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Myosin heavy chain isoform expression in adult and juvenile mini-muscle mice bred for high-voluntary wheel running

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ABSTRACT

The myosin heavy chain (MyHC) isoform composition of locomotor and non-locomotor muscles of mini-muscle mice were assessed at the protein and mRNA levels in both adult and juvenile (21 day old) mice. Mini-muscle mice are one outcome of a replicated artificial selection experiment in which four lines of mice were bred for high voluntary wheel running (HR lines). Two of the lines responded with an increase in frequency of a single nucleotide polymorphism in an intron in the MyHC-2b gene (*myh4*) that when homozygous causes a dramatic reduction in triceps surae mass. We found that both locomotor and non-locomotor muscles of adult mini-muscle mice displayed robust reductions, but not elimination, of the MyHC-2b isoform at both the protein and mRNA levels, with commensurate increases in MyHC-2x and sometimes MyHC-2a, as compared with either a line of HR mice that does not display the mini-muscle phenotype or inbred C57Bl6 mice. Immunohistochemical analyses revealed that locomotor muscles of mini-muscle mice contain fibers that express the MyHC-2b isoform, which migrates normally in SDS-PAGE gels. However, these MyHC-2b positive fibers are generally smaller than the surrounding fibers and smaller than the MyHC-2b positive fibers of non-mini-muscle mice, resulting in characteristically fast muscles that lack a substantial MyHC-2b positive (superficial) region. In contrast, the masseter, a non-locomotor muscle of mini-muscle mice contained MyHC-2b positive fibers that stained more lightly for MyHC-2b, but appeared normal in size and distribution. In adults, many of the MyHC-2b positive fibers in the mini-muscle mice also display central nuclei. Only a small proportion of small MyHC-2b fibers in mini-muscle mice stained positive for the neural cell adhesion molecule, suggesting that anatomical innervation was not compromised. In addition, weanling (21 day old), but not 5 day old mice, displayed alterations in MyHC isoform content at both the protein and mRNA levels, including reductions in MyHC-2b and elevations in the neonatal (a.k.a. perinatal) isoform of MyHC. Collectively, these data demonstrate that the alterations in the expression of MyHC-2b are not restricted to locomotor muscles and therefore are not caused simply by any possible alterations in locomotor activity (e.g., reduced general activity in home cages). The differences in MyHC composition do not appear to result from a defect in innervation of the MyHC-2b fibers, but may result from an inefficient neonatal-to-2b MyHC isoform transition during development and are consistent with a selective lack of maturation of MyHC-2b fibers caused by reduced expression of the MyHC-2b (*myh4*) gene.

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1. Introduction

Alterations in the amount of daily contractile activity can have a profound influence on skeletal muscle. Reductions in neuromuscular activation, such as occur following spinal cord injury, space flight, and hindlimb suspension generally result in fiber transitions towards faster phenotypes (Talmadge, 2000). In contrast, conditions associated with elevations in contractile activity, such as chronic electrical stimulation, exercise, and functional overload are associated with fiber transformation towards slower phenotypes (Pette and Vrbova, 1992; Roy et al., 1991; Schiaffino and Reggiani, 2011).

Although significant strides in understanding the cellular and molecular mechanisms associated with the activity-based regulation of muscle fiber type transformation have occurred over the past two decades, the mechanisms associated with the genetic (and heritable) regulation of adult fiber phenotype have been largely unexplored. A unique animal model for understanding some of the factors that contribute to the genetic regulation of muscle fiber type is the high-activity "mini-muscle" mouse (Garland et al., 2002; Swallow et al., 1998). The high-activity mice (designated as HR for high running) are the product of a replicated artificial selection experiment in which mice were (and continue to be) bred for high voluntary wheel-running activity. The selection protocol entailed the generation of 4 lines of mice that were bred for high wheel running during a 6-day period of wheel access administered at ~6–8 weeks of age. At an apparent selection limit, the HR mouse lines run daily distances that are ~2.5× greater than those of four non-selected control lines (Careau et al., 2013; Garland et al., 2011). Two of the HR lines display a morphological phenotype designated as mini-muscle because the triceps surae muscle mass is approximately 50% of control levels (Garland et al., 2002; Houle-Leroy et al., 2003). This phenotype is inherited as an autosomal recessive (Garland et al., 2002; Hannon et al., 2008; Hartmann et al., 2008) and is caused by a single nucleotide polymorphism (SNP) in the 709-bp intron located between exons 11 and 12 of the 2b-MyHC (*myh4*) gene on chromosome 11 (Kelly et al., 2013). The SNP consists of a C-to-T transition at position 67,244,850 (Kelly et al., 2013). In one of the HR mini-muscle lines (lab-designated as line 3), the mini-muscle morphological phenotype has gone to fixation, i.e., all mice express the phenotype (Syme et al., 2005). The second HR mini-muscle line (designated as line 6) remains polymorphic and follows typical Mendelian inheritance of the mini-muscle phenotype.

