Blood supply of the peroneal tendons

Injection and immunohistochemical studies of cadaver tendons

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ABSTRACT – We studied the vascular pattern of human peroneal tendons with injection techniques and immunohistochemically by using antibodies against laminin.

The main blood supply arises from the peroneal artery. The distal part of the peroneus longus tendon is supplied by branches of the medial tarsal artery. Blood vessels enter the peritenon of both tendons via a mesotenon from the posterior aspect. From the peritenon, the blood vessels penetrate the peroneus brevis and peroneus longus tendons and anastomose with a longitudinally-oriented intratendinous network. The amount of vessels in the tendon substance is consistently less than in the surrounding peritenon. The distribution of blood vessels in the peroneal tendons is not homogeneous. In the region where the peroneus brevis tendon passes through the fibular groove, the longitudinally-oriented intratendinous vascular network is interrupted and the tendon is almost avascular. In this region, the tendon is squeezed between the peroneus longus tendon and the bony slide bearing of the lateral malleolus. The peroneus longus tendon has two avascular zones. In the region where the peroneus longus tendon curves around the lateral malleolus and the peroneal trochlea of the calcaneus, the anterior part of the tendon which is directed towards the pulley is avascular. A second avascular zone is located more distally in the region where the tendon changes direction and wraps around the cuboid.

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Cadaveric studies in the elderly have shown a high frequency of degenerative changes in the peroneal tendons (Sobel et al. 1992). Recent clinical studies have found that degenerative longitudinal splitting of the peroneal tendons may cause chronic pain and need for surgical treatment (Mason and Henderson 1996, Bonnin et al. 1997, Saxena and Pham 1997, Subotnick 1997, Karlson et al. 1998). Reduced vascularity is an important factor contributing to tendon degeneration and possible rupture under strain (Leadbetter 1992, Josza and Kannus 1997). Benjamin et al. (1995) have shown that in the peroneus longus tendon there is also fibrocartilage in the region where the tendon wraps around the medial malleolus and around the cuboid.

Sobel et al. (1992) described the microvasculature of the peroneal tendons with injection techniques. They suggested that the peroneal tendons have a complete vascular network without evidence of avascular zones (Sobel et al. 1992). Histological findings of Benjamin et al. (1995) were different and indicated that fibrocartilage normally is avascular (Tillmann 1998).

Injection techniques may give false positive and false negative results (Rudert and Tillmann 1993, Petersen and Tillmann 1995, Petersen and Hansen 1997). Laminin is a basic component of the basement membrane (Timpl et al. 1979) and immunohistochemical staining detects blood vessels reliably in dense connective tissue (Rudert and Tillmann 1993, Petersen and Tillmann 1995, Petersen and Hansen 1997).

We assessed the blood supply of the peroneal tendons by injection techniques and by immunohistochemical methods in cadavers.

Material and methods

Material

The cadavers studied were from the Departments of Anatomy and Pathology of the Christian-Albrechts-University of Kiel. All tendons studied were free of macroscopically visible degenerative changes.

Injection techniques

In the injection techniques, 10 lower limbs of fresh cadavers (age 45–80 years) were shock-frozen in liquid nitrogen to prevent ice crystal damage of the vessels. The femoral arteries of the limbs were injected with india ink gelatin solution under continuous manual pressure. The injection pressure, which was similar to the arterial blood pressure (120 mm Hg), was controlled by a pressure gauge. The temperature of the cadaver limbs and of the injection medium was 40 °C to prevent premature hardening of the gelatin solution. After the injection, all specimens were fixed in 4% formalin. Then the skin, subcutaneous fat tissue and fascia were removed to show which arteries contributed to vascularization of the peroneal tendons. The tendon sheaths were opened in a longitudinal direction and their retinacula were studied macroscopically. In the investigation of the intratendinous vascularization and measurement of the length of the avascular zones, the tendons were prepared according to Spalteholz (1914). After investigation with a magnifying lens and photographic documentation, all tendons were split in a longitudinal direction to analyze the extension of the avascular zones.

