Horse Soleus Muscle: Postural Sensor or Vestigial Structure?

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ABSTRACT

The soleus muscle of horses is rather diminutive with respect to the overall size of adjacent synergist muscles in the hind limb of the horse. Whether or not such a muscle might be vestigial or may be providing some essential function has not been determined. We have studied the horse's soleus muscle using histochemical (ATPase), immunocytochemical (myosin isoform identification), and SDS-PAGE analysis to demonstrate that it is largely composed of 100% type I, presumed slow-twitch fibers. Only one soleus muscle studied (out of 13 adult horses) contained any type II muscle fibers. Given this consistent high percentage of slow-oxidative fibers, we hypothesized that the soleus muscle could have a significant role in proprioceptive function, essentially functioning as a proprioceptive organ instead of a significant force-generating muscle during locomotion. We tested this by examining three whole soleus muscles and assessing their muscle spindle content, which proved to have a spindle index of about 12. This value provided equivocal support for the hypothesis since it did not approach values reported for other mammalian proprioceptive muscles that were approximately 40–50 spindles per gram of muscle mass. Other parameters, such as motoneuron number and muscle unit size, may be useful in understanding these data. Anat Rec Part A, 288A:1068–1076, 2006. © 2006 Wiley-Liss, Inc.

Key words: muscle; proprioception; soleus; *Equus*; muscle spindles; fiber types

A general tendency of small muscles associated with fine movements, or slow-twitch muscles associated with posture, is that they tend to possess greater numbers of muscle spindles than do larger or faster muscles (Buxton and Peck, 1990; Botterman et al., 1978). Many small muscles show very high counts of spindles per gram of wet muscle tissue, termed the spindle index. Muscles with a high spindle index (approximately 40–50 spindles per gram of wet muscle weight) (cf. Richmond and Abrahams, 1975) are believed to function in a proprioceptive capacity as suggested by their relatively high number of spindles. Do all small, posture-related muscles possess high spindle indexes? At the high end, the cat fifth interosseus muscle had a spindle index of 119 (Barker and Chin, 1960), the guinea pig anconeus and lumbrical 150 and 988, respectively (Buxton and Peck, 1990), and the pigeon coracotriceps 14,582 (that muscle weighed only 0.002 g) (Rosser and George, 1985). The horse articularis humeri had an index of 29 (Lalatta-Costerbosa et al., 1992), while the horse articularis coxae muscle had an index of 26 (Kjaersgaard, 1980). The latter two muscles are diminutive and are associated with specific actions about their affiliated joint capsules. Botterman et al. (1978) summarized results for "mammalian" soleus (rat and cat) as possessing a mean index of 23 (based on Swett and Eldred, 1960; Chin et al., 1962). Muscles with hypothesized dynamic (work-producing) roles tended to have relatively lower spindle indexes: cat lateral gastrocnemius only had a spindle index of 5, whereas that of the cat medial gastro-

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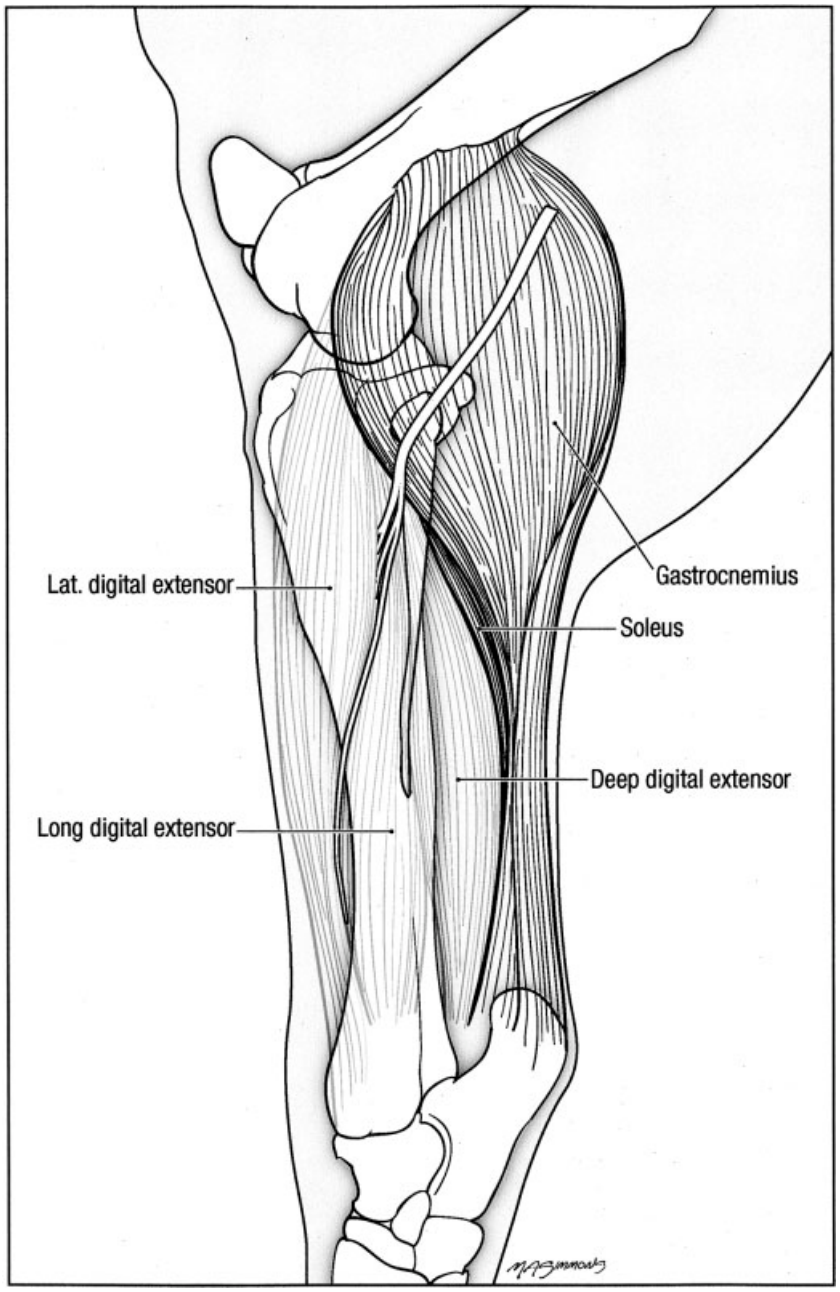

