

Muscle Glycogen Depletion and Repletion Patterns in Horses Performing Various Distances of Endurance Exercise

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Summary

Biopsy samples were collected from the left middle gluteal muscle of horses participating in three competitive endurance rides. Six horses were sampled the day before and 30 minutes after completion of 40 km (Ride 1), nine horses were sampled the day before and 30 minutes after 110 km (Ride 2), and eight horses were sampled 30 minutes after completing 160 km (Ride 3). In addition, muscle samples were collected 24 and 48 hours after Ride 2 and 20 hours after Ride 3.

Serial muscle sections were reacted histochemically for myosin adenosine triphosphatase activity after acid pre-incubation and for glycogen content, using the periodic acid Schiff reaction. When the rides were compared, there was progressive recruitment from I through to IIB fibres, according to the distance completed. This appeared to be independent of the speed of exercise, as horses in all rides were exercised at similar intensities. Elite horses in Ride 3 had higher percentages of type I and type IIA fibres and lower percentages of type IIB fibres than the horses in the two other rides which were considered to be only average competitors. There was, however, considerable variation in muscle fibre types when the nine horses in Ride 2 were sampled repeatedly in the same muscle. This would appear to limit any possible predictive value of muscle biopsy.

Introduction

Endurance rides are one of the most demanding forms of equine competitive exercise. While extensive research work has been undertaken to examine the haematological changes (Carlson 1975; Carlson *et al.*, 1976; Rose *et al.*, 1979; Snow *et al.*, 1982) and the plasma biochemical changes associated with endurance exercise of 80–160 km (Carlson and Mansmann, 1974; Rose *et al.*, 1977; Lucke and Hall, 1978; Lucke and Hall, 1980a, 1980b; Snow and Rose, 1981; Rose and Sampson, 1982; Snow *et al.*, 1982), the changes occurring in muscle have received less attention (Snow *et al.*, 1981; Snow *et al.*, 1982).

In man, it is known that varying workloads alter metabolic requirements in skeletal muscle (Essén 1978), and reports of muscle glycogen depletion patterns have shown variations associated with the intensity and duration of exercise (Costill *et al.*, 1973; Essén 1978). While there have been some reports of glycogen depletion in horses undergo-

ing endurance exercise (Lindholm *et al.*, 1974; Lindholm 1979; Snow *et al.*, 1982), none of these studies involved distances greater than 80 km. This investigation was undertaken to examine muscle glycogen depletion and repletion patterns in horses undertaking competitive endurance exercise of varying distances up to 160 km.

Materials and Methods

Muscle biopsy samples were collected from horses participating in three competitive endurance rides. Six horses were sampled the day before and 30 minutes after completion of 40 km (Ride 1), nine horses were sampled the day before and 30 minutes after 110 km (Ride 2), and eight horses were sampled 30 minutes after completing 160 km (Ride 3). In addition, muscle samples were collected 24 and 48 hours after Ride 2 and 20 hours after Ride 3.

All rides were held over rugged bush terrain, with Ride 1 being less demanding than Rides 2 and 3. Rides 1 and 3 were held in winter (June), while Ride 2 was conducted in summer (December).

All muscle samples were collected percutaneously with a 5 mm biopsy needle from the left middle gluteal muscle 15 cm caudodorsal to the tuber coxae at a depth of 10 cm, using the technique described by Lindholm and Piehl (1974). This muscle was selected as it has been shown to be active at all exercise intensities (Lindholm 1973). Where more than one muscle biopsy was collected from the same horse, these samples were taken within a 2 cm² area of the first sample.

Upon collection, part of the muscle sample was prepared for histochemical analysis. The sample was mounted on to cork blocks with methylcellulose and frozen in isopentane cooled to -80°C in dry ice. All samples were stored at -196°C .

Histochemical analysis involved cutting transverse serial sections on a cryostat microtome at -20°C and mounting the sections on cover slips for staining. Sections (10 μm) were reacted for myosin adenosine triphosphatase (ATP-ase) after acid buffer pre-incubation at pH 4.6 (Essén *et al.*, 1980). To assess muscle glycogen content, sections (20 μm) were stained using the periodic acid Schiff (PAS) method. This stain has been shown to correlate well with total muscle glycogen (Snow *et al.*, 1981). Photomicrographs of each section were taken and muscle fibres identified as type I, IIA or IIB according to their characteristic myosin ATP-ase staining patterns (Brooke and Kaiser, 1970). By comparing these with serial sections stained for PAS, the relative glycogen content of individual fibres was determined. Glycogen content in each of these fibres was classified into one of five categories: very high (+++), high (+++), moderate (++), low (+) and negligible (0). At least 300 fibres were counted in each sample.