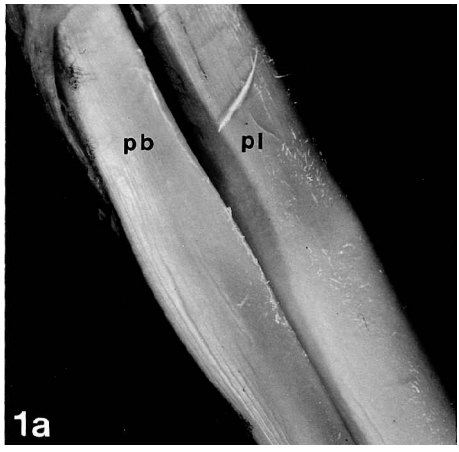
Immunohistochemistry

In the immunohistochemical investigations, 20 peroneus longus and 20 peroneus brevis tendons together with their bony attachment sites were obtained at autopsies performed within 48 hours after death (age of subjects: 39–80 years). The tendons were divided into 2 cm segments (Figure 1). All samples were shock-frozen in liquid nitrogen. Longitudinal frozen sections of each segment were cut with a cryostat at –30 °C and mounted on gelatin-coated slides. For immunohistochemistry, frozen sections were pretreated with testicular hyaluronidase (Boehringer, Ingelheim, Germany) in tris-buffered saline (TBS) in a moist chamber at 37 °C for 30 minutes. The sections were washed three times with TBS and incubated with goat serum for 45 minutes at room temperature. Incubation with the primary antibody was carried out for 60 minutes at room temperature. We used a monoclonal laminin antibody (Medac, Hamburg, Germany). Sections were labeled with the respective secondary antibody, fluorescein thiocyanate conjugated (FITC) goat anti-rabbit IgG (Medac, Hamburg) for 45 minutes. Control sections were incubated only with the FITC-conjugated antibody. Positive controls including a tissue with defined antigen sites (skeletal muscle) were used. 5 sections of each segment were evaluated qualitatively with a microscope equipped for epifluorescence. For the exact localization of the immunoreactions, each section was analyzed with polarized light. The presence of positive immunoreactions in each quarter of the tendon diameter was noted (Figure 1). If there was one positive immunoreaction, we counted it as a positive result.

Results

Injection studies

The injection specimens showed that the major blood supply for the peroneal tendons came from the posterior peroneal artery. The branches of the posterior peroneal artery reached the tendons via a mesotenon from the posterior aspect (Figures 1 and 2). The major part of the tendons was covered by a peritenon, where the blood vessels formed a web-like network. From the peritenon, the blood vessels penetrated the tendon tissue and anastomosed with an intratendinous arterial network. In the tendons, most of the blood vessels were oriented longitudinally along the course of the tendon fibers. The intratendinous distribution of blood vessels was not homogeneous (Figures 2 and 3). In the region where the peroneus brevis tendon passes through the fibular groove, the longitudinally-oriented intratendinous vascular network was interrupted and the tendon was nearly avascular (Figure 2). In this region, the tendon is squeezed between the peroneus longus tendon and the bony slide bearing of the lateral malleolus (Figure 1). The avascular zone of the peroneus



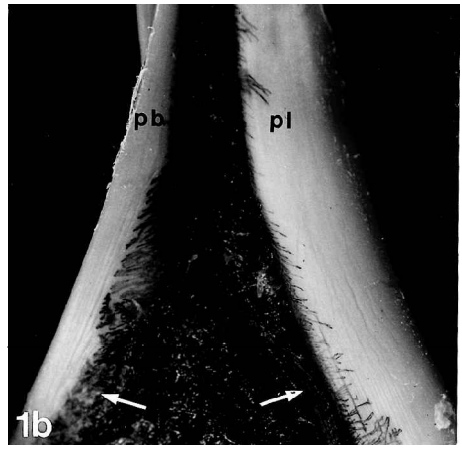


Figure 1. A. India ink injection of a peroneus longus tendon (detail from the region where the tendons wrap around the lateral malleolus. View from anterior aspect). The peroneus brevis tendon (pb) is squeezed between the peroneus longus tendon (pl) and the lateral malleolus. In this region both tendons lack a peritenon. Age: 45 years, female.

