

## Myosin heavy chain expression in peripheral muscles of male and female prairie voles *Microtus ochrogaster* \*

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**Abstract** Previous research has reported skeletal muscle protein expression characteristics for laboratory strains of various rodents, such as rats and mice. However, we do not know the muscle protein expression or sexual dimorphism characteristics of skeletal muscle for other rodents such as voles, for which the behavior, morphology, and physiology have been documented. Myosin heavy chain (MHC) content in skeletal muscle is related to functional characteristics. This study investigated sex characteristics (male,  $n = 6$ ; female,  $n = 8$ ) for MHC expression for triceps brachii, tibialis anterior, gastrocnemius, and soleus muscles in prairie voles. Relative (%) MHC protein expression was determined via SDS-PAGE for types I, II a, II d/x, and II b MHC isoforms. Male voles had greater soleus wet weight and greater II a MHC expression for tibialis anterior as compared to those of female voles. Skeletal muscle mass and MHC protein expression were not sexually dimorphic. Differences in circulating testosterone titers did not appear to influence these characteristics of mature peripheral skeletal muscle. Prairie voles, however, exhibited much more heterogeneity in MHC expression as compared to previous reports on rats, rabbits, and mice. It is likely that this is due to differences in the natural history and functional requirements of voles and other small mammals [*Acta Zoologica Sinica* 54 (1): 104–110, 2008].

**Key words** Sexual dimorphism, Skeletal muscle, Protein isoforms, Sex

## 雌、雄草原田鼠外周骨骼肌肌球蛋白重链的表达 \*

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**摘要** 已往的研究对于实验室应用的各种啮齿类动物, 如大鼠和小鼠骨骼肌蛋白表达的特性已有报道。然而, 至今不清楚其它啮齿类动物如野生鼠骨骼肌蛋白的表达或性双态性的特征, 而这些野生鼠的行为学、形态学及生理学特点均已有报道。已知骨骼肌的肌球蛋白重链 (MHC) 成分与其功能特性有关。我们研究了草原田鼠的肱三头肌、胫骨前肌、腓肠肌和比目鱼肌 MHC 蛋白表达的性别特性。应用 SDS 聚丙烯酰胺凝胶电泳法测定 MHC I 型、II a 型、II d/x 和 II b 型的蛋白表达相对含量。结果表明: 与雌鼠相比, 雄鼠的比目鱼肌湿重较大, 胫骨前肌的 MHC II a 蛋白量表达较高。未见骨骼肌重量及 MHC 蛋白表达含量在雌雄鼠间的性别差异。血中睾酮的浓

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度差异可能不影响外周骨骼肌蛋白的表达特性。然而,与过去在大鼠、兔和小鼠中的已报道的结果相比,草原田鼠骨骼肌 MHC 的表达显示了更多异质性。推测这可能与草原田鼠和其它小型哺乳类动物生存的自然环境和功能需要有关 [动物学报 54 (1): 104-110, 2008]。

关键词 性二态性 骨骼肌 蛋白同分异构 性别

Myosin heavy chain (MHC) weighs approximately 200 kD and constitutes part of the myosin contractile protein in skeletal muscle (Nguyen et al., 1982). Four isoforms of MHC have been isolated in mature rodent skeletal muscle, types I, II a, II d/x, and II b (Aigner et al., 1993; Hämaläinen and Pette, 1993, 1995; Bär and Pette, 1998; Pette and Staron, 1997, 2000; Termin et al., 1989). The relative content of MHC protein is related to the percent area of muscle fiber types (Staron, 1991; Staron and Johnson, 1993; Staron and Pette, 1986; Termin et al., 1989), as well as the functional characteristics (Asmussen et al., 2003; Degens et al., 1998; Fry et al., 1994; Galler et al., 1997; Schilling et al., 2005) of both animal and human muscle. Differences in MHC expression are associated with altered muscle shortening velocities, which could affect functional capacities of the muscle (Degens et al., 1998). Furthermore, muscle fibers readily respond to changes in functional demands, resulting in altered phenotypic expression (Pette and Staron, 1997, 2000). Other animals (e.g., C57 mice, rabbits, guinea pigs) show sexual dimorphism in the MHC expression of masticatory muscles (i.e., masseter m., temporalis m.) (Lyons et al., 1986; English et al., 1999; Eason et al., 2000a). Furthermore, sex differences in hormonal regulation of MHC expression are apparent for rat peripheral muscles (Larsson and Yu, 1997; Yu et al., 1998) and rabbit masseter muscle (Eason et al., 2000b). In each case where sexual dimorphism of MHC expression has been reported, the species involved are also sexually dimorphic in other aspects of their biology, such as males and females differing in body weight and size.