One interesting feature of the "mini-muscle" mice is that they show a pronounced reduction in the fast glycolytic (i.e., type 2B) fiber type in locomotor muscles that normally contain this fiber type (Bilodeau et al., 2009; Guderley et al., 2006; McGillivray et al., 2009b). Accompanying the reduction in the 2B fiber type is a corresponding reduction in expression of the myosin heavy chain (MyHC)-2b isoform (Bilodeau et al., 2009; Guderley et al., 2006; McGillivray et al., 2009b).

It is clear that the MyHC isoform expressed in a single muscle fiber is a primary factor associated with determining the speed-related contractile properties of that fiber (Canepari et al., 2010), and is highly correlated with other metabolic properties of the fiber (Rivero et al., 1998, 1999). Four adult MyHC isoforms are generally expressed in adult rodent muscle fibers: (from slowest to fastest) types MyHC-I (gene designation *myh7*), MyHC-2a (*myh2*), MyHC-2x (*myh1*), and MyHC-2b (*myh4*). Muscles with high maximal contractile speeds, including fast muscles like the gastrocnemius and tibialis anterior, generally have a high complement of MyHC-2b fibers which are typically located at the superficial portion of the muscle, whereas muscles with low maximal contractile speeds, including the soleus and adductor longus, generally have a high compliment of MyHC-I and MyHC-2a fibers. In association with the reduction in the 2B phenotype, significant alterations in muscle contractile performance (McGillivray et al., 2009b; Syme et al., 2005) and in whole-animal performance (reduced maximal sprint-running speed and an increased cost of transport (Dlugosz et al., 2009)) are observed in the mini-muscle individuals.

At present, it is not known if the alterations in fiber type in the mini-muscle mice are restricted to locomotor muscles or if they occur more widely (among most or all muscles of the body). Because HR mice, including mini-muscle individuals, have increased locomotor activity in home cages when housed without wheels (Malisch et al., 2009), it is theoretically possible that the alteration in locomotor muscle fiber type could result from elevated locomotor muscle contractile activity during their ontogenetic development. If so, then the cause of the mini-muscle phenotype would be viewed as an indirect pleiotropic effect, rather than a direct genetic effect. The reduced triceps surae mass of mini-muscle individuals becomes apparent only at approximately two weeks of age, which is before much locomotion occurs (Middleton et al., 2008), but mice do move before this age, e.g., when trying to gain access to nursing. Therefore, to determine if the fiber type differences in the mini-muscle mice are restricted to locomotor muscles or are more generalized, this study assessed MyHC isoform expression at the protein and mRNA levels in several locomotor and non-locomotor muscles in adult mini-muscle mice and two control groups, an HR line that does not express the mini-muscle phenotype (line 8) and inbred C57Bl6 mice from an outside source. This study also assessed if the changes in MyHC isoform expression were restricted to adult mice or observable at earlier ages, specifically 5 day (neonates) and 21 day old (juvenile) mice.

2. Materials and methods

2.1. *Animals and experimental procedure*

The groups included: (1) Line 3 HR mini-muscle mice; (2) Line 7 and 8 HR (non-mini-muscle) mice; and (3) C57Bl6NHsd mice obtained directly from Harlan Laboratories (Indianapolis, IN, USA). For analyses in adult mice, six mice from each group were utilized. For analyses of neonatal (p5) and juvenile (p21), three to six mini-muscle (line 3) mice and three to six non-mini-muscle mice (lines 7 and 8) were utilized. Muscles and muscle groups representing locomotor muscles (including the triceps surae, quadriceps, soleus, and trapezius) and non-locomotor muscles (including the tongue, masseter, and diaphragm) were dissected, cleaned of connective tissue, and frozen in isopentane chilled by liquid nitrogen. The triceps surae and quadriceps were chosen as representative muscles in-