Ultrasonography in the assessment of tendon disease

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Ultrasonography in the assessment of tendon disease: Methodology and diagnosis

PhD thesis

2008

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PhD thesis
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Acknowledgment

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Merete Juhl Kønig
April 2008
Preface and original manuscripts

The research underlying this PhD thesis was carried out in the period 2004 – 2007 at the Parker Institute, Frederiksberg Hospital, at the Department of Sports Medicine, Frederikssund Sygehus, and on location in Athletion Sports Arena, Århus.

The PhD thesis is based on the following four studies, which will be referred to by their roman numerals. The thesis was planned to include anterior knee tendons only, however, lack of patients with anterior knee problems made it necessary to include other tendons than the anterior knee tendons, and therefore a study of the normal Achilles tendon is part of the thesis.


Introduction

In rheumatology and sports medicine high-resolution ultrasound (US) is currently gaining popularity as an imaging modality for musculoskeletal diseases, both as a routine extension of the clinical examination, and as a research tool. US is commonly accepted as the method of choice to evaluate changes in patellar tendon disease. Most musculoskeletal US is performed using GS US, which has enabled the detection of morphological changes in tendon structures. Ultrasound with Doppler makes it possible to visualize the perfusion in tendon structures and may be of advantage in evaluation of jumper’s knee. This PhD study deals with Doppler US in relation to tendon disease. In the present literature, there is a lack of basic knowledge on methodology, definitions and diagnostic criteria for evaluating tendons when using US Doppler in clinical practice.

Aim

The aim of this PhD study was to evaluate the possible advances in the assessment of tendons by using US with Doppler. The aim was pursued by investigation of:

The perfusion in the normal Achilles tendon.
The effect of different knee positions on the US Doppler findings in patellar tendon.
The presence and distribution of possible intratendinous flow in the anterior knee tendons of elite badminton players before and after a match.
The clinical findings compared with US Doppler in the diagnosis of jumper’s knee

Hypotheses

The four studies had the following hypotheses:

I.
Normal Achilles tendons have vessels that can be visualized by the use of US contrast agent. The resistive index (RI) of these vessels is 1.

II.
Changes in patient position will influence Doppler findings. The more tension on the tendon, the less intratendinous Doppler activity.
III.
Painful anterior knee tendons are common among elite badminton players.
Elite badminton players have intratendinous color Doppler activity in the anterior knee tendons.
Doppler activity increases after match.
Players with painful tendons have more color Doppler activity than players with painfree tendons.

IV.
An extensive test of clinical signs will uncover more clinical tests that may point at jumper’s knee.
US with Doppler is a sensitive tool in the diagnosis of jumper’s knee.

Ethical considerations
The local Ethical Committee has approved the protocols for all studies in this thesis. All studies were performed according to the Helsinki declaration.
Informed consent was obtained form all the subjects and patients included.
**Terminology**

Overuse of the anterior knee tendons may lead to pain and functional deficit. This overuse condition has been called many names: jumper’s knee, patellar tendonitis, patellar tendinitis, patellar tendinosis, patellar tendonosis, patellar tendon disorder, patellar apicitis, patellar enthesitis, insertion tendinitis of the patellar tendon, quadriceps tendinitis, partial rupture of the patellar tendon and patellar tendinopathy (1-4).

The many terms reflect differences in interpretation of etiology and pathogenesis.

The suffix – osis indicates a degenerative etiology, whereas –itis indicates an inflammatory etiology, and the term tendinopathy simply indicates that there is a pathological process in the tendon without referring to the etiology.

The articles underlying this PhD thesis are based on clinical research, which is why we choose to refer to the clinical term jumper’s knee. Alternatively, we use the ultrasound-diagnostic term patellar tendon hyperemia.
Background

The anterior knee tendon complex

Anatomy

The quadriceps muscle is the extensor of the knee. The muscle is the largest in the body and provides knee stability, and transmits the force created within the muscle to the bone insertion at the tibial tuberosity (5). The quadriceps muscle is made up from four distinct muscles: rectus femoris, vastus medialis, vastus lateralis, and vastus intermedius. The tendons of the different portions of the quadriceps unite at the lower part of the thigh to form a single strong tendon, the quadriceps tendon. The fibers of the rectus femoris and the vastus intermedius insert almost perpendicular to the superior part of the patella, whereas the vastus medialis and vastus lateralis insert obliquely (6). The patellar tendon is an extension of the quadriceps tendon, primarily generated from central fibers of the rectus femoris tendon that continue over the anterior surface of the patella, and from fibers from the distal part of the patella. The patella may be regarded as a sesamoid bone, developed in the quadriceps tendon. The patellar tendon inserts at the upper part of the tibial tuberosity, continues past the tubercle to blend with the extension of the tendon sheath of the iliotibial tract on the anterior surface of the tibia (6).

The appearance of the tendon is dependent on the degree of tension to the tendon. In the resting state the tendon has a wavy configuration that appears as regular bands across the surface of the tendon. When the tendon is stretched, the wavy pattern disappears (7). With the knee flexed (the tendon stretched) the quadriceps tendon is mean 6.8 cm long, 4.1 cm wide and 4.8 to 5.2 mm thick (6). The patellar tendon is hourglass shaped, flat and broad in the proximal part, slim in the mid-portion of the tendon, and generally becomes thicker and narrow distally. The length is about 4.3 cm., 3.2 cm. wide at the apex of the patella and 2.7 cm. at the tibial tuberosity, 2.6 to 4.2 mm. thick at the apex (8) and 2.6 to 3.1 mm. at the tibial tuberosity (6;9). Athletes have slightly thicker tendons (10).
**Blood supply**

The arterial blood supply to the extensor mechanism of the knee arises from the femoral, the popliteal, and the anterior and posterior tibial arteries, with the popliteal artery as the main contributor.

The vessels are divided in three vascular levels: arterial pedicles, anastomotic arches and tendon arterioles.

Six main arterial pedicles are placed symmetrically around the anterior portion of the knee forming an arterial circle. The superiors are located at the upper part of the patella just in front of the attachment of the quadriceps tendon, the middles are located in the femorotibial interarticular space and the inferiors just proximal to the tibial tuberosity. The anastomotic arches connect the pedicles both transversely and longitudinally. Three main arches are identified: the superior (placed anterior to the quadriceps tendon), the middle (in the posterior part of the patellar tendon) and the inferior (anterior to the tibial tuberosity). The peritendinous tissues are supplied from both pedicles and arches. The peritendinous tissue is characterized by a large number of arterioles, mainly located in the anterior part and with the highest density at the tibial tuberosity.

The anastomotic arches give rise to arterioles from which the tendons are supplied. Vessels enter the tendons at three sites. The quadriceps tendon is pierced from the superficial side, the upper part of the patellar tendon from the posterior side and the tendon at the tibial tuberosity from the superficial side. In the patellar tendon, the arterioles travel along the tendon fibers and anastomose in the middle third (5:11-14).

Figure 2. The extensor mechanism of the knee
Jumper’s knee

Etiology
The etiology of JK is unknown (3;15-21). There are many theories on the etiology of JK. The three prevailing theories are: the mechanical (stress resulting in mechanical overload), the vascular (compromised blood supply), and latest the neural (series of observation of changes in neural transmitters and mediators)(22). All three theories have strengths and weaknesses and tendinitis/tendinosis is far from fully understood. The theories are extensive, however the most predominant is the mechanical theory, an overuse injury of the anterior knee tendons (15;23-26). Although there are multiple theories on the cause of disease, it is unlikely that a single element causes JK (27).

Predisposing factors
Theories on predisposing factors are extensive, though centered on pathologic findings in the patella or the muscles around the knee (2;4;15;23;28-30). It is believed that the development of JK is a result of the interplay of two groups of factors – extrinsic and intrinsic factors (31). Which factor is the most important is debatable: There are favors of both intrinsic (24) and extrinsic factors (23).

Table 1. Suggested predisposing factors associated with jumper’s knee.

<table>
<thead>
<tr>
<th>Intrinsic risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patellar hypermobility (2;15;24;28;32)</td>
</tr>
<tr>
<td>Patella alta (2;4;15;29;33)</td>
</tr>
<tr>
<td>Tendency to patellar subluxation (2;15;24;29)</td>
</tr>
<tr>
<td>Abnormal patella tracking (32)</td>
</tr>
<tr>
<td>Increased length of the patella (34)</td>
</tr>
<tr>
<td>Abnormal knee-alignment (2;23;24;29)</td>
</tr>
<tr>
<td>Reduced antero-posterior patella tilt angle (35)</td>
</tr>
<tr>
<td>Hyperlaxity syndrome (29;33)</td>
</tr>
<tr>
<td>Leg length discrepancy (4;24;29;36)</td>
</tr>
<tr>
<td>Decreased flexibility of the quadriceps and hamstrings muscles (24;36-38)</td>
</tr>
</tbody>
</table>
Reduced ankle dorsiflexion range, abnormal range of motion in the ankle joint (30)
A combination of high ankle inversion-eversion moments, high external tibial rotation and plantar flexion moments, large ground reaction forces, and high rate of knee extensor moment development (39;40)
Impingement of the inferior pole of the patella on the patellar tendon in flexion (17)
Iliotibial band tightening (29)
Knee instability (23;29)
Abnormal patella position (2)
Pelvis and hip disease (4)
Muscular imbalance or insufficiency (23;24;29;41)
Hyperpronation of the foot (29)
Long patellar tendon (4;29)
Increased rotation of femur and tibia (2;23)

<table>
<thead>
<tr>
<th>Extrinsic risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Playing on hard surface (23;33;42)</td>
</tr>
<tr>
<td>More than four training sessions per week (23)</td>
</tr>
<tr>
<td>Height and weight: Increased height (24;28), increased weight (24;29;43), and increased BMI (36)</td>
</tr>
<tr>
<td>High vertical jump ability (38;43;44)</td>
</tr>
<tr>
<td>Stiff landing strategy (45)</td>
</tr>
<tr>
<td>Excessive load on the body (type of movement, speed of movement, number of repetitions, footwear (29)</td>
</tr>
<tr>
<td>Training errors (too long distance, too high intensity, too fast progression, and too much hill work (29;33;42)</td>
</tr>
<tr>
<td>Monotonous, asymmetric and specialized training only (29)</td>
</tr>
</tbody>
</table>

Unfortunately, the scientific support to these theories is sparse, and further research on a prospective randomized basis is needed.
Epidemiology

Jumper’s knee is a clinical syndrome commonly associated with sports activities. Although volleyball is particularly well described, many sport disciplines have been associated with JK, table 2.

