Exercise-Induced Superficial Digital Flexor Tendon Hyperthermia and the Effect of Cooling Sheets on Thoroughbreds

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Flexor tendonitis in racehorses which is caused by racing or training diminishes their running ability. In the present study, the involvement of hyperthermia in the development of flexor tendonitis is investigated. When fibroblasts isolated from the superficial digital flexor tendon of a horse are cultured, their survival rate decreases after 1 hour of exposure to a temperature of 43°C. When a racehorse runs on a dirt track, the center of the tendon runs a fever of 43°C or more. This finding suggests that the fever occurring during running can be a cause of flexor tendonitis. The study also indicates that cooling the distal ends of the fores after racing is effective in preventing flexor tendonitis.

Key words: *exercise, fibroblast, hyperthermia, cooling sheet, flexor tendonitis*

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Flexor tendonitis is most common in the fores of racehorses and greatly impairs their running ability. Flexor tendonitis is superficial in most Thoroughbreds with tendonitis. The incidence of superficial digital flexor tendonitis in racehorses is 7% [5, 6, 12, 14]. When a horse develops tendonitis, it generally presents with acute inflammation, swelling, fever, and tenderness at the affected site, and the horse limps. The horse limps immediately after completing a race when the inflammation is severe, and horses with a mild inflammation begin to limp within 48 hr of completing the race. The site of inflammation is soft early during the course of the inflammation and becomes increasingly hard. Heat and tenderness decrease with time. Tendonitis often recurs when the training of a horse is begun before the affected tendon is cured. Flexor tendonitis arises from tendon fiber rupture due to overtension of the tendon. An extreme strain of the tendon due to excessive exercise or overtension, contusion or bruise of fores hit by hinds, an inappropriate posture due to incorrect tethering, shoeing, or incorrect hoof trimming seems to induce flexor tendonitis. Almost all horses with superficial digital flexor tendonitis are found to have a reddish local change at the tendon center on gross inspection [7, 9, 11]. This inflammatory change is often observed in Thoroughbred without a history of superficial digital flexor tendonitis [15]. The mechanism of development of this change is not known, but exercise-generated heat, hypoxia of the blood vessel-deficient tendon center, and load concentration on the tendon center seem responsible. Heat is generated in the tendon as it extends and contracts repeatedly during exercise [8, 13]. The cooling mechanism of blood flow does not operate sufficiently because the tendon is deficient in blood vessels at its center. Wilson *et al.* predicted by thermodynamic modeling that the temperature of the superficial digital flexor tendon is higher by 11°C at its center than at its periphery. As a matter of fact, the temperature at the tendon center reached 45°C when a horse was allowed to gallop on a treadmill. There was a difference of 5°C between the center and the periphery of the tendon [16]. Such a high temperature can not only damage the fibrous tissue but also affect the survival and nature of

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fibroblasts that are required to repair the injured tendon tissue [2, 10].

In the present study, fibroblasts of a superficial digital flexor tendon of a horse were exposed to high temperature, and the change in the survival rate was determined. Furthermore, to simulate heat generation in the superficial digital flexor tendon in a horse competing in a race, a jockey-mounted racehorse was allowed to run on a dirt track at Tokyo Racecourse. The temperature at the tendon center was measured after running. A cooling sheet was applied to the fore of the racehorse to determine its cooling effect.

Materials and Methods

Isolation and incubation of fibroblasts

Distal ends of the fores of 6 slaughtered horses were obtained. The history of flexor tendonitis was unknown. They were cooled in ice when transported. Fibroblasts were isolated from the tissue within 12 hr of slaughter. The palmar side of the metacarpal region was clipped free of hair and disinfected. With a biopsy trephine (Kai Industries, Gifu, Japan), pieces of tissue were obtained to isolate fibroblasts. An incision was made in the metacarpal region to remove the superficial digital flexor tendon. Small pieces of tissue weighing several milligrams each were harvested from the center of the tendon to culture fibroblasts. The tissue sample was washed with Hank's balanced salt solution (HBBS(−): GIBCO, Rockville, MD), transferred to a Petri dish, and to it were added fetal bovine serum (10%), penicillin (100 U/m*l*), streptomycin (100 μg/m*l*), and amphotenricin B (5 μg/m*l*). The tissue sample was cultured on Dulbecco's modified eagle medium: nutrient mixture F-12 (D-MEM/F-12, GIBCO). The tissue sample was kept pressed against the surface of the Petri dish with the aid of a coverslip (Nunc, Rochester, NY) and a weight until fibroblasts appeared. The Petri dish was cultured at 37°C under 5% CO_2. When the cells became confluent, they were suspended in trypsin-EDTA solution (GIBCO) to be subcultured.

