

# Effect of differences in tendon properties on functionality of the passive stay apparatus in horses

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**Objective**—To determine the effect of differences in structural and mechanical tendon properties on functionality of the passive stay apparatus in horses.

**Sample Population**—5 forelimbs each from nondwarf Friesians, dwarf Friesians, and ponies.

**Procedures**—Harvested forelimbs were loaded to test the passive stay apparatus. Tendons that stabilize the distal portion of the limb (superficial digital flexor tendon, deep digital flexor tendon, and tendo interosseus [suspensory ligament]) were isolated, and force-elongation data were obtained. Bone lengths, initial tendon lengths, and initial tendon cross-sectional areas were measured, and Young moduli were calculated. A model was used to determine whether joint angles could be explained by these 4 factors only.

**Results**—Dwarf limbs were unable to stand passively under loading because tendons that prevent overextension of the distal limb joints were too long and compliant to prevent overextension. Tendon properties of limbs of nondwarf Friesians appeared to be intermediate between those of ponies and dwarf Friesians.

**Conclusions and Clinical Relevance**—Dysfunction of the passive stay apparatus in dwarf Friesians could be related to differences in structural and material properties of the tendons that result in hyperextension of the joints under loading. Nondwarf Friesians had intermediate tendon properties, which might be a breed-specific variation. Results indicated that certain tendon properties were associated with load failure of the stay apparatus and provided additional information about the functionality and requirements of the passive stay apparatus. (*Am J Vet Res* 2011;72:xxx-xxx)

Horses are capable of standing with limited muscle activity as a result of the so-called passive stay apparatus in the forelimbs and hind limbs.<sup>1-4</sup> This is an important energy-saving mechanism for animals that stand for prolonged periods. The distal joints of the forelimb (carpal, metacarpophalangeal [fetlock], proximal interphalangeal [pastern], and distal interphalangeal [coffin] joints) and hind limb (fetlock, pastern, and coffin joints) are stabilized during standing by tendinous structures on the palmar and plantar sides of the limbs (Figure 1).<sup>2-6</sup> The main structures contributing to the distal part of the passive stay apparatus are the SDFT, DDFT, TI (suspensory ligament), and associ-

## ABBREVIATIONS

CSA	Cross-sectional area
DDFT	Deep digital flexor tendon
GJM	General linear model
SDFT	Superficial digital flexor tendon
TI	Tendo interosseus

ated distal ligaments of the proximal sesamoid bones (ie, the sesamoid ligaments). The sesamoid ligaments in combination with the TI prevent hyperextension of the metacarpophalangeal and metatarsophalangeal (fetlock) joints. The combination of the proximal accessory ligaments and the SDFT bridges the carpus, fetlock joint, and proximal interphalangeal (pastern) joint and passively prevents overextension of these joints. The distal accessory ligament in combination with the DDFT forms a tendinous bridge that passively protects the fetlock, pastern, and coffin joints from overextension.

Although the passive stay apparatus is a well-known structure and is described in textbooks,<sup>2-4</sup> little is known about its physical limitations or requirements for proper functioning. One reason for this lack of knowledge is that the distal tendons not only are elements of the passive stay apparatus, but also act as an energy-storage mechanism and contribute to vibration reduction during locomotion.<sup>7-9</sup>

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Apart from trauma, only 2 types of clinical dysfunction of the passive stay apparatus are observed, and these are the result of muscle weakness<sup>10</sup> or do not affect all the limbs equally.<sup>11</sup> These clinical dysfunctions cannot be linked directly to changes in the passive stay apparatus and therefore provide no useful additional functional information on this apparatus.

In Friesians, a disorder (most likely genetic) resulting in dwarfism is characterized by reduced limb length and overextension of the distal limb joints, indicating a

failing passive stay apparatus.<sup>12</sup> These dwarf Friesians could therefore provide a good model to study the functional demands of the equine passive stay apparatus.

Overextension of the distal limb joints can be explained by 2 hypotheses based on differences in structural and mechanical properties of the tendons. The first hypothesis suggests that the tendons in dwarf Friesians are relatively longer, compared with the length of the bones, than those in clinically normal (nondwarf) horses. Because the tendons limit extension, longer tendons will result in greater extension of the distal limb joints. Obviously, long tendons (relative to the limb length) can result from elongation of the tendon or a reduction of the limb length.

A second hypothesis suggests that the tendons have the same proportions (equal resting length and cross-sectional area), but the stiffness of the tendons are different. When the tendon material is less stiff, loading of the tendon will result in greater elongation and therefore overextension of the joint.

The purpose of the study reported here was to determine differences in structural and mechanical tendon properties that are associated with changes in functionality of the passive stay apparatus in horses by examining clinically normal Friesians, dwarf Friesians, and ponies.

## Materials and Methods

**Experimental groups**—Three groups comprised of 5 horses each were used in this study: a group of Friesians with dwarfism, a group of nondwarf Friesians, and a group of ponies. All horses ranged in age from several months to adult age (2.5 to 38 months; mean  $\pm$  SD, 20.7  $\pm$  14.6 months) without significant ( $P < 0.05$ ) age differences among the groups. The dwarf Friesians were euthanized for clinical reasons, such as being no longer able to stand or move, but no abnormalities other than those associated with dwarfism<sup>12</sup> were found. The nondwarf Friesians were used as a direct control group within the Friesian breed. Because of the large size difference between nondwarf Friesians and dwarf

Table 1—Mean  $\pm$  2 SEM values of the absolute lengths and CSAs of components of the forelimbs of dwarf Friesians, nondwarf Friesians, and ponies (n = 5/group).

