Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part B 149 (2008) 490–500
CBP
www.elsevier.com/locate/cbpb

Altered fibre types in gastrocnemius muscle of high wheel-running selected mice with mini-muscle phenotypes

Helga Guderley [a,*], Denis R. Joanisse [b], Sophie Mokas [a], Geneviève M. Bilodeau [a], Theodore Garland Jr. [c]

[a] Département de biologie, Université Laval, Québec QC, Canada G1K 7P4
[b] Division de kinesiologie, Département de Médicine Sociale et Préventive, Université Laval, Québec QC, Canada G1K 7P4
[c] Department of Biology, University of California, Riverside, Riverside, CA 92521, USA

Received 13 July 2007; received in revised form 25 November 2007; accepted 26 November 2007
Available online 14 December 2007

Abstract

Selective breeding of mice for high voluntary wheel running has favoured characteristics that facilitate sustained, aerobically supported activity, including a "mini-muscle" phenotype with markedly reduced hind limb muscle mass, increased mass-specific activities of oxidative enzymes, decreased % myosin heavy chain IIb, and, in the medial gastrocnemius, reduced twitch speed, reduced mass-specific isotonic power, and increased fatigue resistance. To evaluate whether selection has altered fibre type expression in mice with either "mini" or normal muscle phenotypes, we examined fibre types of red and white gastrocnemius. In both the medial and lateral gastrocnemius, the mini-phenotype increased activities of oxidative enzymes and decreased activities of glycolytic enzymes. In red muscle samples, the mini-phenotype markedly changed fibre types, with the % type I and type IIA fibres and the surface area of type IIA fibres increasing; in addition, mice from selected lines in general had an increased % type IIA fibres and larger type I fibres as compared with mice from control lines. White muscle samples from mini-mice showed dramatic structural alterations, with an atypical distribution of extremely small, unidentifiable fibres surrounded by larger, more oxidative fibres than normally present in white muscle. The increased proportion of oxidative fibres and these atypical small fibres together may explain the reduced mass and increased mitochondrial enzyme activities in mini-muscles. These and previous results demonstrate that extension of selective breeding beyond the time when the response of the selected trait (i.e. distance run) has levelled off can still modify the mechanistic underpinnings of this behaviour.

Keywords: Aerobic capacity; Artificial selection; Exercise physiology; Experimental evolution; Muscle metabolic capacities

1. Introduction

Selective breeding involves deliberately choosing individuals with a given trait (or combinations of traits) to produce the next generation. This approach allows the identification of aspects of behaviour, performance, morphology, and physiology that are genetically correlated with the trait under selection (Garland, 2003; Garland and Kelly, 2006). If selection is imposed at the level of behaviour or whole-organism performance, then the mechanistic underpinnings of evolutionary changes in that trait can be elucidated by examination of subordinate traits hypothesised to affect (or permit variation in) the higher-level phenotype (Swallow et al., 2005). As an example, beginning from a base population of outbred laboratory house mice, replicated selective breeding for high voluntary locomotor activity in wheels has resulted in greater running performance and maximal oxygen consumption (VO_{2max}) (Rezende et al., 2006a,b; Swallow et al., 1998a,b), smaller body mass (Swallow et al., 1999), increased insulin-stimulated glucose uptake in extensor digitorum longus muscle (Dumke et al., 2001), more symmetrical hind limb bones and

* Corresponding author. Tel.: +1 418 656 3198; fax: +1 418 656 2043.
E-mail addresses: helga.guderley@bio.ulaval.ca (H. Guderley), denis.joanisse@kin.msp.ulaval.ca (D.R. Joanisse), tgarland@ucr.edu (T. Garland).

1096-4959/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2007.11.012

larger femoral condyles (Garland and Freeman, 2005; Kelly et al., 2006), and greater muscle aerobic capacity in mice that are allowed access to wheels for several weeks (Houle-Leroy et al., 2000). Thus, the four replicate selected lines show several characteristics that appear to favour high levels of sustained, aerobically supported wheel running.

Selection for increased wheel running has also increased the frequency of a peculiar phenotype primarily affecting hind limb muscles in two of the four selected lines (Garland et al., 2002). This phenotype is characterised by a reduction of muscle mass (~50%), accompanied by increased mass-specific oxidative enzyme activities in mixed hind limb muscles (Houle-Leroy et al., 2003). In one of the selected lines, this phenotype has gone to fixation (all individuals now exhibit the phenotype), whereas in another it is still polymorphic (Rezende et al., 2006a,b; Syme et al., 2005). The phenotype is caused by an autosomal recessive allele that was present in the starting population at a frequency of approximately 7% (Garland et al., 2002). That this mini-phenotype only occurs in two of the four selected lines probably reflects the loss of this rare allele by random genetic drift during the early generations of selective breeding (Garland et al., 2002). Differences in the response of replicate lines to selection occur because random genetic processes will alter the gene pool within each line while the adaptive responses to selection are occurring. Although the distance run per day by mice with the mini-phenotype usually does not differ statistically from that of selected mice with normally-sized muscles (Garland et al., 2002; Houle-Leroy et al., 2003; Swallow et al., 2005), mice with the mini-phenotype often run faster (Kelly et al., 2006; Syme et al., 2005) and have a higher VO_{2max} during forced exercise tests in hypoxia (14% O_2) (Rezende et al., 2006a) as compared with selected-line mice with muscles of normal size. Thus, the mini-phenotype seems to be an alternate, but not necessarily superior, phenotype with respect to the artificial selection protocol.