Heating of fibroblasts and counting of surviving cells

The experiment was conducted according to Birch *et al.* [3] after passage through 3–8 subcultures. The confluent fibroblasts were washed with HBSS(−) and suspended in trypsin-EDTA solution. Viability counts were obtained by staining with trypan blue, and the inoculum was adjusted to contain 5×10^5 cells per m*l*. The cell suspensions were warmed in a water bath for 1 hr at 37°C, 39°C, 41°C, 43°C or 45°C and were incubated for 5 min at 37°C after warming. After centrifugation the cells were suspended in equal volumes of fresh media. The surviving cells were counted with a TMCS MTT assay kit (Trevigen, Gaitherburg, MD). A 100-μl aliquot of the cell suspension was put onto a 96-well microtiter plate. The plate was incubated for about 12 hr until cells adhered to the plate. The plate was further incubated for 6 hr while adding 10 μl of MTT reagent. To each plate was added 100 μl of a cytolytic fluid (Trevigen) and allowed to stand at room temperature overnight. Absorbance at 570 nm was measured with a microplate reader (MTP-12, Corona Electric, Gifu, Japan).

Measurement of superficial digital flexor tendon temperature

Two Thoroughbreds offered by the Racehorse Clinic of the Japan Racing Association Ritto Training Center were used. One was a steed weighing 518 kg with a history of right superficial digital flexor tendon rupture. The other was a mare weighing 410 kg which had no history of superficial digital flexor tendon rupture, but had a history of fracture of the proximal seasmoid bone of the right fore. Neither of the horses presented with any clinical symptoms at the time of the experiment. A jockey rode the horse, letting it trot for 30 min in preparation for the experiment. Then the jockey walked the horse on a dirt track, making one round counterclockwise (1878 m). Next, the horse made another round, first cantering and then galloping (about 10 m/s). After track running the horse trotted to the site of the experiment. The horse was laid down on the ground by intravenously administering xylazine and ketamine, and the temperature of the superficial digital flexor tendons of both fores, the palmar skin of the tendons, and the rectum was measured immediately. To take the temperature, a needle type sensor was inserted into the tendon toward its center for 5 mm. The position of the tendon center had been determined on an X-ray film so that the needle sensor could be inserted to a depth of 5 mm. The skin temperature was measured with a disc sensor, and the temperature of the rectum with a bar sensor. The temperature of these sites was measured every min until immediately before the horse came round after the anesthetics. To evaluate the effects of equine leg-cooling sheets (Winback: Saitama Daiichi Pharmaceutical, Kasukabe, Japan), they were

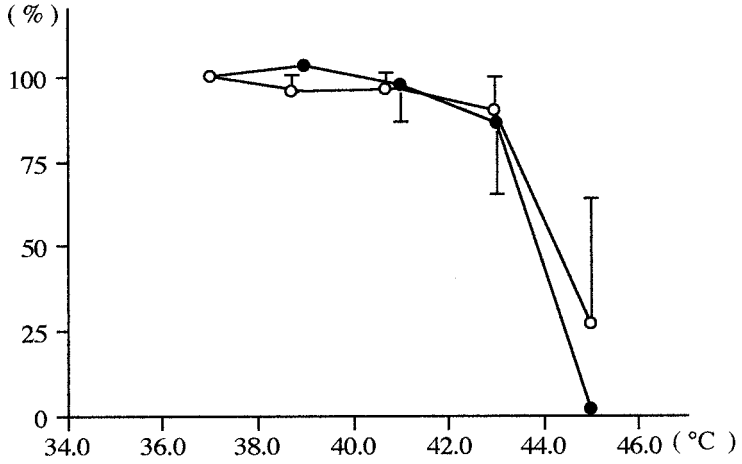

Fig. 1. Changes in the survival rate of fibroblasts exposed to high heat. Viability counts were obtained by the MTT method after 1 hr of exposure to different temperatures. An open circle indicates superficial digital flexor tendon-derived fibroblasts, and a solid circle skin-derived fibroblasts. The error bar indicates a standard deviation for 6 horses.

applied to the right fores of horse 1 and left fores of horse 2. With the same horses as a control, the temperature of the tendon, skin, and rectum was measured in the same manner during resting.