Variable	Dwarf Friesian	Nondwarf Friesian	Pony
Length (cm)			
Radius	24.33 $\pm$ 0.92 <sup>a</sup>	35.26 $\pm$ 1.47 <sup>a</sup>	29.04 $\pm$ 2.91
Metacarpus	20.73 $\pm$ 0.49 <sup>a</sup>	26.50 $\pm$ 0.30 <sup>a,c</sup>	20.84 $\pm$ 2.15 <sup>c</sup>
Proximal phalanx	8.20 $\pm$ 0.20 <sup>a</sup>	10.26 $\pm$ 0.19 <sup>a,c</sup>	7.34 $\pm$ 0.90 <sup>c</sup>
Middle phalanx	4.80 $\pm$ 0.17 <sup>a</sup>	5.62 $\pm$ 0.10 <sup>a,c</sup>	3.88 $\pm$ 0.44 <sup>c</sup>
Length (cm)			
Muscle	26.20 $\pm$ 1.23 <sup>a</sup>	34.48 $\pm$ 1.36 <sup>a,c</sup>	27.28 $\pm$ 2.78 <sup>c</sup>
SDFT	34.20 $\pm$ 1.03 <sup>a</sup>	39.78 $\pm$ 1.09 <sup>a</sup>	28.90 $\pm$ 3.64
DDFT	40.83 $\pm$ 1.65 <sup>a</sup>	47.36 $\pm$ 1.79 <sup>a,c</sup>	33.26 $\pm$ 3.82 <sup>c</sup>
TI	15.50 $\pm$ 1.06	18.78 $\pm$ 1.50 <sup>c</sup>	13.62 $\pm$ 2.01 <sup>c</sup>
CSA (cm <sup>2</sup> )			
SDFT	0.60 $\pm$ 0.14 <sup>a</sup>	1.10 $\pm$ 0.13 <sup>a,c</sup>	0.58 $\pm$ 0.04 <sup>c</sup>
DDFT	1.37 $\pm$ 0.22	1.88 $\pm$ 0.13 <sup>c</sup>	1.27 $\pm$ 0.14 <sup>c</sup>
TI	1.96 $\pm$ 0.16 <sup>a,b</sup>	2.62 $\pm$ 0.23 <sup>a,c</sup>	1.36 $\pm$ 0.18 <sup>b,c</sup>

Muscle is the length of the combined muscle mass of the SDF and DDFT muscles.

<sup>a-c</sup>Values with the same superscript letter are significantly ( $P < 0.05$ ) different.

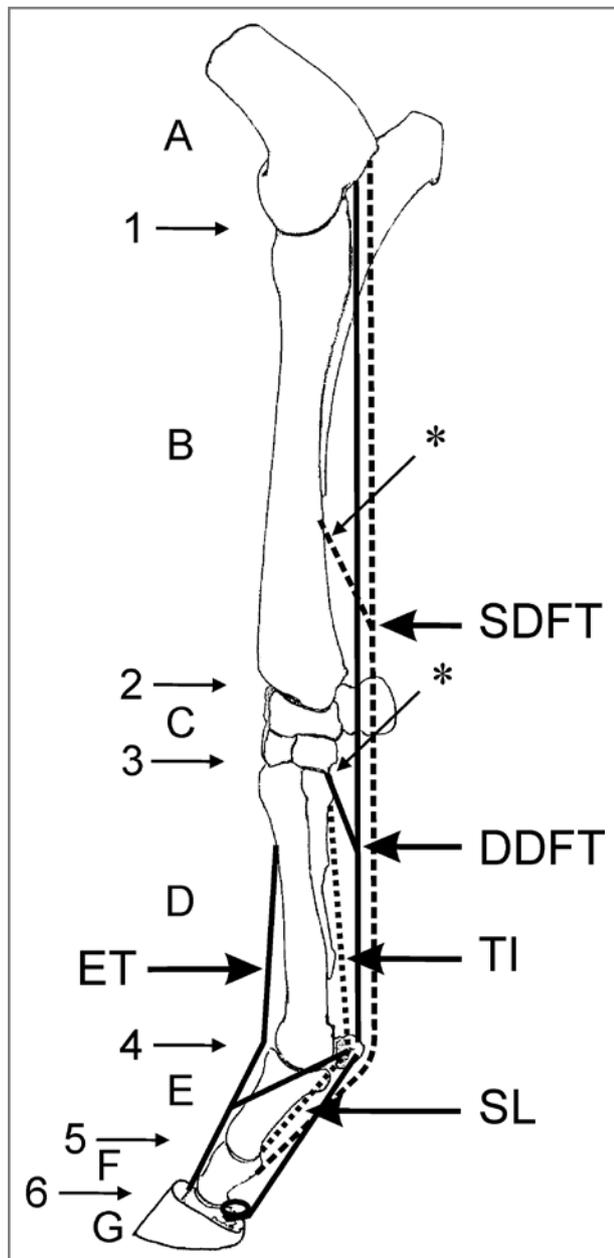


Figure 1—Schematic diagram of the passive stay apparatus in the distal portion of the forelimb in a horse. 1 = Elbow joint. 2 = Antebrachioacral joint. 3 = Carpometacarpal joint. 4 = Metacarpal-phalangeal (fetlock) joint. 5 = Proximal interphalangeal (pastern) joint. 6 = Distal interphalangeal (coffin) joint. A = Humerus. B = Ulna and radius. C = Carpus. D = Metacarpus. E = Proximal phalanx. ET = Extensor tendon. F = Middle phalanx. G = Distal phalanx. SL = Sesamoid ligaments. Asterisks indicate accessory ligaments.

Friesians, a group of 5 ponies with no significant differences in age ( $25.6 \pm 15.8$  months), body weight ( $235.0 \pm 74.2$  kg), and limb size (Table 1), compared with the dwarf Friesians (age,  $18.9 \pm 13.8$  months; body weight,  $214.0 \pm 37.6$  kg), was also analyzed. The non-dwarf Friesians and ponies died from natural causes or were euthanized in the Utrecht University Veterinary Clinic for clinical reasons not associated with the stay apparatus. In each group, 1 forelimb of each horse was collected and stored at  $-20^{\circ}\text{C}$ . This freezing procedure does not affect the biomechanical properties of the tendons.<sup>13,14</sup> For testing, the limbs were thawed while fully submerged in water at  $21^{\circ}\text{C}$ . After removal of the skin, the muscles and tendons were kept moist by wrapping them in wet tissues at all times, except during testing.

**Testing of the passive stay apparatus and determination of joint angles**—Prior to the tendon experiments, the passive stay apparatus (Figure 1) was tested and joint angles and moment arms during stance were determined. For this test, the humerus was cut midway between the epicondyle and the humeral head, and muscles related to the humerus were removed, except for the origins of the flexor and extensor muscles on the epicondyles of the humerus. The skin was removed to facilitate exact measurement of moment arms of the tendons around the joints, and external markers were placed on the center of rotation of the joints. The limbs were then placed in a pneumatic limb-loading device to test the passive stay apparatus (Figure 2).<sup>15</sup> The limb-loading device consisted of a vertically positioned loading cylinder that produced a vertical load on the upper part of the cut humerus. The load was exerted on the

humerus via a disc with a slightly concave surface and a small rim to prevent the humerus from slipping. The humerus was, however, not fixed to the loading device. Because of the passive stay apparatus, a normal horse forelimb will be able to remain standing in this position passively when loaded. Immediately prior to measurements, each limb was preconditioned by loading it 10 times up to 500 N. The limb was then loaded to 1,000 N, which is a value near the typical loading value for horses with a body weight equal to that of ponies and dwarf Friesians when standing.<sup>16,17</sup> Because we were interested in differences attributable to tendon properties alone, loading was kept constant among the 3 groups. Although the loading value was lower than the typical physiologic loading value in horses with the weight of nondwarf Friesians, the use of constant loading provided the opportunity to directly compare the characteristics of the tendons in the 3 groups. After the limb was in a stable position, a digital photograph was obtained from the lateral aspect of the limb, from which joint angles and moment arms were measured. Because the distal phalanx was inside the hoof, it was impossible to accurately measure the angle of the coffin joint.