Typically, laboratory strains of rodents have considerable homogeneity of MHC expression in specific muscles, although there are differences between species and strains. For example, soleus m. in many rodent strains expresses close to 100% of the slow isoform MHC I. On the other hand, other muscles such as tibialis anterior (tibialis cranialis) are primarily comprised of fast isoforms (Aigner et al., 1993; Asmussen et al., 2003; Bar and Pette, 1998; Hämaläinen and Pette, 1993; Staron et al., 1999). Interestingly, exceptions to this pattern are mice which exhibit a more heterogenous expression of MHC in skeletal muscle (Asmussen et al., 2003; Hämaläinen and Pette, 1993). Relative protein expression of MHC has been shown to be related to contractile forces and related performances in both laboratory rodents (Asmussen et al., 2003; Degens et

al., 1998) and humans (Fry et al., 1994, 2003a; Schilling et al., 2005). As such, there is likely a structure-function relationship between different isoforms of contractile proteins and the required muscular contractile performances. Indeed, when muscle activity patterns are distinctly different, such as seen in Japanese waltzing mice, the corresponding MHC isoform expression of the involved musculature is also different (Asmussen et al., 2003). The greater activity levels of these mice are accompanied by a greater expression of slower MHC isoforms, as well as a more heterogenous MHC distribution. Given that relative MHC content is highly related to histochemically determined relative fiber type areas, examination of MHC expression provides insight on not only the content of that particular protein isoform, but also on mATPase fiber type characteristics (Fry et al., 1994; Staron, 1991; Staron and Hikida, 1992; Staron and Pette, 1986; Termin et al., 1989). This relationship is so well established that it has been suggested that expression of the different MHC isoforms are the “most appropriate markers for fiber type delineation” (Pette and Staron, 2000).

Little is known about MHC expression in free-living rodents such as voles, and whether the MHC expression in particular muscles may be also homogeneous. The prairie vole *Microtus ochrogaster* (Wagner) is a monogamous microtine rodent reported to exhibit little sexual dimorphism in their physiology and behavior (Kleiman, 1977; Dewsbury et al., 1980; Tamarin, 1985; Ferkin et al., 2001). On the other hand, female prairie voles are 10% smaller than male prairie voles (Dewsbury et al., 1980) and possess different pelvic, cranial and mandibular dimensions (Huggins and McDaniel, 1984; Severinghaus, 1981). Although there is certainly sexual dimorphism concerning hormonal profiles (i.e., androgens) and the impact on numerous glands and organs, skeletal muscle has not been examined (Stalling, 1990). When the body mass/body volume ratio is used as a measure of fitness or condition, these results predict variations in lean dry mass (Schulte-Hostedde et al., 2001b). Sex differences in lean dry mass have not been described for prairie voles, although these measures for a related species (red-backed voles) indicate that males possess greater lean dry mass compared to females (Schulte-Hostedde et al., 2001a). These data suggest a degree of sexual dimorphism for a related species, although it is not known if these differences would result in sex differences in the functional morphology of peripheral muscles. Peripheral skeletal muscles for free-

living adult prairie voles of both genders are used for reproductive behavior, which is relatively monogamous, as well as daily activities such as locomotion, digging, swimming, runway construction and nest building (Stalling, 1990; Wolff, 1985).

The first purpose of the present study was to determine the phenotypic characteristics of peripheral skeletal muscles in male and female prairie voles. Since these animals were first generation offspring from free-living animals, it was hypothesized that MHC expression would be less homogenous compared to previously reported laboratory rodents, and would instead be similar to reports for mice. The second purpose of the study was to determine if MHC expression in peripheral skeletal muscle exhibited sexual dimorphism. The second hypothesis was that sex differences exist for relative MHC expression for peripheral muscles. Thus, based on endocrine and body mass differences in prairie voles, we predicted that in prairie voles, sexual dimorphism exists for skeletal muscle MHC expression.

## 1 Materials and methods

Subjects were first generation captive adult male ( $n = 6$ ) and female ( $n = 8$ ) prairie voles. The subjects were born and reared under a 14L : 10D photoperiod (lights on at 0700 CST); this day length is prevalent during the breeding season. The parents of these animals were captured in western Tennessee, USA. Each of the subjects was weaned from both parents at 18 days of age, housed with their littermates until they were 38 days of age, and then housed singly in clear plastic cages (36 cm  $\times$  30 cm  $\times$  18 cm) until they were euthanized. Food (Purina mouse chow, 5015, St. Louis, MO, USA) and water were provided ad lib to the animals. When the voles were between 90 – 100 days of age, the subjects were injected with 2.5 mL of a mixture of ketamine and xylazine [100 mg ketamine hydrochloride/ml and 2 mg xylazine hydrochloride/ml (5 : 1)]. Before the animal's heart stopped beating, we collected blood for hormone analysis from the suborbital space in the eye. Immediately following euthanasia, triceps brachii, gastrocnemius, soleus and tibialis anterior mm. were dissected and wet weights recorded. Special care was taken to differentiate the soleus m. from the adjacent plantaris m. based on the proximal attachment on the posterior shaft of the tibia. All procedures for euthanasia were approved by the University of Memphis Institutional Animal Care and Use Committee.

