Pferdeheilkunde 12 (1996) 4 (Juli August) 510–513

Discrimination between endurance horses with different performance records

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Summary

Biopsies from 3 different depths of the gluteus medius muscle were obtained in 36 endurance horses, aged 8.42±2.85 years. Twenty of the horses were considered excellent performers according to their 3 fastest records in endurance events over the past 2 or 3 years. The other 16 horses were moderate performers. Muscle biopsy specimens were analyzed for fibre type distribution (*type I, IIA* and *IIB*), fibre area and relative fibre area. The activities of enzymes citrate synthase, 3-OH-acyl-CoA-dehydrogenase and lactate dehydrogenase were also determined. The data were subjected to discriminant analysis and principal component analysis. It was possible to correctly discriminate all the horses according to their performance records by means of discriminant analyses when the histochemical, morphometric and biochemical data from all 3 muscle biopsies was used. This was not possible with data provided by only a single muscle biopsy of this muscle. This study showed that it was possible to satisfactorily classify an inidivdual horse in a given endurance performance category on the basis of a morphological muscle index.

keywords: horse, endurance, muscle fibres, enzymes, discriminant analysis

Differenzierung zwischen unterschiedlich erfolgreichen Distanzreitpferden

Bei 36 Pferden im Alter von durchschnittlich 8,42 ± 2,85 Jahren, die an Distanzritten teilnahmen, wurden Biopsien aus drei unterschiedlichen Tiefen des M. glutaeus medius entnommen. Zwanzig dieser Pferde wurden anhand ihrer drei schnellsten Zeiten bei Distanzritten in den letzten zwei oder drei Jahren als sehr leistungsstark eingestuft, die anderen sechzehn Pferde fielen in die Kategorie "mäßige Leistungen".
Die Muskelbiopsien wurden auf ihren Gehalt an verschiedenen Fasertypen, auf die Fläche der Faserquerschnitte und den relativen Fasergehalt sowie die Aktivitäten der Enzyme Citrat-Synthase, 3-OH-Acyl-CoA-Dehydrogenase und Laktatdehydrogenase untersucht.
Die erhaltenen Daten wurden einer Diskriminanzanalyse und einer Faktorenanalyse unterzogen. Unter Zugrundelegung der histochemischen, biochemischen und morphologischen Daten aus allen drei Bioptaten konnte jedes Pferd seiner tatsächlichen Leistung zugeordnet werden. Dies gelang jedoch nicht anhand der Daten aus einem einzelnen Bioptat. Die Messung der Enzymaktivitäten war nicht unbedingt nötig, da ihre Aufnahme in die Analysen die Chance, die Pferde nach ihrem Leistungsstand richtig zu klassifizieren, gegenüber einer Analyse auf der Basis der morphologischen und histochemischen Daten nur unwesentlich verbesserte.
Die Studie zeigte, daß es möglich war, jedes einzelne Pferd anhand seines morphologischen Muskelindex in eine vorgegebene Leistungskategorie einzuordnen.

Schlüsselwörter: Pferd, Ausdauer, Muskelfasern, Enzyme, Diskriminanzanalyse

Introduction

Many studies, based on the use of the percutaneous needle biopsy technique (*Lindholm* and *Piehl* 1974), have reported a significant correlation between performance capacity and muscle characteristics in racehorses (*Snow* and *Guy* 1981; *Wood* et al 1988) and endurance horses (*Snow* et al 1981; *Hodgson* et al 1983; *Rivero* et al 1993, 1995). Muscle biopsy as a tool for predicting performance potential and assessing the superior capacity for work of an individual horse is controversial. The problems of reproducibility of results, its invasive nature, the need for highly specialized laboratory techniques and the multifactorial nature of superior race-day performance have all been raised in criticisms of the technique (*Snow* and *Valberg* 1994). However, the obvious importance of muscle in exercise may justify a search for refinements in the analysis of muscle composition that may allow its examination as a predictor for athletic potential.
This study was aimed to answer the following question: Is it possible to satisfactorily classify an individual horse in a given performance category on the basis of the histochemical, morphometric and/or biochemical variables from muscle biopsies?