Fig. 1. Gross structure of the soleus muscle in a left lateral view. The soleus muscle is rather diminutive compared alongside the gastrocnemius muscle complex. In this view, the knee or "stifle" is near the top and the ankle or "hock" is near the bottom. The large gastrocnemius muscle attaches proximally (top) along the femur and attaches distally on the calcaneus. The soleus originates along the lateral fibular head region and becomes attached along the distal, deep surface of the lateral gastrocnemius muscle. The common peroneal nerve (white) is illustrated as it courses over the lateral surface of the gastrocnemius muscle. All thigh muscles were removed for this view.

cnemius was 9 (Chin et al., 1962). The review by Botterman et al. (1978) emphasized the correlation of muscles or muscle compartments characterized by high oxidative muscle potential (referred to as type SO or FOG fibers) with high densities of muscle receptors, including spindles as well as Golgi tendon organs. These authors concluded that muscle spindles were best correlated with muscles (and fiber types) "subserving fine movements" (Botterman et al., 1978: p. 135). There were more spindles located in muscles that control fine movements, such as those effecting head position or joint stability in antigravity situations, than were found in muscles used for coarse and powerful movements. While proprioceptive reflex control mechanisms probably have a role in the control of fast, coarse

TABLE 1. *Equus caballus* specimens used in this study

Animal	Breed	Sex	Age	Soleus mass (g)	Animal body mass (kg)
i	TB	M	1d	4.9	49
ii	TB	M	1d	4.2	41
iii	TB	M	1d	2.4	41
iv	TB	M	1d	2.7	31
1	TB	F	4	6.0	400
2	TB	F	5	8.6	454
3	QH	Mc	6	4.4	590
4	TB	Mc	6	9.1	469
5	TB	Mc	7	7.1	431
6	TB	F	7	7.1	383
7	TB	Mc	9	7.8	590
8	TB	M	12	5.1	558
9	TB	Mc	15	5.6	497
10	QH	F	16	7.4	473
11	TB	Mc	17	6.2	544
12	TB	F	18	4.4	499
13	TB	Mc	25	7.4	394
14	Pony	Mc	10	3.5	123

Breeds included Thoroughbreds (TB) and Quarter Horses (QH) and one pony of unknown genetic origin. Castrated males are indicated by a "c." Age is given in years except for four foals, which were all 1 day old.

movements, these control mechanisms appear to have a more clear relationship to the modulation of slow, fine movements in the limbs. The latter movements were generally associated with muscles or muscle compartments composed primarily of FG (type IIb) fibers. Paradoxically perhaps, the human soleus had a spindle index of less than 1 (Voss, 1971).

We speculated that the equine soleus might prove interesting to study for several reasons. The soleus muscle is extremely small in horses: a 500 kg animal may possess a soleus weighing only about 7 g or less (data not shown). This raises a question about the functional role of the soleus. Although the muscle appears too small to contribute significant force (either in quiet standing or in locomotion), it retains a high type I muscle fiber type profile (nearly 100%) (Meyers et al., 1998), which is characteristic of most mammalian soleus muscles (Ariano et al., 1973). We questioned whether its small size was a byproduct of the equine passive stay apparatus (a mechanism described in the horse's limbs whereby nonfatiguing adaptations of specific muscles facilitate stance or resistance to gravity) (Hermanson and MacFadden, 1996; Dyce et al., 2002). Because muscles containing large areas of slow fibers are known to be correlated with high spindle densities (Botterman et al., 1978; Maier, 1999) and because of the small size of the muscle, we wondered if the horse soleus might be used in a proprioceptive role such as was proposed for the articularis humeri of horses (Lalatta-Costerbosa et al., 1992) or the coracotriceps of the pigeon (Rosser and George, 1985). The soleus extends from the proximal fibula to the cranial border of the lateral gastrocnemius muscle and then to the calcaneal tendon (Sisson, 1975; Nickel et al., 1986). Thus, it crosses the hock (ankle) joint and might function to monitor the spatial orientation of the distal hindlimb and the tarsocrural joint (the hock) in particular (Fig. 1). Alternatively, it could contribute to tension in the calcaneal tendon, which is dominated by the larger medial and lateral gastrocnemius forces as well as several other muscles, or simply monitor the tension in this tendon.

