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Degree of correspondence between contractile and oxidative capacities in horse muscle fibres: a histochemical study

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Summary. Samples taken from the middle gluteal muscle of 95 untrained adult horses of different ages and sex were subjected to histochemical analysis using the myosin adenosine triphosphatase (m-ATPase) and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) staining techniques. Fibres were classified into types I, IIA and IIB according to m-ATPase activity after preincubation at pH 4.4. The percentage of FT (Fast-Twitch Glycolytic) fibres and the proportion of IIB fibres with «high» and «low» oxidative capacity were determined in serial sections stained for NADH-TR.

Statistical analysis revealed a significantly higher proportion of IIB fibres than FT fibres ($P < 0.001$), though both percentages were correlated. Thus, 72.2 +/− 17.6% of type IIB fibres showed low oxidative capacity, but the remaining 27.8 +/− 17.6% showed high aerobic potential, and thus did not correspond to FT fibres.

These results confirm that the contractile capacity of a muscle fibre does not determine its oxidative profile. The different types of muscle fibre should thus be classified solely according to m-ATPase activity, since this characteristic is related to the molecular structure of contractile proteins. Oxidative capacity should be assessed separately, and not be used as a criterion for fibre classification in horses.

Key words: Muscle fibre types, Histochemistry, Horse

Introduction

Histochemical techniques (m-ATPase, NADH-TR, SDH; LDH, GPase, etc.) can be used to determine the contractile and metabolic properties of different skeletal muscle fibres in mammals (Brooke and Kaiser, 1970; Peter et al., 1972). In horses, these fibre types have commonly been classified according to a combination of m-ATPase activity and oxidative and/or glycolytic capacity (Lindholm and Piehl, 1974; Snow and Guy, 1976; Kline and Bechtel, 1983; Raub et al., 1983). Fibres have thus been typified as ST or SO (Slow-Twich Oxidative), FTH or FOG (Fast-Twitch High oxidative or Oxidative Glycolytic) and FT or FG (Fast-Twitch Glycolytic). In addition, m-ATPase activity in equine muscle after incubation at various pHs may be used to identify type I (Slow-Twitch) and type II (Fast-Twitch) fibres, as well as subtypes IIA, IIB and IIC (Essén et al., 1980). At present, these two classification systems are used interchangeably (Cutmore et al., 1985; Raub et al., 1986; Kline et al., 1987; Wood et al., 1988; Bechtel and Lawrence, 1989), despite the fact that various studies have shown that they are not completely identical (Snow and Guy, 1980; Hodgson et al., 1985, 1986; Andrews and Spurgeon, 1986; Valberg et al., 1988). No specific study has been made of this phenomenon, however, and the quantitative and qualitative results reported in some studies have not always coincided.

Oxidative capacity, widely recognised as an important property of horse muscle because of its value as an indication of muscular metabolic response to training (Hodgson et al., 1985, 1986; Valberg, 1986; Valberg et al., 1988), can be determined using some of the techniques already mentioned (NADH-TR, SDH) (Shubber, 1971/72; Lindholm and Piehl, 1974; Snow and Guy, 1980; Essén et al., 1980; Hodgson et al., 1985; Andrews and Spurgeon, 1986). Despite considerable subjectivity in the interpretation of these techniques (Valberg et al., 1988), type I and IIA fibres have substantial oxidative potential, whereas the oxidative capacity of type IIB fibres is highly variable (Essén et al., 1980; Essén-Gustavsson and Lindholm, 1985; Van Den Hoven et al., 1985; Andrews and Spurgeon, 1986). No information is available, however, regarding the extent of these findings in untrained adult horses.

The purpose of this study, therefore, was to clarify the

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degree of correspondence between oxidative capacity and the contractile profile in these types of equine muscle fibres. The variation in oxidative capacity in type IIB fibres was assessed statistically in different breeds of horse. Differences in middle gluteal muscle fibre composition in some breeds have already been evaluated in a previous study (unpublished data).

Materials and methods

Horses

Ninety-five adult horses (mares and stallions) of five different breeds (Andalusian, Thoroughbred, Arabian, Anglo x Arab, Spanish x Breton) and ranging in age from 4-24 years, were examined in this study. Animals were grouped as shown in Table 1. All horses were in a satisfactoy state of health, and of comparable physical fitness (inactive or semi-inactive).

Biopsies

Samples were taken from the ventral portion of the right middle gluteal muscle using the percutaneous needle biopsy technique described by Lindholm and Piehl (1974) and Snow and Guy (1976), although the biopsy needle had an internal diameter of 6 mm (Henckel, 1983). All samples were collected from similar specific anatomical locations, and at a constant depth of 5 cm.