The percentages of type I, IIA and IIB fibres were determined in each horse pre ride and post ride (Rides 1 and 2), the 24-hour and 48-hour post-ride samples from horses in Ride 2, and only the post-ride samples from horses in Ride 3. A comparison of pre-ride and post-ride fibre type percentages was made using a paired Student's 't' test. To compare the fibre type percentages between horses in the three rides, a one-way analysis of variance was performed, and where F values were significant, a calculation of least significant difference was made. In the nine horses in Ride 2, four muscle samples were obtained from each horse. The mean, standard error and coefficient of variation were calculated for the observations of fibre type percentages in each horse.

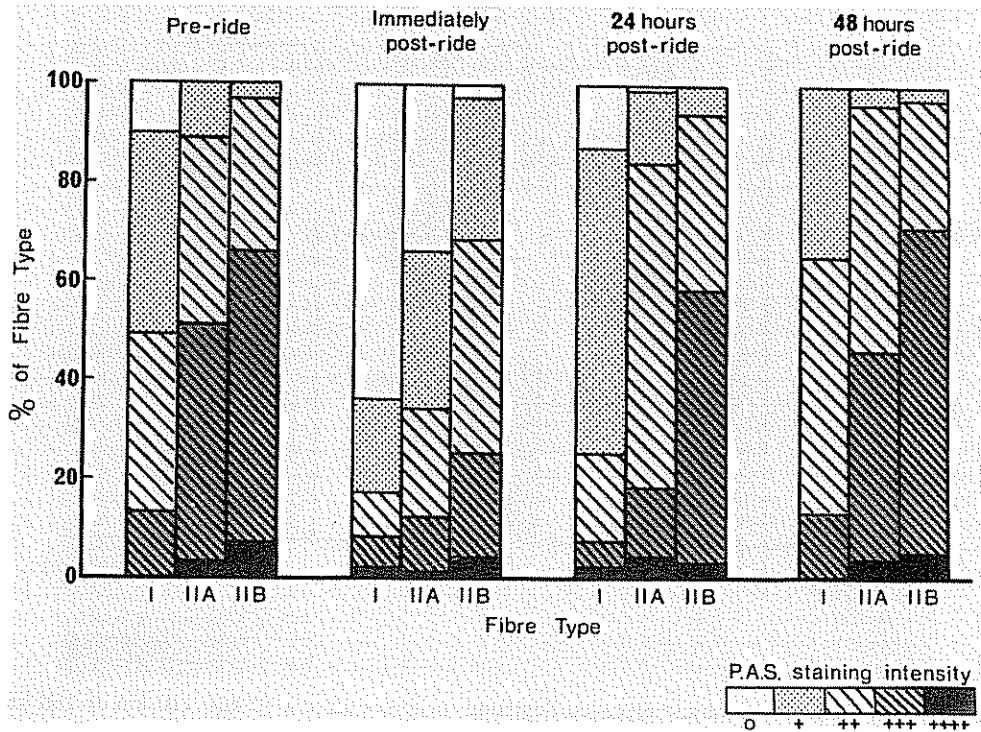


FIGURE 1. Patterns of muscle glycogen depletion and repletion in horses competing in a 110 km endurance ride.

Results

The mean (per cent) results of PAS staining intensity in types I, IIA and IIB fibres before and up to 48 hours after Ride 2 are presented in Fig. 1. A comparison of the mean (percentage) staining intensity in the samples collected 30 minutes post ride is shown for all three rides in Fig. 2.

Although there was some variation in the percentage of type I, IIA and IIB fibres when pre-ride and post-ride samples were compared, this was not significant ($p > 0.05$). Horses in Ride 3 had significantly higher percentages ($p < 0.01$) of type I fibres and significantly lower percentages ($p < 0.02$) of type IIB fibres than horses in Ride 1. Horses in Ride 2 had significantly lower percentages ($p < 0.05$) of type IIA and significantly higher percentages ($p < 0.01$) of type IIB fibres than horses in Ride 3. There were no significant differences in muscle fibre types between Rides 1 and 2. The results (mean \pm SE) for the percentage fibre types in each of the rides are presented in Table 1. The results of variation in fibre type percentages associated with repeated sampling of horses in Ride 2 are presented in Table 2.