Table 2. Sports associated with jumper’s knee.

<table>
<thead>
<tr>
<th>Sport</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volleyball (2;16;23-25;28;46-50)</td>
</tr>
<tr>
<td>Basketball (2;23-25;28;33;38;47-49)</td>
</tr>
<tr>
<td>Track (athletic) (51)</td>
</tr>
<tr>
<td>High jump, long jump and triple jump (2;16;23;25;28;33;47;49)</td>
</tr>
<tr>
<td>Icehockey (25)</td>
</tr>
<tr>
<td>Running (15;16;48)</td>
</tr>
<tr>
<td>American Football (2)</td>
</tr>
<tr>
<td>Tennis (2;24;28;49)</td>
</tr>
<tr>
<td>Climbing (2;15)</td>
</tr>
<tr>
<td>Soccer (24;25;28;47;49;52)</td>
</tr>
<tr>
<td>Gymnastics (24;28)</td>
</tr>
<tr>
<td>Weight-lifting (23;53)</td>
</tr>
<tr>
<td>Cycling (23;25)</td>
</tr>
<tr>
<td>Handball (24;25)</td>
</tr>
<tr>
<td>Skiing (47)</td>
</tr>
<tr>
<td>Ballet dancing (47)</td>
</tr>
</tbody>
</table>

It appears that elite athletes are more affected by JK than recreational athletes (3). Among military recruits, JK accounts for 15 % of the soft tissue injuries, which has been ascribed to an abrupt increase in physical activities (54).

The frequency of JK is reported differently in different sports. It seems that almost any sport with excessive strain on the anterior knee tendons or any repeated overload activity to the knee is able to cause JK.

The prevalence of JK in different sports is mostly unknown, however it seems that the prevalence is high in sports with large demands on speed and power (16;28;55). The overall prevalence of JK among elite athletes in different sports is reported as 14% (25), others
report an incidence of up to 20 % in an athletic population (24;56). The right knee is affected more often than the left knee (25). The prevalence is highest in volleyball players, i.e. 45 % (23;25;47), and in basketball players, 32% (25).

There seems to be some discordance in reported differences in gender prevalence. In handball and football players women have a lower prevalence than men (25). In volleyball players some discordance between studies are found; the same authors report both no gender differences and a higher incidence among female players (23;42).

It seems that there are gender differences in the frequency of unilateral and bilateral JK. Bilateral JK is equally prevalent, unilateral JK is seen twice as often in men than in women (57).

**Classification and grading**

Blazina used the term "jumpers knee" the first time in 1973. He described the symptoms and clinical findings from the infrapatellar- or suprapatellar regions (2). In 1978 Roel described symptoms from the insertion on the tibial tuberosity and suggested the site as a possible addition in the JK diagnosis (15). The tibial tuberosity was added in the classification of jumper’s knee in 1982 by Martens (28). As a result the term jumper’s knee has been used since 1982 to describe pathology in all three locations: the quadriceps tendon, the proximal patellar tendon and the insertion on the tibial tuberosity.

All the abovementioned authors used the history of pain in combination with clinical findings (i.e. pain on palpation). The classification has become differentiated throughout the past 35 years, latest in 1996 by Lian (46).

Table 3. Classification of jumper’s knee according to Lian (46).

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>Pain at the infrapatellar or suprapatellar region after practice or after an event.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2</td>
<td>Pain at the beginning of the activity, disappearing after warm-up and reappearing after completion of activity</td>
</tr>
<tr>
<td>Grade 3a</td>
<td>Pain during and after activity, but the patient is able to participate in sports at the same level</td>
</tr>
<tr>
<td>Grade 3b</td>
<td>Pain during and after activity and the patient is unable to participate in sports at the same level</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Complete rupture of the tendon</td>
</tr>
</tbody>
</table>
Despite the development in grading it does not discriminate between patients with widely differing symptoms, nor to grade recovery. The reliability of the grading has never been tested.

**The clinical presentation of JK**

JK can be a frustrating diagnosis and a therapeutic problem (58). Pain in the anterior knee tendons is the most prominent symptom in JK, and the athletes often present anterior knee pain after sports activity as the initial symptom. Usually the pain disappears after a period of rest, varying from hours to days (2;48). The onset of pain is often insidious as the symptoms fluctuate (24). Other symptoms than pain are weakness, giving away and a feeling of fullness in the affected area (2;48).

In some cases the symptoms are progressive. Athletes who continue to do sports after an episode of JK will often experience that pain begins to appear at the beginning of activity, disappears after warming up, and reappears after activity (48). Further on, the patient may have pain at rest, after walks, during climbing stairs, after prolonged sitting with the leg in the same position, interrupted sleep and eventually progress to constant pain (28;42;48).

At the onset of symptoms and in the initial stage of disease, the patients are often able to continue to do sports without performance impairment. At a later stage the athletes reduce their sports activity or are obliged to give up the activity because of pain (33;42). In rare cases the continuation of intense activity may cause rupture of the tendon (2;42)

**Diagnosis and differential diagnosis of jumper’s knee**

The diagnosis based on clinical findings is generally believed to be straightforward (1;48;59). Most authors include examination of the proximal part of the patellar tendon with focus on localized pain, and a history of anterior knee pain (1;33;50;60).

Objectively, the knee should be examined in full extension (48). The only remarkable sign is a marked tenderness at palpation or by active extension of the knee against resistance (2;28;42). The locations of pain in the quadriceps tendon (Q), the patellar tendon (P), and at the tibial tuberosity (TT), vary in distribution: Q: 6 - 25%, P: 65 - 88% and TT: 10 - 14% (28;33). Other signs are localized puffiness and quadriceps atrophy (2;15;28;33;61). The quadriceps atrophy may be considered a secondary effect to pain (28).

The signs and symptoms are often bilateral (33).
Even though the diagnosis is considered to be easy to establish, JK is frequently overlooked or mistaken for another lesion in the anterior knee region. There are several conditions that resemble the diagnosis JK: chondromalacia patellae, prepatellar or infrapatellar bursitis, Osgood-Schlatter’s disease, inflamed synovial plicae folds, fat-pad entrapment, patellofemoral arthrosis or Sinding-Larsen’s disease (17;59). Imaging is the key factor in depicting abnormalities in the tendons.

**Imaging in jumper’s knee**

Imaging can be used to confirm the clinical diagnosis of JK and provide information on severity. Several imaging modalities are available as diagnostic tools in JK: conventional X-rays, bone scintigraphy, computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound. US is recommended as the initial investigation in the assessment of JK (1;34). This thesis focuses on US, which will be described more extensively.

**Conventional radiography – x-ray**

X-ray is the method of choice for evaluating bone and alignment, but is most often normal in JK-cases. In case of longstanding symptoms abnormalities visible on x-ray may occur. These abnormalities include: soft tissue swelling (15), elongation of the inferior pole of the patella (2;15;28;48), calcifications and increased density within the tendon matrix (28;48), periosteal reaction at the anterior surface of the patella (2), cystic radiolucent zones at the inferior pole of the patella (28), irregular contour at the inferior pole of the patella (2;28), abnormal high-riding patella/patella alta (2;15), genu recurvatum (2), genu valgum (2), external tibial rotation (2), and Osgood-Schlatter disease (2). The conclusion is that x-ray may provide information on predisposing factors and differential diagnosis, but is unfit for use in the diagnosis of JK.

**Bone scintigraphy**

With the use of $^{99m}$Tc-methylene diphosphonate (MDP) it is possible to view scintigraphic images of the patella and the tibial tuberosity. Increased tracer localization predominantly at the inferior pole of the patella and at the tibial tuberosity can be revealed. The use of bone scintigraphy is significantly limited by its inability to demonstrate tendon abnormalities. Thus, bone scintigraphy is able to detect bony abnormalities sooner than x-ray, but is unable to detect soft tissue changes (62).
**Computed tomography (CT)**

CT has its major advantages in bone pathology, but offers a good contrast resolution in soft tissues. The CT findings in JK are: thickening of the tendon, small cystic bone lesions (63), and reduced attenuation (34;63). The areas with low attenuation in JK tendons has 79 Hounsfield units (HU) compared with 120 HU in normal tendons (34). Although CT provides an easy means of confirming the JK diagnosis, it has a drawback because of the ionizing radiation. Furthermore, US and MRI are superior to CT for evaluating tendons.