For MHC analysis, the sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) procedures of Carraro and Catani (1983) and Perrie and Bumford (1984) were used with modifications developed for single muscle fibers (Staron, 1991; Staron and Hikida, 1992). Briefly, muscles were mounted and frozen in isopentane cooled to  $-60^{\circ}\text{C}$  by liquid nitrogen. Samples were stored at  $-80^{\circ}\text{C}$  until later analysis. Ten

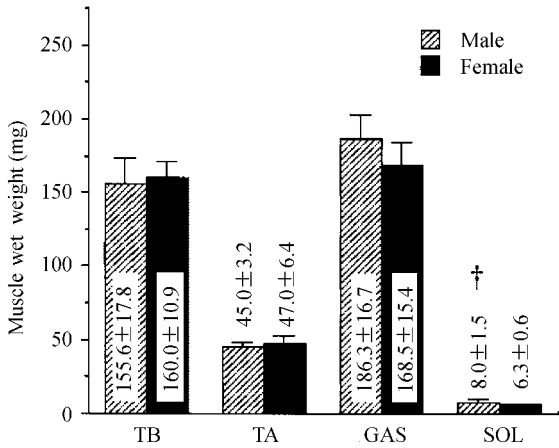
to twelve serial sections 12  $\mu\text{m}$  thick were collected in a cryostat after the samples were warmed to  $-20^{\circ}\text{C}$ . Sectioned samples were placed in 0.5 ml of lysing buffer [10% (w/v) glycerol, 5% (v/v)  $\beta$ -mercaptoethanol, and 2.3% (w/v) SDS in 62.5 mmol  $\cdot$  L $^{-1}$  Tris/HCl, pH 6.8] and heated to  $60^{\circ}\text{C}$  for 10 min. Small amounts of the extracts (5 – 15  $\mu\text{l}$ , depending on the muscle) were loaded on a 7% – 10% gradient gel with a 4% stacking gel, and were electrophoresed at a constant current of 120 mV for 20 hrs. Gels were stained with Coomassie blue and analyzed for relative MHC content using NIH Image v1.62 software. MHC isoform bands were verified with marker proteins in adjacent lanes, and from the consistent isoform migration patterns from other species in the Muridae family (Termin et al., 1989; Pette and Staron, 1990; Hämaläinen and Pette, 1993; Staron et al., 1999; Wada et al., 1995).

Serum samples were collected from all voles and stored at  $-80^{\circ}\text{C}$  until analyzed to ascertain the different hormonal profiles for both male and female prairie voles (Leonard et al., 2001). We determined serum concentrations of sex steroids by enzyme immunoassays (EIA) for testosterone and 17- $\beta$  estradiol titers for both male and female voles (Diagnostic Systems Laboratories, Webster, TX; DSL-10-4000 and DSL-10-4300).

Paired  $t$ -tests (SPSS for Windows version 10.0) were used to compare sex differences for muscle wet weights and hormonal titers. Two-way ANOVAs (sex  $\times$  type) were used for MHC isoform analyses. If the ANOVA was statistically significant, we performed Tukey's multiple pairwise comparisons to determine differences among the MHC isoforms. All data are reported as mean  $\pm$  SE. Statistical significance was accepted at  $\alpha \leq 0.05$ .

