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Variability of muscle fibre composition and fibre size in the horse gluteus medius: an enzyme-histochemical and morphometric study

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ABSTRACT

To determine the variability in fibre types and fibre sizes in the equine gluteus medius muscle, biopsy specimens were removed from 5 sites, at 4 different depths, within the right and left muscles of 3 Andalusian stallions. The percentage, lesser fibre diameter and cross-sectional area of the various fibre types were measured systematically in myosin ATPase and NADH-tetrazolium reductase-stained, serial cryostat sections of these multiple samples. Significant differences in muscle fibre type composition were recorded, with a lower percentage of type I fibres (high myosin ATPase activity at pH 4.5) being observed towards superficial regions of the muscle and a greater percentage towards the deep areas. Type IIB fibres (moderate myosin ATPase activity at pH 4.5), including both IIB nonoxidative (low NADH-TR activity) and IIB oxidative (moderate NADH-TR activity), displayed the opposite tendency, and the percentage of type IIA fibres (low myosin ATPase activity at pH 4.5) did not change with depth. Types I and IIA fibres in the deep regions were larger than superficially, whereas the IIB fibres in the deep regions were smaller than in the superficial parts of the muscle. The results also imply that type I fibres tend to be larger than type II fibres in the deep regions. The size of type I fibres is more homogeneous in the deep parts than in the superficial regions of the muscle, while IIB fibres vary more in size in the peripheral portions than in deep regions. A single biopsy taken from the gluteus medius muscle of the horse is therefore a poor representative of the whole muscle and care should be exercised in sampling and interpreting data obtained from limited biopsy of this muscle. The pattern of variation in fibre types and fibre sizes between the different depths of the muscle probably reflect different functional demands on the gluteus medius muscle.

INTRODUCTION

Fibre-type proportions are known to vary widely within individual human muscles (Nygaard & Sánchez, 1982; Lindman et al. 1991) and within animal muscles (Gunn, 1978; Newsholme et al. 1988; Bredman et al. 1990). Similarly, mean fibre size may also vary from site to site within a muscle (Armstrong & Phelps, 1984; Lexell & Taylor, 1989, 1991).

The proportion and size of different fibre types in equine skeletal muscle, particularly in the gluteus medius, are frequently measured to investigate muscle performance, growth, training and pathology. Muscle biopsies have often been used to obtain tissue when killing the animal is not desired, and information about the whole muscle has been deduced from these samples. However, there is now sufficient evidence that the fibre composition of this muscle varies extensively, especially as a function of depth (Kai, 1984; Bruce & Turek, 1985; Kline et al. 1987; Kline & Bechtel, 1988; López-Rivero et al. 1992*a*). Little information exists on intramuscular variation in the size of histochemically determined fibre types in horse skeletal muscle. Likewise, few studies have been designed to investigate the homogeneity of muscle fibre type composition over a specific area and depth (Wood et al. 1988). Homogeneity in this sense would be advantageous, in that it would allow repeated sampling over a small area of muscle throughout a period of training or experimentation, while avoiding

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the danger of sampling too close to a previous site, always providing that the sampling depth remains the same.

The main objective of the present study was to ascertain whether the distribution of fibre types and fibre sizes in the gluteus medius muscle of adult horses is homogeneous over a confined area and depth, as well as at different depths. Secondly, since there is a correlation between the histochemical profile, motor unit contraction speed, and fatiguability, the fibre size and composition of the muscle provides additional information about its function.

MATERIALS AND METHODS

Experimental design

Three Andalusian stallions in good nutritional state were used. They were all of similar age (12, 13 and 9 y), body weight (505 kg, 566 kg and 531 kg; Horse 1, Horse 2 and Horse 3, respectively) and conformation, and were not exercised over the year prior to the experiment. None had a history of neuromuscular disease.

Muscle biopsies were taken from 5 sites in the right and left gluteus medius according to the technique described by Lindholm & Piehl (1974), except that the needle had an outer diameter of 6 mm and was further modified with finger and thumb rings (Henckel, 1983). The initial biopsy site (central area) was identified by measuring 10 cm (at an angle of 45°) dorsocaudal to the tuber coxae of the ilium. The remaining 4 sites were determined by measuring 4 cm dorsal, caudal, ventral and cranial from the initial site. At each site, muscle samples were removed at depths of 2, 4, 6 and 8 cm below the fascia.