Materials and methods

Horses
The study was carried out retrospectively on 36 endurance horses of several breeds (17 Anglo-arabians, 9 purebred Arabians and 10 cross Arabians), comprising one stallion, 17 geldings and 18 mares, all between 4 and 17 years (mean±sd, 8.42±2.85 years). All horses had entered national and/or international long distance events for 2–10 years, according to the age of each individual. They had generally been out at pasture under similar environmental conditions and had been regularly endurance-trained for at least 2 years before the experiment.
Mean speeds of the three fastest records for the last 2–3 years for each individual were used to determine whether a horse was

successful or unsuccessful at the competition. Only horses with all 3 records >3.5 m/s (in 120- to 180-km endurance rides), >4 m/s (in 80- to 120-km endurance rides) or >3.75 m/s (in 40- to 60-km endurance rides at novice level) were considered excellent performers: n = 20 horses (7 females and 13 males; mean age, 8.65±3.14 years). The remaining 16 horses (11 females and 5 males; mean age, 8.23±3.42 years), with slower times, were categorized as moderate performance horses. In all three categories of endurance rides considered in the present study, mean speed was statistically different ($P<0.001$) between both performance groups: a) 120–180 km endurance rides, 14.5±1.2 km/h (mean ± sd) in excellent performers *vs.* 11.8±1.2 km/h in moderate performers; 2) 80–120 km endurance rides, 15.5±1.7 km/h *vs.* 12.5±1.3 km/h; and 3) 40–60 km endurance rides, 14.6±0.4 km/h *vs.* 11.3±1.0 km/h.

Muscle biopsies

Through the same incision, biopsy specimens were obtained at depths of 20, 40 and 60 mm from the right *gluteus medius* muscle using the technique described by *Lindholm* and *Piehl* (1974).

Upon collection, the muscle samples were divided into 2 parts, one for histochemical analysis and the other for biochemical analysis. All samples were stored at –80 °C until analyzed.

Collection and organization of the data

Histochemistry: Transverse serial sections (10 µm) were cut on a cryostat at –20 °C and incubated for myofibrillar adenosine triphosphatase (mATPase, Enzyme Comission, EC 3.6.1.3) after acid (pH 4.5-4.6 and 4.2) and alkaline (pH 10.3) buffer pre-incubation (*Dubowitz* 1985). Muscle fibres were classified as *types I* (slow-twitch), *IIA* and *IIB* (fast-twitch) according to staining.

Morphometry: For each biopsy specimen the relative frequencies (%), mean cross-sectional area (µm²) and relative distribution in area (%) of *types I, IIA* and *IIB* fibres were calculated. These variables were obtained by means of a computerized image analysis system (Imago, SIVA Group, Univ Cordoba, Spain).

Biochemistry: Selected muscle enzyme activities were also determined in muscle biopsies from some horses and used as markers of selected metabolic pathways. The activities of the enzymes citrate synthase (CS, Enzyme Comission, EC 4.1.3.7; an indicator of Kreb's cycle), 3-hydroxy-acyl-CoA-dehydrogenase (HAD, EC 1.1.1.35; a marker for lipid oxidation) and lactate dehydrogenase (LDH, EC 1.1.1.27; used as anaerobic marker) were measured using fluorimetric techniques. Enzyme activities were expressed in units of µmol NADH converted/min/g freeze dried muscle.

Statistical methods

Discriminant analysis: The data were analyzed by means of a discriminant analysis according with the DISCRIM procedure developed by the Statistical Analysis System (*SAS Institute,* 1986). From our set of observations, the homogeneity of the within-group covariance matrices was tested. Consequently, discriminant functions used in the present study were based on this classification criterion and prior probabilities of each group of classification were assumed to be equal (e.g., 0.5). Only a probability of a given horse belonging to its real performance category >0.95 was considered as an acceptable index for a correct classification.