B. Same specimen as in A after unfolding the tendons, indicated by the small arrows. Terminal endings of the peroneal artery reach the tendons via a mesotenon from the posterior aspect. pl: peroneus longus tendon, pb: peroneus brevis tendon.

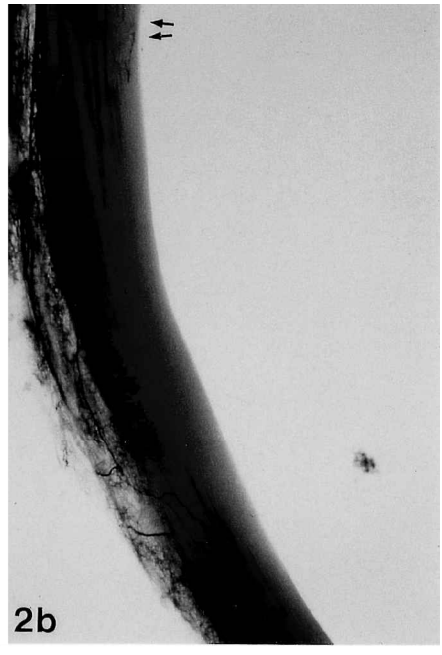


Figure 2. A. India ink injection of a peroneus brevis tendon after clearing of the tissue (detail from the region where the tendon wraps around the lateral malleolus, view from lateral aspect). Terminal endings of the peroneal artery enter the tendon via a mesotenon (arrowheads) from the posterior aspect. The proximal and distal parts of the tendon are covered by a well vascularized peritenon where the blood vessels form a web-like network (small arrows). In the region where the tendon turns around the lateral malleolus, the well vascularized peritenon is absent. In this area, the tendon tissue is avascular. Age: 61 years, male.

B. Longitudinal section of a peroneus longus tendon (detail from the region where the tendon wraps around the lateral malleolus, view from lateral aspect). The proximal and distal parts of the tendon are covered by a peritenon (small arrows). The distribution of blood vessels in the tendon is not homogeneous. The posterior part of the tendon has a complete vascular network. In the anterior part, the longitudinally-oriented intratendinous network is interrupted. In this zone, the tendon tissue is avascular. India ink injection. Age: 45 years, female

Table 1. Results of the injection techniques. Length of an avascular zone, mm

No.	Age	Peroneus brevis tendon	Peroneus longus tendon	
		Lateral malleolus	Lateral malleolus	Cuboid
1	45	29	45	25
2	54	41	61	18
3	61	55	38	24
4	65	31	47	30
5	69	50	51	29
6	71	42	50	31
7	75	37	63	19
8	76	40	56	23
9	78	45	59	26
10	80	45	51	23
Average	67	40	52	25

brevis tendon had a variable longitudinal extension between 29 mm and 55 mm (average length: 40 mm, Table 1).

The peroneus longus tendon had two avascular zones. In the region where the peroneus longus tendon curved around the lateral malleolus and the peroneal trochlea of the calcaneus, the anterior part of the tendon which is directed towards the pulley, was avascular (Figure 2). This avascular zone had a variable length between 38 mm and 63 mm (average length: 52 mm, Table 1). A second avascular zone was located more distally in the region where the tendon changes its direction and wrapped around the cuboid (Figure 3). The length of this avascular zone varied between 18 mm and 31 mm (average length: 25 mm, Table 1).

Detection of blood vessels by immunohistochemical demonstration of laminin

Compared with human skeletal muscle, immunohistochemical demonstration of laminin in the wall of blood vessels was positive in all examined sections. Even small collapsed capillary vessels became visible (Figure 4). The immunohistochemical study of the vascular anatomy confirmed the results of examination with injection techniques. The peroneal tendons were covered with a well vascularized peritenon (Figure 4). In the tendon, the blood vessels were located in the loose connective tissue between the longitudinal fiber bundles.