Enzyme-histochemical methods

The biopsy specimens were mounted on cork sheets using embedding medium (Tissue-Tek II) and oriented so that myofibres could be cut transversely. The specimens were quick-frozen by immersion in isopentane precooled to about −160 °C with liquid nitrogen, and stored at −80 °C until analysis. Serial sections of 10 μm were cut in a cryostat at −20 °C and incubated for Ca^{2+}-activated myofibrillar adenosine triphosphatase (mAPTase) at pH 9.4, after 3 preincubations: pH 10.3, 4.5 and 4.2 (Dubowitz, 1985). Reduced nicotinamide adenine dinucleotide dehydrogenase tetrazolium reductase (NADH-TR) was used to determine the oxidative capacity of the various fibre types. Myofibres were classified into types I, IIA, IIB (Fig. 1) and IIC (Dubowitz, 1985) according to mAPTase staining characteristics at the different levels of preincubation acidity.

Morphometry

The relative frequency of the various fibre types (I, IIA and IIB) was determined from an average of 570 ± 9.3 (mean ± S.E.M.), range 402–895, fibres identified on a basis of acid (pH 4.5) preincubation for mATPase. The presence of IIC fibres was inconsistent and always less than 1–2%, so this fibre type was included in the percentage of type IIB fibres (Essén-Gustavsson & Lindholm, 1985). Type IIB fibres were then redivided into 2 subgroups according to the extent of their staining for NADH-TR. Fibres with low intensity NADH-TR staining (fibres uncoloured on the outside and with an uniform blue-white colour on the inside; Fig. 1) were described as IIB non-oxidative fibres, and the remaining type IIB fibres were classified as type IIB oxidative (López-Rivero et al. 1991).

Measurements of the lesser fibre diameter (LFD) and cross-sectional area (CSA) of types I, IIA and IIB fibres were made from biopsy sections stained for mATPase after preincubation at pH 4.5 (magnification ×200) using a computerised video-display image analysis system, equipped with a digitising tablet (DT-2851, Data Translation) connected to a microcomputer (Mitac MPC 4000 E/F) and a standard morphometric programme (Imago, SIVA Group, University of Cordoba, Spain). Only areas without artefacts, with distinct cell borders and, if possible, located in the central areas of the biopsy specimen were measured. No measurements were made in those areas where the fibres had mostly been sectioned longitudinally (Blomstrand et al. 1984).

The mean (± S.D.) LFD and CSA of type I, IIA and IIB fibres was determined from an average of 130 ± 2.7, range 75–214, fibres/muscle biopsy, including a minimum of 35 for each histochemical type (Blomstrand et al. 1984). The lesser fibre diameter (or minimal diameter) is defined as the maximum diameter across the lesser aspect of the muscle fibre and is designed to overcome the distortion that occurs when a muscle fibre is cut obliquely (Dubowitz, 1985).

Coefficients of variability (C.V.) of individual fibre types in each muscle specimen were calculated as follows: S.D. divided by mean LFD and multiplied by 1000 (Dubowitz, 1985). 'Atrophy' and 'hypertrophy' factors were derived from megahistograms of muscle fibre diameter (Dubowitz, 1985). In calculating these factors, a fibre diameter range of 35 to 75 μm was