**Tendon characteristics**—Three tendons critical for the functionality of the passive stay apparatus were analyzed: the SDFT, DDFT, and TI. Analysis of the TI included the sesamoid ligaments that form the distal continuation of the TI onto the distal aspect of the proximal phalanx and proximal aspect of the middle phalanx.

In the same specimens as used for the loading test, the origin of the tendons was cut and the tendon was

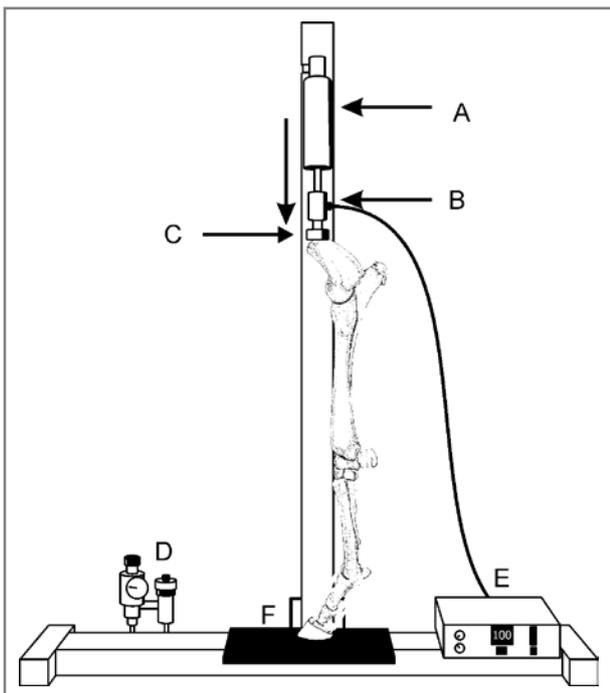


Figure 2—Schematic diagram of a pneumatic loading device. A = Pneumatic loading cylinder. B = Load cell with data output to data logger (E). C = Slightly concave disk to keep the humerus in place. D = Pneumatic control and pressure gauge. F = Flat surface on which the hoof stands. Arrows indicate the direction of force.

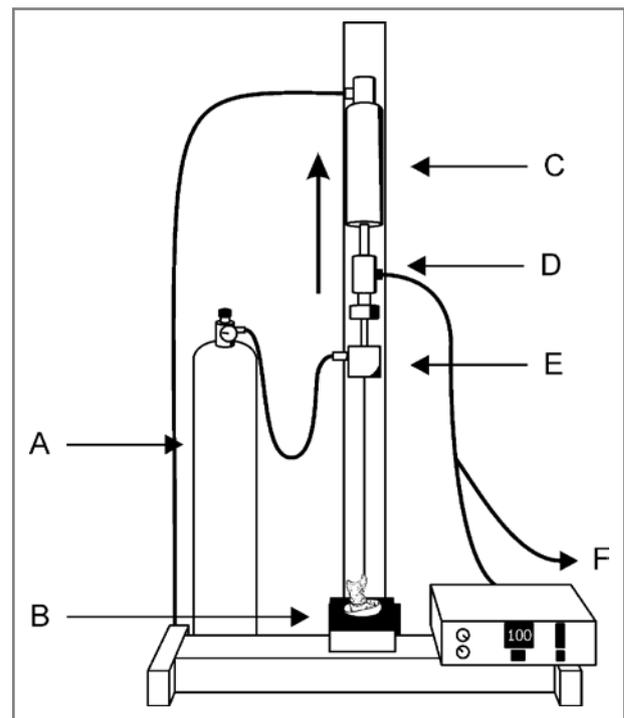


Figure 3—Schematic diagram of a tendon loading device. A = Carbon dioxide cylinder connected to the tendon clamp (E). B = Adjustable hoof clamp. C = Pneumatic loading cylinder. D = Load cell with analogue and digital data-logging. F = Data connection to computer interface. Arrows indicate direction of force.

dissected free from the surrounding tissue, keeping the insertion on the bones intact. The SDFT and DDFT were both cut at the transition from muscle to tendon, and the accessory ligaments were cut from their origin, but the insertion on the main tendon was kept intact. The TI was removed from the metacarpus at its origin. The limb was cut at the fetlock joint, and all structures proximal to this joint, except for the tendons and associated structures under study, were removed.

The stress-strain curve of the tendons was determined by use of a force-transducer<sup>a</sup> attached to a moveable piston that moved at a constant speed of 0.015 m/s (Figure 3). A jaw clamp<sup>18</sup> was fitted to the force transducer in which the tendons could be fixed between serrated jaws that were frozen by use of expanding liquid carbon dioxide; use of the clamp prevents slipping of the tendons.<sup>18,19</sup> The hoof was immobilized in a fixed mechanical clamp in such a way that the whole tendon was in a straight line in the direction of loading when the piston moved upward. Before measurement, tendons were preconditioned by loading them 10 times to approximately 10% of body weight. Before the measurements, the system was preloaded at 10 N to eliminate interference from interphalangeal joint movements. During measurements, each tendon was stretched from its resting length to a load of at least 30%

of body weight, 10 times. During stretching, the load was measured at a rate of at least 1 kHz and data were saved digitally by use of a digital interface.<sup>b</sup> Changes in tendon lengths were calculated as duration of movement of the piston divided by the speed. Speed of the piston during loading was checked by measuring time intervals for movement of known distances during the experiment. Force-elongation curves were analyzed for sudden deviations that might indicate slippage at either end of the system or failure of the tendon. No deviations were observed in any of the force-elongation curves used in the analysis, nor were there signs of slippage visible at the points of fixation.