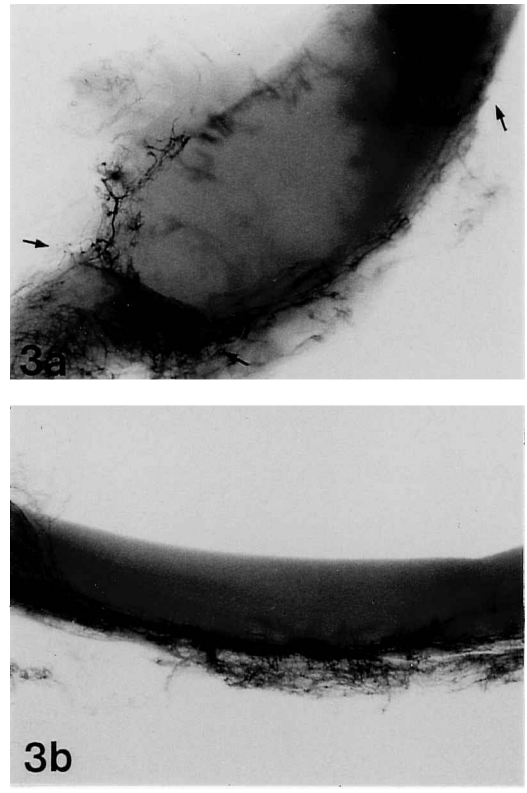


Figure 3. A.India ink injection of a peroneus longus tendon (detail from the region where the tendon wraps around the cuboid, view from above). The proximal and distal parts of the tendon are covered by a peritenon where the blood vessels form a web-like network (small arrows). In the area where the tendon turns around the cuboid, there is an avascular zone. India ink injection. Age: 54 years, female B. India ink injection of a peroneus longus tendon (detail from the region where the tendon wraps around the cuboid, longitudinal section, view from lateral aspect). In this region, the longitudinally-oriented intratendinous network is interrupted and the tissue is avascular. India ink injection. Age: 61 years, male

The distribution of blood vessels in the peroneal tendons was not homogeneous (Figure 6, Table 2). In the peroneus brevis tendon, there was no immunohistochemical proof of laminin in the segment in the peroneal groove of the fibula (segment 2 in Figure 1a). Positive immunoreactions were found only in the posterior quarter of the retromalleolar gliding zone of 6 specimens (Table 2).

In the peroneus longus tendon there was no immunohistochemical proof of laminin in the anterior part of the gliding zone where the tendon turns around the peroneal groove of the lateral malleolus and the calcaneus and in the region where the

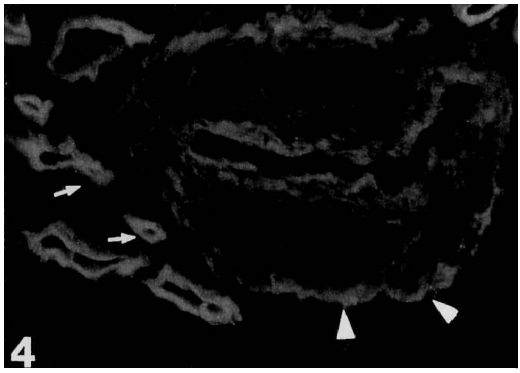


Figure 4. Immunohistochemical proof of laminin in the basement membrane of blood vessels, detail from the peritenon of a peroneus longus tendon. Compared with human skeletal muscle, immunohistochemical demonstration of laminin in the wall of blood vessels was positive in all sections examined. This method allows the detection of blood vessels of different size and diameter. Even small collapsed capillaries become visible (arrowheads: arteriole, small arrows; capillaries). Age 51, male, × 60

peroneus longus tendon turns around the cuboid (Table 2).

Discussion

Only Sobel et al. (1992) have previously reported on the intratendinous blood supply of the peroneal tendons and they did not find a hypovascular zone. They used conventional injection techniques which may lead to false negative or false positive results (Rudert and Tillmann 1993, Petersen and Tillmann 1995, Petersen and Hansen

Table 2. Positive laminin immunoreactivity in the gliding zones of the peroneus longus and peroneus brevis tendons (segment numbers 2 and 4 in Figure 5). Total number of cases with positive immunoreactivity in various quarters of the tendon diameter

	Peroneus brevis tendon n 20	Peroneus longus tendon n 20	
Tendon quarter	Lateral malleolus segment 2	Lateral malleolus segment 2	Cuboid segment 4
1	0	0	0
2	0	1	0
3	0	13	3
4	6	20	18