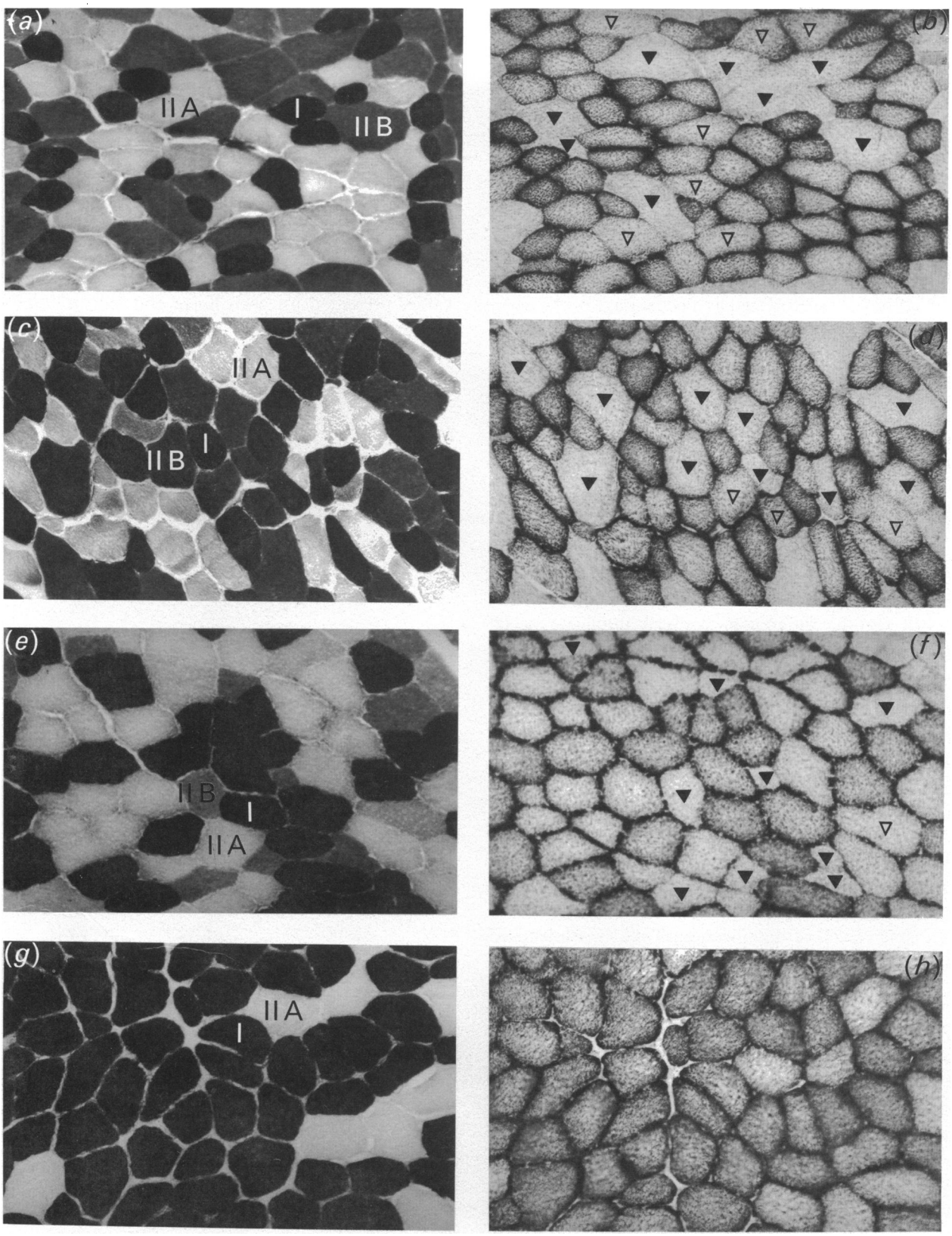

Fig. 1(*a–h*). Myosin ATPase staining after preincubation at pH 4.5 (left column) and NADH-TR activity (right column) of 4 biopsy specimens removed from the gluteus medius of 1 horse at depths of 2(*a*, *b*), 4(*c*, *d*), 6(*e*, *f*), and 8(*g*, *h*) cm. Types I, IIA and IIB fibres are marked. Full triangles, IIB nonoxidative fibres; empty triangles, IIB oxidative fibres. ×200.

selected; these arbitrary numbers were chosen because approximately 85–90% of fibres in the normal histogram were within this range.

To evaluate the technical error in the measurement of both fibre type proportion and fibre size, 30 biopsy specimens were analysed independently by 2 assessors. Moreover, methodological error in the fibre diameter measurement was determined by performing 3 repeated measurements of 20 fibre diameters by 2 assessors at different sites in the biopsy specimens for a total of 15 biopsy specimens. The average standard deviation (S.D.), expressed as a percentage of the mean value of 6 measurements of 20 fibres was 1.65% (range 0.8–3.0). Sampling errors were evaluated by calculating the coefficients of variation of fibre type percentages and fibre sizes from 20 duplicate biopsies obtained from the same location within the gluteus medius.

Statistical methods

Conventional statistical procedures have been used to calculate mean and S.D. The coefficient of variation (C.V.) for the differences between duplicate determinations was calculated according to the formula: C.V. $= [(\Sigma d^2\, 2n)^{1/2}]/\bar{x}$, where '$d$' is the difference between duplicate measurements, 'n' is the number of duplicate analyses and '$\bar{x}$' is the mean value. Student's paired t test was used to test for possible differences between right and left muscles.

Two-way analysis of variance (ANOVA) to compare multiple samples was used to test the hypothesis that no difference is present in fibre type proportion and fibre type sizes within each depth and between the different depths. The null hypothesis was rejected at the 0.05 level of significance. Tukey's studentised range test was performed to compare group means 2×2.

RESULTS

Morphology

Histochemical evaluation was characterised by a mosaic pattern of polygonally shaped fibres (Fig. 1), but in most biopsy specimens originating from the deepest part of the muscle (8 cm), areas with almost exclusively slow-twitch fibres were observed (Fig. 1*g*). In these biopsy specimens II B fibres were frequently (60%) absent. Microscopic examination of the muscle samples revealed marked differences in the proportion of the various fibre types between biopsy specimens taken from different depths (Fig. 1).