Histochemical techniques

Samples were orientated to obtain a transverse section when cut, and immediately frozen in isopentane cooled in liquid nitrogen (Dubowitz and Brooke, 1973). Serial sections 10 μm thick were cut in a cryostat at -20° C and stained for m-ATPase, at pH 9.4 (Padykula and Herman, 1955) after acid preincubation at pH 4.4 (Brooke and Kaiser, 1970), and for NADH-TR in order to determine the oxidative capacity of each fibre type (Novikoff et al., 1961).

Fibre classification and quantitative histochemical analysis

Fibres were classified into types I, IIA and IIB, according to m-ATPase activity after acid preincubation (Brooke and Kaiser, 1970). To determine the staining intensity for NADH-TR, fibres were rated as «high» or «low» according to staining intensity (Snow and Guy, 1980). Only those fibres which had uncoloured borders and internally were of an even blue-white colour were deemed to be «low» staining intensity fibres (Valberg et al., 1988). These fibres were termed type FT (Snow and Guy, 1980).

Percentages of fibre types I, IIA and IIB were calculated by counting at least 500 fibres in a serial section stained for m-ATPase after acid preincubation at pH 4.4. The proportions of IIB fibres with «high» and «low» oxidative capacity were determined using two serial sections (m-ATPase and NADH-TR).

Statistical analysis

Quantitative results are expresed as means (+/−SD). Type IIB and FT fibres were compared for each group using Student's t test and Pearson's correlation coeffi-cient. Diffeences between groups were analysed using Snedecor's F test and Tukey's test.

Results

Qualitative analysis

Figure 1 shows fibre identification and correlation with each of the histochemical techniques used in this study. Types I, IIA and IIB were clearly defined in a section stained for m-ATPase after acid preincubation at pH 4.4 (Fig. 1a). In the section stained for NADH-TR, only the «low» staining or FT fibres could be identi-fied objectively (Fig. 1b). Type I and IIA fibres generally showed some degree of oxidative capacity, while most IIB fibres showed low oxidative capacity and were thus classified as FT. Nevertheless, the oxidative capacity of IIB fibres was not constant, since a considerable proportion of these fibres showed some reaction to NADH-TR staining (Fig. 1b).

Quantitative analysis

Table 2 shows the results obtained for each group. In all groups, the percentage of IIB fibres was always significantly greater than that of FT fibres ($P < 0.001$). These two percentages were correlated in groups 1, 2 and 3 and in the total ($P < 0.001$), though not in groups 4 and 5. The proportion of IIB fibres with high oxidative capacity was significantly higher in group 2 than in groups 1 and 5, and was higher in 3 than in 5, but not similar relationship was observed between groups 3-1, 3-4 and 4-5 (Tukey test).

Discussion

The fibre reaction observed with the NADH-TR stain, consisting of intracellular blue formazan deposits, is the result of the oxidation of reduced NADH by the enzyme tetrazolium reductase, which is bound to the mitochondrial membrane (Valberg et al., 1988). Thus, this technique makes it possible to assess the mitochondrial density of a muscle fibre and thereby obtain some information regarding its aerobic potential (Hoppeler et al., 1983; Andrews and Spurgeon, 1986). However, NADH-TR stains are open to highly subjective interpre-tations (Valberg et al., 1988), and allow only a qualitative (Hodgson et al., 1985) or semi-quantitative (Essén et al., 1980; Valberg et al., 1988) determination of the oxidative capacity of different fibre types (Surway, 1980). It is felt that the solution proposed by Snow and Guy (1980) in order to overcome this drawback (the consideration of only two staining intensities -«high» and «low») makes these techniques more objective. Even,

Table 1. Grouping of horses used in this study.

GROUP	BREED	STALLIONS	MARES	TOTAL	MEAN AGE (Yr)
1	Andalusian	17	15	32	11.7
2	Thoroughbred	9	17	26	10.9
3	Arabian	8	8	16	12.5
4	Anglo x Arab	5	5	10	12.3
5	Spanish x Breton	11	0	11	7.8

Table 2. Percentages of fibre types and Pearson correlation coefficients between percentages of type IIB and FT fibres for all groups studied and for total number of animals.