**Magnetic resonance imaging (MRI)**

MRI has multiplanar capacity and provides a high spatial resolution, which allows detailed anatomical structures to be visualized (31;64). The MRI findings in JK are: Thickening of the tendon (17;19;58;65), poor definition of the posterior tendon margin (59;64), inhomogeneous focally or diffusely increased intensity of signal on T1WI and T2WI (T2 brighter than T1) (19;58;59), tear (58;59;65), and abnormal signal in the patella (T2-weighted STIR(short T1inversion recovery) and fast SE(spin echo) sequences) (19). On T2*-weighted GRE (gradient rephrasing) imaging increased intensity has also been noticed in asymptomatic subjects (66;67). Two semi-quantitative grading systems have been proposed, both based on signal intensity (17;59) and bone involvement (59). None of them have been correlated to clinical findings. Small lesions or mild cases of JK may exhibit diagnostic problems (68). Both modalities are investigator dependent. The advantage of MRI compared to US is the spatial resolution. The disadvantages are availability, financial costs and the limitation of only being able to examine one region at a time. MRI is considered the second imaging method in the assessment of JK.

**Ultrasound**

Around 1985 US became an important and established imaging technique in tendon imaging (61;69-71). At first US was restricted for rare or difficult cases assessed in the radiology department. Now US has become an integrated part of daily practice in rheumatology and sports medicine (52;72;73).

Most musculoskeletal US is done with gray-scale (GS) US. GS US in tendon disease is considered a valid procedure and the inter-observer reliability is good (74;75). GS US has enabled clinicians to detect morphological changes in tendon structures (69-71), and substantial advances in the technology have improved the image quality to a degree where almost all anatomically named soft tissue structures are visible. Unfortunately, the morphological abnormalities are more or less permanent once they have developed
Despite the permanent character of the changes, the optimized GS image is important, since it serves as the basis for the Doppler examination. At present the use of US Doppler in the evaluation of musculoskeletal disease is increasing. With color or power Doppler, areas with increased blood flow may be found and the amount of color may be quantified. The amount of color Doppler (CD) activity in Achilles tendinosis and JK has been found to correlate well with disease activity. This is the case even though no standardized scoring methods have been developed so far, neither for GS nor for Doppler US.

The continuing technical advances have made US a sensitive diagnostic aid, which challenges other imaging modalities such as MRI. The advantages of US are many. The examination is non-invasive and preparation time for the patient and examiner is limited. The equipment is normally user friendly and easy to handle. Pre-installed optimal machine settings (presets) enable the examiner to perform well, without extensive knowledge on image generation. In addition, most equipment is equipped with an auto-optimizer function, which automatically optimizes to the best image quality when activated. In contrast to MRI, it is possible to make dynamic examinations, and thereby evaluate the site of possible pathology in a more natural way (.. it only hurts when I do this...). The US images are real-time examinations and can be performed in multiple planes and locations in short time. The superficial position of soft musculoskeletal structures on the extremities along with the direct imaging control during needling, makes US the perfect tool to guide interventional procedures.

The disadvantages are few. Most of them can be minimized by increased knowledge of US physics, machine settings, proper training and standardizations of image acquisitions.

**Gray-scale ultrasound – B-mode**

B-mode is the most common imaging mode and in musculoskeletal US it is the system default. The B-mode produces a two-dimensional sectional image of the tissues investigated. To generate an image, a transducer element transmits a short pulse at regular intervals. Between the pulses, the system listens for echoes. By measuring the time interval between the transmitted pulses and the returned echoes, the distances to the tissues that created the echoes can be calculated. From the echoes received, the system can construct an image that represents the relative locations of all echoic structures.

A 4 cm transducer array may have 128, 256 or 512 individual elements. The number of elements determines the number of possible scan lines and thereby the image quality. The
more scan lines, the larger lateral resolution (the ability to distinguish two points beside each other in the same distance from the transducer).

As the US wave propagates through tissues, the signal is attenuated. Only a minimum of the signal is reflected back to the transducer, most of the energy is lost to tissue absorption. The US echoes are generated when the propagating wave encounters a difference in acoustic impedance. The strength of the reflected echo is proportional to the difference in impedance of the two tissues or structures. If there is a large difference, the reflection is large, smaller differences reflect less. Good reflectors such as bone appear white in the image, whereas homogeneous structures, such as most fluid collections or cartilage, appear black. Tendons, connective tissues and synovial tissues appear in varying shades of gray.

**Transducers**

The transducer is built from piezoelectric material, which has the ability to produce and receive specific resonant frequencies. These properties allow the transducer to serve both as emitter and receiver of the US pulse. In musculoskeletal US the most frequently used transducer is a high frequency, linear array transducer. The high frequency transducer is dedicated for examination of superficial structures; it ensures a high resolution in the superficial layers on the expense of tissue penetration. Examination of deeper structures is performed at lower transducer frequencies with a better penetration at the expense of image resolution. In practice the choice of transducer is a compromise between good resolution at higher frequencies and good tissue penetration at lower frequencies (87). In the studies of this thesis a 14 MHz linear transducer was used.

**Axial and lateral resolution**

Two types of resolution are of importance in US: axial and lateral (horizontal). Axial resolution is the ability to distinguish two points directly above each other (aligned along the length of the US beam) as separate. The transducer frequency determines the axial resolution. The higher the frequency, the better the axial resolution (because the pulse is shorter). Lateral resolution is the ability to distinguish two points beside each other in the same distance from the transducer. The beam width determines the lateral resolution. The beam can be optimized with focal zones. In a focal zone the pulse is at its narrowest. It is therefore of importance to focus in the area of interest to optimize the image quality (88).
Doppler ultrasound

Tissue perfusion seems to be an integral part of tendon disease (76;80;89-92), and with US the Doppler technique is the only available method to assess and grade changes in the tendon perfusion.

The Doppler effect is a change in wavelength (frequency) of sound resulting from motion of a sound source, receiver or reflector (93). The source and receiver of the waves is the transducer, which is stationary. The reflectors are moving blood cells, primarily the erythrocytes. When a pulse is reflected, the frequency of the wave differs from that which is transmitted. This difference is known as the Doppler shift, named after the Austrian physicist and mathematician Christian Andreas Doppler, who first described the Doppler effect for light in 1843 (94).

When the US pulse is being reflected, two successive Doppler shifts are involved. The first shift occurs when the sound pulse from the transducer is received by the moving erythrocytes. The second shift occurs when the pulse is emitted (latter half of the reflection) from the moving erythrocytes back to the transducer. These two Doppler shifts account for the factor 2 in the Doppler equation:

\[ f_D = f_t - f_r = 2 f_t \frac{v \cos \theta}{c} \]

Where: \( f_D \) is the Doppler shift, \( f_t \) is the transmitted frequency, \( f_r \) is the received frequency, \( v \) is the blood velocity, \( \theta \) is the insonation angle (the angle between the US beam and the blood flow), and \( c \) is the speed of sound. Thus the Doppler shift is directly proportional to the velocity of the flow, \( v \), the insonation angle, \( \theta \), and the transmitted frequency of the US, \( f_t \) (93).

The range of the Doppler shifts, \( f_D \), is in the range of audible frequencies up to 15 KHz. The transmitted frequency (ft) from the transducer used in the studies of this thesis was 7 MHz.

Insonation angle, Doppler angle

The insonation angle is the angle between the path of the Doppler pulses and the direction of flow in the vessels (in practice the angle between the vessel axis and the Doppler pulse). The angle is of importance since it is part of the Doppler equation. If the angle is \( 90^0 \), there will be no Doppler shift, as can be seen from the equation: \( \cos (90^0) = 0 \). The maximum Doppler
shift is obtained when the flow moves directly towards or away from the transducer: \( \cos (0^\circ) = 1 \).

All newer equipments report blood velocities (both in spectral Doppler and on the color bar in the image) assuming that the Doppler angle is zero, which in most cases is misleading, since it often will move in some angle (87;95). During spectral Doppler analyses, the machine can be informed about the insonation angle (angle correction), in order to calculate the blood velocity from the Doppler shift. Angle correction is not used in musculoskeletal US, the only issue is to receive a un-aliased signal which can be index analyzed. Neither is the velocity of importance in musculoskeletal US, since it is the amount of flow present in the ROI which is important, and not the velocity of the flow.

**Color Doppler**

Color Doppler (CD) images present the frequency shift data by converting the information into a spectrum of colors. The result is a real time presentation of flow information superimposed on the GS image (93). In CD, Doppler analysis is performed in the color box, which is divided into cells. Each cell behaves as an independent Doppler gate where a separate Doppler analysis is carried out. The mean frequency shift for each cell is computed and displayed as a color. The colors primarily indicate direction of flow, to a lesser extent velocity. Generally, red hues indicate flow towards the transducer (positive Doppler shifts) and blue hues indicate flow away from the transducer (negative Doppler shifts). Lighter hues are used to indicate higher Doppler shifts (86).

The size of the color box influences the frame rate. A wide color box demands more color processing and causes a decrease in frame rate unless (and unacceptably) either the line density is reduced, which means degrading the lateral resolution, or the number of pulses per scan line is decreased, thus degrading the sensitivity and accuracy of the color estimate (87). Expanding the box in superficial direction until it reaches the skin surface does not affect the frame rate noteworthy because no extra Doppler lines are added and no extra travel distance for the Doppler pulses are required.

