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Age-related changes in metabolic properties of equine skeletal muscle associated with muscle plasticity

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Abstract

The purpose of the present study was to determine the age-related changes in myosin heavy chain (MHC) composition and muscle oxidative and glycolytic capacity in 18 horses ranging in age from two to 30 years. Muscle samples were collected by excisional biopsy of the semimebranosus muscle. MHC expression and the key enzymatic activities were measured.

There was no significant correlation between horse age and the proportions of type-IIA and type-IIX MHC isoforms. The percentage of type-I MHC isoforms decreased with advancing age. Muscle citrate synthase activity decreased, whereas lactate dehydrogenase activity increased with increasing age. Muscle 3-OH acyl CoA dehydrogenase activity did not change with ageing. The results suggest that, similar to humans, the oxidative capacity of equine skeletal muscle decreases with age. The age-related changes in muscle metabolic properties appear to be consistent with an age-related transition in MHC isoforms of equine skeletal muscle that shifts toward more glycolytic isoforms with age.

Keywords: Ageing equine skeletal muscle; Citrate synthase; 3-OH acyl CoA dehydrogenase; Lactate dehydrogenase; Myosin heavy chain

1. Introduction

The ageing process typically is responsible for physical impairments such as a reduction in muscle force generating capacity and impaired mobility in mammals. This phenomenon seems to be primarily due to a decrease in the ability of muscle to generate and sustain power output in association with changes in muscular structure and function during ageing (White, 1995; Janssen et al., 2000). A decrease in muscle mass and a loss of motor unit number partially induce the age-related reduction in functional capacity of muscle. The slow (type I) to fast twitch (type II) fibre ratio increases with advancing age. The age-related shift in fibre types appears to be concomitant with a shift in myosin heavy chain (MHC) isoforms with age in horses (Rivero et al., 1997). In humans, the type-I to type-II MHC isoform ratio appears to be greater in aged muscles than in young muscles due to a shift from type-II to type-I MHC isoforms during the ageing process (Welle et al., 2000).

As age increases, the oxidative capacity of human skeletal muscle also declines (Rooyackers et al., 1996; Conley et al., 2000). Evidence of the decreased oxidative capacity is seen in both the rate of mitochondrial protein synthesis and activity of oxidative enzymes such as cytochrome *c* oxidase and citrate synthase (Rooyackers et al., 1996; Houmard et al., 1998). The reduced oxidative capacity appears to be related to the age-related decrease in aerobic capacity and muscle performance (Houmard et al., 1998; Conley et al., 2000). There is also evidence of diminished glycolytic capacity in aged skeletal muscle (Welle et al., 2000). These age-related

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adaptations are due to inevitable physiological changes and exacerbated by physical inactivity during the ageing process (Devor and Faulkner, 1999).

Marked ageing process changes in MHC distribution and activity of enzymes indicative of both carbohydrate and fat oxidation in humans and laboratory animals have only been superficially examined in horses (Rivero et al., 1993), and it is not well established whether the changes do occur in the horse, which is naturally athletic species. The majority of previous equine studies in this subject have either focused on trained horses, involve the interaction of training and ageing, or used only horses younger than 10 years of age (Essen et al., 1980; Rivero et al., 1991; Barrey et al., 1999; Roneus and Lindholm, 1991; Roneus, 1993).

Little is known about age-related changes in skeletal muscle characteristics of untrained horses (Rivero et al., 1993). As there is an increase in population of retired and ageing pet horses, it is important to define age-related physiological adaptations in sedentary horses. Therefore, the purpose of the present study was (1) to define the characteristics of muscle of sedentary aged horses, and in particular to characterize the age-related changes in the expression of MHC isoforms and the activity of marker enzymes of energy metabolism and (2) to determine whether that age-related changes in horses would mimic those of humans given the conservation of skeletal muscle structural and function characteristics across a wide range of mammalian species (Charette et al., 1991). Our working hypothesis was that age-related adaptations in the expression of MHC isoforms and marker enzymes of fuel utilization in equine skeletal muscles would be similar to those observed in humans and other mammalian species.

2. Materials and methods

2.1. Animals

In order to define the characteristics of skeletal muscle of ageing horses, 18 horses 2–30 years of age were obtained by either donation to the Veterinary Teaching Hospital, horses from the teaching herd, or in response to a request made to clients of the Equine Ambulatory Service. Upon the approval by the Veterinary Teaching Hospital Executive Committee and the Institutional Laboratory Animal Care and Use Committee (ILACUC) at The Ohio State University, an informed consent was provided to all clients and obtained prior to their participation.

Horses were selected to represent light breeds (Thoroughbred, Standardbred, Quarterhorse, cross breeds, and derivatives of these breeds) because of their genetic and ancestral similarity. Heavy horse breeds (Percherons, Clydesdales, and Belgiums) and ponies or Miniature horses were not sampled. Horses selected were not in active athletic training. No horse had a history of lower extremity joint, bone, muscle trauma, or disease.

2.2. Experimental protocol

Muscle samples were collected by excisional biopsy of the semimebranosus muscle either immediately (<2 min) after euthanasia by intravenous injection of barbiturate or under sedation and local anaesthesia. The biopsy was performed after induction of local anaesthesia by injection of 10 mL of mepivicaine hydrochloride and surgical preparation of the area. All biopsy samples were collected from the mid-belly region of the semimembranosus muscle in approximate sizes of 1.5 cm length, 1 cm width, and 1 cm thickness. A 3-cm skin incision was made and all biopsy samples were collected from approximately 7 mm ± 2 mm depth under the skin. The muscle samples were immediately frozen in liquid nitrogen and stored at −80 °C for subsequent biochemical analyses. The 3-cm skin incision was closed with interrupted skin sutures and dressed with antimicrobial ointment. Horses were administered tetanus prophylaxis and sutures were removed in 7–10 days.

2.3. Biochemical analyses

2.3.1. Myosin heavy chain electrophoresis

Myosin heavy chain electrophoresis was performed following the protocol of Rivero et al. (1997). A standard sample of adult rat costal diaphragm muscle (Sigma) was included in gels and used as a control, which has shown to contain four different MHC isoforms identified as type I, IIA, IIX, and IIB (Talmadge and Roy, 1993). Approximately 10 mg of isolated muscle sample was homogenized in a 1:100 dilution in Tris buffer (62.5 mM Tris, pH 6.8).

Electrophoresis was performed in 8% separating gel with 30% (w/v) glycerol and 14% stacking gel with 30% glycerol. Based on pre-determined total protein concentrations by protein assay, 10 μL aliquots of diluted myosin, which contained 5 μg of protein, were subjected to electrophoresis for 26 h at 285 V and 4 °C with 20-cm long vertical gels. Separating gels were stained with Coomasie blue and to determine the optimal density and percentage of each MHC isoform (type I, IIA, and IIX), the gels were scanned with the Mode GS-700 Imaging Densitometer (Bio-Rad, Hercules).

2.3.2. Enzyme analyses

Muscle tissue stored at −80 °C was weighted at −20 °C. The activity of citrate synthase (CS) as a measure of citric acid cycle activity was determined at 37 °C using spectrophotomic techniques (Chi et al., 1983). The activities of 3-OH-acyl-CoA dehydrogenase (HAD) as a measure of lipid oxidation and lactate dehydrogenase