Methodological error and comparison between right and left muscles

Comparative data concerning the analyses of the same biopsy specimens carried out by the 2 assessors, the duplicate samples from the same location of the gluteus medius muscle, and the muscle biopsy samples from the left and right muscles are presented in Table 1. In this material no significant differences in fibre type proportions and fibre sizes were obtained between the right and the left legs ($P > 0.05$). The values presented for each horse and for the total in Tables 2 and 3 represent pooled means for the right and left sides.

Variation between and within sampling depths

Both the effect of sample depth and site effect were highly significant with respect to the relative proportion, mean lesser fibre diameter (LFD) and mean cross-sectional area (CSA) of the various fibre types (Tables 2, 3). However, the degree of variation in data

Table 1. *The variations in muscle fibre type composition (%) and fibre sizes (lesser fibre diameter and cross-sectional area) of the same 30 biopsy specimens carried out independently by 2 assessors (observational bias), between 20 duplicate biopsy specimens obtained from the same location within the gluteus medius (sampling errors), and between the right and left leg for the whole material (variation between contralateral muscles)*

	Observational bias		Sampling errors		Variation between contralateral muscles	
	n	C.V.	n	C.V.	n	C.V.
Fibre type						
I	30	0.098	20	0.076	60	0.202
II A	30	0.103	20	0.109	60	0.188
II B	30	0.113	20	0.170	60	0.192
II Bno[1]	30	0.116	20	0.163	60	0.372
II Box[2]	30	0.199	20	0.369	60	0.511
Lesser fibre diameter						
I	30	0.051	20	0.114	60	0.083
II A	30	0.062	20	0.101	60	0.104
II B	24	0.089	16	0.087	47	0.126
Cross-sectional area						
I	30	0.101	20	0.174	60	0.145
II A	30	0.103	20	0.137	60	0.151
II B	24	0.109	16	0.148	47	0.209

n, Number of duplicate analyses; C.V., coefficient of variation for the differences between duplicate measurements; [1] type II B non-oxidative fibres; [2] type II B oxidative fibres.

Table 2. *Mean values are shown for relative frequency (% ± S.D.) of various fibre types at different depths in the right and left gluteus medius muscle of 3 adult horses. Data derived from 10 sample sites at the same depth*

Sampling depth	Horse	Type I % ± S.D.	Type IIA % ± S.D.	Type IIB % ± S.D.	Type IIBno[1] % ± S.D.	Type IIBox[2] % ± S.D.
2 cm	1	13 ± 3	37 ± 7	50 ± 8	32 ± 7	18 ± 8
	2	28 ± 7	36 ± 5	36 ± 8	25 ± 6	11 ± 7
	3	24 ± 4	36 ± 3	36 ± 3	25 ± 4	11 ± 4
	Total	21.5 ± 8	36.5 ± 5	42 ± 9	27 ± 6	15 ± 7
4 cm	1	27 ± 7	37 ± 5	36 ± 3	25 ± 4	11 ± 4
	2	39 ± 7	34 ± 3	27 ± 7	19 ± 5	8 ± 4
	3	40 ± 9	35 ± 4	26 ± 8	17 ± 6	9 ± 3
	Total	35.5 ± 9	35 ± 4	29.5 ± 8	20 ± 7	9.5 ± 6
6 cm	1	45 ± 6	39 ± 5	16 ± 6	12 ± 8	4 ± 3
	2	49 ± 6	32 ± 6	19 ± 6	11 ± 5	8 ± 9
	3	55 ± 11	32 ± 8	13 ± 9	7 ± 7	6 ± 4
	Total	49.5 ± 10	34 ± 7	16.5 ± 9	10 ± 7	6.5 ± 6
8 cm	1	59 ± 13	36 ± 10	5 ± 6	1 ± 1	4 ± 6
	2	57 ± 5	39 ± 5	4 ± 6	2 ± 4	2 ± 3
	3	70 ± 15	27 ± 11	3 ± 5	1 ± 2	3 ± 4
	Total	61.5 ± 12	34 ± 10	4.5 ± 6	1.5 ± 3	3 ± 4
F values[3]	F depth	88.18***	0.96	124.14***	113.86***	21.36***
	F site	3.67**	1.11	4.43*	6.83***	1.51

[1] Type IIB nonoxidative fibres; [2] type IIB oxidative fibres; [3] variance ratios of a 2-way ANOVA testing variation in fibre types attributable to different sampling depths (F depth) and different sample site (F site); *** means that a ratio is significant at $P < 0.001$ level; ** $P < 0.01$; * $P < 0.05$.