For each of the 10 repeats of the individual tendon stretch experiments, a second-degree polynomial curve was fitted through the obtained force-elongation data by use of a least-squares algorithm. The second-degree polynomial curve was used because this provided the best fit for the observed curves, as determined by use of the highest mean  $R^2$  value in regression analyses performed with commercial statistical software.<sup>c</sup> The Young modulus ( $E$ ) of the tendon was calculated by multiplying the first derivative of the force-elongation curve ( $F/\Delta l$ ) by the initial length of the tendon divided by the initial cross-sectional area of the tendon ( $L_0/A_0$ ). The Young modulus was calculated for a stress ( $\sigma$ ) of 35 MPa, which is within the linear phase of the force-elongation curve where collagen fibers have straightened and the true stiffness of the material can be determined.<sup>20</sup> This stress of 35 MPa is much less than the failure stress of tendons.<sup>21</sup> Because the tendon stiffness in literature is often calculated ( $F/\Delta l$ ; without taking into account the properties of the tendons), we also calculated the tendon stiffness for a load of 2,000 N, which is in the range of previous measurements.<sup>22</sup>

**Properties of tendons and bones**—After determination of the force-elongation curves, the tendons were dissected free from all other tissues and from their insertion. Their initial (resting) lengths were measured from the origin of the tendon to the insertion without applying any force. For the SDFT and DDFT, the length was determined from the origin of the distal accessory ligament because that is the part that restricts the flexion of the distal limb joints.<sup>9</sup> The length of the com-

Table 2—Mean  $\pm$  2 time SEM values of the relative (scaled) lengths and CSAs of components of the forelimbs of dwarf Friesians, nondwarf Friesians, and ponies (n = 5/group).

Variable	Dwarf Friesian	Nondwarf Friesian	Pony
Scaled length (cm)			
Radius	0.42 $\pm$ 0.01 <sup>a,b</sup>	0.45 $\pm$ 0.01 <sup>a,c</sup>	0.48 $\pm$ 0.00 <sup>b,c</sup>
Metacarpus	0.36 $\pm$ 0.01	0.34 $\pm$ 0.01	0.34 $\pm$ 0.00
Proximal phalanx	0.14 $\pm$ 0.00 <sup>b</sup>	0.13 $\pm$ 0.00 <sup>c</sup>	0.12 $\pm$ 0.00 <sup>b,c</sup>
Middle phalanx	0.08 $\pm$ 0.00 <sup>a,b</sup>	0.07 $\pm$ 0.00 <sup>a,c</sup>	0.06 $\pm$ 0.00 <sup>b,c</sup>
Muscle part	0.45 $\pm$ 0.01	0.44 $\pm$ 0.01	0.45 $\pm$ 0.02
SDFT	0.59 $\pm$ 0.02 <sup>a,b</sup>	0.51 $\pm$ 0.02 <sup>a</sup>	0.47 $\pm$ 0.02 <sup>b</sup>
DDFT	0.69 $\pm$ 0.02 <sup>b</sup>	0.61 $\pm$ 0.03	0.54 $\pm$ 0.02 <sup>b</sup>
TI	0.27 $\pm$ 0.02 <sup>b</sup>	0.24 $\pm$ 0.02	0.22 $\pm$ 0.01 <sup>b</sup>
Scaled CSA ( $\times 10^{-2}$ )			
SDFT	1.30 $\pm$ 0.13	1.31 $\pm$ 0.08	1.28 $\pm$ 0.11
DDFT	1.94 $\pm$ 0.16	1.76 $\pm$ 0.04	1.89 $\pm$ 0.14
TI	2.33 $\pm$ 0.14 <sup>b</sup>	2.08 $\pm$ 0.09	1.79 $\pm$ 0.14 <sup>b</sup>

See Table 1 for key.

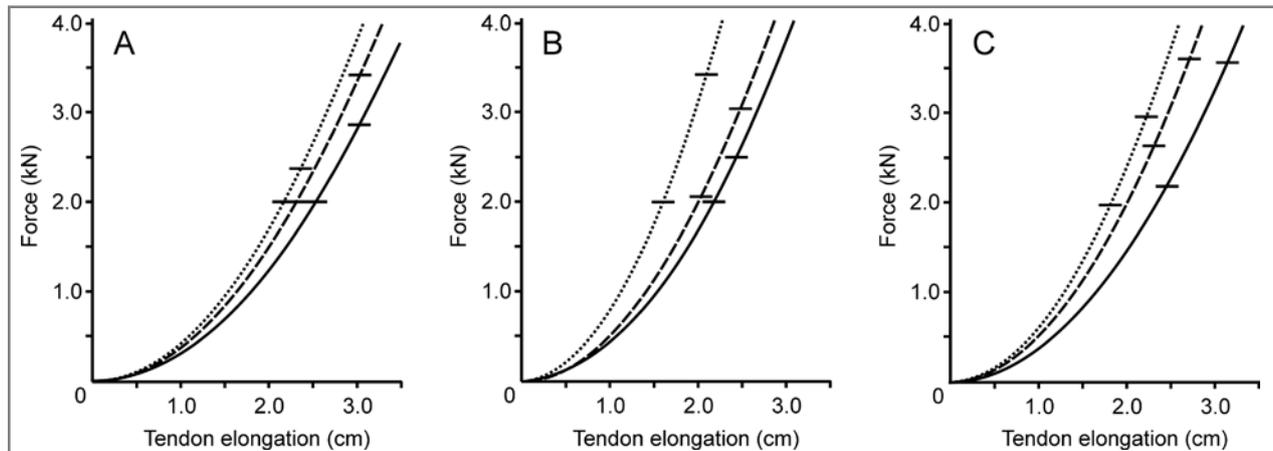


Figure 4—Mean fitted force elongation curves of the SDFT (A), DDFT (B), and TI (C) in dwarf Friesians (solid lines), nondwarf Friesians (dashed lines), and ponies (dotted lines). Horizontal lines indicate the applied maximum and minimal load during repeated trials.

bined muscle mass of the SDF and DDF was measured in the same way from origin to insertion on the tendon, where it is combined with the accessory ligaments. The cross-sectional areas of the tendons were measured at their most slender point. Because there were no apparent differences in tendon form among groups, all measurements were taken at the same relative positions. The tendons were cut at the following positions: SDFT, halfway between the musculotendinous junction and the point where it passes the proximal sesamoid bones; DDFT, halfway between the insertion of the distal accessory ligaments and the point where it passes the proximal sesamoid bones; and TI, halfway between its origin on the metacarpal bone and its insertion on the proximal sesamoid bones.