Results

Change in the survival rate of tendon-derived fibroblasts after exposure to heat

The change in the survival rate of tendon-derived fibroblasts isolated from 6 tissue samples was determined by the MTT method after 1 hr of exposure to heat. The absorbance of fibroblasts exposed to different temperatures was measured in relation to that of fibroblasts exposed to a temperature of 37°C which was arbitrarily given a value of 100 (Fig. 1). The relative absorbances of fibroblasts exposed to temperatures of 39°C, 41°C, 43°C and 45°C were 0.96, 0.96, 0.90 and 0.27, respectively. The survival rate decreased steeply in a temperature-dependent manner, especially at 43°C and 45°C. When the time of exposure was extended to 2–4 hr, the survival rate decreased in an exposure time-dependent manner at 43°C and 45°C (data not shown). A similar experiment was conducted with skin-derived fibroblasts of 6 tissue samples (Fig. 1). The relative absorbances of the 6 samples were 1.03, 0.98, 0.86, and 0.02 at temperatures of 39°C, 41°C, 43°C, and 45°C, respectively. The survival rate again decreased steeply in a temperature-dependent manner, especially at 43°C and 45°C. When the time of exposure was extended to 2–4 hr, the survival rate decreased in an exposure time-dependent manner at 43°C and 45°C (data not shown). When the temperature sensitivity of tendon- and skin-derived fibroblasts was compared, tendon-derived fibroblasts tended to show more resistance to higher temperatures than skin-derived ones.

Temperature measurement during resting

The horse was laid down on the ground by anesthetization during resting, and the temperature of the superficial digital flexor tendons of both fores, the palmar skin of the tendons, and the rectum was measured. Horse 1 was laid down on the ground in about 1 min so that temperature measurement could be started 5 min after anesthetization. The temperature of the superficial digital flexor tendon of the left fore, superficial digital flexor tendon of the right fore, the central palmar skin of the metacarpus of the left fore, the central palmar skin of the metacarpus of the right fore, and the rectum was 33.5°C, 33.8°C, 32.7°C, 32.3°C, and 38.2°C, respectively. An equine leg cooling sheet was then applied to the central palmar skin of the metacarpus of the right fore at 7 min after anesthetization, and temperature measurement was continued (Fig. 2A). The temperature of the skin and

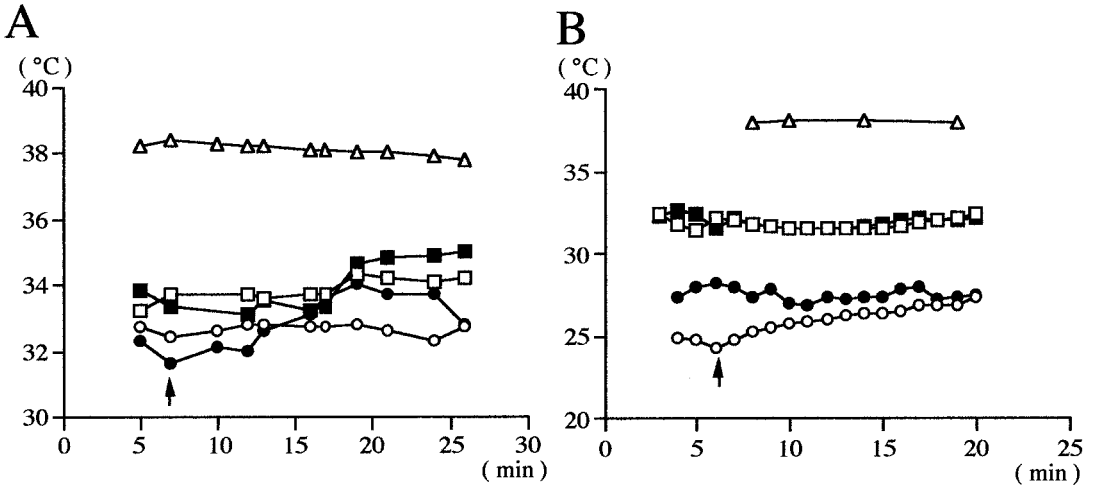

Fig. 2. Body temperatures of 2 horses measured during resting. A and B indicate horses 1 and 2, respectively. The time lapsed after anesthetization is laid off along the horizontal axis. The symbols △, □, ■, ○, and ● indicate the temperature of the rectum, the left superficial digital flexor tendon, the right superficial digital flexor tendon, the palmar skin of the left metacarpus, and the palmar skin of the right metacarpus. The ↑ symbol indicates the time of cooling sheet application (at 7 min after anesthetization). The cooling sheet was applied to the right fore of A, and the left fore of B.

tendon of the treated fore tended to rise 12 min or more after starting temperature measurement, but no other appreciable change was observed. In horse 2, temperature measurement could be started 3 min after anesthetization. The measurements obtained are shown in Fig. 2B. The equine leg-cooling sheet was applied to the left fore of horse 2 at 7 min after anesthetization. The temperture of the skin of horse 2 was lower than that of horse 1, reflecting the lower air temperature when the experiment was carried out. The temperature of the skin and tendon of the treated fore tended to rise as in horse 1.