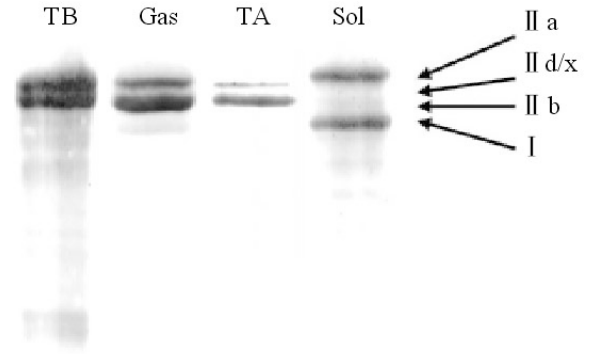
## 2 Results

Muscle wet weight differed between male and female voles for only the soleus m., which was heavier for males than for females ( $t = -2.482$ ,  $df = 12$ ,  $P < 0.05$ ; Fig.1). Muscle wet weights for the gastrocnemius, tibialis anterior, and triceps brachii mm. were not significantly different between males and females (each comparison;  $t \leq |0.805|$ ;  $df = 12$ ;  $P > 0.05$ ; Fig.1). Fig.2 illustrates the separation and resolution resulting from the SDS-PAGE for a sample exhibiting all four MHC isoforms. Significant differences for % MHC IIa isoform content between male and female voles were observed for only the tibialis anterior m. ( $F = 4.01$ ;  $df = 3, 10$ ;  $P < 0.05$ ). This isoform was present only in males (Fig.3). The other MHC isoforms were not significantly different between males and females (each comparison;  $F < 3.86$ ;  $df = 3, 10$ ;  $P > 0.05$ ; Fig.3). We also found sex differences in the titers of estradiol and testosterone for the voles, thus illustrating the different hormonal profiles for each. Testosterone titers for males were 12.2-



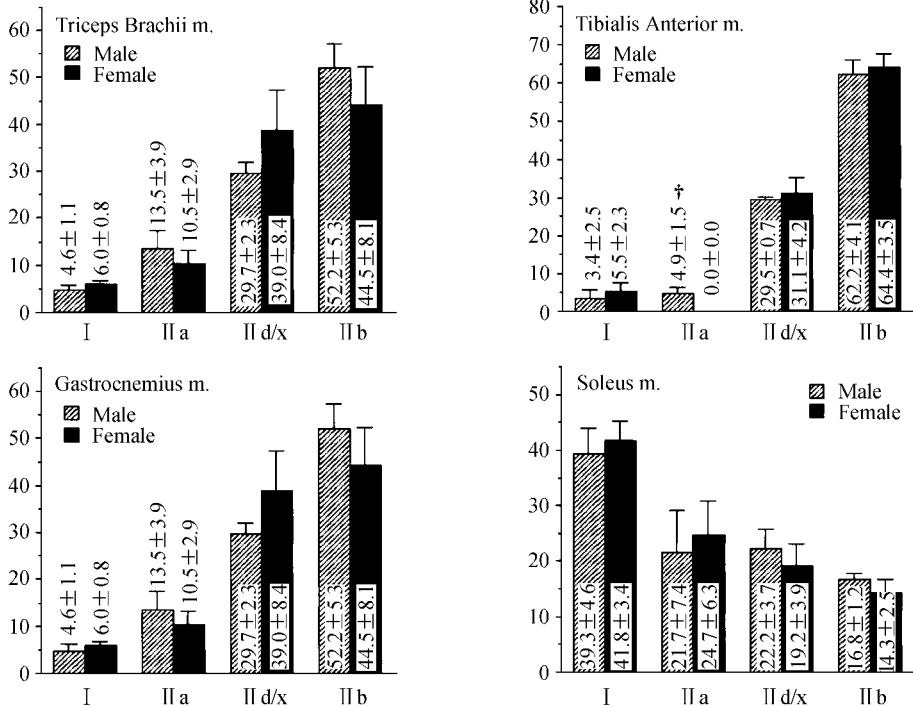
**Fig. 1** Muscle wet weights (Mean ± SE; mg) for male and female prairie voles *Microtus ochrogaster*

TB: triceps brachii, TA: tibialis anterior, GAS: gastrocnemius, SOL: soleus. † Different from females ( $P < 0.05$ ).



**Fig. 2** Representative examples of electrophoretic isoform separation of the four myosin heavy chain (MHC) isoforms using a 7% – 10% gel gradient with a 4% stacking gel

TB: triceps brachii, Gas: gastrocnemius, TA: tibialis anterior, Sol: soleus, I: MHC I, II a: MHC II a, II d/x: MHC II d/x, II b: MHC II b.



**Fig. 3** Relative myosin heavy chain (MHC) expression for triceps brachii, tibialis anterior (tibialis cranialis), gastrocnemius, and soleus muscles (Mean ± SE; %) in male and female prairie voles *Microtus ochrogaster*

I: MHC I isoform, II a: MHC II a isoform, II d/x: MHC II d/x isoform, II b: MHC II b isoform. † Different from females ( $P < 0.05$ ).

fold greater than for females [males =  $3.00 \pm 1.49$  nmol  $\cdot$  L<sup>-1</sup> ( $0.87 \pm 0.43$  ng/ml), females =  $0.25 \pm 0.21$  nmol  $\cdot$  L<sup>-1</sup> ( $0.07 \pm 0.06$  ng/mL)] ( $t = 5.400$ ,  $df = 12$ ,  $P < 0.05$ ), and estradiol titers for females were 12.0-fold greater than for males (males =  $3107 \pm 1554$  pmol  $\cdot$  L<sup>-1</sup> [ $847 \pm 421$  pg/ml], females =  $37358 \pm 32150$  pmol  $\cdot$  L<sup>-1</sup> [ $10176 \pm 8758$  pg/ml]) ( $t = -2.640$ ,  $df = 12$ ,  $P < 0.05$ ).