Principal component analysis: The data were also studied by means of a principal component analysis (PCA) according with the FACTOR procedure (SAS Institute 1986). This analysis describes the relationships among parameters and compares the performance capacity of each horse by considering all the quantitative data obtained from muscle biopsies. Plotting the observations (horses) relative to the various principal components gave an overall view of their performance capability.

Results

In general, there were systematic and significant differences in fibre type composition, fibre sizes, relative areas, and enzyme activities between the two performance categories of endurance horses (Tab. 1). The significance of these differences have been discussed in our previous studies (*Rivero* et al 1993, 1995).

Discriminant analysis

In general, a low proportion of horses was adequately classified using data from a single gluteus medius muscle single biopsy (Fig. 1). Moreover, fourteen horses (40 %) correctly classified according to a single biopsy sample were misclassified by computing data derived from another one (or both) of the remaining two biopsies. Similarly, a low percentage of horses correctly classified was still present when discriminant analyses were performed by mixing data from 2 different biopsies. However, when the discriminant analysis was carried out with the mixed data from all the 3 muscle biopsies, 100 % of horses were correctly classified with a probability >0.95 (Fig. 1).

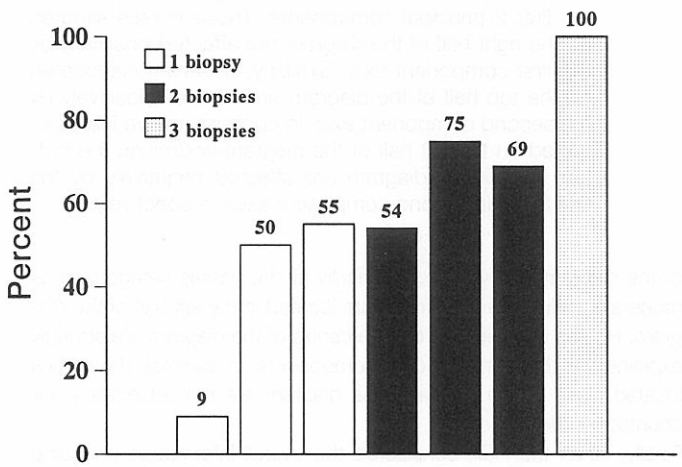

Fig. 1: Percents (%) of endurance horses correctly classified (with a probability >0.95) in their performance category by discriminant analyses according to data derived from 1, 2 and 3 muscle biopsies.

Principal component analysis (PCA)

When the PCA was based on histochemical and morphometric data from the 3 muscle biopsies, the first component axis included the most relevant muscle parameters describing the athletic potential of endurance horses. The percentages and relative areas of *types I* and *IIA* fibres were affected positively, whereas the mean cross-sectional area of *type I* fibres was affected negatively by the first component axis. The second component axis distinguished horses according to the mean area of *type II* fibres and percentage and relative area of *type IIA* fibres. The mean area of types IIA and IIB fibres from 40- and 60-mm-muscle sampling sites were not clearly explained by the first 2 components.

The PCA also gives the coordinates of each observation (horse) on the principal component axes (Fig. 2). The majority of horses classified as excellent endurance performers were situated in the right-half