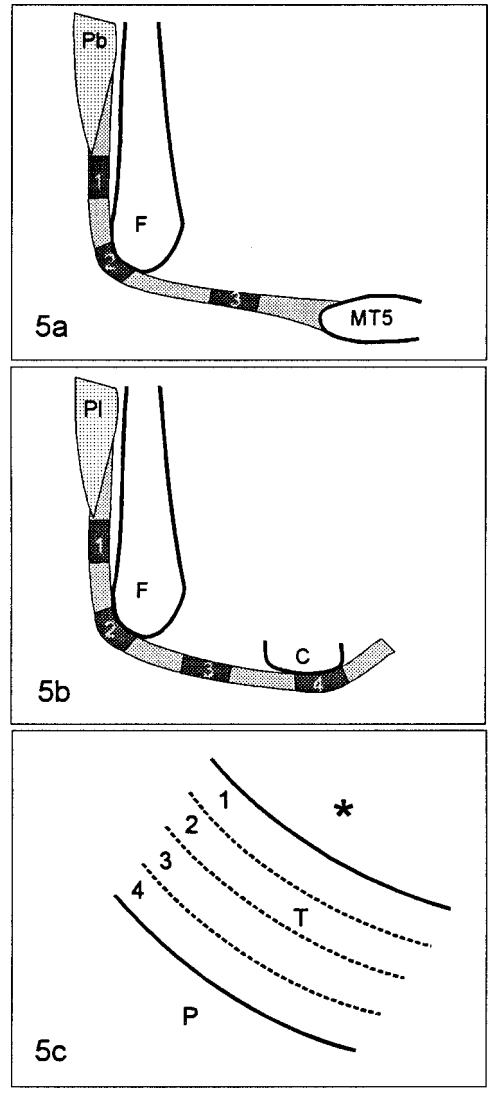
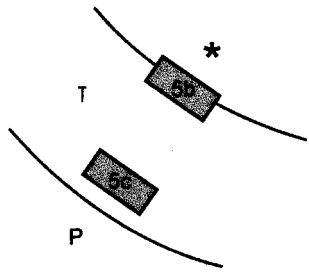


Figure 5. A. Location of 3 segments (dark-marked) obtained from the peroneus brevis tendon (Pb: peroneus brevis muscle) for immunohistochomical proof of laminin (F: fibula, MT5: fifth metatarsal). Segment 2 came from the region where the peroneus brevis tendon wraps around the lateral malleolus. The segments measured 2 cm.
B. Location of biopsies (dark-marked) from the peroneus longus tendon (Pl: peroneus longus muscle) for the immunohistochemical investigations. From each tendon 4 segments (length: 2 cm) different regions were (F: fibula, C: cuboid).
C. In the immunohistochemical analysis of the blood supply (proof of laminin in the wall of vessels), the presence of positive immunoreactions in different quarters of the tendon were noted: 1. anterior quarter adjacent to the gliding surface, 2 and 3. middle quarters, 4. peripheral quarter (Table 2). The asterisk marks the location of the bony pulley (T: tendon, P: peritendineum).



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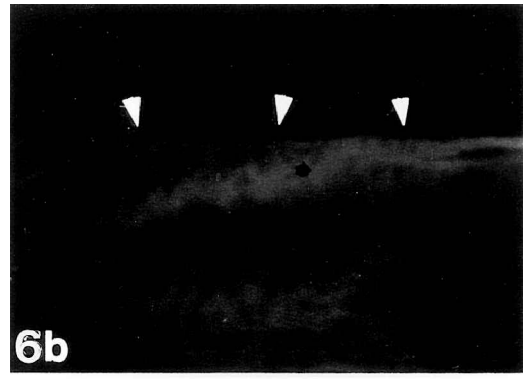