### 3 Discussion

Male and female prairie voles seem to be relatively similar in their MHC isoform expression. The almost complete lack of sex differences for MHC expression suggests a lack of sexual dimorphism in prairie voles extending to the molecular level for skeletal muscle. It is likely that the lack of sexual dimorphism for MHC expression is due to a similarity of typical physical activity patterns for both male and female prairie voles (Stalling, 1990; Wolff, 1985). Such a lack of sexual dimorphism may also be related to the fact that prairie voles possess a

monogamous mating system, which thus influences their behavior (Kleiman, 1977; Zeveloff and Boyce, 1980). The present data do not support the hypothesis that sex differences exist in relative MHC expression for peripheral muscles. Although female prairie voles are approximately 10% smaller than their male counterparts, likely due in part to a smaller lean mass (Schulte-Hostedde et al., 2001a), the differences in body mass are not extended to the tissue, cell and molecular levels, as evidenced by the wet weights and relative MHC expression.

Although there was a statistically significant difference in % II a MHC expression in tibialis anterior, this difference may be of little physiological significance due to the low relative amounts of this MHC isoform. Examination of Fig. 3 illustrates the primary contributing MHC isoforms for the tibialis anterior m. in prairie voles is undoubtedly II d/x and II b. In contrast to the present data for prairie voles, relative expression of MHC in the tibialis anterior for inbred C57BL/6J mice and for New Zealand White rabbits indicates no MHC I or II a is present (Hämäläinen and Pette, 1993). Furthermore, while male Wistar rat and C57BL/6J mice gastrocnemius contain predominantly MHC IIB (Hämäläinen and Pette, 1993; Harjola et al., 2000), the present data for prairie voles indicates considerable expression of all four MHC isoforms. Since the gastrocnemius for these prairie voles was not separated into superficial and deep components due to the small muscle size, the relative MHC expression was similar to what has been reported for New Zealand white rabbits, and Wistar or Fisher 344 rats (Hämäläinen and Pette, 1993; Staron et al., 1999).

Of particular interest was the heterogeneity for MHC isoform expression in the soleus muscle of prairie voles. This is considerably different from the soleus muscle in Wistar and Fisher 344 rats which exhibit almost exclusively MHC I expression. These data suggest that prairie vole skeletal muscle tends to exhibit more heterogeneity than many other small mammals that have been studied, which tend to exhibit greater homozygosity and reduced variation. Since it has been established that functional and performance properties are related to MHC expression (Staron, 1991; Staron and Johnson, 1993; Fry et al., 2003a, 2003b; Schilling et al., 2005), it is possible that the greater variability in skeletal muscle observed for prairie voles is related to activity and behavior patterns necessary for survival in the wild. Such activity and behavior patterns may differ for prairie voles, which are socially monogamous, with males assisting in parental care. In contrast, rats and mice are polygynous, and males do not offer paternal care (Kleiman, 1977; Zeveloff and Boyce, 1980; Bamshad et al., 1993). For example, in monogamous species, like prairie voles, mating competition is less frequent, and it may be less necessary for either sex to evolve traits that make them more suited for attracting mates as compared to species

that are non-monogamous (Kleiman, 1977; Zeveloff and Boyce, 1980).

As previously indicated, relative MHC expression is highly correlated with relative cross-sectional areas of the major fiber types (i. e., I, II A, II D, and II B) (Staron, 1991; Fry et al., 1994). Moreover, the almost complete lack of sex differences in relative MHC expression in the prairie vole suggests that muscle protein expression, and the related contractile characteristics, may be similar to the lack of sexual dimorphism found in other aspects of their gross morphology, such as size and weight as adults (Tamarin, 1985). These data also indicate that the sexual dimorphism for MHC expression for masticatory muscles of some rodents is not evident in the peripheral musculature of prairie voles (English et al., 1999; Eason et al., 2000b).