Our goals, therefore, were to examine the equine soleus muscle with respect to its fiber type composition, and number and distribution of muscle spindles, to assess if this muscle possesses high density of spindles consistent with other studies examining proprioceptive muscles. A preliminary discussion of this study was presented by Meyers et al. (1998). In light of the rather drastic reduction of many muscles to ligamentous (such as the equine hindlimb's superficial digital flexor or peroneus tertius muscles) (Dyce et al., 2002), we sought to characterize the status of the equine soleus muscle. Is this muscle vestigial or on a vestigial trajectory, or does it have the potential to provide important afferent feedback regarding knee position?

MATERIALS AND METHODS

Soleus muscles from 1 pony, 4 foals, and 13 adult horses (*Equus caballus*) were obtained at postmortem within 2 hr of death (Table 1). All animals were euthanized by an intravenous overdose of barbituate euthanasia solution because of medical or surgical considerations that excluded locomotory abnormalities. Muscle samples were collected from the right hindlimb. Samples used in histochemistry were trimmed and positioned in a cold slurry of 5% gum tragacath and mounted on a cork block. The sample was immersed in isopentane cooled to about −150°C in liquid nitrogen. The frozen samples were stored in a freezer at −80°C until used.

Transverse serial sections were cut at 10 μm thickness in a freezing microtome (IEC, Needham, MA) and mounted on clean glass slides for histochemistry, or on clean gelatin-coated glass slides for immunocytochemistry. Histochemical procedures followed that of Hermanson and Hurley (1990). Briefly, sections were stained for myofibrillar ATPase following acid (pH ranges from 4.0 to 4.6) and alkaline (pH 10.3) preincubation. Identification of three fiber types (type I, IIa, and IIb/x) in horse muscles could generally be obtained following acid preinculation at pH 4.45. Serial sections were also stained for nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), a marker of oxidative potential, and for α-glycerolphosphate dehygrogenase (α-GPD), an indicator of glycolytic or anaerobic potential.

Immunocytochemical protocols followed that of Serrano et al. (1996). Sections were blocked in 2% normal goat serum for 30 min. Primary antibody (about 50–100 μl) was applied to the sections for 12–14 hr at 4°C. Primary antibodies used included S58, an antislow myosin antibody provided by Dr. Frank Stockdale; MY 32, an antifast myosin antibody from Sigma Chimical (St. Louis, MO); NCL-MHCs, an antislow myosin antibody; and NCL-MHCf, an antifast myosin antibody from Novacastra Laboratories (Newcastle Upon Tyne, U.K.). The slides were then rinsed in 0.05 M PBS with 0.85% NaCl solution and washed for 10 min in the same solution. This was followed by incubation with a biotinylated rabbit antimouse secondary antibody for 10 min at room temperature. Subsequent washes in PBS were followed by reaction with a strepavidin enzyme conjugate and another PBS wash. Final

TABLE 2. Summary of horses and foals used in this study to document the fiber type composition and sizes in the proximal and distal region of the equine soleus muscle

	Proximal region				Distal region			
Animal	Percent type I	Type I diameter	Percent type II	Type II diameter	Percent type I	Type I diameter	Percent type II	Type II diameter
i	100	45	0	–	100	37	0	–
ii	100	39	0	–	100	40	0	–
iii	100	31	0	–	100	28	0	–
iv	100	35	0	–	100	37	0	–
1	100	26	0	–	100	26	0	–
2	100	41	0	–	100	40	0	–
3	100	35	0	–	100	33	0	–
4	94	50	6	53	89	51	11	–
5	100	28	0	–	100		0	–
6	100	40	0	–	100	41	0	–
7	99	37	1	45	100	29	0	–
8	100	30	0	–	100	30	0	–
9	100	33	0	–	100	39	0	–
10	100	33	0	–	100	31	0	–
11	100	38	0	–	100	41	0	–
12	100	28	0	–	100	42	0	–
13	100	38	0	–	100	40	0	–
14	100	32	0	–	100	34	0	–

All diameters are means for the individual, given in μm.

staining was achieved with a DAB reaction (Invitrogen, Chicago, IL).

