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Histochemical properties and enzyme activities of skeletal muscle in Chilean draught horses

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Summary

Biopsies of the gluteus medius muscle were taken at three different depths from 16 adult (5 to 15 years old) Chilean crossbred draught horses (8 males, 8 mares). The biopsy samples were analysed for muscle fibre type composition, fibre sizes, and the activities of the enzymes citrate synthase (an indicator of citric acid cycle activity), lactate dehydrogenase (an indicator of anaerobic metabolism) and creatine kinase (used as a marker of the metabolism of high energy phosphates). The percentage of type I fibres (high myosin adenosine triphosphatase activity at pH 4.5) increased by 100% and the proportion of type IIB fibres (moderate myosin adenosine triphosphatase activity at pH 4.5) decreased by 67% going from the superficial to the deep sampling sites of the muscle. The percentage of type IIA fibres (low myosin adenosine triphosphatase activity at pH 4.5) did not present variations as a function of sampling depth. There were no significant differences in the lesser fibre diameter of fibre types between sampling sites. The activity of the enzyme citrate synthase increased by 100% from the superficial to the deep samples, whereas lactate dehydrogenase activity was 50% lower in the deepest portion of the muscle. In general, these results indicated that Chilean crossbred draught horses had a high aerobic capacity and a relatively low anaerobic capacity in the gluteus medius muscle, expressed by a high percentage of type I fibres, a low proportion of type IIB fibres and high activity of the enzyme citrate synthase, used as a marker of end terminal oxidative capacity. These muscle characteristics are highly consistent with the sort of work carried out by these animals, prolonged and sustained exercise of low intensity.

Keywords: muscle fibre, histochemistry, exercise, enzyme activity

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Histochemische Merkmale und Enzymaktivitäten im Skelettmuskel Chilenischer Zugpferde

Von 16 Chilenischen Zugpferden im Alter von 5 bis 15 Jahren (8 männliche und 8 weibliche Tiere) wurden aus dem M. glutaeus medius Muskelbiopsien aus drei verschiedenen Tiefen entnommen (3, 6 und 9 cm). Die Proben wurden analysiert hinsichtlich der Verteilung der Musklefasertypen, der Muskelfasergröße sowie der Aktivität der Enzyme Citrat-Synthase (Indikator für die Aktivität des Citratzyklus), Laktat-Dehydrogenase (Indikator des anaeroben Stoffwechsels) und Creatin-Kinase (Indikator des Stoffwechsels energiereicher Phosphatverbindungen). Der prozentuale Anteil an Typ I Fasern (hohe Aktivität der Myosin-Adenosin-Triphosphatase bei pH 4,5) nahm um 100% zu, der Anteil an Typ IIB Fasern (mittlere Aktivität der Myosin-Adenosin-Triphosphatase bei pH 4,5) verringerte sich um 67% und zwar von der oberflächlichen hin zur tiefen Entnahmestelle des Muskels. Der prozentuale Anteil der Typ IIA Fasern (niedrige Aktivität der Myosin-Adenosin-Triphosphatase bei pH 4,5) zeigt keine Veränderungen in Abhängigkeit von der Entnahmetiefe. Der kleinste Durchmesser der verschiedenen Fasertypen war zwischen den drei Entnahmetiefen nicht signifikant verschieden.
Die Aktivität der Citrat-Syntase nahm um 100% zu, und zwar von der oberflächlichen hin zur tiefen Entnahmestelle, wohingegen die Aktivität der Laktat-Dehydrogenase im tiefsten Teil des Muskels um 50% geringer war. Insgesamt betrachtet weisen diese Ergebnisse darauf hin, daß der M. gluteus medius Chilenischer Zugpferde über eine hohe aerobe und relativ niedrige anaerobe Kapaztität verfügt, was sich in einem hohen Prozentsatz an Typ I Fasern, einem niedrigen Anteil an Typ IIB Fasern und der hohen Aktivität des Enzymes Citrat-Synthase, welches als Indikator für die oxidative Kapazität eingesetzt wird, ausdrückt. Diese Muskelcharakteristika reflektieren auch die Art von Arbeit, für die diese Tiere eingesetzt werden: Dauerbelastungen bei niedriger Intensität.