**Power Doppler**

Power Doppler US (PD) is an imaging technique that displays the power of all the Doppler shift in each cell, rather than the mean frequency shift as in CD (96). This gives PD a theoretical advantage over CD in sensitivity. When PD came on the market it was less angle dependent, without aliasing, the image quality is less influenced by noise and more sensitive
to flow compared to color Doppler. The only disadvantage was that direction and velocity information was lost (96).

**Choice of Doppler Mode**

In musculoskeletal US the issue of Doppler is to detect any flow that may be present, disregarding direction and velocity. Thus, sensitivity to flow is the most important. In newer high-end machines the theoretical advantage of PD has disappeared, and CD seems more sensitive than PD. In medium range equipment (or older high-end), PD is likely to be more sensitive. The choice between Doppler modes depends on the type of equipment available (86). Figure 3.

![Figure 3. Color versus power Doppler in a jumper’s knee. Longitudinal image of the proximal part of the patellar tendon with color Doppler (top image) and power Doppler (bottom image). The patella is seen in the left side of the image (P). The images illustrate that the color Doppler is more sensitive than power Doppler on our High-end equipment (GE logic 9). Both the color Doppler and power Doppler settings are optimized to maximum sensitivity for low flow. The transducer is kept immobile and with unaltered pressure while the Doppler is toggled between the color and power Doppler mode. Both modalities show blooming artifacts.](image)

**Spectral Doppler**

Spectral Doppler is a real-time graphic representation of relative blood velocities from within the Doppler gate (the measurement interval on the Doppler image) throughout the cardiac cycle (97). The gate size is adjustable and is limited by 2 cursors. Numerous indices are proposed in the quantification of vascular resistance in vessels although, the most commonly used index in musculoskeletal US is the RI, or Pourcelot Index (98).
The RI is defined as:

\[
RI = \frac{(\text{Peak systolic velocity} - \text{End diastolic velocity})}{\text{Peak systolic velocity}}
\]

The RI is directly proportional to peripheral vascular resistance. The RI values usually are a number between 0 and 1 in direct ratio to resistance. Because the intratendinous vessels are very small, both the artery and its concomitant veins are often sampled simultaneously even with the smallest possible Doppler gate. RI values higher than 1 are seen in vessels where the blood reverses during diastole. A flow reversal, which is normal in musculoskeletal tissues, will then go unnoticed because the reversed arterial flow will drown in the venous signal. In order to obtain uniform measurements, we have therefore limited the spectral measurements to the arterial side of the Doppler line and thereby defined 1.00 as the maximum for RI.

Correction of the insonation angle is not necessary when using indices, since RI as other indices is independent of the angle (87).

With spectral Doppler analyses it is possible to evaluate the type of flow, i.e. low resistance versus high resistance. Normal resting musculoskeletal tissues have a high peripheral resistance with little or no flow in the diastole (99). In inflammatory conditions this changes to low resistance with flow throughout the cardiac cycle (79;100;101). Low resistant flow profile may be present in other conditions than inflammation e.g. in muscle tissues during and after a workload.

**Doppler parameters**

Several Doppler parameters are of importance when applying Doppler in musculoskeletal US. The most important are listed below.

**Doppler frequency**

The Doppler frequency is selectable for each transducer. Lower Doppler frequencies will allow more penetration, which will improve Doppler sensitivity. The lower frequency, however, might decrease sensitivity toward slowest flow according to the Doppler equation. A higher Doppler frequency will have lower penetration, which will decrease Doppler sensitivity. However, according to the Doppler equation the higher frequency will increase sensitivity toward slow flow. All in all, it cannot be predicted at what Doppler frequency a given transducer on a given system will have optimum Doppler performance. This must be tested in
practice (86). The most sensitive Doppler frequency setting for the Siemens Acuson Sequoia 14 MHz linear transducer, type 15L8W was the lowest possible – 7 MHz.

**Color box**
The color box is the area of the image in which the color processing takes place. The color processing is demanding on processing power and will cause the frame rate (the number of images displayed per second (Hz)) to drop. The size of the box influences the frame rate, and since the frame rate decreases when the color is added, it is generally recommended to work with the smallest possible color box (87). However, to be aware of reverberation artifacts, the box should always include the top of the image (86).

**Pulse Repetition Frequency (PRF) - Doppler scale**
PRF is the Doppler sampling frequency of the transducer. The maximum Doppler shift frequency that can be sampled without aliasing is equal to PRF/2. This is called the Nyquist limit (87). The Nyquist limit can be presented either as maximum Doppler shift (Hz) or as a velocity (m/s). If the blood velocity exceeds the Nyquist limit, the machine will misinterpret the velocity and display is as reverse flow, i.e. aliasing (93).

The sensitivity of the Doppler is affected by PRF adjustments. In most Doppler systems the manufacturer has implemented linked controls between PRF and wall filter as well as other parameters. When choosing a high PRF the machine assumes that the operator is interested in detecting high velocities. By means of linked controls, low velocities and noise artifacts are removed by filters, which render the system insensitive to low velocities. By choosing a low PRF, the detection of slow flow is optimized, but increases the likelihood of aliasing (87). In musculoskeletal US, a high sensitivity to any flow is important; therefore a low PRF is mandatory. Aliasing is not an issue and efforts to avoid it should not be made.

**Color priority (threshold)**
When color information is obtained, GS information may be present in the same image. The machine must then determine whether to show the GS or the color information in a given pixel. The color priority determines the threshold i.e. the minimum amplitude a Doppler signal must have before it is displayed (87). The function allows valid GS information to override false Doppler information e.g. if the surrounding tissues has a high gray level, the GS information overrides the color signal. On the other hand it also allows valid Doppler information to override false GS information e.g. inside vessels color override GS information. (86).
The GS gain may be of importance, since the increase in gray level may influence the threshold values so that less color is displayed. In tendon imaging the important structures are small vessels that often are not visible on GS US. The vessels are present in relatively hyperechoic parts of the image and the color priority must be 100% in favor of color in order to make the vessels appear.

**Filters – Wall filters**

All Doppler instruments have a high-pass filter, which eliminates the lowest Doppler shift from the display. The Doppler shifts originate from moving vessels and soft tissues. These unwanted Doppler shifts are referred to as clutter or motion artifacts. The filters may also eliminate signals from low-velocity blood flow. Thus, to avoid removal of flow information, the filters should be kept at the lowest possible (88). In most Doppler systems, the PRF and the wall filters are linked controls (87;102).

**Gain**

The Doppler gain is independent of GS gain (88). The gain setting determines the system’s sensitivity to flow. If the gain-setting is too low, valuable flow information may be lost (103). A low gain-setting will prevent noise and motion artifacts (104). If the gain-setting is too high, it will result in random noise and furthermore blooming artifacts (where the colors bleed beyond the vessel wall) will be more pronounced (103). The correct setting of gain is therefore dependent of noise. The Doppler gain is set by turning up until random noise is present in the image, and then lowering the gain until the noise disappears (104).

**Persistence - frame averaging**

This function controls the averaging of the color information between frames. A higher persistence means longer afterglow of color information – The highest levels result in all vessels being filled with color. A low persistence is more real-time and displays the pulsatile nature of tissue perfusion. In musculoskeletal US the pulsatory variation is important in image evaluation – high resistance flow versus low resistant flow. According to this, the persistence should be set as low as possible (86).

**Focus**

In contrast to the GS examination, only one focal point is possible in the Doppler examination. The focal point is where the US beam is thinnest. The energy is thereby concentrated in a smaller volume meaning higher spatial energy. The echoes generated from
the focal point and immediately above and below will therefore have higher amplitudes. Higher amplitudes translate into higher signal to noise ratio, which again means higher Doppler sensitivity. The performance of the Doppler is therefore very dependant on focus position. Substantial differences in detected perfusion will be seen as the focal point is moved (86).

**Doppler settings in musculoskeletal US**

In musculoskeletal US, where the goal is to detect as much flow as possible, the settings must be set to fulfill this requirement. In our clinic the settings exist as a pre-set, to ensure that the settings are the same for all examinations. The only features to adjust are focus and GS gain. The suggested settings are summarized in table 4.

Table 4.
The recommended Doppler settings in musculoskeletal US (86).

<table>
<thead>
<tr>
<th>Doppler parameter</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doppler frequency</td>
<td>Lowest or highest depending on the machine</td>
</tr>
<tr>
<td>PRF</td>
<td>Lowest possible*</td>
</tr>
<tr>
<td>Color priority</td>
<td>Maximum priority to color</td>
</tr>
<tr>
<td>Filter</td>
<td>Lowest possible</td>
</tr>
<tr>
<td>Persistence</td>
<td>Lowest possible</td>
</tr>
<tr>
<td>Gain</td>
<td>On the threshold to noise</td>
</tr>
<tr>
<td>Focus</td>
<td>Placed where highest sensitivity is required</td>
</tr>
</tbody>
</table>

Doppler artifacts

Many artifacts are induced by errors in scanning technique or machine settings. Most of them are easily recognized once the operator is aware of them, and the circumstances in which they may be encountered (93).

**Random noise**

Random noise is produced in all electrical circuits. In the US Doppler image it is seen as color foci appearing randomly in the image. The noise artifacts are easily recognized as such, because the noise generally does not reappear in the same location opposite to true flow.
Random noise is closely related to gain and is removed by lowering the gain. However, if the gain is too low, valuable slow flow information may be lost and the gain should therefore be highest possible with no or minimal noise. A too high gain results in a cluttered image with random noise (103).