Temperature measurement immediately after making a round of a dirt track

Horse 1 made a round of a dirt track in 3 min and 24 sec. The animal was anesthetized 56 sec after running, and temperature measurement could be started 3 min after anesthetization. The temperature of the superficial digital flexor tendon of the left fore, superficial digital flexor tendon of the right fore, the central palmar skin of the metacarpus of the left fore, and the central palmar skin of the metacarpus of the right fore was 40.4°C, 39.4°C, 37.1°C, and 37.9°C, respectively. The temperature of the superficial digital flexor tendon was higher in the left fore than in the right, presumably reflecting the fact that the left fore came under a greater load when the horse was galloping. The equine leg-cooling sheet was applied to the left fore at 4 min after anesthetization. The change in tendon temperature determined after cooling sheet application is shown in Fig. 3A. There was no appreciable difference between the left and the right fores in tendon temperature, but the skin temperature of the treated left fore was clearly lower.

Horse 2 made a round of the dirt track in 3 min and 9 sec and was anesthetized 31 sec after running. Temperature measurement could be started 3 min after anesthetization. The temperature of the superficial digital flexor tendon of the left fore, superficial digital flexor tendon of the right fore, the central palmar skin of the metacarpus of the left fore, and the central palmar skin of the metacarpus of the right fore was 40.0°C, 35.8°C, 37.3°C, and 35.7°C, respectively. The temperture of the skin of horse 2 was lower than that of horse 1, reflecting the lower air temperature when the experiment was carried out. The temperature of the superficial digital flexor tendon of horse 2 was clearly higher in the left fore than in the right, as in horse 1. These results strongly suggest that the temperature of the superficial digital flexor tendon materially differs from the left fore to the right when a horse is galloping. The equine leg-cooling sheet was applied to the right fore at 3 min after

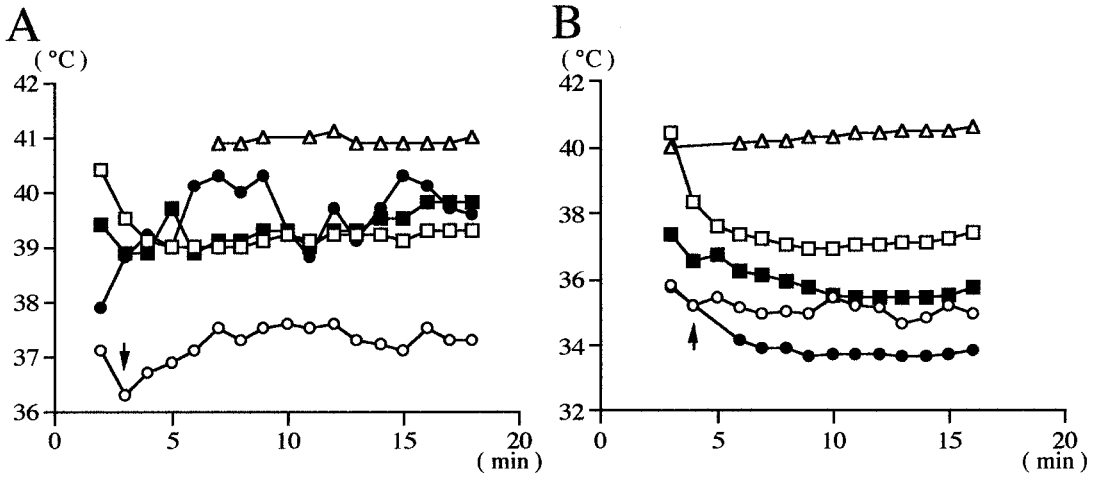

Fig. 3. Body temperate of 2 horses measured after galloping. A and B indicate horses 1 and 2. The time lapsed after anesthetization is laid off along the horizontal axis. The symbols △, □, ■, ○ and ● indicate the temperature of the rectum, the left superficial digital flexor tendon, the right superficial digital flexor tendon, the palmar skin of the left metacarpus, and the palmar skin of the right metacarpus. The ↑ symbol indicates the time of cooling sheet application (at 4 min after anesthetization). The cooling sheet was applied to the left fore of A, and the right fore of B.

anesthetization. The change in tendon temperature determined after cooling sheet application is shown in Fig. 3B. The temperature of the superficial digital flexor tendon was consistently lower in the left fore, and the skin temperature was clearly lower in the treated right fore.