Discussion

In all three endurance rides studied, the mean speed of exercise was similar. Therefore, the observed patterns of muscular glycogen depletion would appear to be related solely to

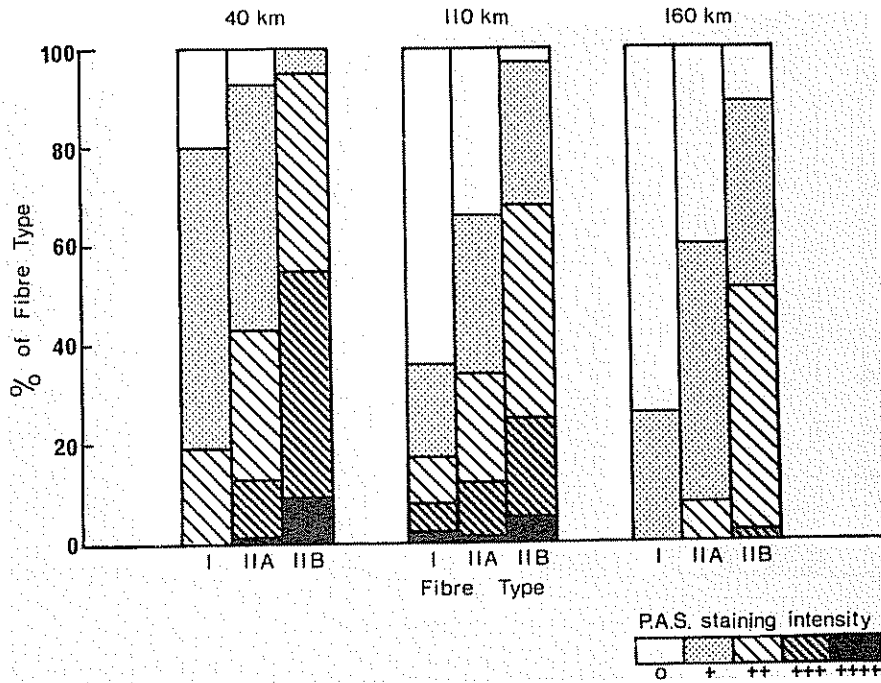


FIGURE 2. Patterns of muscle glycogen depletion in horses after a 40 km, 110 km or 160 km endurance ride

the variations in the ride distance. In all resting samples, type I fibres had lower glycogen contents than either the IIA or IIB fibres, confirming observations of Lindholm *et al.* (1974), Snow *et al.* (1981) and Snow *et al.* (1982). The only previous observations of glycogen depletion patterns in endurance horses are those of Snow *et al.* (1981) and Snow *et al.* (1982) in which twelve and four horses, respectively, were studied after 80 km rides. In this study, observations of progressive patterns of glycogen depletion with increasing distances of exercise were possible. The results showed that in all rides type I fibres

TABLE 1 Percentages of muscle fibre types (mean \pm SE) in horses competing in three different endurance rides

Fibre type (%)	Ride 1 (40 km) n = 6	Ride 2 (110 km) n = 9	Ride 3 (160 km) n = 8
I	20.7 \pm 3.5 ***	25.1 \pm 1.3	31.8 \pm 3.6
IIA	44.3 \pm 4.1	36.4 \pm 1.6 *	46.4 \pm 2.5
IIB	35.8 \pm 7.3 **	38.4 \pm 1.6 ***	20.6 \pm 4.5

* p < 0.05
 ** p < 0.02
 *** p < 0.01 } Significant differences from Ride 3.

TABLE 2. Variations in muscle fibre types associated with repeated sampling of the left middle gluteal in horses competing in a 110 km endurance ride.

Horse	Fibre types (%)					
	I		IIA		IIB	
	Mean	CV	Mean	CV	Mean	CV
Sandi	23.3	8.9	43.8	6.8	23.0	6.1
Kelly	24.0	10.2	34.8	10.9	41.3	12.4
Tasha	21.5	21.0	40.3	8.2	40.0	8.9
Smokey	24.8	12.3	38.3	4.5	36.8	6.0
Gideon	18.8	31.5	37.3	8.3	44.0	19.7
Tira	21.3	24.7	40.0	14.7	38.8	12.5
Freckles	20.5	26.0	30.3	1.7	49.3	11.5
KB	19.8	33.9	40.3	8.2	40.0	8.9
Snake	25.3	5.9	35.0	7.0	39.8	8.1

CV: coefficient of variation.

showed the most glycogen depletion, with the most extensive depletion found in horses after the 160 km ride. In these horses, more than 70% of type I fibres showed zero glycogen content. In three horses ridden 25 km to attend the 110 km ride, glycogen depletion was found almost exclusively in the type I fibres in samples collected pre ride. As the ride distance increased, progressive recruitment of type IIA and IIB fibres was evident. However, after both the 40 km and 110 km rides the IIB fibres still contained substantial amounts of glycogen. While the same relative patterns of depletion existed in horses after the 160 km ride, the IIB fibres showed substantial glycogen depletion. In one horse, all three fibre types showed almost complete absence of glycogen. These findings of progressive fibre type recruitment from I through to IIB have been found in horses after shorter distances of exercise (Snow *et al.*, 1982), while similar findings have been reported in rats during prolonged swimming (Armstrong *et al.*, 1975) and in men performing heavy bicycle exercise (Andersen and Sjogaard, 1976). While some recruitment of IIB fibres was evident in the present study, after the 40 km ride almost 60% of these fibres still exhibited a high glycogen content.