Table 3. *Mean values are shown for lesser fibre diameter (μm ± S.D.) and cross-sectional area (μm² · 100 ± S.D.) of various fibre types at different depths in the right and left gluteus medius muscle of 3 adult horses*

Sampling depth	Horse	n	Type I		Type IIA		Type IIB	
			μm ± S.D.	μm² · 100 ± S.D.	μm ± S.D.	μm² · 100 ± S.D.	μm ± S.D.	μm² · 100 ± S.D.
2 cm	1	10	46 ± 3	23 ± 3	44 ± 4	24 ± 3	59 ± 3	41 ± 4
	2	10	56 ± 4	35 ± 3	58 ± 6	40 ± 8	63 ± 6	49 ± 7
	3	10	46 ± 5	24 ± 4	50 ± 4	31 ± 3	60 ± 5	48 ± 5
	Total	30	49 ± 6	27 ± 6	51 ± 7	32 ± 8	61 ± 5	46 ± 6
4 cm	1	10	46 ± 5	24 ± 3	46 ± 6	26 ± 6	54 ± 5	37 ± 8
	2	10	60 ± 6	38 ± 3	59 ± 6	40 ± 5	59 ± 4	45 ± 7
	3	10	51 ± 8	29 ± 6	57 ± 6	38 ± 5	63 ± 5	48 ± 6
	Total	30	52 ± 8	30 ± 8	54 ± 8	35 ± 8	58 ± 5	43 ± 8
6 cm	1	10	54 ± 3	33 ± 3	50 ± 5	31 ± 6	51 ± 4	31 ± 4
	2	10	61 ± 4	42 ± 5	59 ± 5	43 ± 5	52 ± 8	37 ± 10
	3	9	56 ± 6	36 ± 7	59 ± 8	42 ± 9	59 ± 10	44 ± 11
	Total	29	57 ± 5	37 ± 6	56 ± 6	38 ± 9	54 ± 8	37 ± 10
8 cm	1	5	57 ± 6	34 ± 6	48 ± 3	29 ± 4	48 ± 3	30 ± 3
	2	5	63 ± 6	45 ± 6	59 ± 7	44 ± 6	57 ± 5	45 ± 7
	3	3	61 ± 6	43 ± 7	61 ± 4	42 ± 7	59 ± 10	46 ± 11
	Total	13	60 ± 6	41 ± 8	56 ± 8	40 ± 10	54 ± 7	39 ± 10
F values[1]		F depth	14.55***	21.77***	2.51	5.25**	7.42**	5.90**
		F site	2.44**	2.89*	2.02	1.82	1.01	0.77

n, Number of data referred to mean type IIB fibres LFD and CSA; for each horse n = 10 and for each sampling depth n = 30. [1] Variance ratios (2-way ANOVA); ***, **, * significant $P < 0.001$, < 0.01 and < 0.05, respectively.

derived from these 2 factors differed markedly. In general, the combined interaction depending on both factors was not significant ($P > 0.05$).

A 3-fold increase in the percentage of type I fibres was found between the most superficial and the deepest sampling site ($P < 0.001$). There was a 90, 95 and 77% reduction of proportion IIB, IIB non-oxidative and IIB oxidative fibre types, respectively,

Table 4. *Variance ratios of 4 2-way ANOVAs carried out within each sampling depth to test the possible significance of any variation in percentages, minimal diameters and cross-sectional areas of the various fibres types which might be attributable to differences between horses (F horse) and between sample sites (F site)*

Sampling depth	Fibre type	Frequency		Minimal diameter		Cross-sectional area	
		F horse	F site	F horse	F site	F horse	F site
2 cm	I	24.19***	1.81	11.85***	0.08	40.89***	0.16
	IIA	0.15	0.80	17.96***	1.72	48.10***	2.85
	IIB	11.42***	2.19	1.72	1.25	7.38**	2.12
	IIBno	6.95**	4.58*	—	—	—	—
	IIBox	3.57	1.44	—	—	—	—
4 cm	I	12.27***	4.61*	14.45***	2.11	16.02**	2.88
	IIA	1.13	0.18	15.48***	1.25	16.75***	0.57
	IIB	8.51**	5.45**	6.80**	0.69	5.03*	0.30
	IIBno	5.14*	6.55**	—	—	—	—
	IIBox	1.41	0.71	—	—	—	—
6 cm	I	7.73**	11.08***	9.84**	3.52*	9.41**	3.36*
	IIA	7.04**	2.31	6.94**	0.77	8.32**	1.23
	IIB	3.00	5.24**	4.05*	0.73	8.03**	1.35
	IIBno	2.21	1.79	—	—	—	—
	IIBox	1.55	1.29	—	—	—	—
8 cm	I	4.12*	1.51	2.72	2.00	7.98**	2.06
	IIA	5.42*	1.02	22.73***	2.34	45.00***	3.68*
	IIB	0.21	0.77	—	—	—	—
	IIBno	0.69	0.78	—	—	—	—
	IIBox	1.08	0.95	—	—	—	—

***, **, * Significant at $P < 0.001$, 0.01 and 0.05, respectively. IIBno, type IIB nonoxidative fibres; IIBox, type IIB oxidative fibres.