**Aliasing**
Aliasing occurs when the blood velocity results in a Doppler shift higher than PRF/2, the Nyquist limit. Aliased signals are displayed with wrong direction, the peak flow appears on the opposite side of the baseline, i.e. a high positive flow appears as a negative flow (105). In musculoskeletal use, aliasing is only important in spectral Doppler because an unaliased signal is a prerequisite for measurements. Aliasing can be overcome in several ways, they include: increasing PRF, increasing Doppler angle, lowering transducer frequency or shifting the baseline (106). The methods of choice to overcome aliasing are moving the baseline (first) and increasing the PRF (second).

Aliasing is not a problem and must be accepted when evaluating tendon structures with color Doppler. To remove aliasing from the color image, the PRF would have to be increased resulting in decreased sensitivity to slow flow.

**Motion**
Movement of any kind; the patient, the operator, the transducer, or movement of the tissues or vessels under investigation; produces Doppler shifts (103). The movements are slow and produce low frequency Doppler shifts that appear as random short flashes (97). The manufacturer decreases motion artifacts by means of filters. The filters only allow Doppler shifts above a certain threshold to be displayed, and is closely linked to color priority

**Mirror**
Mirror artifacts occur at highly reflective surfaces such as bone. The mirror artifact is easily detected as such when the image contains both the true image and the mirror. If a vessel close to the bone surface is mirrored, flow will falsely appear inside the bone. The spectral Doppler will show a flow curve since it is a mirror of true flow and cannot be used to rule out an artifact (103;107).

**Blooming**
The blooming artifact is the phenomenon that the color bleeds outside the vessels, so that the vessels appear larger than they are (108). Blooming is dependant of gain, a lower gain-
setting decreases the artifact (104). However, if the gain is decreased in order to minimize blooming, some true flow may be removed from the image. Therefore, in order to detect all actual flow in the ROI, blooming must be accepted as a systematic error.

Reverberation
Reverberation artifact behaves the same way in GS and Doppler examinations. The reverberation occurs when US is repeatedly reflected between the transducer and an interface or between interfaces. Simple reverberation occurs when the pulse reverberates between one interface and the transducer and the interface is repeated equidistally down through the image. Complex reverberation occurs when the pulse passes through a series of interfaces and reverberates between any two interfaces. This creates a showering of repetitions below the interfaces. The intensity of these artefactual echoes decreases with distance from the object.

Complex reverberation in the superficial and deep wall of a superficial vein may give rise to a shower of CD activity into the tissues below. In this way, vessels superficial to a tendon structure may give the false impression of intratendinous flow.

Pressure
Soft tissue flow information can be misinterpreted if the applied transducer pressure is too high. The pressure will affect the haemodynamics resulting in decreased flow. When scanning a convex surface with a linear array transducer, it is very tempting to press the surface flat to obtain good acoustic contact. This should be avoided. Scanning should be performed with a light pressure and with the use of generous amounts of gel.

Patient positioning
The patient must be positioned comfortably, with the area under investigation completely relaxed. Otherwise the muscles and tendons produce a slight tremor resulting in movement artifacts on Doppler. Some patients should be asked not to speak during the Doppler examination because the voice itself may produce movement artifacts, and because some patients cannot talk without gesticulation. This of course also applies to the examiner. The scanning arm must be relaxed and comfortably resting (86).

Scanning technique
The subjects in this thesis were examined in the prone position with the probe placed parallel to the tendon fibers to avoid anisotropy (69). Scanning was performed in longitudinal and
transverse planes (109). In the Doppler examinations we took care to avoid transducer pressure.
The same scanning technique was used in all studies of this thesis.

**Normal and pathological appearances of tendons**
Lack of definitions is a major problem when assessing tendons with US. In the literature from the past twenty years, ultrasonic normality is not defined, but it appears that many authors agree on defining a normal tendon as: a clearly demarcated tendon with homogeneous fibrillar architecture, without change of shape and without Doppler activity (71;110). On the other hand, a tendon is defined as pathological if it displays change in fiber structure, has blurred demarcations towards the surrounding tissues, change of shape, and CD activity (111). The GS US appearance of a diseased patellar tendon in a JK patient is a hypoechoic area in a thickened tendon. This finding is generally used as an ultrasound diagnostic criterion for JK (1;46;52;90;112-115). The concept of normality is being challenged by the development in equipments where more and more morphological details become visible and Doppler activity is found in many normal tendons (91;116).

**Image review – film clips**
The machines are able to store film clips. A film clip of 4 seconds typically contains 38 images in our equipment, with our settings. The stored clip may be viewed frame by frame. The function is highly functional for selecting images for further analyses. In the studies for this thesis we stored film clips whenever there was intratendinous Doppler activity. From each film clip the image with maximum CD activity was selected for further evaluation.

**Quantification of color Doppler perfusion**
A method to quantify tendon vascularisation is a prerequisite when the importance of vascularity in painful tendons is explored. Quantification of vascular changes over time may allow a better understanding of the role of vascularity in tendon morbidity. Valid scoring systems are difficult to establish. At present there are four methods of quantifying tendon vascularity: length of vessels, color fraction (CF), measurement of resistive index (RI) and semi-quantitative scoring systems.

**Length of vessels**
This method was described by Cook in 2005 (82). In the longitudinal image a ROI is selected in the area with maximum CD activity. The tendon perfusion is measured by connecting the
intratendinous color spots to form lines that express the length of the vessels. Tortuous vessels are measured with a maximum of three lines and summed to an overall score. The score can be estimated during the examination or processed later. The limitations of calculating the vessels’ length are that it is impossible to know whether closely spaced color spots belong to the same vessel or if it represents two or more vessels. Neither can confluent areas be expressed adequately with lines.

The scoring system reports a number and seems well suited to measure change over time.

**Calculation of color fraction (CF)**
Rubin first described this method in 1995 (117). The CF is an exact measurement of the tendon hyperemia. The CF is calculated as color pixels/total pixels in a defined ROI (76;81). The measurement is made off-line using the longitudinal image. The image with maximum CD activity is used representing worst-case scenario. Several programs may be used to compute CF, e.g. DataPro (Noesis, Courtaboeuf, France). This program reports the total number of pixels and the number of color pixels and calculates the CF. The limitations of using CF is that shrinking of the tendon during the healing process may give a falsely high CF. The feature for calculating pixels is not yet available on the machines, and CF has to be calculated off-line.

The scoring system reports a number and seems well suited to measure change over time.
Resistive Index – RI

The flow curve generated from the spectral Doppler analysis is the basis for quantitative determination of the vascular resistance in vessels. The RI has been shown to correlate with disease activity in Achilles tendons and in arthritis (79;100;101;118). The RI value is calculated in a single vessel. If possible, three intratendinous arteries are measured. From the three RI-values the mean value is calculated and the resulting value is noted as the RI of the tendon. The limitation of using RI is that it is time consuming. The scoring system reports a number and seems well suited to measure change over time.
Semi-quantitative scoring systems

These methods are the easiest to apply, but unfortunately also the most imprecise. The methods are often based on clinical experience. Several authors simply refer to the tendons as vascular or non-vascular (91;119). With the present knowledge this scoring is of limited importance, since detectable intratendinous perfusion has been reported in healthy and non-painful tendons (116;120).

In 2005 Alfredson suggested a scoring system for patellar tendons ranging from 0 to 4+ referring to the hyperemia inside the tendons. 0 represents the non-vascular tendon, 1+ a tendon with one or two visible vessels, and 2+ to 4+ with several vessels (80). There are several limitations to the semi-quantitative scoring system. It is very investigator dependent since they have not defined thresholds between classes, and there are no differentiations between “normal” and “pathological”. There are no exact values for tendon perfusion.

Contrast agents

Contrast- enhanced US is a relatively new application in musculoskeletal US. The contrast agents in form of micro-bubbles enhances the US signal from blood and thereby makes it easier for the Doppler to detect its movement. The bubbles cause a 100 % reflection because medical US cannot propagate in gas.

Micro-bubbles are designed to be smaller than 7 µm in diameter, to enable them to cross capillary beds. With the US contrast agents, vessels as small as 40 microns in diameter have been detected (121).

With contrast ultrasound phase inversion technique is used to remove the tissue background and only see the bubbles using harmonic technique. With present agents only very low frequencies can be applied. This makes musculoskeltal contrast studies a challenging technique where high frequency to image the morphology has to be coupled with low frequency to image the bubbles. It may be that new contrast agents will overcome some of the difficulties.

The use of contrast agents in the musculoskeletal sphere is naturally sparse.
Safety considerations
US is sound waves and is not an ionizing radiation technique. US has no contraindications in adults. A thermal effect due to tissue heating has been reported as a possible side effect, however this is assumed to be of importance only in fetal US examinations (93). US contrast agents are generally safe and well tolerated. Adverse reactions are rare, serious reactions are even more rare. Minor reactions e.g. headache, nausea and sensation of heat are self-resolving (122;123).