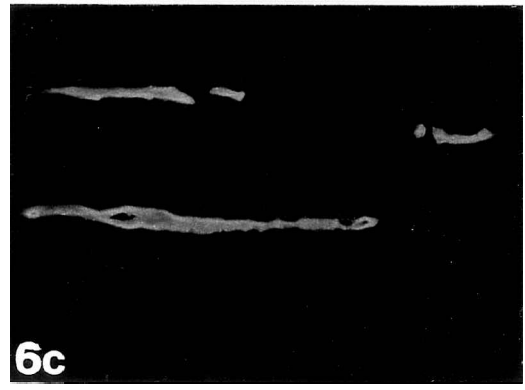

Figure 6. A. Immunohistochemical proof of laminin in the region where the peroneus brevis tendon turns around the lateral malleolus (segment 2, according to Figure 1B, asterisk: lateral malleolus, T: tendon). This schematic drawing shows the histological details in Figures 5B and 5C.
B. Immunohistochemical proof of laminin (detail of the anterior quarter of the tendon, see Figure 5 A). Arrowheads mark the tendon surface. In this region, the immunohistochemical proof of laminin was negative in all 20 tendons (compare to Table 2). × 20, age: 63 years, fitc conjugated antibody.
C. Immunohistochemical proof of laminin (detail of the posterior quarter of the tendon, see Figure 5 A). In the posterior part, the immunohistochemical proof of laminin was positive in all 20 tendons investigated (compare to Table 2), age 63, fitc conjugated antibody. × 20.

1997). A false positive result may be caused by injecting medium into the paravasal space, due to a high injection pressure or when a vessel is damaged due to postmortal degradation. A false negative result may be caused by microembolism, by insufficient filling of the vessels due to a low injection pressure, or when the injection medium hardens before it reaches the terminal arteries.

Our observations about the gross blood supply of the peroneal tendons agree with previous findings (Huber 1942, Sobel et al. 1992, Tillmann 1998). The peroneal tendons receive their major blood supply from the posterior peroneal artery and branches of the medial tarsal artery. The blood vessels enter the tendons from the posterior aspect via a mesotenon.

In contrast to the findings by Sobel et al. (1992), our results show that the distribution of blood vessels in the peroneal tendons is not homogeneous. In the peroneus brevis tendon, there is an avascular zone in the region where the tendon turns around the lateral malleolus. The peroneus longus tendon has two avascular zones. One is located in the region where the tendon turns around the lateral malleolus and the peroneal trochlea of the calcaneus, the other avascular zone is located where the tendon turns around the cuboid. In all three avascular zones demonstrated by injection techniques, the immunohistochemical demonstration of laminin remained negative. The discrepancy between our results and those of Sobel et al. (1992) may arise from the accuracy of the methods used.

Avascular zones consisting of fibrocartilage occur in several gliding tendons in regions where the tendons run around a bony pulley (Ploetz 1938, Tillmann et al. 1991, Benjamin et al. 1995, Koch and Tillmann 1995, Stein et al. 1999). The peroneus longus tendon is a typical gliding tendon and Benjamin et al. (1995) have shown that the regions where this tendon changes its direction also consist of fibrocartilage. The location of the avascular zones found in this study corresponds with the location of the fibrocartilaginous zones described by Benjamin et al. (1995). Our finding indicating that the gliding zones of the peroneus longus tendon are avascular is supported by the findings of Benjamin (1995) because fibrocarti-

lage normally is avascular (Tillmann 1998). The peroneus brevis tendon which is squeezed between the peroneus longus tendon and the bony pulley is stressed from two sides by compression. This may explain why in this region the entire tendon is nearly avascular and not only the anterior part which is directed towards the bony pulley.

Although there are individual differences in the extension of the avascular zone in the anterior portion of the peroneal tendons, we found no relation to age (Table 1).

Tenosynovitis of the peroneal tendons is related more to sports than work (Josza and Kannus 1997). Burman (1934) described three regions with an increased incidence of tenosynovitis: 1. posterior to the lateral malleolus, 2. at the peroneal trochlea of the calcaneus (peroneus longus), and 3. under the cuboid (peroneus longus). Our study has shown that the blood supply of the tendon is critical in all three regions.

The most frequent type of rupture in both peroneal tendons is longitudinal splitting in the region behind the lateral malleolus (Sobel et al. 1992). This location corresponds well to the avascular region of the gliding zone behind the lateral malleolus.

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