Table 5. *Differences in fibre type frequency between the different areas of the muscle within each sampling depth. Tukey's test**

Sampling depth	Muscle area	Muscle area				
		Central	Dorsal	Caudal	Ventral	Cranial
2 cm	Central	—	—	—	IIBno	IIBno
	Dorsal	—	—	IIBno	IIBno	IIBno
	Caudal	—	—	—	IIBno	—
	Ventral	—	—	—	—	—
	Cranial	—	—	—	—	—
4 cm	Central	—	I	—	IIB, IIBno	—
	Dorsal	IIB, IIBno	—	IIBno	IIBno	IIB, IIBno
	Caudal	—	I	—	IIB, IIBno	—
	Ventral	—	I	I	—	—
	Cranial	—	I	—	IIB, IIBno	—
6 cm	Central	—	I	IIB	IIB	—
	Dorsal	IIB	—	IIB	IIB	IIB
	Caudal	I	I	—	—	I
	Ventral	I	I	—	—	I
	Cranial	—	—	—	—	—
8 cm	Central	—	—	—	—	—
	Dorsal	—	—	—	—	—
	Caudal	—	—	—	—	—
	Ventral	—	—	—	—	—
	Cranial	—	—	—	—	—

* The fibre types which were significantly more frequent ($P < 0.05$) in the areas of the muscle listed in the second column compared with those listed horizontally are shown in the table. IIBno, type IIB nonoxidative fibres.

going from the most superficial to the deepest sampling site ($P < 0.001$). The proportion of IIA fibres was similar in all depths ($P > 0.05$). Between the most superficial and the deepest sampling site, the mean type I fibres size increased (24% for LFD, $P < 0.001$; and 52% for CSA, $P < 0.001$) and the

mean type IIB fibres size declined (LFD, 10%, $P < 0.001$; CSA, 24%, $P < 0.01$). Significant changes were not recorded between sampling depths for the mean LFD of IIA fibres ($P > 0.05$), but the mean type IIA fibres CSA increased significantly (28%; $P < 0.01$). Thus the hypothesis of homogeneity of fibre type composition and fibre size at different depths of the muscle can be rejected.

In general, no significant difference ($P > 0.05$) was observed in fibre sizes between sample sites (Table 3). However, muscle fibre type composition was not completely homogeneous (Table 2). Because the individual horses differed markedly with respect to the relative frequency and size of the various fibre types (Tables 2, 3), a 2-way ANOVA with horse and sample site as factors was applied to the data within each sampling depth (Table 4). Differences of significance in the percentage of fibre types, at various depths are shown in Table 5. The maximal significant differences in percentage of type I (18.5%), IIB (13.5%) and IIB nonoxidative (13.5%) fibres in biopsy specimens originating from the same absolute depth were recorded between the dorsal and the ventral zones of the muscle. Generally, at the same absolute depth, biopsy specimens from the dorsal zone contained a significantly lower percentage of type I and higher percentage of IIB and IIB nonoxidative fibres than those originating from the ventral area of the muscle (Table 5). Therefore, the hypothesis that the distribution of fibre types is independent of the intramuscular location of the biopsy specimens over a specific area and depth must also be rejected.

Differences in size between the fibre types

The differences between the mean LFD of the various fibre types (i.e. IIA minus I; IIB minus I; IIB minus IIA) were calculated for each biopsy specimen. The relationships between sampling depth and these differences are presented in Figure 2. There was a strong linear relationship for all 3 differences ($P < 0.001$).