Additional samples were obtained from each muscle adjacent to the histochemistry samples and were snap-frozen (−196°C) in liquid nitrogen and stored at −80°C for electrophoretic analysis of myosin heavy chain isoforms. Myosin heavy chain isoforms were studied with the protocol of LaFramboise et al. (1990) and modifications derived from Blough et al. (1996). A sample of rat costal diaphragm or of little brown bat (*Myotis lucifugus*) pectoralis muscle was run on each gel as a control to assess differences in mobility between gels. The rat muscle was useful because it provided three or four MyHC isoforms (type I, IIa, IIx, and in many cases IIb).

Myosin was extracted from minced muscle on ice for 30 min in four volumes of a high salt buffer (300 mM NaCL, 100 mM NaH_2PO_4, 50 mM Na_2HPO_4, 1 mM $MgCl_2$, 10 mM $Na_4P_2O_7$, and 10 mM EDTA: pH adjusted to 6.5). Resultant extracts were centrifuged at 13,000 rpm for 30 min at 2°C. Supernatants were diluted in nine volumes of 1 mM EDTA buffer, vortexed, and allowed to precipitate for 12 hr at 4°C. The sample was again centrifuged and the resulting pellet dissolved in 0.5 M NaCl and 10 mM $NaPO_4$ and denatured by heating to 100°C for 2 min. The samples were diluted 1:100 in SDS buffer [62.5 mM Tris/HCL, 2% (w/v) SDS, 10% (v/v) glycerol, and 0.001% (w/v) bromophenal blue: pH adjusted to 6.8]. Electorphoretic separation was performed in a 4.8% separating gel with 30% (v/v) glycerol and a 3% stacking gel without glycerol. Ten to 15 μl aliquots of the diluted mysoin sample were placed in each lane and electrophoresed for 22 hr at 120 V at 14°C. The separating gels were silver-stained following Oakley et al. (1980). Gels were dried between two layers of cellophane and sealed for long-term storage.

Black-and-white photographs were obtained from identical regions of soleus muscles from serial sections treated for acid preincubation mATPase, α-GPD, NADH-TR, one of the antislow myosin antibodies, and an antifast myosin antibody. Myofibers were classified as type I, IIa, IIb/x following Rivero et al. (1996a, 1996b). A minimum of 1,000 fibers were sampled to assess fiber type percentages, while 40 fibers of each fiber type were measured per muscle sample to determine fiber types sizes (Hermanson and Hurley, 1990).

Whole soleus muscles were obtained from three adult horses and cut into blocks approximately 1–2 cm long. These in-series blocks were mounted for histochemistry or histology as described above, and the entire muscle was sampled to study muscle spindle density. To identify intrafusal fibers, mATPase after acidic preincubation was used on samples from two of the animals. A hematoxylan stain was employed on one pony muscle and the position of the spindles was tracked through this muscle.

Finally, one whole soleus muscle was dissected free from one pony and fixed for 10 days in 10% formalin solution. The muscle was then chemically dissected in nitric acid (3–15%, depending on the state of the dissection) over 2 days (based on gross appearance of the fibers as they became free from the connective tissue matrix). Once the muscle fiber bundles became disassociated, the solution was replaced with 50% glycerol (v/v) and finally a 100% glycerol. Fiber bundles were placed in a Petri dish with glycerol and examined under a dissecting microscope. Individual fibers from eight bundles representing different portions (ranging from proximal to distal) of the muscle were teased apart and measured. Eighty fibers were measured in total. Fiber lengths were then standardized by measuring the mean length of sarcomeres viewed under a microscope: 100 sarcomeres were measured and this value was normalized to a sarcomere length of 2.5 μm.

RESULTS

All foals examined contained 100% type I (slow-twitch) muscle fibers. While most of the adult muscles examined contained 100% type I fibers, samples from 2 (out of 14) ani-