The mechanism for the progressive fibre type recruitment appears to be related to fibre contractile properties. In a controlled study of the human medial gastrocnemius muscle, intramuscular microstimulation was used to study the various motor units (Garnett *et al.*, 1979). It was found that type I and IIA fibres were employed at low force levels, while IIB fibres were recruited in stronger contractions. A similar deduction can be made from the work of Lindholm (1979) where progressive recruitment of type I, IIA and IIB fibres was apparent when increasing speeds of exercise were examined in Standardbred horses.

From the evidence of these combined studies, it would appear that there are two separate mechanisms for muscle fibre recruitment. Increased exercise intensity will lead to progressive involvement of the faster contracting, more powerful fibres. However, if prolonged lower intensity exercise is performed, progressive recruitment from I through to IIB fibres will take place.

Glycogen repletion occurred in the reverse pattern to depletion, there being preferential repletion of type IIB relative to type I fibres. This confirms a previous study by Snow *et al.* (1982) of four horses and is similar to a report in man (Piehl 1974). In this study we found that after 24 hours, the IIB fibres had a similar glycogen content to their pre-ride levels in Ride 2 horses. However, in horses in Ride 3, sampled 18 to 20 hours after the 160 km ride, repletion was apparently slower, with no IIB fibres being classified in the high intensity category for PAS staining. Repletion in type I and IIA fibres in horses in Ride 2 was not complete until 48 hours post exercise. This is similar to the findings of Piehl (1974) where complete glycogen repletion took 46 hours, following exhaustive exercise in man.

The preferential repletion of type IIB fibres has been related to differing glycogen synthetase (GS) activity in type II fibres (Snow *et al.*, 1982). GS is present in human muscle in two forms, a highly active I-form and a less active D-form. After exercise-induced muscle glycogen depletion, a rapid increase in the I-form of GS occurs without concomitant increases in total GS levels (Piehl *et al.*, 1974). Therefore, the preferential glycogen repletion found in equine type IIB fibres, following exercise, could be related either to higher total GS levels in type IIB fibres or to an adaptational response to produce the more active I-form of GS in these fibres. A differential pattern of GS activity or difference in total GS levels between type I and type II fibres could explain their relative differences in resting glycogen content.

When the muscle fibre types were compared for the horses in the three rides, there were significant differences between Ride 3 and Rides 1 and 2. Ride 1 was a low-standard endurance ride for inexperienced competitors, while Ride 2 was of intermediate standard. Ride 3 is the most difficult ride in Australia and considered to be the premier competitive endurance ride. Furthermore, whereas the horses in Rides 1 and 2 were only average competitors, the eight horses in Ride 3 finished within the first 13 places out of a field of 65 horses and could therefore be considered to be elite endurance horses. In general, these elite horses showed higher percentages of type I and IIA fibres with lower percentages of type IIB fibres in their middle gluteal muscles than the average competitors in Rides 1 and 2. In human endurance events, there have been several reports equating a high proportion of slow twitch fibres in active muscles with superior performance (Costill *et al.*, 1976; Jansson and Kaijser, 1977). In 16 horses studied at an 80 km endurance ride, Snow *et al.* (1981) also found a higher percentage of slow twitch fibres in the middle gluteal muscle of the best performers. Whether genetic factors or training influence the distribution of fibre types within muscle is still unresolved.

To have any useful predictive value, the muscle biopsy sample must be representative of the particular muscle sampled. However, in the nine horses in Ride 2, each sampled on four occasions, there was a substantial coefficient of variation in some horses, particularly with regard to type I fibres. In two horses the coefficients of variation were greater than 30% for the type I fibres. This was despite the fact that all horses were sampled in the same 2 cm² area of the middle gluteal muscle and all samples were collected at the same depth. This variation in fibre type percentages with repeated sampling is contrary to a previous report by Lindholm (1973) and requires further investigation on a large sample of horses.

This study demonstrates that there is a direct correlation between the extent of muscle glycogen depletion and the duration of exercise, with progressive recruitment of type I,

type IIA and finally type IIB fibres. Repletion of glycogen occurs in IIB muscle fibres first but may not be complete in all fibres for as long as 48 hours. Exercise should therefore be restricted in this post-ride period to allow maximal repletion of muscle glycogen stores.

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