance training on 3-month-old Diversity Outbred mice (J:DO, 009376). Three groups of mice were included in the study as follows: (1) three control (C), (2) two low resistance runners (LR), and (3) three high resistance runners (HR). All mice were placed in individual cages with running wheels (Ergometric mouse running wheel, Columbus Instruments, Ohio; see Figure 1). For C mice, the running wheel was locked. For LR mice the running wheel was unlocked, accessible, and included a 0% resistive load (low resistance). For HR mice, the running wheel included a 15% resistive load (0.003 Nm of torque, high resistance). Wheel revolutions were quantified daily for 10 weeks. After 10 weeks, the mice were euthanized and the hindlimb Gastrocnemius-plantaris muscles were dissected, cleaned of connective tissue, and frozen in liquid nitrogen. The Gastrocnemius-plantaris complex is a fast muscle. Thus, any increase in slow muscle protein in this complex would indicate a fast to slow transition. All animal procedures were performed at the University of California, Riverside and samples shipped to Cal Poly Pomona for analysis. The animal studies were approved by the University of California, Riverside Animal Care and Use Committee in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

To assess for expression of slow troponin isoforms as a result of training, the slow isoforms of troponin I and C were determined using chemiluminescence-based western blots. Thus, we explored the effect of early-life exposure to voluntary exercise, coupled with different resisting loads, on phenotypic properties of mouse skeletal muscle.

*Specific methodology.* The muscles were stored at -80ºC until analysis. Myofibrillar protein content was extracted from the frozen muscles and quantified using the Bradford assay. The extracts were then diluted to a final concentration of 2.5 μg/μl. Ten μl (25 μg/lane) of myofibrillar protein were subjected to standard western blotting analysis using 12% SDS-PAGE gels and antibodies directed against the slow/cardiac isoforms of troponin I and C.

**Figure 1.** Running wheel cages used in the experiment.

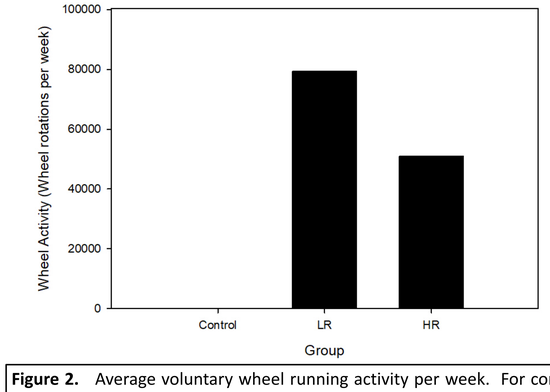


**Figure 2.** Average voluntary wheel running activity per week. For control mice, the wheel was locked and unable to turn. For low resistance running (LR) mice, the running wheel was unlocked with no resistance. For high resistance running (HR) mice, the wheel was unlocked, but with a 15% resistive load (0.003 Nm of torque).

## Results

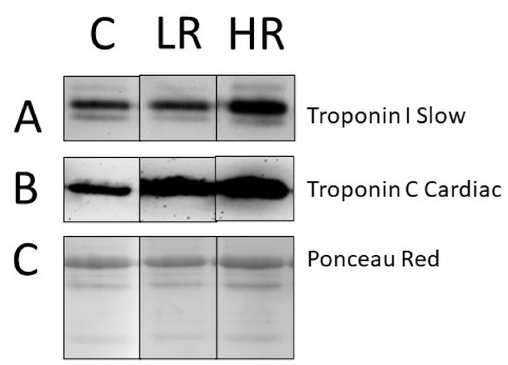


**Figure 3.** Representative images of western blots for A) troponin I slow; B) troponin C cardiac; and C) Ponceau red stain for total protein (α-skeletal actin) normalization. Lane 1, control (C); lane 2, low resistance (LR); lane 3, high resistance (HR). The identification of bands was performed by assessment of band position at the appropriate molecular weight using molecular weight markers (not shown).

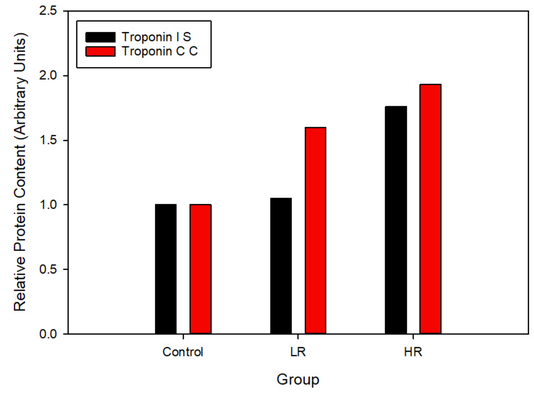


**Figure 4.** Relative amounts of troponin I slow (Troponin I S, black) and troponin C cardiac (Troponin C C) isoforms in the gastrocnemius muscles of control, LR, and HR mice as determined by western blot quantification of band intensities. The n = 2 - 3 per group, thus statistical analyses are not appropriate for this preliminary set of data.

## Summary & Conclusion

1) Low resistance runners had greater voluntary running distances per week compared to high resistance runners (nearly +50%)

2) The slow isoform of troponin I is elevated only in the high resistance running mice which is consistent with a fast to slow transition due to endurance training in the HR group.

3) The cardiac isoform of troponin C is elevated in both running groups, but slightly more so in the high resistance mice. This is consistent with a fast to slow transition due to endurance training in both LR and HR groups.

4) Overall, despite a 50% lower amount of voluntary running activity, HR running mice showed a greater muscular adaptation, i.e., increase in slow phenotypic protein expression compared to LR running mice (particularly for troponin I slow). This supports the idea that muscular load (contractile resistance) is an important variable in determining the adaptations induced by training (elevations in contractile activity).

5) Young mice have the capacity to produce muscular adaptations to increases in muscular activity (voluntary wheel running).

**Overall, in young mice contractile load, i.e., the running wheel resistance, may be more important than the volume of activity (total amount of muscular contractions), i.e., the amount of distance run, for inducing muscular adaptations (an increase in slow phenotypic protein expression).**

## References

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