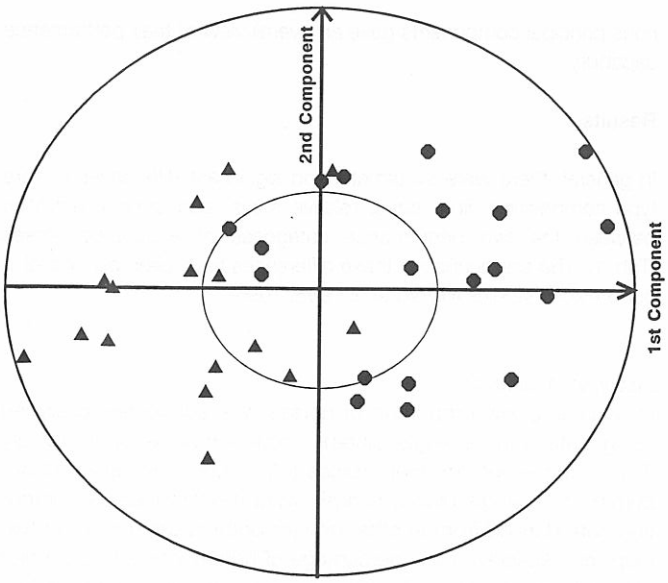

Fig. 2: Coordinates of each individual horse on the first 2 principal components according to the principal component analysis. Circles, excellent endurance performers; triangles, moderate performers. Those horses situated far from the centre of the diagram are strongly explained by the first 2 principal components. By contrast, the horses situated inside of the inner circle are poorly explained by the first 2 principal components. Those horses situated on the right half of the diagram are affected positively by the first component axis. Similarly, observations located on the top half of the diagram are affected positively by the second component axis. In contrast, the variables situated on the left half of the diagram and/or on the bottom half of the diagram are affected negatively by the first and the second component axes, respectively.

of the diagram. Conversely, practically all the horses categorized as moderate endurance performers are located in the left half of the diagram. Horses situated far from the centre of the diagram are strongly explained by the first 2 principal components. In contrast, the horses located close to the centre of the diagram are not adequately accounted for these axes.

Finally, when the PCA considered the overall information (including muscle enzyme activities) from 2 different muscle biopsies, the distribution of muscle parameters was similar in pattern to those previously described. In addition, the first component axis positively included CS and HAD muscle enzyme activities. The activity of the LDH muscle enzyme was affected negatively. Thus, all these analyses also permitted an adequate discrimination between endurance horses with different performance records.

Discussion

Results from the present study substantiate those of *Snow* et al (1981), *Hodgson* et al (1983) and *Rivero* et al (1993, 1995). All these studies reported a substantial correlation between certain muscle properties and performance in endurance horses. In general, higher percentages and larger sizes of *types I* and *IIA* muscle fibres, lower proportions of *IIB* fibres and greater oxidative capacities were found in excellent than in moderate performers. The current material, however, differs from that of previous studies in that information can now be provided regarding the interrelationship among several muscle properties as a tool in the assessment of fitness in endurance horses.

Discriminant analysis

Discriminant analyses used in the present study permit a numeric individual value to be calculated for each horse. This value is the result of a linear function of an intragroup index derived from the covariance matrices and observations of each muscle variable for that individual horse. The individual values of a group of horses (performance category) should show a gaussian distribution. Thus, from the individual value of a given horse, the analysis also provides the probability of that horse belonging to a defined performance category. Our results clearly showed an optimal discrimination of the horses according to their performance category, particularly when the overall information from 3 different gluteus medius muscle biopsies were computed (Fig. 1).

In this study, a high percentage of horses was misclassified using data from a single muscle biopsy. Moreover, there was a lack of relationship among results from different discriminant analyses using data from a single muscle biopsy. These findings clearly show that a single biopsy from this muscle is not sufficient for discriminating between horses with different performance records. The considerable variability of *gluteus medius* muscle characteristics in association with depth and position (*Rivero* et al 1992) means that a single muscle biopsy is a poor estimator of fibre type characteristics of the whole muscle. *Lexell* et al (1985) concluded that it is possible to minimize the variance involved in the procedure of sampling muscles taking from each individual multiple biopsies from different depths of the muscle. This conclusion is confirmed by the present results. Moreover, the rate of change with increasing sampling depth of many characteristics of this muscle also varies significantly from horse to horse (*Rivero* et al 1993).