The cross-sectional cut surface of each tendon was coated with ink, and its corresponding image was transferred 6 times without force onto graph paper. The images were digitized (approx 22,500 pixels/cm<sup>2</sup>), and areas were determined on the basis of the number of pixels by use of image analysis software.<sup>d</sup>

The lengths of the 4 bones in the distal part of the limb (radius, metacarpus, proximal phalanx, and middle phalanx) were determined by clamping the bones between 2 blocks and measuring the distance between the blocks. The distal phalanx was not included in the analysis because the tendons investigated in this study insert only on the palmar-proximal side of the bone.

For comparison among groups, the length data of bones and tendons were scaled to the summation of the total measured bone lengths. Cross-sectional area, which is a surface measure, was first transformed to a length measure by taking the square root and then also scaled to the summation of the total measured bone lengths.

Table 3—Mean ± SD tendon stiffness (kN/cm) calculated at 2,000 N of the SDFT and DDFT in dwarf Friesians, nondwarf Friesians, and ponies (n = 5/group) and in Thoroughbreds (4/group).

Tendon	Dwarf Friesian	Nondwarf Friesian	Pony	Thoroughbred*
SDFT	1.39 ± 0.24	1.68 ± 0.15	1.73 ± 0.25	2.73 ± 0.18
DDFT	1.82 ± 0.16	1.95 ± 0.10	2.44 ± 0.20	3.22 ± 0.20

\*Data from reference 22.

**Model**—Because the distal portion of the limbs of horses consists of many more elements than the 3 tendons and the 4 measured bones, it is possible that the observed overextension cannot be explained by the measured variables alone. To test whether the differences in the measured variables alone were sufficient to result in the observed limb postures, a simple model was used to estimate the joint angles in a loaded limb on the basis of the initial length and elongation of the tendons under loading. For ease of comparison, it was assumed there was an equal load on all tendons and an equal loading for all groups. In mammalian quadrupeds, the forelimbs support approximately 60% of the body weight during standing,<sup>16,17</sup> which means that the load during standing for dwarf Friesians and ponies (body weight, approx 215 kg) was much smaller than that for nondwarf Friesians (body weight, approx 430 kg). Because of this large difference in physiologic loading among the groups and because calculations were used for direct comparisons only, an intermediate loading value of 1,000 N was chosen.

For all groups and tendons separately, the length of each tendon under a load of 1,000 N was calculated by adding the tendon elongation to the mean initial length of each tendon. The tendon elongation was calculated by use of the following equation:

$$\Delta l = \frac{F \cdot L_0}{E \cdot A_0}$$

where F is the force of 1,000 N, L<sub>0</sub> is the mean initial length, A<sub>0</sub> is the mean cross-sectional area, and E is the mean Young modulus, as calculated for each tendon and each group separately. These lengths were used to determine the maximum distance each tendon could span, which determined the maximum joint angles. To predict the joint angle proximal to the accessory ligaments, the muscle bellies of the SDFT and DDFT were also included. The lengths of the muscle bellies were assumed to be constant because the architecture of the muscle allows for a maximum elongation of only 6 mm.<sup>9</sup> Joint angles were described on the basis of their normal position, which means that a joint that is extended during standing will have positive values for extension, whereas a joint that is flexed during standing will have positive values for flexion. When a joint

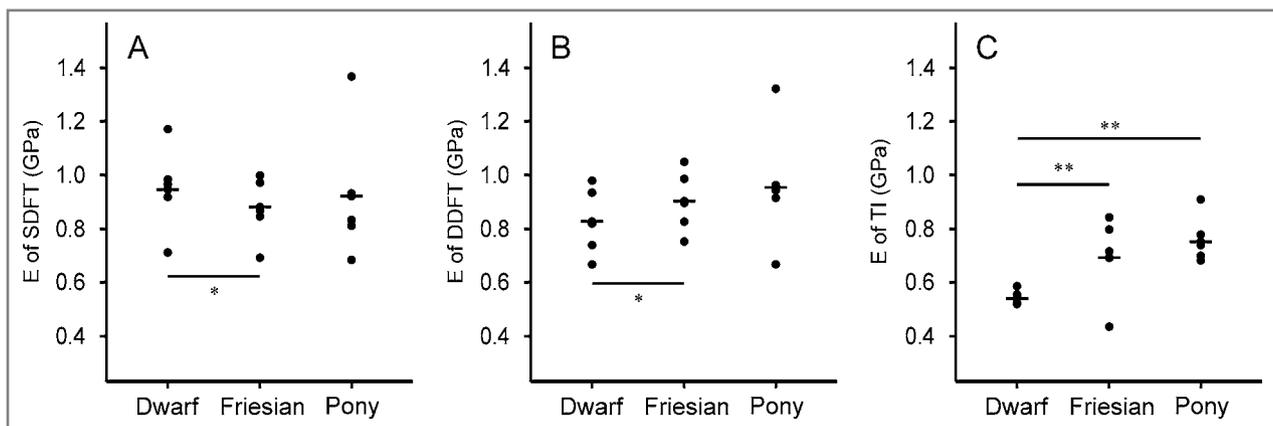


Figure 5—Young modulus values of tendons of dwarf Friesians, nondwarf Friesians, and ponies (n = 5/group) calculated at a tendon stress of 35 MPa. Dashed circles indicate the means. A—SDFT. B—DDFT. C—TI. \*P < 0.05. \*\*P = 0.01.

is straight, the angle is described as 0°. All angles described are the angles between connected bones and do not refer to any earth-bound frame of reference.

After the lengths of the 9 tendons were calculated, the data were used to calculate the joint angles of the distal limb joints. Calculation of the joint angles was based on the same principles that influence joint angles in vivo. Under the influence of loading, the joint angles will increase, thereby elongating the tendons that span the joints. When an equilibrium is reached, the length of the tendon is equal to the length of the bony elements it spans plus the distance it travels around the joints it bridges. In the model used here, the tendon length was calculated as described and the bone lengths were known, so the discrepancy in length between these 2 lengths corresponded to the joint angles.

For example, the TI, including the sesamoid ligaments, spans the distance from its origin (distal carpus and proximal metacarpal bone) to its insertion on the proximal side of the middle phalanx. Its length is therefore equal to the lengths of the metacarpal bone, proximal phalanx, and distance around the fetlock joint. The distance around the fetlock joint can easily be converted to a joint angle when the length of the tendon to the rotation point is known because the distance the tendon travels around the joint can be described as part of a circle with a radius equal to the distance to the rotation point. The lengths from the tendons to the rotation points were measured in the lateral photographs obtained when the limbs were in the loading device, and the length of the bony elements was the mean of the actual bone lengths (Table 1).