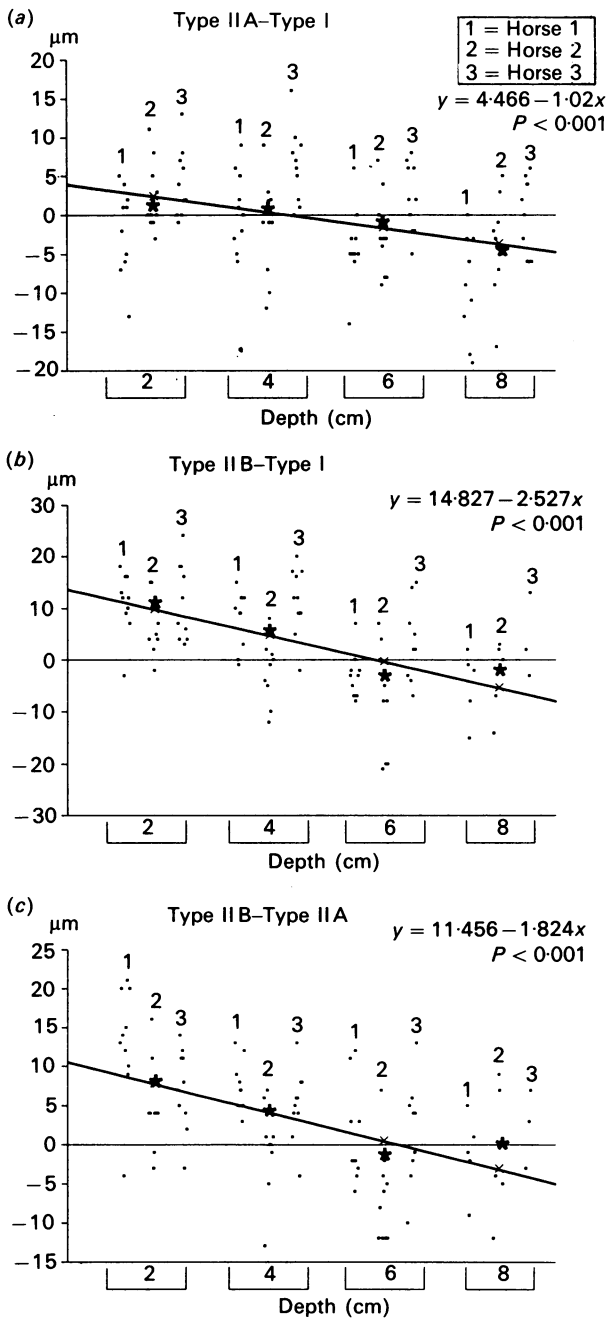

Fig. 2(*a–c*). Differences between the mean lesser diameter (µm) of the various fibre types [(*a*) type IIA-type I; (*b*) type IIB-type I; (*c*) type IIB-type IIA] for 10 biopsy specimens/horse (dots) together with the mean (asterisks) differences for whole sampling depths of the gluteus medius muscle from 3 adult horses. The regression line and the significance level give the relationship between the mean differences (asterisks) and sampling depth.

Variability coefficients and atrophy and hypertrophy factors

The histographic distribution of fibre type diameters was unimodal in all biopsy specimens. In most instances, mean value for atrophy and hypertrophy factors was < 100. Moreover, the S.D. of the mean LFD in biopsy specimens was generally less than a fourth of the value of the mean diameter, i.e. variability coefficients for types I, IIA, and IIB fibres were < 250 in 94%, 92%, and 84%, respectively, of biopsy specimens analysed. A significant negative correlation between C.V. and percentage of type I fibres ($r = -0.45$; $P < 0.001$) was observed for the whole material. In contrast, there was a significant positive correlation between C.V. and percentage of IIB fibres ($r = 0.23$; $P < 0.001$).

DISCUSSION

In this study, biopsy specimens from both left and right muscles were taken in order to obtain duplicate data. Variation in fibre types has been shown to be small when samples have been taken from the same location (Kai, 1984; López-Rivero et al. 1991) or from contralateral sites (Essén-Gustavsson et al. 1989) within the gluteus medius of the horse. The most marked variation, seen in the percentages of fibre IIB subtypes (IIB nonoxidative and IIB oxidative; Table 1), could be due to the spectrum in reaction intensities for NADH-TR in the fast twitch fibres, and subjective decisions must be made about where to draw the dividing line between IIB nonoxidative and IIB oxidative fibres (Armstrong & Phelps, 1984).

Results from this study are consistent with the histochemical findings from other previous studies (Kai, 1984; Bruce & Turek, 1985; Kline et al. 1987; Kline & Bechtel, 1988; López-Rivero et al. 1992*a*). However, information can now be provided regarding intramuscular variation in the size of various fibre types within equine muscle. Type I and IIA fibres in the deep regions are larger than in superficial regions, while IIB fibres in the deep regions are smaller than in the superficial parts of the muscle. Our results also imply that type I fibres tend to be larger than type II (A and B) fibres in the deep regions. Moreover, it is believed that the size of type I fibres is more homogeneous in the deep parts than in the superficial regions of the muscle; by contrast, type IIB fibres vary more in size in the superficial areas than in deep regions. No previous study has detected any clear systematic variation in fibre size within an equine muscle.