	Group 1	Group 2	Group 3	Group 4	Group 5	Total
FIBRE TYPES (%)	**ANDALUSIAN**	**THOROUGHBRED**	**ARABIAN**	**ANGLO x ARAB**	**SPANISH x BRETON**	**TOTAL**
I	29.0 (6.9)	22.3 (7.0)[a]	37.0(11.0)[a,b]	29.2 (5.3)	28.4 (5.5)[c]	28.5 (8.7)
IIA	38.3 (7.0)	48.6 (7.6)[a]	35.7 (8.1)[b]	44.6 (9.3)[c]	34.8 (5.2)[b,d]	40.9 (9.1)
IIB	32.7 (5.8)	29.1(10.3)	27.3 (5.4)	26.2 (5.3)	36.8 (4.5)b,c,d	30.6 (7.7)
"H"	21.6(14.2)	36.3(16.3)[a]	34.8(16.4)	30.7(18.8)	12.7(16.2)[b,c]	27.8(17.6)
"L"	78.4(14.2)	63.7(16.3)[a]	65.2(16.4)	69.3(18.8)	87.3(16.2)[b,c]	72.2(17.6)
FT	24.5 (5.4)	18.0 (6.5)[a]	17.9 (6.3)[a]	18.1 (5.5)[a]	31.6 (4.6)[a,b,c,d]	22.1 (7.5)
PEARSON CORR. COEFF. between %IIB and %FT	0.66***	0.77***	0.69***	0.50	–0.24	0.70***

Data are expressed as mean (+/– SD)

***P < 0.001

a, b, c and d: statistically different compared to group 1, 2, 3 and 4, respectively

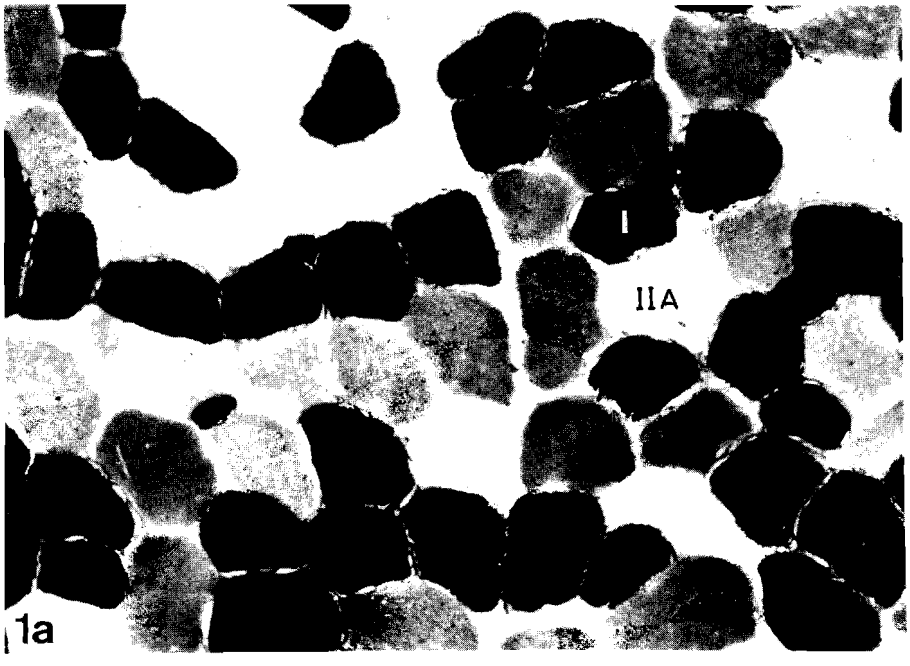
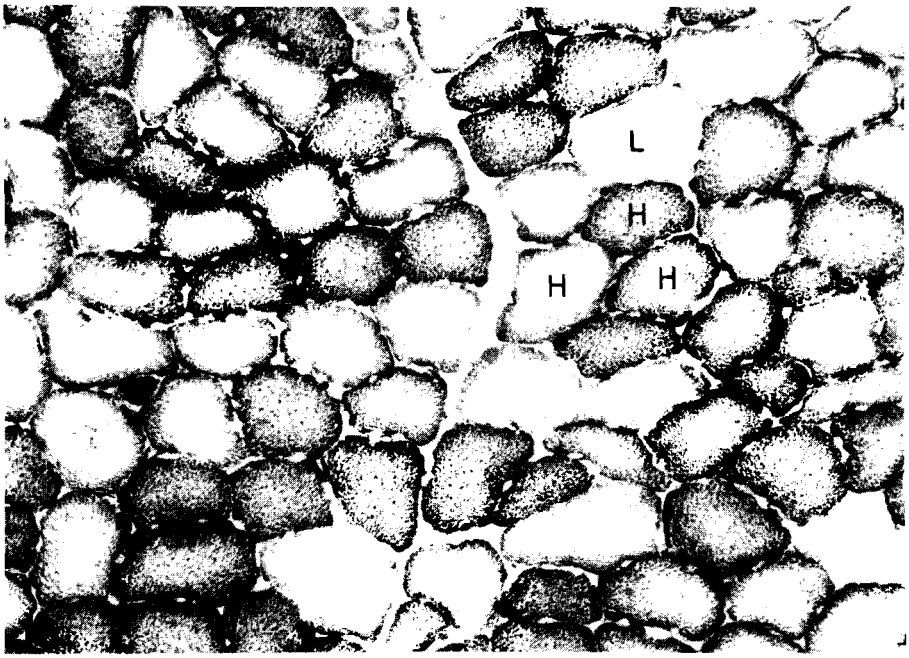