It is interesting to note the similar MHC expression for both males and females occurred despite very different hormonal environments. These results present the possibility that myosin heavy chain isoform expression in peripheral skeletal muscle of adult prairie voles is not under testosterone regulation to the extent that masticatory muscles are in other species (Lyons et al., 1986; Yu et al., 1998; Larsson and Yu, 1999; Eason et al., 2000b). In general, these previous studies have suggested that testosterone contributes to a shift to faster MHC isoforms in masticatory muscles (Lyons et al., 1986; English et al., 1999; Eason et al., 2000a, 2000b). The lack of sex differences in the present study, however, are by supported Harjola et al. (2000) who failed to see any effect of testosterone on peripheral muscle mRNA and protein expression in mature rats. It is now evident that hibernation and torpor appear to influence MHC expression in peripheral skeletal of some small rodents (Rourke et al., 2004), and that the role of testosterone depends on seasonal variations as indicated by changes in the photoperiod (Ferkin and Gorman, 1992). Although voles do not hibernate, they display positive correlations between activity patterns and testosterone concentrations (Perrot-Sinal et al., 1998). Furthermore, during development, testosterone titers are positively related to increases in body mass (Dobrowolska and Gromadzka-Ostrowska, 1984). As such, it is possible that across time, skeletal muscle of prairie voles may be under some influence from gonadal hormones. Regardless, it is clear that testosterone titers certainly influence reproductive physiology and behaviors (Ferkin and Johnston, 1993; Ferkin et al., 1994), but future study is necessary to more clearly determine the role of the sex steroid hormones on mature peripheral skeletal muscle, especially as it relates to the different annual seasons. Additionally, much of the related literature above is taken from studies on related species (Schulte-Hostedde et al., 2003). As such, more study is necessary to effectively examine the relevant physiology of

the prairie vole.

Overall, male and female prairie voles seem to be relatively similar in their wet muscle mass. The muscle wet weights in prairie voles for tibialis anterior, gastrocnemius, and soleus, were similar to those reported for mice (Bishop and Milton, 1997; Toursel et al., 2000). The large difference in wet weights between the two plantar flexors (soleus approx. 4% of gastrocnemius) suggests that the gastrocnemius is the primary mover for plantar flexion, while the soleus functions in a purely synergistic manner. Since the gastrocnemius is a two-joint muscle, it is also possible that this muscle contributes significantly to knee flexion during locomotion in the prairie vole. We have found no comparable data for wet weights of triceps brachii m. in other small mammals. It would be interesting to know if differences in lean mass would have possible implications for functional differences of the peripheral muscles, some of which may be under hormonal influences in prairie voles as they are in other animals (Lyons et al., 1986; Yu et al., 1998; Larsson and Yu, 1999; Eason et al., 2000b).

In summary, adult prairie voles do not exhibit sexual dimorphism for relative expression of skeletal muscle MHC isoforms or muscle wet weights. In general, these voles possessed considerable MHC isoform heterogeneity in the muscles examined. Although it was beyond the scope of the present study, future work is necessary to examine sexual dimorphism in this species for skeletal muscle physiology during different times of the reproductive year when the endocrine environment is altered.