Schlüsselwörter: Muskelfaser, Histochemie, Belastung, Emzymaktivität

Introduction

Most equine skeletal muscles are composed of fibre types with different contractile and metabolic properties. The use of histochemical techniques has allowed the characterisation of these fibres, the method most extensively used in the horse being based upon actomyosin adenosine triphosphatase myofibrillar (mATPase) activity. According to mATPase activity, it is possible to identify type I fibres (slow twitch) and type IIA and IIB fibres (fast twitch) (*Brooke* and *Kaiser,* 1970). The percutaneous needle biopsy technique described by *Lindholm* and *Piehl* (1974) has been extensively used in the horse for collecting muscle samples, and a great number of studies have been performed over the last two decades. The muscle most commonly examined has been the gluteus medius, because of its great volume, its relatively superficial location, but mainly because it plays an important role in the propulsion of the hindquarters of the horse (*Lindholm* and *Piehl* 1974).

Histochemical and biochemical characteristics of various locomotor muscles vary significantly between breeds of horses, according to the type of work for which the breed has been selected (*Snow* and *Guy* 1980; *López-Rivero* et al. 1989), and this may influence the physical ability of individual horses. Numerous studies have shown that many factors affect muscle properties: breed, age, sex, training, individual genetical factors, etc. (see *Snow* and *Valberg,* 1994 for a review). Crossbred draught horses are routinely used in Chile for agricultural purposes, but our knowledge about their muscle characteristics is very scarce. Since a consistent breed-related pattern of muscle composition has been demonstrated in horses, it is reasonable to hypothesise that these animals have particular muscle properties and that they are related to the specific type of work they perform. The purpose of this study was to examine muscle fibre type composition, muscle fibre sizes and activities of selected aerobic and anaerobic enzymes of the gluteus medius muscle in adult Chilean draught horses.

Materials and methods

Sixteen Chilean crossbred draught horses (8 males, 8 mares) between 5 and 15 years old were used. They were all of comparable conformation, body weight (mean±SD, 650±40 kg), and had all the hippometric characteristics of the Chilean crossbreed draught horses described by *Pérez* et al. (1993). All horses were maintained in a resting state for 90 days and they were fed with the same dietary components: natural pasture supplemented and improved with clover hay during the winter.

Muscle biopsies

Muscle biopsy specimens were taken at depths of 30 mm (superficial sampling site), 60 mm (middle sampling site) and 90 mm (deep sampling site) below the gluteal fascia, through the same incision, from the right gluteus medius muscle by the method of *Lindholm* and *Piehl* (1974). The biopsy site was identified as being a site on the line at an angle of 45° from the tuber coxae of the ilium. The exact location of the biopsy sample ranged, according to conformation of each horse, between 150 and 200 mm from this point along the line previously described. After collection, muscle samples were oriented so that myofibres could be cut transversely, frozen by immersion in isopentane kept at freezing point in liquid nitrogen, and stored in liquid nitrogen until analysed.

Histochemical analyses

Transverse serial sections of 10 µm were cut in a cryostat microtome (Micron HM 500) at -20 °C. The sections were incubated for mATPase activity at pH 9.4 after 3 preincubations at pH 10.3, 4.5 and 4.2 according with *Dubowitz* (1985). A semiquantitative evaluation of the oxidative capacity of fibre types was obtained from serial sections stained with reduced nicotinamide adenine dinucleotide dehydrogenase tetrazolium reductase (NADH-TR) (*Dubowitz* 1985). The myofibres were classified into type I, IIA and IIB (*Brooke* and *Kaiser* 1970) according to the mATPase staining patterns. Type IIB fibres were further subdivided into 2 subgroups according to the extent of the NADH-TR reaction: IIB oxidative and IIB non-oxidative (*López-Rivero* et al. 1991).

Morphometry

Serial sections stained for mATPase after preincubation at pH 4.5 and NADH-TR were systematically photomicrographed (magnification x160). A representative area (range 250,000 to 500,000 µm^2), containing a minimum of 200 fibres, was systematically examined in each biopsy. The relative frequencies of types I, IIA and IIB and of subtypes IIB oxidative and IIB non-oxidative fibres were determined on photomicrographs by typing at least 200 fibres (*Snow* and *Guy* 1980). Measurements of the lesser fibre diameter of fibre types were made on photomicrographs from biopsy sections stained for mATPase (preincubation pH 4.5) with a computerised video display analysis system, equipped with a standard morphometric programme. Values derived were averaged according to fibre type.

Biochemical analyses

The muscle tissue was dissected free of blood, fat and connective tissue. A weighed portion of muscle (15 mg) was homogenised in ice-cooled phosphate buffered saline (PBS) and centrifuged (3 times) at 1,000 rpm for 10 min. Then, the supernatant was eliminated and 10 ml of PBS were added to the sediment, which was homogenised again for 30 seconds at 8,000 rpm in a ultra turrax T-25. The homogene was sonicated in a Trassonic C-460 for 30 sec. The activities of the enzymes citrate synthase (CS, used as an indicator of end-terminal oxidative capacity in the Krebs's cycle), lactate dehydrogenase (LDH, used as an indicator of anaerobic capacity), and creatine kinase (CK, used as a marker of the metabolism of high energy phosphates) were determined by spectrophotometric techniques following *Cooney* et al. (1981). The activity of each enzyme was calculated in units of µmol NAHD converted/minute/mg of total protein.