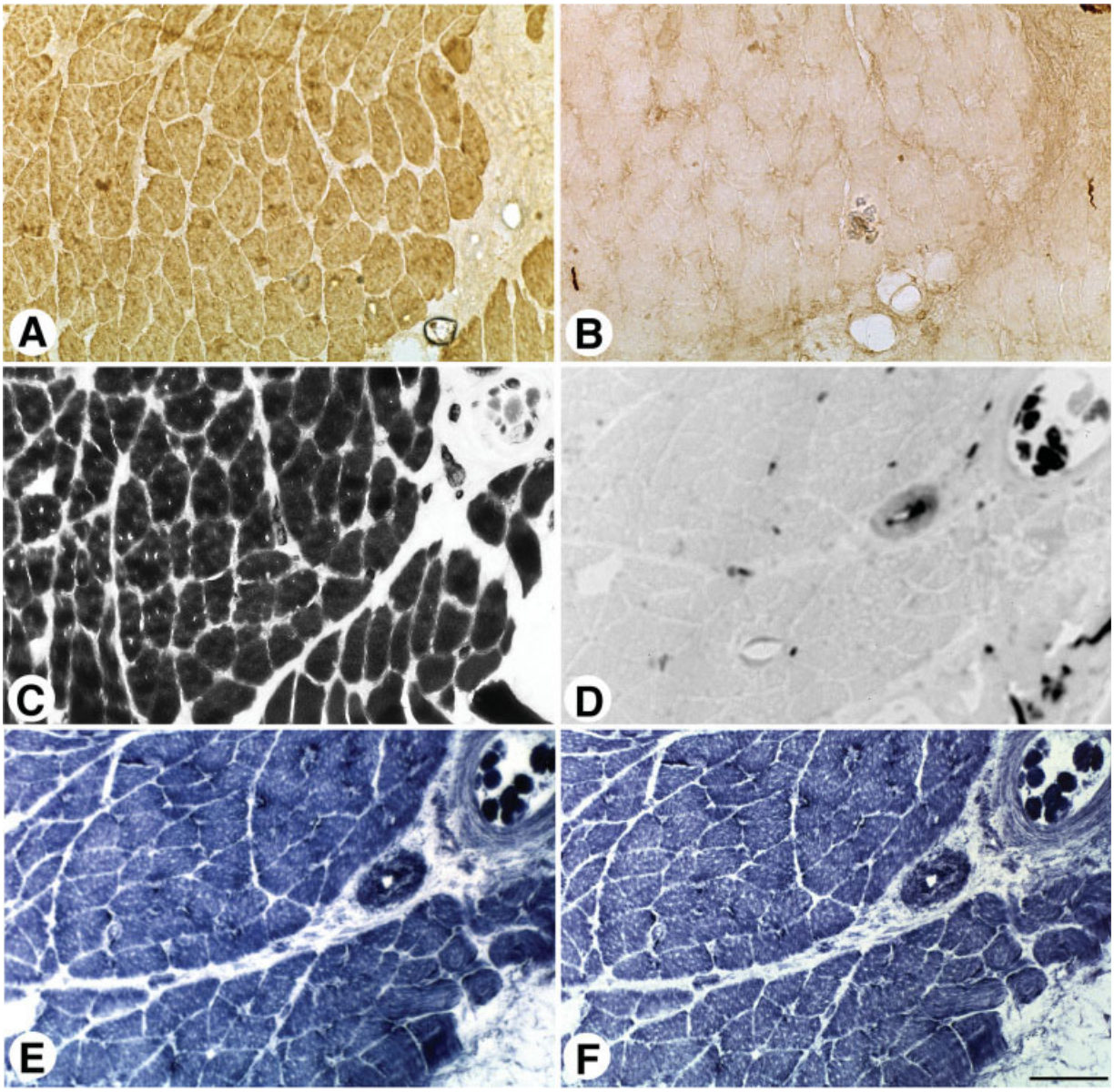

Fig. 2. Serial sections of the soleus muscle from a horse, *Equus caballus*. **A:** Antislow antibody reaction with red-stained fibers being positive for type I myosin. **B:** Antifast antibody reaction against MY32 showing a lack of positive reaction in muscle fibers. **C:** mATPase histochemistry after preincubation at pH 4.3 with dark fibers being type I, presumed slow-twitch fibers. **D:** mATPase reaction after preincubation at pH 10.3 and showing the only positive (dark stain) reactions in intrafusal fibers at the top right, and in some vascular structures (arterioles and capillaries). **E** and **F:** α-GPD and NADH-TR studies showing a nearly uniform staining intensity among all fibers. Scale bar = 100 μm.

mals contained a relatively small proportion of type II fibers (1–11%) (Table 2). All fibers demonstrated strong oxidative potential based on the NADH-TR assay, and little or no anaerobic potential based on weak staining in the α-GPD protocol. Correlation of these observations with reaction against either antislow myosin heavy chain antibodies confirmed the histochemical interpretations (Fig. 2). Thus, for example, all type I fibers identified with the mATPase protocol were also strongly reactive against the antislow myosin antibodies. Finally, SDS-PAGE analysis of the same muscles yielded agreement in that single bands were observed on the gels that comigrated with type I myosin we have seen from other horse muscles (Rivero et al., 1996b; Serrano et al., 1996) and rat costal diaphragm (Fig. 3).

Muscle spindles were counted and measured in whole muscles from three animals (Table 3). We noted some variance between the three animals. The mean number of spindles observed was 52.7 (SD = 7.6), and the mean spindle index for the three muscles was 11.9 (SD = 4.6). Spindle length was measured by following the fibers as

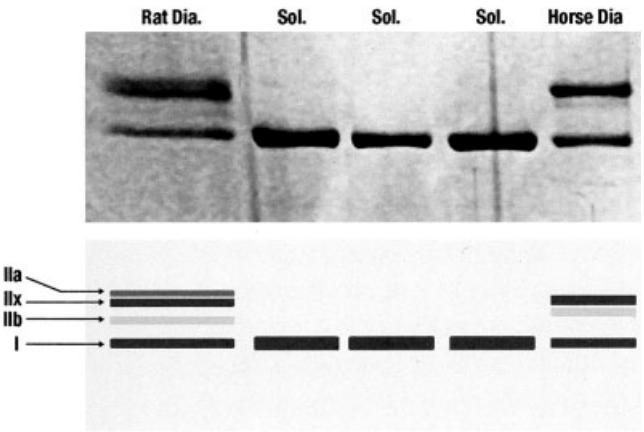

Fig. 3. SDS-PAGE showing single isoform in soleus. Rat diaphragm exhibiting four myosin heavy chain isoforms is shown for comparison. Horse diaphragm shows a predominance of type I and IIa isoform, as well as one isoform that comigrates with IIx myosin, but exhibits no type IIb isoform. Horse soleus exhibits a single myosin heavy chain isoform comigrating with type I MyHC isoform of the other two muscles.