Fig. 1. Seried migrographs showing fibre type identification in middle gluteal muscle. × 160. **a.** Myosin adenosin triphosphatase activity (m-ATPase after acid preincubation -pH 4.4). **b.** Nicotinamide-adenine dinucleotide-tetrazolium reductase (NADH-TR).



so, a more objective quantitative assessment of the oxidative capacity of each fibre type may be obtained using a microspectrophotometer to assess this histochemical reaction (White and Snow, 1985).

The qualitative results obtained in this study generally coincided with those obtained in previous studies of the horse by other authors (Essén et al., 1980; Hodgson et al., 1985; Andrews and Spurgeon, 1986). Wide variations in the oxidative capacity of IIB fibres led Hoppeler et al. (1983) to considerer two IIB subtypes in the horse semitendinosous muscle, according to oxidative capacity: IIB-oxidative and IIB-glycolytic. However, some horses were found to have no IIB fibres with low oxidative capacity in a recent study (Valberg et al., 1988). This is not surprising, since horse muscle has an extremely high aerobic potential compared to humans and other animals (Essén-Gustavsson, 1985).

The quantitative results obtained here also coincide with those reported by other authors (Hodgson et al., 1985, 1986). However, Essén et al. (1980) have reported medium oxidative capacity in a proportion of IIB fibres almost twice as high (nearly 60%) as the proportion found in the present study. In untrained adult horses of different breeds, it was confirmed that the classification of fast-twitch fibre subtypes proposed by Lindholm and Piehl (1974) (FTH and FT) did not fully correspond to that proposed by Essén et al.(1980) (IIA and IIB). In our study, 72.2 +/− 17.6% of type IIB fibres had low oxidative capacity in all animals sampled, but the remaining 27.8 +/− 17.6% showed high oxidative capacity, and thus did not correspond to FT fibres. In this respect, our results differ from those of Valberg (1986), who reported that in untrained horses FTH and FT fibres correspond to IIA and IIB fibres, respectively.

Breed did not have a decisive influence on the oxidative capacity of IIB fibres. Only the throughbred had a significantly higher proportion of IIB fibres with high oxidative capacity than the other breeds studied. The Spanish x Breton had a significantly higher proportion of glycolytic IIB fibres than the other breeds. This may be related to the temperament and functional character of the breeds concerned, although the possibility that these levels of statistical significance are the result of methodological errors due to the difference in IIB fibre size between breeds cannot be ruled out. A large fibre may be rated as being of «low» staining intensity with the NADH-TR technique, even though it might contain as many mitochondria as a fibre with a smaller cross-sectional area (Valberg et al., 1988). Thoroughbreds generally have significantly smaller IIB fibres than Andalusian or Arabian horses (unpublished data). Moreover, Spanish x Bretón horses usually have extremely large IIB fibres (unpublished data).

The large variation in enzyme activity in skeletal muscle fibre (Pette and Spamer, 1986) means that contractile capacity does not automatically determine metabolic profile, so that fibre classification systems based on enzyme activity may contain considerable implicit errors (Pette, 1985). This alone accounts for the unsuitability of muscle fibre classification systems based wholly or partly on metabolic characteristics (Howald, 1982). Moreover, the oxidative capacity of horse muscle fibres has been shown to increase with age and/or training (Lindholm and Piehl, 1974; Henckel, 1983; Hodgson et al., 1985, 1986). Since oxidative capacity reflects mitochondrial density (Hoppeler et al., 1983), which may be modified regardless of fibre m-ATPase activity (Barnard et al., 1971; Kugelberg, 1973), different fibre types should be classified solely according to m-ATPase activity, because this property is closely related to the molecular structure of contractile proteins. Moreover, it has recently been reported that the contractile and metabolic properties of muscle fibre may be independently regulated (Pette, 1985). Each muscle fibre thus constitutes a separate metabolic compartment, so that fibre subtypes IIA and IIB cannot be classified into different metabolic groups.

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