Principal component analysis (PCA)

The PCA using fibre types, fibre areas and relative fibre areas from 3 different sampling depths is also a good alternative for assessment of endurance performance in equine athletes. The results of this study also substantiate the relationship between parameters describing the oxidative capacity of the muscle and the performance of endurance horses (*Snow* and *Valberg* 1994). The combination of a high proportion of slow- to fast-contracting fibres and a high *type IIA*-to-*type IIB* fibre ratio had a positive effect in distinguishing the best performing endurance horses. This finding corresponds optimally with myofibre recruitment during exercise and should be very beneficial for endurance horses. Studies on muscle glycogen depletion patterns after exercise have reported that in general the order of recruitment of fibres during most types of exercise is *I→IIA→IIB* (*Snow* et al 1982; *Hodgson* et al 1983).

The inclusion of muscle enzyme activities in PCAs did not substantially improve the accuracy of the analysis. Accordingly, biochemical analyses of muscle biopsies could be omitted in the assessment of endurance performance in well-trained horses.

In conclusion, this study shows that an optimal discrimination between endurance horses with different performance records is possible by applying multivariate statistical procedures that take into account the overall information derived from multiple *gluteus medius* muscle biopsies. The precision of the estimate can be greatly improved by computing multiple biopsies removed at different depths of this muscle. The inclusion of muscle enzyme activities in the analysis of muscle biopsies did not further increase the discrimination. As muscle characteristics are factors limiting performance in endurance events, methods originally described in this report could become a useful aid for the comparison and subsequent selection (or rejection) of horses for this discipline.

Tab. 1: Mean (±sd) are shown for fibre type composition (%), mean cross-sectional areas (µm²), relative cross-sectional areas (%), and enzyme activities (µmol/g/min) in the 3 sampling depths of the gluteus medius muscle of 20 excellent endurance performers and 16 moderate performers

	20 mm		40 mm		60 mm	
Variable	Excellent	Moderate	Excellent	Moderate	Excellent	Moderate
Fibre types						
I	22 (5)	18 (5)	36 (6)	26 (6)***	51 (9)	37 (12)****
IIA	41 (6)	38 (5)	45 (6)	41 (4)*	46 (8)	44 (6)
IIB	37 (8)	44 (8)**	19 (7)	33 (7)***	3 (6)	19 (12)
Fibre areas						
I	2453 (510)	2407 (487)	2938 (396)	2757 (466)	3454 (699)	3068 (764)
IIA	3412 (545)	3275 (432)	3692 (613)	3417 (4492)	3926 (730)	3638 (767)
IIB	4609 (912)	4739 (846)	4199 (738)	4587 (853)	4239 (497)	4256 (810)
Relative areas						
I	14 (5)	11 (4)*	30 (6)	20 (5)***	48 (10)	32 (11)****
IIA	38 (7)	32 (6)*	48 (7)	38 (5)***	49 (10)	45 (8)
IIB	48 (9)	57 (8)**	22 (6)	42 (9)***	3 (5)	23 (11)***
Enzyme activities						
CS	19 (7)	15 (4)	24 (7)	17 (6)*	31 (8)	24 (7)*
HAD	26 (8)	23 (5)	36 (7)	28 (8)**	46 (12)	40 (14)
LDH	958 (315)	1173 (261)	938 (339)	898 (148)	768 (165)	884 (321)

*, **, ***, P< 0.05, P<0.01, P<0.001 compared to excellent performers, respectively

Acknowledgments

This work has been jointly supported by the I+D Spanish Programme (DEP91-0465), the University of Cordoba (Spain) and the National Institute of Animal Science (Denmark). The author thanks owners of endurance horses who so willingly cooperated and allowed muscle samples to be taken from their horses. Gratitude is expressed to Mss. *Marianne Andersen* for technical assistance, to Dr. *Poul Henckel* who allowed me to develop part of this investigation in his lab, and to Dr. *Arno Lindner* and Dr. *David H. Snow* for their comments on the manuscript.

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