Statistical analysis

The values are expressed as mean±SD. Differences among sampling sites were examined by means of a one-way analysis of variance. Correlation coefficients for muscle enzyme activities and muscle fibre types and fibre diameters were also calculated.

Results

In general, there were no significant differences in fibre composition, fibre diameters and enzyme activities between sexes. In contrast, there were systematic and significant differences among the three sampling sites. The values in the Table and Figure below are therefore presented separately for each muscle sampling depth, but they represent pooled means for both sexes.

As is shown in the table, the percentage of type I fibres increased by 100% ($P<0.001$) and the proportion of type IIB fibres decreased by 67%($P<0.001$) going from the superficial to the deep sampling sites. This rate of change was approximately similar in IIB oxidative (68%, $P<0.001$) and IIB non-oxidative (66%, $P<0.001$) fibre subtypes (fig. 1). The percentage of type IIA fibres did not present significant variations as a function of sampling depth ($P>0.05$; Table).

There were no significant differences in the lesser fibre diameters of fibre types among sampling sites. When the data were

Tab.: Mean (±sd) are shown for fibre type composition (%), lesser fibre diameter (µm) and activities (µmol/min/mg protein) of the enzymes citrate synthase (CS), lactate dehydrogenase (LDH) and creatine kinase (CK) at the 3 sampling depths of the gluteus medius muscle of 16 Chilean crossbreed draught horses

	30 mm	60 mm	90 mm	Overall
Fibre types				
I	30±9[a]	49±14[b]	62±10[c]	
IIA	24±7	25±4	24±4	
IIB	46±21[a]	27±12[b]	15±9[c]	
Fibre diameter				
I	46±4	48±5	50±4	48±5[a]
IIA	53±6	53±4	56±4	54±5[b]
IIB	57±4	54±5	55±3	55±4[b]
Enzymeactivities				
CS	566±231[a]	904±435[b]	1083±490[c]	
LDH	0.432±0.18[a]	0.426±0.08[b]	0.248±0.09[c]	
CK	0.359±0.16	0.314±0.12	0.394±0.175	

Means with a different letter within a row or within a column are statistically different (P<0.05 at least).

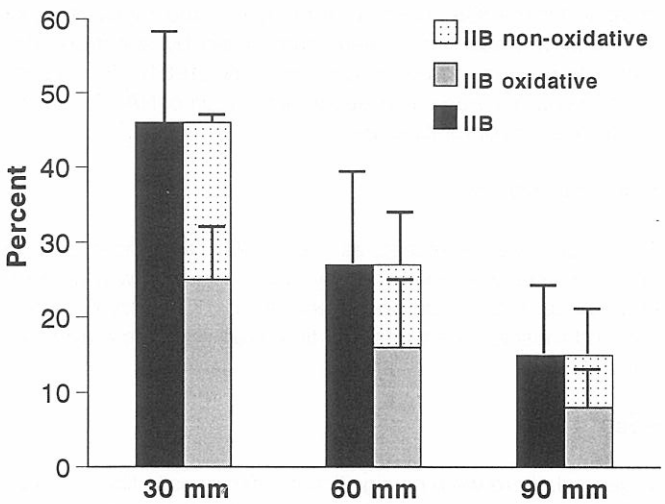

Fig.: Mean (±sd) percentage of type IIB fibres, including both subtypes (IIB oxidative and IIB non-oxidative) at the 3 sampling depths of the gluteus medius muscle of 16 Chilean crossbred draught horses.

considered as a whole the fast twitch fibres (types IIA and IIB) had a greater lesser diameter than the slow twitch fibres (type I).

The activities of the enzymes CS and LDH changed significantly with sampling depth. Citrate synthase increased by 100% from the superficial to the deep samples, whereas LDH activity was 50% lower in the deepest portion of the muscle. The activity of the enzyme CK was similar in the three regions of the muscle (P>0.05; Table).