Assessment of tendon disease – Methods
Study I.
Ultrasound Doppler of the Achilles tendon before and after injection of an ultrasound contrast agent - findings in asymptomatic subjects

The sensitivity of US Doppler has reached a level at which perfusion can be detected even in normal, resting musculoskeletal tissues. To be able to distinguish normal from abnormal flow, the RI determined by spectral Doppler may be of value. To be able to measure RI values it is essential that vessels are visible. In the clinical setting this is the case if there is an ongoing inflammation. When the joint or tendon improves, these vessels may no longer be seen. It has been assumed that the resistance in the vessels is normal, i.e. RI = 1, when Doppler cannot detect the flow. To test this assumption we used contrast agent on tendons with no Doppler activity in order to increase the sensitivity in detecting intratendinous vessels. Theoretically, increased sensitivity may result in visualization of arterioles very close to the capillary bed and may have low-resistant flow, i.e. an RI lower than 1.00. Does this increase result in detection of vessels with possible low-resistant flow, or does it result in high resistant flow as for normal musculoskeletal tissue with an RI= 1?

We tested the following hypotheses
Normal Achilles tendons have vessels that can be visualized by the use of US contrast agent. The resistive index (RI) of these vessels is 1.00.

Material and methods
In the search for five normal tendons, we had to examine 22 tendons in twelve subjects recruited among the staff of the institute. None of the subjects had a history of Achilles
tendon problems whatsoever. A normal tendon was defined as a clearly demarcated tendon with homogeneous fibrillar architecture, without spindle shape and without Doppler activity (71;110;111;121). All subjects were examined with US scanning and clinical examination (124;125). The five tendons, which fulfilled the criteria of US normality, were examined with US contrast agent.

Results and discussion
In all the five normal tendons, intratendinous flow was detected with color and spectral Doppler after contrast injection. The flow profile was normal without diastolic component and therefore with an RI of 1.00. Our hypotheses were confirmed, intratendinous vessels can be detected and the RI=1.00. All the excluded tendons had US abnormalities both on GS and color Doppler.
It was surprisingly difficult to find a normal tendon by US among healthy asymptomatic subjects. The US definition of a normal tendon may be too strict, since the clinician categorized eighteen tendons as normal.

We excluded 17 tendons because of minor morphological changes and intratendinous Doppler activity.

In an US context these tendons could be categorized as having tendinopathy. Instead, we acknowledge that normal subjects may display changes that most investigators till now have considered associated with tendinopathy.

**Knowledge gained from this study**

Achilles tendinopathy should be diagnosed with caution if it is solely based upon GS findings and minor intratendinous Doppler activity. Age-stratified normal materials are needed to define normality.

The increased sensitivity caused by the contrast agent resulted in detection of flow beneath the normal threshold for identification of flow. The resulting flow displayed the same high resistance flow as for normal musculoskeletal tissue, RI= 1. In normals, RI can be recorded as 1.00 in the absence of CD activity.

**Study II.**

**Effect of Patient Position on Ultrasound Doppler Findings in the Patellar Tendon**

An Achilles tendon loses its Doppler activity when it becomes stretched during eccentric training (126). This finding indicates that the Doppler examination of any tendon may be affected by strain on the tendon during the examination. This could be of importance when examining patients with painful anterior knee tendons, since the examination is often performed with a small pillow under the knee for comfort during a scanning session. Different scanning positions are used in the evaluation of the anterior knee tendons. They range from fully stretched to a 90 degree flexed position (60;73;75). Some investigators do not report the knee position (1;38). The aim of the study was to investigate to what extent the tension on the patellar tendon affects the Doppler findings.

**We tested the following hypotheses**

Changes in patient position will influence Doppler findings.

The more tension on the tendon, the less intratendinous Doppler activity (decreased CF).
The more tension on the tendon, the higher intratendinous vascular resistance (increased RI).

Material and methods
Thirty patients with clinical and US signs of JK formed the study group. The patients were scanned in three different positions: fully extended, 15° flexed and in 20° flexion. The patients were randomized to begin scanning in either the fully extended or 20° flexed knee, the medium flexed was always second.

On US we defined a patellar tendon ROI from 0.5 cm proximal to the apex of the patella and 1.5 cm distal. The image with maximum intratendinous CD activity obtained in each of the three knee positions was stored for calculation of CF. With spectral Doppler, three intratendinous arteries were sampled in each knee position and the RI was measured.

Results and discussion
All 30 patients, evaluated individually, had the highest CF in the fully extended knee position compared to 20° flexion, regardless of the initial position. Five patients (17%) changed from having Doppler activity in the fully extended position to no Doppler activity at 20° flexion. All but one patient, 29 (97%), evaluated individually, had the lowest mean RI value in the fully extended knee position compared to 20° flexion, regardless of the initial position. Nine (30%) patients changed from having RI < 1 in the fully extended position to either no detectable flow or RI = 1 at 20° flexion.
The main finding of this study is that Doppler studies of the patellar tendon should be done on an extended knee. Any flexion of the knee during examinations may lead to reduced blood flow, and thus result in false findings.

Figure 9. Effect of knee position on color fraction and resistive index. The graphs with filled boxes are with full extension as initial position. Values are presented as (least-squares) means ± standard error of the mean (SE).

Left: Color fraction (CF) as function of knee position. The decrease in CF with increasing flexion was highly significant (P for trend <0.0001).

Right: Resistive index (RI) as function of knee position. The increase in RI with increasing flexion was highly significant (P for trend <0.0001).
We demonstrated that RI, statistically significant, and CF, with statistic tendency, were dependent of the position of the knee in the initial scan. When the 20° flexed position was scanned first, higher perfusion was seen in all three positions, demonstrating higher CF values and lower RI values. A possible explanation might be that tissue in the tendon at flexion position does not receive adequate blood flow due to intratendinous pressure, resulting in relative hypoxia. Then (as stretched position is obtained), tissue pressure diminishes and normal perfusion is possible. Elevated perfusion will then result for some time until oxygen debt has been paid whereafter steady state is established at a lower rate of perfusion.

When extension was the initial scan position, a low steady state was first established. The following scan of the flexion position resulted in a lower rate of perfusion than if flexion had been scanned first. Our study did not account for time spent in each scan position, which may have had an influence on the result, allowing for steady state to be established. Nevertheless, in our study the patient always spent the longest time in the initial position, and we believe that steady state was established only for initial position.

There was a highly significant association between mean RI and CF ($P<0.0001$), indicating that both methods are able to detect changes in perfusion caused by changes in knee position.

Our hypotheses, that changes in patient position will influence Doppler findings and that
increased tension on the tendon will lead to a decrease of intratendinous Doppler activity, were confirmed.

**Knowledge gained from this study**
The results are important for the choice of positioning of patients during US Doppler examinations of the patellar tendon. Care must be taken to ensure that there is absolutely no tension on the tendon, which requires examination on fully extended and relaxed knees. If the slightly flexed position is used, the tendon perfusion is diminished resulting in underestimation of the flow and an overestimation of the peripheral vascular resistance. It is also obvious that the same position must be used from one examination to the next.

**Assessment of tendon disease – Diagnosis**
**Study III.**
**Ultrasound Doppler of the anterior knee tendons in elite badminton players.**
**Color fraction before and after match.**

In order to investigate the variation in tendon appearance and response to exercise we found it of value to investigate the absolute extreme: elite athletes in a sport with obvious strain to knee. Jumper’s knee is a common disease in athletes, but not reported as a problem among badminton players.

We were fortunate to get the opportunity of examining world top elite badminton players at an international badminton tournament. Despite the popularity of badminton worldwide, little research has been made on the anterior knee tendons in badminton players (127-131), and the present study is the first of its kind. The study took place on location, in a locker room, a setting far from what is usual for an ultrasound study.

The study group was very difficult to recruit; the players and coaches were reluctant to participate in anything that takes focus away from the upcoming match. Also, some nations were not interested in allowing medical insight into the injury profile and ongoing problems of their national team players, especially Asian countries. This had implications on the recruiting rate of players for the study; 23% accepted an interview and 14 % had an ultrasound examination of the anterior knee tendons before and after match.

The aim of this study was to clarify characteristics of the elite badminton player with an interview and investigate the presence of and distribution of possible intratendinous flow in the anterior knee tendons in elite badminton players.
We tested the following hypotheses
Painful anterior knee tendons are common among elite badminton players.
Elite badminton players have intratendinous color Doppler activity in the anterior knee tendons.
Doppler activity increases after match.
Players with painful tendons have more color Doppler activity than players with painfree tendons.

Material and methods
All the 320 players in Denmark Open 2004 were invited to participate in the study. 72 accepted an interview about basic characteristics, the amount and type of training, and the amount of tournaments. The players were specifically questioned about present and former symptoms or injuries in the anterior tendons of the knee. 46 of the players were scanned in three anatomical locations: The quadriceps tendon (Q), the superior part of the patellar tendon (P), and the patellar tendon insertion (TT), both before and after match. We defined and evaluated three ROIs, figure 11:

Figure 11. The three regions of interest.
A: The quadriceps tendon. The ROI extends 1 cm proximally and distally to the most superior part of the patella.
B: The proximal part of the patellar tendon. The ROI extends 0.5 cm proximally and 1.5 cm distally to the apex.
C: The insertion on the tibial tuberosity. The ROI extends 1 cm proximally and distally to the most superior contact between bone and tendon.
The image with maximum intratendinous CD activity obtained in the three scanning positions was stored for calculation of CF.

**Results and discussion**

The interview disclosed that pain in the anterior tendons of the knee is a common problem among elite badminton players. Of the 72 interviewed players, 37 (51%) had previous or present pain in the anterior tendons of the knee in the previous three years, 14 players (19%) had present pain at the interview. There were no statistically significant differences in age, weight, years playing badminton and self-reported training loads between players with and without anterior knee pain. Male players had significantly more painful anterior knee tendons than female players. No differences were found in respect to single or double players, or racket side.