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## References

- Aigner S, Gohlsch B, Hämäläinen N, Staron RS, Uber A, Wehrle U, Pette D, 1993. Fast myosin heavy chain diversity in skeletal muscles of the rabbit: heavy chain II d, not II b predominates. *Eur. J. Biochem.* 211 (1–2): 367–372.
- Asmussen G, Schmalbruch I, Soukup T, Pette D, 2003. Contractile properties, fiber types, and myosin isoforms in fast and slow muscles of hyperactive Japanese waltzing mice. *Exp. Neurol.* 184 (2): 758–766.
- Bamshad M, Novak MA, De Vries GJ, 1993. Sex and species differences in the vasopressin innervation of sexually naïve and parental prairie voles *Microtus ochrogaster* and meadow voles *Microtus pennsylvanicus*. *J. Neuroendocrinol.* 5 (3): 247–255.
- Bar A, Pette D, 1998. Three fast myosin heavy chains in adult rat skeletal muscle. *FEBS Letters* 235: 153–155.
- Bishop DL, Milton RL, 1997. The effects of denervation location on fiber type mix in self-innervated mouse soleus muscles. *Exp. Neurol.* 147: 151–158.
- Carraro U, Catani C, 1983. A sensitive SDS-PAGE method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. *Biochem. Biophys. Res. Commun.* 116: 793–802.
- Degens H, Yu F, Li X, Larsson L, 1998. Effects of age and gender on shortening velocity and myosin isoforms in single rat muscle fibers. *Acta Physiol. Scand.* 163 (1): 33–40.
- Dewsbury DA, Baumgartner DJ, Evans RL, Webster DG, 1980. Sexual dimorphism for body mass in 13 taxa of murid rodents under laboratory conditions. *J. Mammalogy* 61: 146–149.
- Dobrowolska A, Gromadzka-Ostrowska J, 1984. Age and androgen-related changes in morphological parameters, haematological indices and serum protein fraction in common vole (*Microtus arvalis* Pall.) growing in different photoperiods. *Comp. Biochem. Physiol. A, Comp. Physiol.* 79 (2): 241–249.
- Eason JM, Schwartz G, Pavlath GK, English AW, 2000a. Sexually dimorphic expression of myosin heavy chains in the adult mouse masseter. *J. Appl. Physiol.* 89 (1): 251–258.
- Eason JM, Schwartz G, Shirley KA, English AW, 2000b. Investigation of sexual dimorphism in the rabbit masseter muscle showing different effects of androgen deprivation in adult and young animals. *Arch. Oral Biol.* 45 (8): 683–690.
- English AW, Eason J, Schwartz G, Shirley A, Carrasco DI, 1999. Sexual dimorphism in the rabbit masseter muscle: myosin heavy chain composition of neuromuscular compartments. *Cells Tiss. Org.* 164 (4): 179–191.
- Ferkin MH, Gorman MR, 1992. Photoperiod and gonadal hormones influence odor preferences of the male meadow vole *Microtus pennsylvanicus*. *Physiol. Behavior* 51 (5): 1087–1091.
- Ferkin MH, Johnston RE, 1993. Roles of gonadal hormones in control of five sexually attractive odors of meadow voles *Microtus pennsylvanicus*. *Horm. Behavior* 27 (4): 523–538.
- Ferkin MH, Mech SG, Paz-y-Mino C, 2001. Scent marking in meadow voles and prairie voles: a test of three hypotheses. *Behaviour* 138: 1319–1336.
- Ferkin MH, Sorokin ES, Renfro MW, Johnston RE, 1994. Attractiveness of male odors to females varies directly with plasma testosterone concentration in meadow voles. *Physiol. Behavior* 55 (2): 347–353.
- Fry AC, Allemeier CA and Staron RS, 1994. Correlation between myosin heavy chain content and percent fiber type area in human skeletal muscle. *Eur. J. Appl. Physiol.* 68: 246–251.
- Fry AC, Staron RS, Hageman FC, Hikida RS, Thrush JT, 2003a. Muscle fiber characteristics and performance correlates of elite Olympic-style weightlifters. *J. Str. Cond. Res.* 17 (4): 746–754.
- Fry AC, Webber JM, Weiss LW, Harber MP, Vaczi M, Pattison NA, 2003b. Muscle fiber characteristics of elite power lifters. *J. Str. Cond. Res.* 17 (2): 402–410.
- Galler S, Hilber K, Pette D, 1997. Stretch activation and myosin heavy chain isoforms of rat, rabbit and human skeletal muscle fibres. *J. Muscle Res. Cell Motil.* 18 (4): 441–448.
- Hämäläinen N, Pette D, 1993. The histochemical profiles of fast fiber types IIB, IID, and IIA in skeletal muscles of mouse, rat and rabbit. *J. Histochem. Cytochem.* 41: 733–743.
- Hämäläinen N, Pette D, 1995. Patterns of myosin isoforms in mammalian skeletal muscle fibres. *Microsc. Res. Tech.* 30 (5): 381–389.
- Harjola VP, Jankala H, Harkonen M, 2000. Myosin heavy chain mRNA and protein distribution in immobilized rat skeletal muscle are not affected by testosterone status. *Acta Physiol. Scand.* 169: 277–282.
- Huggins JA, McDaniel VR, 1984. Intraspecific variation within a southeastern population of the prairie vole *Microtus ochrogaster* (Rodentia). *The Southwestern Naturalist* 29: 403–406.
- Kleiman DG, 1977. Monogamy in mammals. *Quarterly Rev. Biol.* 52: 39–69.
- Larsson L, Yu F, 1997. Gender-related differences in the regulatory influence of thyroid hormone on the expression of myosin isoforms in young and old rats. *Acta Physiol. Scand.* 159 (1): 81–89.
- Leonard ST, Ferkin MH, Johnson MM, 2001. The response of meadow voles to an over-mark in which the two donors differ in gonadal hormone status. *Animal Behaviour* 62: 1171–1177.
- Lyons GE, Kelly AM, Rubinstein NA, 1986. Testosterone-induced changes in contractile protein isoforms in the sexually dimorphic temporalis muscle of the guinea pig. *J. Biol. Chem.* 261 (28): 13278–13284.
- Nguyen HT, Gubits RM, Wydro RM, Nadal-Ginard B, 1982. Sarcomeric myosin heavy chain is coded by a highly conserved multigene family. *Proc. Natl. Acad. Sci.* 79 (17): 5230–5234.
- Perrie WT, Bumford SJ, 1984. Correlation of myosin heavy chains with