From the available literature, there is sufficient evidence that the regional distribution of fibre types and fibre sizes within a muscle appears to reflect structure-function interrelations (e.g. Armstrong & Phelps, 1984; Bredman et al. 1990). The distribution of fibre types in the gluteus medius therefore indicates a functional differentiation of the muscle. The deeper regions of the muscle seem best suited for posture maintenance and lower-level but longer-duration activity. Muscle fibre size is known to be related to muscle use, training increasing (López-Rivero et al. 1992*b*) and inactivity decreasing (Nicks et al. 1989) the fibre size. As different parts of the muscle are used during different phases of movement, the functional demands on the fibre population differ and the fibres develop different properties. Thus the high proportion of type I fibres in the deeper regions of the gluteus medius muscle, as an indication of a more postural function, corresponds well with the larger size in this part of the muscle noted in the current study. Conversely, the fibre type distribution and size of the most superficial parts of the muscle indicates a completely different pattern of use. These regions of the muscle seem to be more involved with short duration, rapid, propulsive force generation.

In areas of muscle with a large proportion of type I fibres, individual I fibres were also relatively large. This suggested that there may be a direct proportionality between the frequency of occurrence of fibres of a given type in the muscle and the size of the fibres. To study this relationship the mean LFD and CSA of each of the fibre types in all biopsy specimens were regressed on the percentages of the fibre type in the respective biopsy specimens. For type I fibres there was a close relationship (LFD, $r = 0.69$; CSA, $r = 0.77$; $P < 0.001$). For IIB fibres, there was also a statistically significant relationship ($P < 0.05$), but the correlation coefficient was relatively low (LFD, $r = 0.22$; CSA, $r = 0.24$). Type IIA fibre sizes were not related to IIA fibre populations (LFD, $r = -0.13$; CSA, $r = -0.14$; $P > 0.05$). Thus in those parts of the gluteus medius muscle with larger populations of either I or IIB fibres, the respective fibres were of relatively larger size.

Results from a previous study showed that no significant differences in fibre proportions existed within a defined area and depth on the equine gluteus medius muscle when biopsies were taken at an absolute depth of 5 cm (Wood et al. 1988). The systematic, and significant, difference in the percentage of fibre types recorded in this study between dorsal and ventral sites of the muscle, at the same absolute depth (Table 5), could be due to the fact that this muscle has two distinct anatomical regions, dorsal and ventral, separated by a thick tendinous sheet, and differentiated by architecture and nerve innervation patterns (Bruce & Schurg, 1990). However, in a previous report, we found that both regions contain a similar proportion of slow-twitch and fast-twitch fibres at the same relative depth (López-Rivero et al. 1992*a*). Because the muscle mass in changing, another possible explanation could be that a biopsy taken from the ventral region has a position within the gluteus medius muscle relatively deeper than a biopsy specimen taken from the dorsal region at the same absolute depth.

Although our horses were of the same breed and gender, of similar age and body weight, had received the same diet and had lived in the same conditions, differences in fibre types and fibre sizes between individuals were significant (Table 4). Clearly, this

background variation, possibly related to genetic factors, must be heeded in the design of cross sectional studies involving this muscle. This variability is in accordance with results from human (Lindman et al. 1991) and other animal studies (Newsholme et al. 1988; Bredman et al. 1990), including those of the horse (Wood et al. 1988; López-Rivero et al. 1991).

It is widely documented that type II fibres are larger than type I fibres in the gluteus medius muscle of the adult horse (Essén-Gustavsson et al. 1989; López-Rivero et al. 1992*b*). In the study reported here, this concept was not entirely confirmed. Type I fibres tended to be larger than type II (A and B) fibres in the deep regions (Fig. 2). Similarly, IIA fibres showed a clear tendency to be larger than IIB fibres in the deep regions. Thus results regarding the predominance in size of a given histochemical fibre type must be treated with some caution, unless several biopsy specimens are taken or the biopsy site is clearly defined.

The more homogeneous size of fibre types in the deep parts compared with superficial regions of muscle, may also be the result of the way the 2 portions of the muscle are used. Muscle fibres in the deep parts are probably only recruited for posture maintenance, but the most peripheral regions are used in a wider range of dynamic activities (walking, trotting, cantering, galloping). According to this argument, the difference in functional demands would then cause a greater variation in the LFD of types I and IIB fibres in the superficial regions, expressed by a larger coefficient of variation.

In conclusion, our results confirm that a single biopsy taken from the gluteus medius of the horse is a poor representative of the whole muscle, that care should be exercised in sampling and interpreting data obtained from limited biopsy of this muscle, and that the sample site must always be well defined. The pattern of variation of our morphometric findings probably indicates the functional differences between various parts of the muscle and the capacity of the fibre population to adapt to the different physical demands.

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