In an epidemiological view, badminton can be added as a sports discipline associated with JK.

As in other studies of elite sport (91;119;132;133), many of our participants accepted pain as part of the game and this did not keep them from training or participating in competitions. Eighty-six percent accepted pain as part of the game, 50% played with ongoing pain and 21% used painkillers, mainly nonsteroidal anti-inflammatory drugs (Nsaid), to be able to play.

At baseline, the majority of anterior knee tendon complexes (Q, P and TT), 85 of 92 (92%) had CD flow in at least one scanning position (of the 3 possible). After the match, 85 of 92 (92%) anterior knee tendon complexes had CD flow in at least one scanning position, thus the US findings were unchanged. The high incidence of intratendinous CD activity could be explained by the high training activity by the players. With an average of 18 hours training per week, we expect that these tendons are in a regenerative phase most of the time, i.e. a continuous “after match” state.

Most subjects had CD-flow in all three of the anterior knee tendons, both before and after match; this included overall results (all tendons as an entity, undivided in sub-groups), dominant- and non-dominant side, single- and double players.

![Overall before match](image1.png)

![Overall after match](image2.png)

**Figure 12.** Distribution of color Doppler activity in the three regions of interest. The three ROIs are described in figure 11. Q: quadriceps ROI. P: Patellar ROI. TT: Tibial tuberosity ROI. As can be seen, a large portion of tendon complexes has flow in all three regions of interest.
Quantitatively: Both before and after match, the majority of tendons (Q, P, TT) had little or no CD activity, e.g. 59% of the examined locations had CF lower or equal to 5%.

The CF values were related to self-reported pain both before and after match. Pain was reported in one of three categories: Present pain, previous pain and never pain. A significantly higher mean maximum CF was found in the group with present pain compared to both previous pain and never pain. This was the case both before ($p=0.04$ and $p=0.002$) and after match ($p=0.02$ and $p=0.02$). The difference between previous pain and never pain was not significant, neither before nor after match.

We tested the ability of CF to predict knee pain by plotting fraction of players with knee pain as a function of CF threshold. For each CF threshold value e.g. 5, 9, 14, we calculated the number of tendons with a CF equal to or higher than the given value, the fraction of tendons with knee pain were plotted. Despite the association between CD flow and pain, we were not able to establish a useful cut-off value to distinguish between painful and non-painful tendons (figure 13).

![Positive predictive values for Color Fraction and pain before match](image)

Figure 13. The graph illustrates positive predictive values of color fraction (CF) in predicting knee pain. No useful threshold to distinguish painful tendons from non-painful could be defined in elite badminton players, e.g. only approximately 50% of players with CF close to 30% suffer from pain in their anterior knee tendons.
Examples of CF close to 30% are given in figure 14.

Our hypotheses, that painful anterior knee tendons are common among elite badminton players and that elite badminton players have intratendinous CD activity in the anterior knee tendons, were confirmed. It was also confirmed that players with painful tendons have more CD activity than players with painfree tendons.

Our hypothesis, that Doppler activity increases after match, was not confirmed.

Knowledge gained from this study:

According to the interview, a large proportion of elite badminton players had experienced anterior knee tendon problems and play regularly with knee pain. Thus pain in the anterior knee tendons was a prominent problem for elite badminton players. Badminton is an epidemiological factor in JK.

US Doppler can detect intratendinous Doppler activity in most players both before and after a match, also in players without symptoms. High levels of Doppler activity were associated with self-reported ongoing pain. The future challenge is to be able to distinguish between physiological CD activity and possible pathological activity. It is apparent from this study that the anterior knee tendons in elite badminton player are very different from a normal population regarding intratendinous Doppler activity. We need to be able to differentiate elite athletes from untrained individuals. Threshold values are needed.
Study IV.
Evaluation of signs, symptoms and Doppler ultrasound in the diagnosis of jumper’s knee

The diagnosis of JK based on clinical findings is generally believed to be straightforward (1;48). Despite that, it is possible to misinterpret other pain conditions in the anterior knee as JK.

We have undertaken a detailed prospective study of a group of JK patients in the search for parameters characterizing the disease. We have focused on the evaluation of CD findings that until now have not been integrated in the diagnosis of JK.

We tested the following hypothesis
An extensive test of clinical signs will uncover more clinical tests that may point at JK.
Ultrasound with Doppler is a sensitive tool in the diagnosis of jumper’s knee.

Material and methods
Forty patients with clinical and GS US diagnosis of JK formed the study group. The GS US diagnosis of JK was defined as: thickening of the tendon with at least 15% compared to the unaffected side and/or presence of a granuloma.

The patients underwent a thorough examination including: interview, VISA (91;134) and KOOS questionnaires (135-137), evaluation of pain (figure 15) (138), measurement of isokinetic muscle strength (139;140), clinical examination, blood screening and an extensive US examination.

On US three ROIs were defined, figure 11. The image with maximum intratendinous CD activity obtained in the three scanning locations was stored for calculation of CF. With spectral Doppler three intratendinous arteries, if possible, were sampled in each location, and the RI was measured.
Results and discussion

Clinical parameters

Our patients were healthy; normal weighted, non-alcoholic, non-smoking and sports active. Most patients had a clear opinion on what caused their knee problems: overuse. Our study revealed a variety of non-specific symptoms, with pain as the most prominent. 63% of the patients had daily pain even at rest and 88% felt impeded by pain in their daily life. VAS at rest was mean 18 (range 0-63) and VAS at activity was mean 61 (range 6-95). To objectify the sensation of pain, the patients marked the location of pain on a schematic drawing of the knee (figure 16).
The clinical examination of the leg from back to toes was very detailed. The abnormalities found were relatively sparse. As expected (because of the inclusion criteria) all tendons had pain at palpation. Pain at palpation was not limited to the primary site of discomfort. Pain at palpation was present at all quadriceps and apex patellae primary sites. The apex patellae seems to be involved in all types of JK since palpatory pain at the apex was found in 5 of 7 patients reporting quadriceps or the tibial tuberosity as the primary site. Surprisingly, only 25% of the patients who reported primary pain at the tibial tuberosity had pain at palpation in that location. On the day of inclusion (which is not the day of examination in the study) these patients had pain at their primary site (or else they could not have been included).

Findings besides the knee-related, were that a substantial percentage of patients had pes planus (40%) and/or hyperpronation of the foot (23%).

**Ultrasound parameters**

**Gray-scale**

All 40 patients had a granuloma at the primary site of pain, two patients had two lesions at the primary site. In addition, we found 7 lesions in painfree tendons sites. It is not surprising to find these extra JK-changes since our patients had a diagnosed overload condition in their extensor complex. The question remains to be answered whether these lesions represent active disease, or merely changes after prior clinical or subclinical attacks. 94% of the lesions had Doppler activity.

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**Figure 17. Solid lesions.**

The images illustrate the proximal part of the left patellar tendon, all from the same patient. The right images are transverse scans with lateral oriented right. The left images are longitudinal scans with patella oriented left. P = patella. The top images are grayscale images of a solid lesion showing the hypoechoic jumper’s knee changes. In the middle images the solid lesions are marked with arrows. The bottom images show the color Doppler information. The Doppler activity was located inside the area with grayscale changes.
Doppler
We found CD activity in 53 of 120 tendon sites. All 40 patients had Doppler activity at their primary site of pain. This indicates a 100% sensitivity of CD in diagnosing JK. The amount of Doppler activity in these 40 sites was substantial – the lowest CF was 14%. Pain was reported at 79% of the sites with Doppler activity. 82% of the painfree sites with Doppler activity were located at the tibial tuberosity. The Doppler findings at the tibial tuberosity were very pronounced, and further studies of this phenomenon are needed to find an explanation and to estimate the importance of the Doppler in this region.
The CF in painful tendons (mean 55%) was significantly higher than CF in pain-free tendons (mean 10%) (P=<0.001). The CF values indicate that with our equipment and our settings, values around 10% would be a possible threshold.
All painful tendons had an RI equal to or lower than 0.85. The RI in painful tendons (mean 0.68) was significantly lower than RI values in pain– free tendons (mean 0.81) (p=0.02). The value of 0.85 may be a threshold between low- and high- resistance musculoskeletal flow. The hypothesis that US with Doppler is a sensitive parameter in the diagnosis of jumper’s knee was confirmed.

Inflammation, hyperemia, and neovascularisation
The cardinal signs of macroscopic inflammation are: calor, dolor, rubor, tumor and loss of function (141-143). Calor and rubor are signs seen on US Doppler as hyperemia. Detection of hyperemia is used in inflammatory disorders, where hyperemia is an integral part of the condition e.g. arthritis, bursitis, enthesitis, and tendonitis. The suffix -itis is controversial when added to a tendinous structure because it is debated whether inflammation is part of the disease. It is, however, unquestionable that hyperemia is present. Another finding pointing in the direction of an inflammatory component are the RI data with RI values < 1.00.
In spite of the macroscopic inflammatory signs, the general opinion is that JK is a degenerative condition (1;144;145), because it has been difficult to identify inflammatory cells (microscopic inflammation) (146-148). The difficulties are not surprising, since most biopsies are performed during surgical procedures, and not in the acute stages. With the absence of inflammatory cells as background, some authors advocate that the “tendinitis myth” should be abandoned (149) and that the terminology should be changed in favour of a degenerative etiology (150).
In the description of intratendinous CD activity, we use the term “hyperemia” instead of the frequently used “neovascularisation”. The distribution of Doppler activity in the three tendon
sites matches the normal perfusion, i.e. is located where the normal vessels enter the tendon, figure 18.