Hind limb muscles from mice with the mini-phenotype differ in their contractile, metabolic, and structural properties from those in mice with muscles of normal size. The reduction in mass with the mini-phenotype is greatest in muscles that normally contain a high proportion of glycolytic fast-twitch type IIB fibres in the normal phenotype (Guderley et al., 2006), with a greater mass reduction in the gastrocnemius than in the plantaris and an increased mass in the soleus. At the same time, the increase in mass-specific activities of mitochondrial enzymes with the mini-phenotype (Houle-Leroy et al., 2003) is greater in the gastrocnemius than the plantaris (Guderley et al., 2006). Gastrocnemius muscles of individuals with the mini-phenotype also exhibit increased glycogen concentrations as compared with those found in the normal phenotype (Gomes et al., 2004). For the plantaris muscle, fibre number and size do not systematically differ between individuals with the two phenotypes in a given line, but plantaris muscles from individuals with the mini-phenotype show many small, minimally differentiated myofibril-containing cells in their surface layers (Guderley et al., 2006). Despite its increased mass, the contractile properties of the soleus muscle did not differ between the normal and mini-phenotypes (Syme et al., 2005). However, the medial gastrocnemius muscle of mice with the mini-phenotype has slower twitches, a more curved force–velocity relationship, produces about half the mass-specific isotonic power, 20–50% of the mass-specific cyclic work and power, and fatigues at about half the rate of normal muscles (i.e. has greater endurance) (Syme et al., 2005). In the gastrocnemius, the % myosin heavy chain (MHC) IIb isoform was markedly reduced in mice with the mini-phenotype, whereas the proportions of MHC IIa and I isoforms were enhanced (Guderley et al., 2006). Together these results suggest that mice with the mini-phenotype have decreased the proportion of type IIB fibres in their hind limb muscles, leading muscles that typically have a high proportion of these fibres to reduce their mass, increase their mass-specific aerobic capacity, reduce their power production, and increase their endurance. The current study examines this hypothesis, by comparing the fibre types of the gastrocnemius muscles from mice that express the mini- and the normal phenotypes.

Specific genetic backgrounds can modulate the expression of particular genes, such as the allele that causes the mini-muscle phenotype. For example, the expression of the mini-phenotype differs somewhat between lines, with the decrease in % MHC IIb, the increase in % MHC IIa, and the increase in mitochondrial volume density being greater in one than the other (Guderley et al., 2006). In analogy, the "normal" phenotype in selected lines may demonstrate specific fibre type combinations that favour high levels of wheel running. In mammals, muscle fibres range from small oxidative, slow-twitch, type I red fibres to oxidative IIA fibres and large glycolytic, fast-twitch, type IIB white fibres (Torgan and Daniels, 2001). In rodents, type IIA fibres are the most oxidative and also the smallest, followed by type I (Azpiazu et al., 2000). The type IID (also known as IIX) fibres, assumed to exist only in small mammals, are intermediate between types IIA and IIB (Hamalainen and Pette, 1993). With this range of fibre types, multiple specific combinations could, in principle, enhance sustained wheel-running ability. Although the fibre types of medial gastrocnemius muscle were not statistically changed by 10 generations of selection for high voluntary wheel running (Zhan et al., 1999), the subsequent 25 generations of selective breeding could have differentiated the lines.

To evaluate whether mice with the mini-muscle phenotype have decreased the proportion of type IIB fibres and to assess whether the selection protocol has led to line-specific responses in fibre composition, we examined the medial (MG) and lateral (LG) sections of the gastrocnemius in mice after 35–36 generations of selection for voluntary wheel running. In rodents, both the MG and LG have mixed fibre compositions and possess "red" and "white" sections. The LG is somewhat richer in type IIB fibres than the MG (Burkholder et al., 1994), suggesting that metabolic and fibre type changes with the mini-phenotype should be more pronounced in the LG. We first examined the metabolic capacities of these portions to confirm the increased oxidative capacity that accompanied the mini-phenotype in previous generations (Guderley et al., 2006; Houle-Leroy et al., 2003). Further, we examined the fibre types in samples of red and white muscles from the gastrocnemius to assess whether the two types of muscles showed similar changes with selection and the mini-phenotype. We used the