Discussion

The gluteus medius muscle is often selected for this type of research because of its importance in hindlimb propulsion (*Lindholm* and *Piehl* 1974). Fibre type composition of this muscles is, however, extremely heterogeneous (*López-Rivero* et al. 1992) and it may be asked to what extent one small sample obtained with a biopsy needle can be representative of the composition of the muscle as a whole. The differences in muscle parameters recorded in the present study among the 3 sampling depths of the muscle substantiate former studies (*Kline* and *Bechtel* 1988; *López-Rivero* et al. 1992; *Rivero* et al. 1995) and probably reflect different functional demands on the gluteus medius muscle. As in the study by *Kline* and *Bechtel* (1988) and *Rivero* et al. (1995), a gradient was detected in fibre types and enzyme activities in the gluteus medius muscle in which the superficial portion of the muscle had a higher percentage of type IIB fibres, a higher activity of the glycolytic enzyme LDH, a lower percentage of type I fibres and a lower activity of the oxidative enzyme CS. Conversely, the activity of CS and the percentage of type I fibres increased in the deeper region of the muscle whereas the activity of LDH and the percentage of type IIB fibres decreased very significantly. These results provide further evidence that the superficial portion of the equine gluteus medius muscle is more glycolytic and less aerobic in its metabolism than the deeper regions and that as a result a single biopsy taken from this muscle is a poor estimator of the metabolic activity of the whole muscle. The deeper region of the muscle which has a large proportion of type I fibres and a predominantly aerobic metabolic capacity seems best suited for the maintenance of posture and the longer term performance of less strenuous activities. In contrast, the histochemical composition of the superficial part of the muscle, which has a high proportion of large type IIB fibres, and its predominantly glycolytic metabolism, indicate that it has a completely different pattern of use. These regions of the muscle seem to be more involved with the generation of rapid, propulsive forces for short periods.

The Chilean crossbreed draught horses presented a percentage of type I fibres higher and a proportion of type II fibres lower than Thoroughbred racehorses and Andalusian horses (*Snow* and *Guy* 1980; *López-Rivero* et al. 1989). These results are compatible with the kind of work that draught horses must carry out. These animals are specifically adapted for exercise requiring low intensity but long duration. A high percentage of type I fibres, which have a high oxidative capacity, is an optimal muscle property for this type of exercise.

The percentage of type IIA fibres was similar in the three sampling sites of the muscle and, in general, its value was lower than those observed in Thoroughbred racehorses and Arabian horses (*Snow* and *Guy* 1980; *López-Rivero* et al. 1989). These fibres are characterised by their high oxidative capacity and a great contraction velocity, a characteristic necessary for carrying out high speed and resistance work. The low percentage of type IIA fibres found in Chilean crossbred draught horses is also compatible with the type of work that they do.

The values obtained in the present study for the lesser fibre diameter were of similar range as those reported for other breeds of horses (*López-Rivero* et al. 1990), and no differences were observed as a function of sampling depth. The lesser fibre diameter is an important morphometric property of the

muscle, because of its relationship to the isometric force generated during muscle contraction (*Hill* 1950). Types I and IIA muscle fibres are smaller than type IIB fibres. As those fibres are also more capillarised in relative terms than type IIB fibres, they have a greater capacity both for the diffusion of oxygen and substrates toward the interior of the cells, and for the elimination of lactate and other metabolites (*Essén-Gustavsson* et al. 1989).

The pattern of variation of enzyme activities of the gluteus medius muscle with depth were consistent with changes in muscle fibre type composition. In the superficial region of the muscle, the high activity of the enzyme LDH was consistent with the high percentage of type IIB non-oxidative fibres recorded in this area of the muscle. These fibres show a high capacity for glycolytic metabolism, so they might be involved in the generation of propulsive force of high intensity and short duration (*Snow* and *Valberg* 1994). By contrast, in the deepest muscle biopsies taken, the high percentage of type I fibres is reflected by an increase in the activity of the enzyme CS. This observation indicates the use of a preferably oxidative metabolism. These results are similar to those observed by *Rivero* et al. (1995) in endurance horses. Additionally, in the present study there was a positive correlation ($P<0.05$) between the proportion of type I fibres and the activity of the enzyme CS.

The high activity of the enzyme CS seen in the present study would permit a better regulation of substrate flow towards fibres during submaximal exercises of long duration and low intensity. This mechanism could improve efficiency in the use of energy within the muscle fibre, diminishing the risk of muscular fatigue during endurance exercises (*Snow* and *Valberg* 1994; *Rivero* et al. 1995).

In conclusion, results from the present investigation indicated that locomotor muscles of Chilean crossbred draught horses have a very high oxidative capacity, expressed by a high proportion of slow-twitch fibres (type I) and high activity of the enzyme used as marker of end terminal oxidative capacity. These muscle properties are highly consistent with the kind of work routinely carried out by these animals, agricultural labour of low intensity but long duration.

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