By use of this method, the angle of the fetlock joint was determined under the assumption that it is mainly limited by the TI and sesamoid ligaments.<sup>3,4</sup> The calculated length of the TI limits the joint angle such that the joint is prevented from extending to the point that it would touch the ground. After the joint angle of the fetlock joint was determined, the joint angles of the proximal and distal interphalangeal joints were calculated on the basis of the length of the DDFT from the distal accessory ligaments to its insertion on the proximal portion of the distal phalanx. Because multiple nonphysiologic solutions can be calculated, the following limitations on the model were implemented on the basis of prior observations.<sup>23</sup> Measurements of equine joint angles indicate that the angles of the proximal interphalangeal joint are small; therefore, in the model, this angle was restricted to a maximum of 15°. The angle of the carpal joints was calculated on the basis of the length of the SDFT from the origin of the proximal accessory ligament to the insertion of the SDFT on the middle phalanx. Previous observations indicate that the carpal joint behaves like a single hinge joint at the level of the carpometacarpal joint and is almost straight during standing.<sup>23</sup> Movement in the carpometacarpal joint was therefore limited to a maximum of 10°, and all other carpal joints were considered unmovable. With all other joint angles determined, the angles of the antebrachio-carpal joint and the elbow joint were calculated on the basis of the length of the muscular parts of the SDF and DDF, under the assumption that of the remaining restricting muscle-tendon length, 10% would con-

tribute to the flexion of the antebrachio-carpal joint and 90% to the flexion of the elbow joint. These values were based on the observed joint angles of these 2 joints in the loading experiment. The results of the model were used to determine whether the variables measured in this study were sufficient to predict the differences in joint angles in the distal portion of the limb of the different groups and thus the aberrant limb postures in dwarf Friesians.

**Statistical analysis**—Variables were tested for differences among experimental groups by use of commercial statistical software.<sup>c</sup> A GJM was used, with age and mass as covariates, to test whether these factors influenced the variables. Because of the low numbers of individuals in each group, the nonparametric Mann-Whitney *U* test was used for direct comparisons between pairs of groups. A value of  $P < 0.05$  was considered significant.

## Results

Although all limbs of ponies and nondwarf Friesians were able to stand passively under loading in the limb-loading device, only a limb from one of the dwarf Friesians could stand passively under loading. When force was applied to the other dwarf limbs, a large flexion of the elbow joint of  $> 100^\circ$  was observed, and limbs were ejected from the device when only a small part of the intended loading was achieved.

No significant differences among groups were found in bone lengths, although the difference between metacarpal bone length in ponies versus the other 2 groups nearly reached significance ( $P = 0.054$ ). No significant differences among groups in age were observed. The absolute lengths of the bones of the distal part of the limb clearly revealed the obvious difference between dwarf and nondwarf Friesians (Table 1). The bones of the dwarf Friesians were approximately 20% shorter than those of nondwarf Friesians. Limb length of the ponies was not significantly different from the limb length of the dwarf Friesians, indicating that the ponies were a suitable control group.

The GLM for the different tendon lengths revealed no significant effect for group, age, or body weight. The lack of group effect was not confirmed by direct comparisons of the tendon lengths in different groups, in which significant differences between dwarf Friesians and nondwarf Friesians and between nondwarf Friesian and ponies were found (Table 1).

Because use of the scaled lengths of the bones and tendons removed the size effect, differences were detected more easily among groups. For all bone and tendon lengths, except the length of the TI, a significant group effect was observed. No age or body weight effects were observed, except for an age effect for the metacarpal bone length. Regarding the relative contribution of the different bones to the total length of the distal portion of the limb, only the relative length of the metacarpus did not differ among groups, indicating that in different groups, the contribution of different bones to the total length of the distal portion of the limb was not the same (Table 2).

There were no significant differences in relative tendon lengths between nondwarf Friesians and ponies (Table 2). All relative tendon lengths of dwarf Friesians were significantly greater than those of ponies. Nondwarf Friesians had intermediate relative tendon lengths, but differences between nondwarf Friesians and other groups were not significant.

Because the joint angles of the limb are determined by the total length of muscle and tendon, it might be possible that the longer tendons of the dwarf Friesians were compensated for by shorter muscles. Analysis of the relative muscle length via GLM and direct comparison revealed no significant differences that might compensate for differences in relative tendon lengths among the 3 groups (Table 2).

In all instances, nondwarf Friesians had larger relative tendon lengths than did ponies, which might indicate that long tendons relative to the size of the animal is an intrinsic character of the Friesian breed and that this is more strongly expressed in the dwarf phenotype. Although not all differences in tendon length among groups were significant, longer tendons contributed to greater overextension of the joint angles in the distal portion of the limb.

**CSA**—A significant group effect was found for the cross-sectional area of all tendons, although no age or body weight effect was observed. As expected, the larger size of the nondwarf Friesians resulted in larger cross-sectional areas of all tendons, compared with the other 2 groups, although this difference was not significant for the DDFT of the dwarf Friesians (Table 1). Values in dwarf Friesians and ponies were similar, with only a significant difference in the cross-sectional area of the TI.

When the data were scaled by dividing the square root of the CSA by the total bone length, no significant effects were detected by use of the GLM. In direct comparison, the only significant difference in relative cross-sectional area was observed in the TI, for which the relative cross-sectional area of dwarf Friesians was significantly larger than that of ponies. Just as for the relative tendon lengths, the nondwarf Friesians had an intermediate relative cross-sectional area, compared with dwarf Friesians and ponies (Table 2).

**Young modulus**—Young moduli (E) were obtained by use of the first derivative of the linear phase of the force-elongation graphs, the unloaded tendon length, and the tendon cross-sectional areas. The force-elongation graphs (Figure 4) clearly revealed that under equal loads, the dwarf Friesians had the greatest tendon elongation, whereas ponies had the least tendon elongation. Nondwarf Friesians had intermediate tendon elongation. This can also be seen in the tendon stiffness data ( $F/\Delta l$ ) calculated for a load of 2,000 N (Table 3).