far as they could be visualized in either polar direction. Total length was estimated for most spindles, although some were truncated when they occurred at the interface between the individual frozen muscle blocks and could not be traced across the gap. Those spindles that were truncated were not included in statistical analysis of spindle lengths. Mean spindle length was 3,582 μm (SE = 265; n = 45) and the mean number of intrafusal fibers per spindle was 8.6 (SE = 0.3). The distribution of spindles appeared uniform throughout the length of the muscle (Fig. 4), although we were unable to sample the ends of the muscles we cut serially and may have missed a concentration of spindles, or a likely representation of tendon organs in the end-regions. Of the intrafusal fibers sampled, there was a mean of 2.44 bag fibers and 6.1 chain fibers per spindle (Fig. 5). The range of intrafusal fibers per spindle was 1–15. At least three pairs of spindles were associated in tandem arrays (Richmond and Abrahams, 1975) and occasionally in association with what appeared to be Golgi tendon organs. While Golgi tendon organs were noted, we focused on muscle spindles because of their ease of visualization, particularly in the capsular region. Further, without appropriate silver staining, we may have missed some of the Golgi tendon organs in our analysis.

We measured 80 isolated muscle fibers from proximal and distal regions of the muscle, obtained after one pony soleus muscle was chemically digested in nitric acid. Fibers were approximately 45 mm long (mean, 45.4 ± 12 mm). While the fibers were generally parallel in their alignment along the longitudinal axis of the soleus, they could not extend from one end to the other of this muscle since the overall muscle length was about 70 mm. Thus, fibers had to terminate intrafascicularly throughout the muscle or along the distal aponeurosis that merged into the lateral gastrocnemius muscle and the calcanean tendon. While small-diameter fibers were noted throughout the soleus muscle, we did not quantitate them or describe their distribution relative to specific regions within the muscle. These small fibers could represent the tapered ends of fibers as they blend with perimysium within the muscle.

DISCUSSION

Almost all muscle fibers in the adult and newborn equine soleus are type I, presumed slow-twitch fibers. This is similar to the situation reported in many other mammalian species (Ariano et al., 1973; Burke et al., 1974; Armstrong and Phelps, 1984; Wigston and English, 1992) with regard to the soleus muscle. One study reported that equine soleus consists of only 22.8% type I fibers (Rome et al., 1990). That study utilized biopsy samples taken from two Thoroughbred horses and was primarily concerned with contractile testing of skinned single fibers. We cannot ascertain why so few type I fibers were reported by Rome et al. (1990), but, as a result of their study, we were extremely careful with our sample collection (taken on postmortem specimens) and analysis.

The soleus is generally a postural muscle, responsible for maintaining limb position during periods of quiet standing or slow locomotion (Smith et al., 1977, 1980; Rasmussen et al., 1978). It is assumed that the effect of the soleus, relative to that of the medial and lateral gastrocnemius muscles, is diminished during periods of rapid and high force production such as occurs during higher-speed movements, especially during a trot or gallop in quadrupeds, or during a jump (Smith et al., 1980). Measurements of twitch contraction profiles in soleus in other mammals generally indicate a slow rise time (to peak tension) as well as a more gradual reduction in tension subsequent to peak tension application (Burke et al., 1974; Burke, 1978). In contrast, the medial gastrocnemius muscle is both larger and faster contracting than soleus muscles, and the former produces significantly greater amounts of force (Walmsley et al., 1978). Indeed, in the horses we studied, the lateral and medial gastrocnemius muscles were approximately 200× larger (by mass) than soleus. Although a small number of fast fibers were found in two adult horse soleus muscles studied, the proportion of fast fibers remains insignificant (less than 1%). No fast fibers were observed in any of the juvenile muscles we examined, suggesting that some transformation of slow to fast myosin isoforms may occur during ontogeny. It is not clear if this is a response to functional demands or not. This also seems counter to observations in rat muscle in which fast fibers present at birth appear to transform into slow (type I) fibers during ontogeny (Wigston and English, 1992). Sufficient data regarding the training history of our horses were not available to make any conclusions about these few fast fibers.