- ATPase staining of skeletal- and cardiac-muscle fibers. *Biochem. Soc. Trans.* 12: 825 – 826.
- Perrot-Sinal TS, Innes D, Kavaliers M, Ossenkopp KP, 1998. Plasma testosterone levels are related to various aspects of locomotor activity in wild-caught male meadow voles *Microtus pennsylvanicus*. *Physiol. Behavior* 64 (1): 31 – 36.
- Pette D, Staron RS, 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. *Rev. Physiol. Biochem. Pharmacol.* 116: 1 – 75.
- Pette D, Staron RS, 1997. Mammalian skeletal muscle fiber type transitions. *Int. Rev. Cytol.* 170: 143 – 223.
- Pette D, Staron RS, 2000. Myosin isoforms, muscle fiber types, and transitions. *Microsc. Res. Tech.* 50 (6): 500 – 509.
- Rourke BC, Qin A, Haddad F, Baldwin KM, Caiozzo VJ, 2004. Cloning and sequencing of myosin heavy chain isoform cDNAs in golden-mantled ground squirrels: effects of hibernation on mRNA expression. *J. Appl. Physiol.* 97 (5): 1985 – 1991.
- Schilling BK, Fry AC, Chiu LZ, Weiss LW, 2005. Myosin heavy chain isoform expression and *in vivo* isometric performance: a regression model. *J. Str. Cond. Res.* 19 (2): 270 – 275.
- Schulte -Hostedde AI, Millar JS, Hickling GJ, 2001a. Sexual dimorphism in body composition of small mammals. *Can. J. Zool.* 79 (6): 1016 – 1020.
- Schulte -Hostedde AI, Millar JS, Hickling GJ, 2001b. Evaluating body condition in small mammals. *Can. J. Zool.* 79 (6): 1021 – 1029.
- Schulte -Hostedde AI, Millar JS, Hickling GJ, 2003. Intraspecific variation in testis size of small mammals: implications for muscle mass. *Can. J. Zool.* 81 (4): 591 – 595.
- Severinghaus WD, 1981. Sexual dimorphism in the pelvic girdle of the prairie vole *Microtus ochrogaster*. *J. Tenn. Acad. Sci.* 56: 129 – 130.
- Stalling DT, 1990. *Microtus ochrogaster*. *Mammalian Species* 355: 1 – 9.
- Staron RS, 1991. Correlation between myofibrillar ATPase activity and myosin heavy chain composition in single human muscle fibers. *Histochemistry* 78: 33 – 39.
- Staron RS, Hikida RS, 1992. Histochemical, biochemical, and ultrastructural analyses of single human muscle fibers with special reference to the C fiber population. *J. Histochem. Cytochem.* 40: 563 – 568.
- Staron RS, Johnson P, 1993. Myosin polymorphism and differential expression in adult human skeletal muscle. *Comp. Biochem. Physiol.* 106B (3): 463 – 475.
- Staron RS, Kraemer WJ, Hikida RS, Fry AC, Murray JD, Campos GER, 1999. Fiber type composition of four hindlimb muscles of adult Fisher 344 rats. *Histochem. Cell Biol.* 111 (2): 117 – 123.
- Staron RS, Pette D, 1986. Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. *Histochemistry* 86 (1): 19 – 23.
- Tamarin RH, 1985. *Biology of New World Microtus*. Special Publication No. 8. The American Society of Mammalogists, Lawrence, KS.
- Termin A, Staron RS, Pette D, 1989. Myosin heavy chain isoforms in histochemically defined fiber types of rat muscle. *Histochemistry* 92 (6): 453 – 457.
- Toursel T, Bastide B, Stevens L, 2000. Alterations in contractile properties and expression on myofibrillar proteins in Wobblers mouse muscles. *Exp. Neurol.* 162: 311 – 320.
- Wada M, Hämäläinen N, Pette D, 1995. Isomyosin patterns of single type II B, II D and II A fibres from rabbit skeletal muscle. *J. Muscle Res. Cell Motil.* 16 (3): 237 – 242.
- Wolff JO, 1985. *Behavior*. In: Tamarin RH ed. *Biology of New World Microtus*. Special Publication of the American Society of Mammalogy 8: 340 – 372.
- Yu F, Degens H, Li X, Larsson L, 1998. Gender- and age-related differences in the regulatory influence of thyroid hormone on the contractility and myosin composition of single rat soleus muscle fibers. *Pflug. Arch.* 437 (1): 21 – 30.
- Zeveloff SI, Boyce MS, 1980. Parental investment and mating systems in mammals. *Evolution* 34: 973 – 982.