In the GLM analysis, no age or body weight effect was observed for the values of the Young modulus. For the SDFT, dwarf Friesians had significantly higher values than did the nondwarf Friesians; values in the ponies were not significantly different from values in the other 2 groups (Figure 5). For the DDFT and TI, the Young modulus of the dwarf Friesians was lowest among the 3 groups and that of the ponies was highest. No significant differences were found between the ponies and the other 2 groups for the SDFT and DDFT, which was probably attributable to the large variation in the data caused by a single pony specimen with high modulus values for both tendons. The lower values for the dwarf Friesians and nondwarf Friesians indicated that the tendon material was less stiff, compared with the ponies, which, if all structural variables were the same, would contribute to larger elongations of the tendons at the same loads and larger overextension of the distal limb joints.

**Model**—The estimated joint angles of the ponies and nondwarf Friesians indicated that the model gave a good estimation of the observed joint angles (Table 4). This was a clear indication that joint angles in the distal portion of the limb were mainly determined by the variables evaluated in the study and that the passive stay apparatus was also defined by these variables. Results from the model also revealed that large differences in joint angles occurred when only the variables measured in this study were varied (Figure 6). Because there were no differences among groups in relative CSA for the SDFT and DDFT, the differences in joint angles were fully attributable to the differences in length and stiffness of the tendons.

Table 4—Calculated stretched tendon lengths (cm) and estimated (Est) and observed (Obs) joint angles (degrees) in forelimbs of dwarf Friesians, nondwarf Friesians, and ponies (n = 5/group).

Variable	Dwarf Friesian		Nondwarf Friesian		Pony	
Length						
SDFT	35.02		40.30		29.44	
DDFT	41.28		47.64		33.52	
TI + sesamoid ligaments	31.28		37.21		27.30	
Joint angle	Est	Obs*	Est	Obs	Est	Obs
Elbow	101	41	40	39	26	27
Antebrachiocondylar	30	9	6	3	4	3.5
Carpometacarpal	10	5	10	11	5	4.5
Fetlock	51	34	34	9	13	13
Pastern	15	15	15	14.5	5	4
Coffin	-30	ND	-18	ND	23	ND

For all joints except the elbow joint, extension has positive values.  
 \*Data obtained from a single horse capable of passive standing; all other dwarf Friesians were unable to stand passively.  
 ND = Not determined.

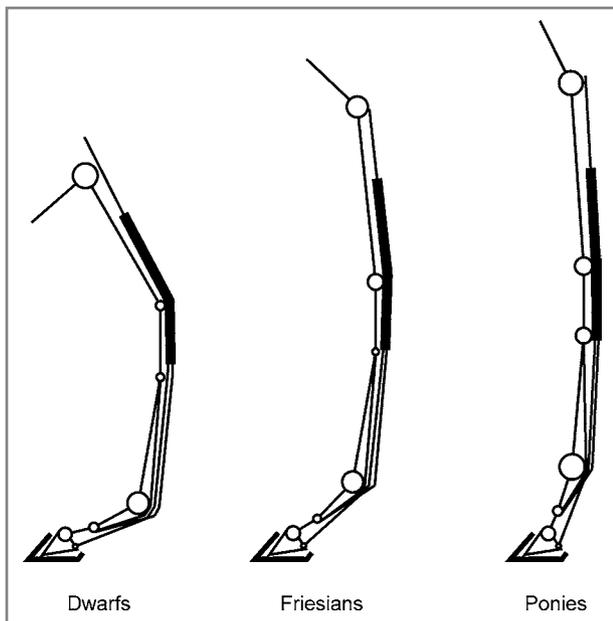


Figure 6—Schematic diagram of predicted limb postures of dwarf Friesians, nondwarf Friesians, and ponies based on joint angles calculated by use of a model. The 3 limbs are scaled to the same total bone length.

## Discussion

The passive stay apparatus of horses is a complex mechanism that differs substantially between the forelimb and the hind limb with regard to the proximal joints of the limbs (ie, the elbow joint and femorotibial joint), but is similar for the 5 most distal joints and consists mainly of 3 tendons that bridge the limbs on the palmar and plantar aspects.<sup>2-4</sup> The efficiency of the stay apparatus differed largely among the 3 groups investigated here, as judged on the basis of the angles of the distal limb joints. Dwarf Friesians had a dysfunctional passive stay apparatus that was unable to support a passive stance in the limb loading device, and most limbs were ejected from the device. A probable cause for this ejection was the large flexion of the elbow joint that was observed in these animals. When the elbow joint is flexed to extreme angles, the work line of the load force shifts forwards, resulting in a torque in combination with the ground reaction force, finally resulting in ejection of the limb. This was a clear demonstration that the passive stay apparatus in most dwarf Friesians is dysfunctional, although in living animals, this dysfunction is probably compensated by muscle action. The muscle action, however, cannot completely compensate all of the elongation in the tendons, which results in extreme joint angles in the distal portion of the limb.

The nondwarf Friesians had greater distal joint angles than did the ponies, indicating that the passive stay apparatus could not stabilize the lower portion of the limb without excessive flexion in the joints. Analysis revealed that the difference in joint angles could be related to relatively longer tendons and less stiff tendons, both contributing to a longer tendon length under loading and resulting in greater joint angles.

The Young modulus was the main mechanical property investigated in the study, and although there was variation among groups, values for the SDFT and DDFT of all groups were near or within the reference range for horses weighing up to 500 kg (0.9 to 1.8 GPa)<sup>20</sup> and were similar to values in Thoroughbreds.<sup>22</sup> Remarkable, however, was the low Young moduli of the TI, which were outside the range given by Pollock and Shadwick<sup>20</sup> (0.9 to 1.8 GPa) for each group. The reason for these low values was hard to determine because little data about Young moduli of the TI are available. A possible reason for its low stiffness might be that the TI plays a major role in absorbing impact forces during landing.<sup>24</sup> Because thick tendons are less susceptible to rupture under heavy loads than are thin tendons, it might be a functional advantage to increase the thickness of the TI. However, increasing thickness (cross-sectional area) of a tendon would reduce strain on the tendon and cause a limitation on the flexion of the joints. Therefore, to increase thickness without considerably reducing strain on the tendon, it is necessary to reduce the Young modulus, exactly as observed in all experimental groups. Therefore, it seems plausible that the TI is adapted for large force absorption by increased thickness and decreased Young modulus.

Unfortunately, little is known about the material properties of tendons in other equine breeds because most authors only determine tendon strain without discriminating between the effects of material (Young modulus) versus structural (length and cross-sectional area) properties. Swanstrom et al<sup>22</sup> calculated the Young modulus and tendon stiffness in Thoroughbreds for loads between 1,500 and 3,000 N. Those values can be compared with tendon stiffness values calculated for loads of 2,000 N in the groups of the present study (Table 3). This comparison revealed that the tendons of the Thoroughbreds were stiffer than those of all groups in the present study, whereas the Young moduli were similar. This is a clear indication that tendon stiffness is highly variable among breeds, which might be the effect of differences in loading attributable to the effects of different breed-specific locomotion patterns or gaits.