Our initial impression was that the equine soleus contained a large number of muscle spindles. This was based on our experience with a number of other horse muscles examined over the past few years, including M. biceps brachii (Hermanson and Hurley, 1990), forearm flexor muscles (Hermanson and Cobb, 1992), elbow extensor muscles (Ryan et al., 1992), and a carpal extensor muscle (Hermanson, 1997). In all of these muscles, it had been notable when we encountered a single muscle spindle during the course of study. In contrast, most sections we examined in the soleus appeared to contain at least one or more spindles. Thus, based on the high oxidative potential (Botterman et al., 1978), slow profile, and seem-

TABLE 3. Spindle numbers and morphology in *Equus caballus*

	No. of spindles	M mass (g)	Spindle index	Mean number intrafusal fibers	Intrafusal fiber length (μm)
Horse 3	61	4.3	14	10	2447
Horse 7	46	6.8	6	10.4	3193
Horse 14	51	3.5	11	8.4	3377

Spindles were counted in three soleus muscles and the mean number of intrafusal fibers and intrafusal fiber lengths are presented. The spindle index is the number of spindles per gram muscle wet weight.

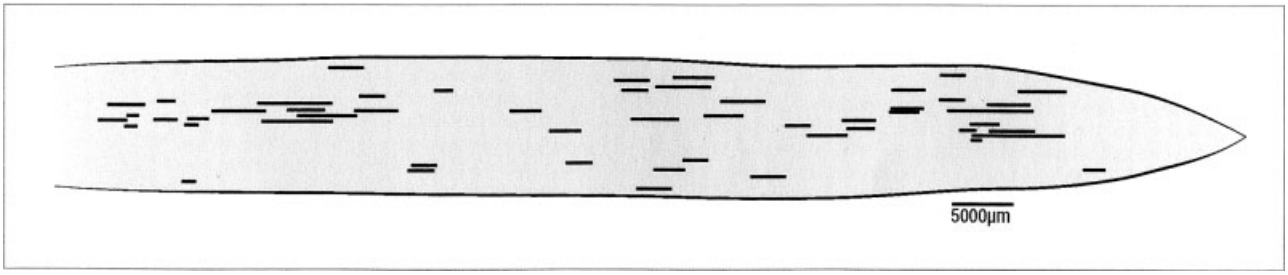

Fig. 4. Schematic of distribution of spindles within a horse soleus muscle. Note the relatively even distribution of spindles throughout this muscle, both across the width of the muscle and along the length of the muscle. The proximal and distal ends of the muscle were truncated. Proximal is to the left.

ingly high numbers of spindles, we hypothesized that the equine soleus might be an important proprioeceptive muscle in the same manner as the M. articularis humeralis as proposed by Lalatta-Costerbosa et al. (1992). The mean spindle index of about 12 for soleus does not appear significantly different from the value of 9 reported for feline medial gastrocnemius by Chin et al. (1962). Unfortunately, there are no comparable data for muscles such as the equine medial and lateral gastrocnemius, although work underway in our laboratory has indicated that there are not nearly as many spindles per gram in those muscles (data not shown). However, it is impractical to expect that something like an equine medial or lateral gastrocnemius will ever be studied with the level of resolution necessary to quantify their spindle indexes. These muscles are too large for such an effort to be undertaken. Spindles in soleus were unremarkable, containing a mean of almost nine intrafusal fibers per muscle spindle and extending a mean length of 3,582 μm.

It is possible that the spindle index is not a critical tool for assessing the relative role of a muscle in providing proprioceptive feedback. For example, it follows that a muscle containing a high number of spindles may contribute more afferent feedback about muscle shortening than a muscle containing few or no muscle spindles. However, the spindle index may not scale in a linear fashion as muscles or animals become larger. Other considerations that could alter interpretation of a muscle spindle index include the distribution of afferent inputs to central motoneurons. If afferent neurons from a single spindle synapse upon a larger number of motoneurons in some animals (in larger animals), this would negate the need to have an ever increasing spindle index in these larger animals. The feedback from a relatively small number of spindles would suffice to inform the motoneuron pool of ongoing stretch within a muscle. It would be useful to understand the size of motor units, or specifically whether or not muscles have fine control properties (many motoneurons innervating a muscle vs. the coarse control that would be effected if a smaller number of motoneurons innervated the same muscle). Thus, the present study was really intended to focus on the basic muscular properties of the equine soleus muscle, and to provide a reference point regarding the actual number and distribution of spindles in this intriguing muscle.

Horses have a number of vestigial muscles. Examples include important components of the hind limb passive stay apparatus such as the peroneus tertius and the superficial digital flexor muscles (Nickel et al., 1986). These two muscles are largely reduced to connective tissue bands and only the superficial digital flexor contains any muscle fibers. Similarly, the equine interosseus was previously described as a muscle that "loses" its constituent muscle fibers with ontogeny (Callegari, 1968; Nickel et al., 1986). Recent work suggests that there is a small but significant population of working muscle fibers in the interosseus (Wilson et al., 1991; Soffler and Hermanson, 2006). The muscle fibers of equine interosseus are remarkable because of their length in the range of 600–800 μm (Soffler and Hermanson, 2006). In contrast, the equine soleus is simply a small muscle (if considered as a proportion of the animal's body mass) that has retained a fleshy muscle belly with muscle fibers spanning a mean of 45 mm, which is not the entire length of the muscle belly. Tapering fibers observed in the muscle support the idea that fibers must terminate intrafascicularly, or on a tendon of insertion in such a way that fibers do not pass from origin to insertion of the muscle. Recent work on human soleus muscles demonstrated a complex architecture with pennate fibers inserting on a posterior aponeurosis and a median septum that continue to the Achilles (calcaneal) tendon (Hodgson et al., 2006). In horses, the diminutive size of the muscle