Discussion

The tendon not only connects the muscle and bone dynamically during exercise but stores elastic energy, thus increasing the efficiency of exercise [1, 4]. A major fraction of the stored elastic energy is converted back to kinetic energy, and 5% to 10% of the remaining elastic energy is released as heat. The center of the tendon is deficient in blood vessels so that the cooling mechanism does not operate sufficiently so that the tendon is prone to accumulate heat. In the present study, a jockey ran a horse on a dirt track to simulate the condition of competition in an actual race. As a result, it was found that heat was generated in the tendon center and that the tendon temperature differed from the left fore to the right in the present study, presumably reflecting a difference in exercise load between the fores according to the lead of gallop or running direction on a round race course. For technical reasons the temperature of the tendon could not be measured earlier than 3 min after running. The temperature of the tendon center was higher than 40°C 3 min after running, and showed a decrease of about 1°C 1 min after the first temperature measurement. Assuming that the tendon center is cooled by heat conduction alone without the mediation of blood flow, the temperature of the tendon center at the end of exercise should be 43°C or more when Fourier's rule of heat conduction is considered. In an actual race the exercise load would be greater, so that the temperature of the tendon center would be higher still. In the present study, fibroblasts were isolated from the superficial digital flexor tendon and their sensitivity to heat was determined. As a result, it was found that the number of dead cells was increased after 1 hr of exposure to a temperature of at least 43°C and that almost all cells were dead after 1 hr of exposure to a temperature of 45°C. Tendon-derived fibroblasts were a little more resistant to heat than skin-derived ones. As a matter of fact, the temperature of the tendon center would not remain at 43°C for 1 hr, but may exceed 45°C, though for a brief period, and the tendon center may form a hypoxic environment because it lacks blood vessels. This environment may adversely affect not only fibroblast proliferation but also physiological function associated with the maintenance and repair of the

tendon. In fact, we observed that gelatinase activity in the equine fibroblasts was decreased when they were exposed to a temperature of 43°C (data not shown). The damage done by heat to fibroblasts seems like to be the primary cause of tendon center degeneration that is seen even in horses without flexor tendonitis.

What should be done to prevent flexor tendonitis? The direct cause of flexor tendonitis is tendon fiber rupture caused by running, and tendon center degeneration is behind it. If a degenerative change in the tendon center accounts for much of heat generated there, then direct treatment of temperature reduction in the superficial digital flexor tendon will be effective in the prevention of flexor tendonitis. In the present study, we made an attempt to cool the superficial digital flexor tendon through topical application of an equine leg-cooling sheet. When it was applied during resting, the skin temperature showed a slight decrease as the cooling sheet deprived the skin of its heat immediately after application, but the skin temperature tended to rise thereafter. It seems that when the temperature of the surrounding skin is clearly lower than the body temperature, a compensatory mechanism that derives its drive mainly from blood flow operates to keep the skin temperature constant. On the other hand, when the skin temperature was relatively high after exercise, the temperature of the skin of the treated fore showed a clear decrease. The tendon center is deficient in blood vessels so that heat conduction accounts for much of the cooling effect. Since heat conductivity is proportional to the difference between the tendon center and the surrounding skin temperature, the cooling of the surrounding skin provides an effective means of cooling the tendon center. The cooling of the surrounding area of the superficial digital flexor tendon lacking blood vessels should be of significance unlike in well-vascularized muscles.

In the present study, heat generated in the tendon during running was noted as a cause of tendon center degeneration. The equine leg-cooling sheet used in this study was found to be safe, for it had no excessive cooling effect during resting. It would inhibit heat generation in the superficial digital flexor tendon when worn during or immediately after running. Nevertheless, large-scale studies are warranted to demonstrate an inhibitory effect of leg cooling on the development of flexor tendonitis. A significant improvement in riding grounds would be most effective in the prevention of flexor tendonitis, but for the time being it seems worthwhile to cool legs as a relatively easy means to prevent the development of flexor tendonitis.

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