Although there were large differences in body weight among the groups, this cannot explain the difference in the Young modulus because in a wide range of species, a relationship between body weight and tendon properties is not found.<sup>20</sup> There is, however, evidence that during aging, tendons become stiffer under the influence of increased loading,<sup>25</sup> which might have contributed to the variation among individual horses. This, however, cannot explain the difference between the nondwarf Friesians and dwarf Friesians because the horses in these groups were in approximately the same age range (3 to 36 months) and no age effects were observed. This was also confirmed by the fact that nondwarf Friesians do not have clinical problems, whereas foals and dwarf Friesians do.<sup>12</sup> This indicates that the Young modulus of young dwarf Friesians was less than that of young nondwarf Friesians. The lower Young modulus of nondwarf Friesians, compared with that of ponies, also indicated that a relationship between body size and Young modulus is not plausible because horses

with a larger body weight would benefit more from a large Young modulus than from a small one.

Another factor that reduces tendon stiffness is reduced loading on the tendons.<sup>26,27</sup> There was, however, no indication that the reduced tendon stiffness in dwarf Friesians was the effect of reduced loading. First, most dwarf Friesians are able to stand, and although the joints are highly overextended, this still means that the tendons are fully loaded. Second, the overextension of the distal limb joints is observed directly after birth,<sup>12</sup> when no difference in mechanical loading on the limbs between dwarf Friesians and other horses has occurred. Therefore, it is clear that the differences in Young modulus described here were not related to body weight, age, or reduced loading.

The factors that cause the differences in Young modulus among tendons are presently unknown and need to be investigated. One factor could be a difference in the concentrations of glycosaminoglycans among tendons because it has been found that concentrations are higher in more compliant tendons.<sup>5</sup> Differences in the matrix of the tendons can affect the collagen fibril composition,<sup>28</sup> but how glycosaminoglycans and collagen fibrils affect the biomechanical properties of a tendon is probably complex and needs further investigation.<sup>5,29</sup> Another factor that may influence stiffness of the tendon is water content, and because this may be genetically determined, this might explain variations among breeds. There are also other indications that the properties of tendons are breed specific,<sup>22</sup> so it can be assumed that having weak tendons is a character of the Friesian breed as a whole. This may be more pronounced in dwarf Friesians and, in combination with their relatively long bones, may result in a dysfunctional passive stay apparatus.

Interestingly, the nondwarf Friesians had greater overextension in the distal limb joints than did the ponies. Because no differences were found in relative CSA, this confirmed that the lower Young moduli and larger tendon lengths in the nondwarf Friesians did contribute to greater joint angles and a less-effective passive stay apparatus. The comparison under equal loads revealed that the naturally observed large joint angles of the nondwarf Friesians were the result of tendon factors that reduced the effectiveness of the passive stay apparatus, and not the result of higher loading because of their greater body weight. This might be an indication that a less-effective passive stay apparatus is a characteristic of the Friesian breed in general. It must be kept in mind that the naturally observed overextension of the distal joints in nondwarf Friesians will be even larger than predicted here because the load on a single forelimb in a natural situation during standing can be as high as 1,400 N (30% of body weight<sup>16,17</sup>).

The model also revealed that the combination of relatively long tendons, low Young moduli, and small CSAs, as observed in the dwarf Friesians, results in severe overextension of the joints. However, the model predicted a larger overextension of the distal joints than observed and a flexion in the elbow joint that was twice as large as observed (101°; Table 4). Although the predicted values seem to conflict with the observed values, the predicted values might actually be accurate because the specimen used for the joint angle calculation was not representative of the whole group. In the

limb-loading experiment, only 1 limb was able to stand passively, and the observed joint angles were obtained from that specimen. For the model, the mean values of all limbs were used, including the ones that were unable to stand. In those cases, we observed a large flexion in the elbow joint before the limb was ejected from the loading device. This observation was consistent with the results from the model.

The functional characteristics of the passive stay apparatus can be described by a simple model and a limited number of variables, including material properties as described, bone length, and structural properties (initial length and cross-sectional area) of the 3 main tendons. Although these variables are sufficient for a good description of the stay apparatus, nondwarf and dwarf Friesians deviated from the model in the angle of the fetlock joint. In both, the estimated joint angle was greater than the observed angle, indicating less overextension than predicted by the model, which cannot be the effect of the palmar tendons. Additional structures that might contribute to stabilizing the fetlock joint are the extensor branches that connect the TI to the extensor digitoralis communis muscle on the dorsal aspect of the limb (Figure 1). These branches limit the flexion of the fetlock joint alone and were not included in the model because findings in other breeds indicated that their role in the stance phase of locomotion is minimal<sup>15</sup> or limited to high-velocity limb displacement only.<sup>24</sup> Although the extensor branches play a limited role in other breeds, their contribution might be more substantial in horses with flexor tendons that are less efficient because they are longer and more compliant, as observed in the nondwarf Friesians. The extensor branches could be more important functionally because the tendons of extensor muscles typically have a higher Young modulus than those of flexor muscles.<sup>5</sup> This could mean that in ponies and other breeds, extension of the fetlock joint is limited by the stiffer TI and sesamoid ligaments, whereas in the 2 Friesian groups with more compliant TIs, the extensor branch alone is the limiting factor for the extension of the fetlock.

Although the molecular causes for the change in tendon stiffness are still unknown, we conclude that small changes in tendon length, CSA, or Young modulus can have large effects on the efficiency of the passive stay apparatus. The effects of difference in length and stiffness between breeds were clearly demonstrated in the comparison between ponies and nondwarf Friesians. The relatively longer and less-stiff tendons of the nondwarf Friesians resulted in a less-effective passive stay apparatus and increased extension of the distal limb joints. Further investigation of the role of the TI and the extensor branches, as well as the study of differences in tendon properties at the cellular and molecular level, is probably necessary for a full understanding of this system.

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- a. TCLP-2B, TML Sokki Kenkyujo Co Ltd, Tokyo, Japan.
  - b. LabView, National Instruments, Austin, Tex.
  - c. SPSS 15, SPSS Inc, Chicago, Ill.
  - d. QWin, Leica, Cambridge, England.
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