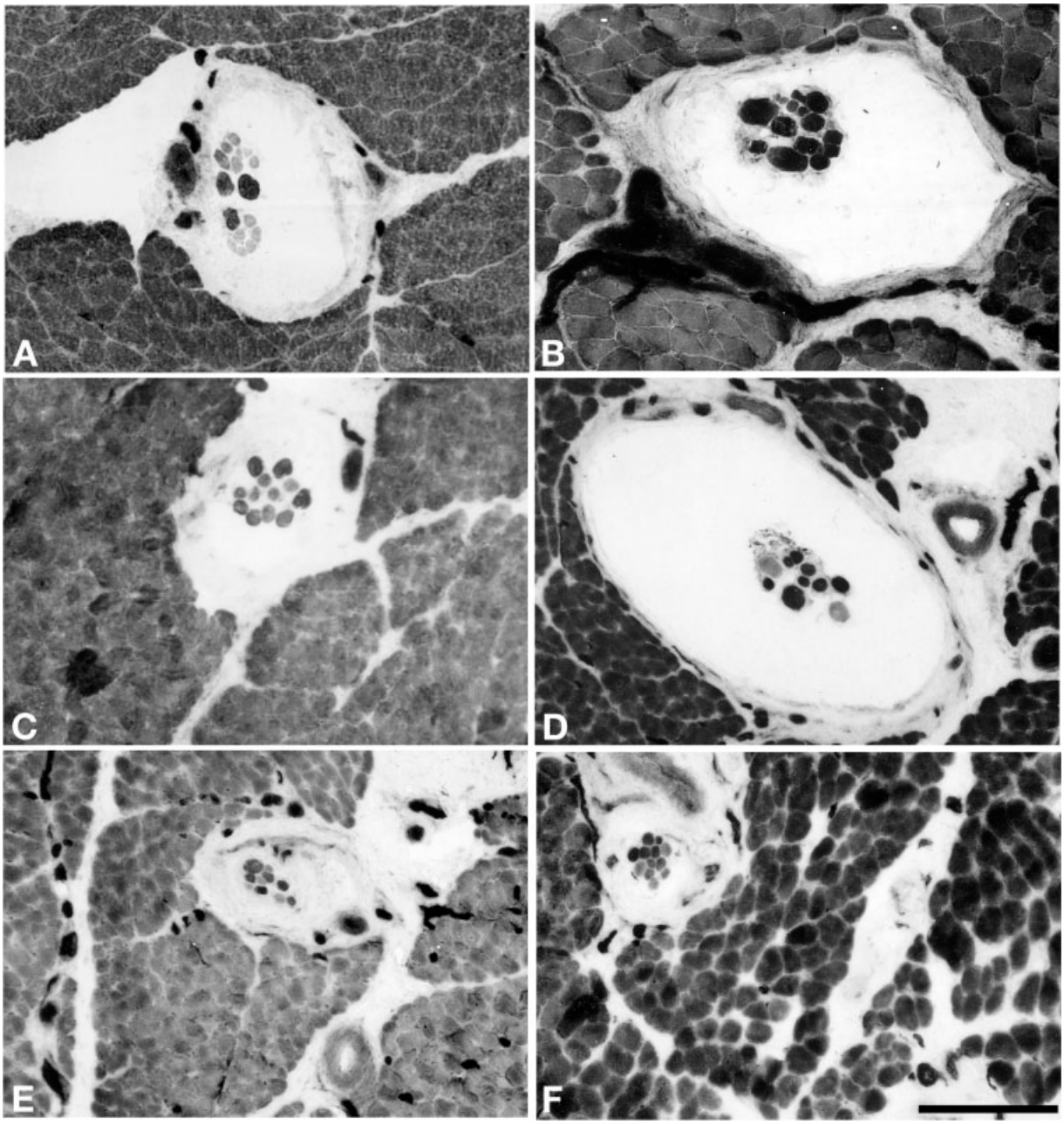

Fig. 5. **A-F**: Six representative cross-sections of horse soleus muscle spindles at the level of the spindle capsule. These sections were all stained after reaction for mATPase at pH 9.4 following pH 4.3 pre-incubation. Dark fibers are presumed slow-twitch fibers. These six images demonstrate variation in capsule size and intrafusal fiber content. Scale bar = 200 μm.

suggests that it cannot contribute significantly to the forces within the calcaneal tendon, at least in contrast to the medial and lateral heads of the gastrocnemius and the superficial digital flexor. It remains a good possibility that the soleus retains an important proprioceptive function as indicated by the number of muscle spindles observed. However, characterization of this muscle as being an important proprioceptive organ would be overstated but remains an attractive hypothesis.

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