We have no evidence to support that these vessels are new. They may just as well be dilated pre-existing vessels. The papers using the term neovascularisation have illustrations with Doppler activity at the exact same sites as normal perfusion (80;151;152). The evidence for stating that the intratendinous Doppler activity (a macroscopic finding) is in fact neovascularisation is sparse (146;147;153). We advocate the use of the term hyperemia until further evidence supports one or the other.

Knowledge gained from this study
In this material, pain at palpation was the only clinical parameter with a diagnostic capability. It therefore seems acceptable to perform a somewhat limited number of tests when JK is suspected.
US Doppler is highly sensitive in diagnosing jumper’s knee by detecting intratendinous hyperemia. There is a clear association between knee tendon pain and Doppler activity.
US Doppler makes it possible to distinguish between active and dormant disease. A threshold value for CD activity, expressed as CF, is suggested to be 10 % and for RI below 0.85.

We advise to use US CD in the evaluation of jumper’s knee. The Doppler findings may be quantified and graded for use in follow-up.
Considerations for the future

The mapping of US Doppler changes in tendons is far from completed. The technology and the improved equipment make it possible to view many details, but despite much effort there is still a need for agreement on what is normal and what is pathological. Normal materials and agreement on definitions will help overcome this difficulty. In this respect it will be necessary to develop normal materials stratified on age and activity level.

With continuing improvement of high-end equipment the Doppler sensitivity it will be increasingly possible to detect the normal perfusion of tendon structures. In the evaluation of Doppler findings it will then be important to determine threshold values to distinguish normal and pathology.

We used two different quantitative analyses of Doppler activity – CF and RI. The CF is an exact measure, but currently not available on the machine for analysis in daily practice. However this feature may be incorporated in the machines in the future, enabling the calculation of the CF during the examination.

The RI measures the resistance in single vessels over time, e.g. during a heart cycle. The vessels chosen for measurements are randomly selected in the ROI. The mean value of three measurements represents the RI of the tendon ROI as a whole. The RI seems a promising tool in the quantification of Doppler activity in tendon disease.

An evaluation of CF variation over the cardiac cycle could be interesting and relatively easy to investigate. As with RI, the peak systolic and end diastolic values of CF could be measured. Large differences between these two measurements of CF could mean minor or no flow in the diastole (high CF/RI value), indicating little inflammation. A smaller difference could indicate flow in the diastole (low CF/RI value), indicating more pronounced inflammation.

The outcome of an US examination may be influenced by numerous factors. The knee’s position while sitting in the waiting area, the extent of physical activity in the days before the examination, the examination time of the day, smoking habits, heat, cold, skin temperature, accompanying diseases, e.g. diabetes, etc. These factors may affect tendon perfusion and should be investigated. If they prove to affect the outcome of the examination they should be incorporated in the standardizations of an US Doppler examination.

The US examinations performed in the studies of this thesis are all two-dimensional imaging. Three-dimensional US imaging (3D) is a new modality in the musculoskeletal sphere. The benefits may be the exact calculation and evaluation of volume and the ability to analyze the
tissues investigated in any plane after the investigation. The relevance to musculoskeletal imaging and the exact benefits of 3D has to be examined.

3D imaging and the use of contrast agents offer new perspectives in understanding the perfusion of tendon structures, e.g. feeding arteries or the architecture of the vessels in a lesion.

Image fusion is another future aspect of tendon imaging. The fusion of MRI and US images is a new application that may provide additional and clarifying information in the evaluation of tendons. Studies are needed on this application.
**English Summary**

Over the past decades US has been developed into a highly usable diagnostic method for evaluation of tendon structures.

A good measure for disease activity is blood perfusion (hyperemia) in the tendons, measured by Doppler activity. This activity can be assessed and quantified.

The general purpose of this thesis is to assess, quantify and interpret US Doppler findings in tendon structures.

The thesis is written on the basis of four studies in which the Doppler US parameters: presence of Doppler activity, color fraction (color pixels in a region of interest) and resistive index, were assessed, discussed and interpreted.

In **Study I**, we injected US contrast in five normal Achilles tendons. Thereby we demonstrated vessels and measured the resistive index (RI), which was normal and high. The assumption that vessels that cannot be seen without contrast agent have a normal flow profile with an RI = 1, was confirmed. An additional finding was that it was surprisingly difficult to find a normal Achilles tendon (no structural changes and no intratendinous Doppler activity).

In **study II** 30 patients with jumper’s knee diagnosed with US Doppler were examined with the purpose of assessing whether the position of the knee influenced the Doppler findings. We found that the position of the knee is of major importance for the result of the tendon perfusion. An increase in tendon tension, e.g. by a pillow under the knee, resulted in decreased perfusion. Changes due to patient position may hide presence of Doppler activity i.e. disease activity, and thereby result in false negatives. For measurement of maximal Doppler activity the knee should be positioned in the relaxed extended position.

In **study III** 46 elite badminton players at the Denmark Open competition had the anterior knee tendons examined. Badminton and jumper's knee have not previously been epidemiologically associated, but this study adds badminton as a sports discipline associated with JK.

Almost all players had some Doppler activity in their tendons. These findings were not worse after match. Painful tendons had highest CD activity (highest CF).
In study IV, 40 patients diagnosed with jumper’s knee in terms of both clinical and GS US changes were examined. The case history, the symptoms and the clinical examination are not unique for jumper’s knee.

Doppler US, CF and RI, are sensitive US parameters, by means of which detection and grading of JK is possible.

We have proposed threshold values for CF and RI to distinguish normal from pathological Doppler activity. As a result of the US findings we recommend that Doppler US be used in diagnosing jumper’s knee.

**Conclusion**

In normal Achilles tendons without Doppler activity RI is normal. During examination of the knee it should be assured that the extremity is relaxed without tension on the patellar tendon. Increased tension diminishes the blood perfusion in the tendon making it appear normal and resulting in misjudgment.

Not surprisingly, badminton involves strain to the anterior tendons of the knee. We found that many elite players have intratendinous Doppler activity. There is a correlation between the degree of Doppler activity and pain.

Doppler US is a sensitive tool in diagnosing jumper’s knee. Measurement of color fraction and resistive index seem to be useful for diagnosing and grading the disease.
Dansk sammenfatning (Danish summary)

Ultralyd har gennem de seneste årter udviklet sig til en diagnostisk metode der er særlig anvendelig til vurdering af senestrukturer.

Et godt mål for sygdomsaktivitet er blodgennemstrømning (hyperæmi) i senerne, målt ved senens doppleraktivitet. Denne aktivitet kan vurderes og kvantiteres. Det overordnede formål med denne afhandling er at vurdere, kvantitere og tolke ultralyd dopplerfund i senestrukturer. Afhandlingen er skrevet med baggrund i nedenstående fire studier, hvorvi vurdering af doppler-ultralydsparametrene: tilstedeværelse af doppleraktivitet, farvefraktion (% farvepixler i en region of interest) og modstandsindeks (RI) blev vurderet, diskuteret og tolket.

I studie I injicerede vi ultralydskontraststof i 5 raske achillessener. Derved fremkaldte vi synlige kar, og målte modstandsindeks som var normalt og højt. Vi viste dermed at det er korrekt at antage, at modstandsindeks er normalt, når der ikke kan påvises kar i sener. Et bifund var, at det viste det sig overraskende svært at finde en billede- og dopplermæssigt normal achillessene.

I studie II blev 30 patienter med ultralyd-doppler diagnosticeret springerknæ undersøgt for at vurdere om knæets lejring influerede på dopplerfundene. Vi fandt, at knæpositionen havde stor indflydelse på senens blodgennemstrømning, og at en stigning i senens spænding (fx ved at der er en pude under knæet) resulterer i nedsat blodgennemstrømning. Ændring i knæposition kunne fjerne tilstedeværelse af doppleraktivitet, hvilket vil sige tilstedeværelse af sygdom og derved give anledning til fejlvurdering. For at opnå maksimal doppleraktivitet bør knæet der vurderes, lejres i strakt position.


I studie IV blev 40 patienter med klinisk og ultralydssgråtoneforandrede springerknæ undersøgt. Sygehistorien, symptomerne og den kliniske undersølgelse er ikke unikke for springerknæ. Dopplerultralyd, farvefraktion og RI er følsomme ultralydsparametre, der muliggør detektion og gradering af sygdomsaktivitet. Tærskelværdier for patologisk
doppleraktivitet er foreslået. Som resultat af ultralydsfundene anbefales at dopplerultralyd anvendes i diagnostik af springerknæ.

**Konklusioner**

I normale achillessener uden påviselig doppleraktivitet er RI normal. Ved undersøgelse af knæslen skal det sikres at ekstremiteten er afslappet, og at der ikke er træk (spænding) på senen. Øget tension mindsker senens blodgennemstrømning med tilsyneladende normalisering og fejlvurdering til følge.

Badminton er en sportsgren der belaster knæets anteriore sener. Mange elitespillere har påviselig doppleraktivitet. Der er en sammenhæng mellem graden af doppleraktivitet og smerte.

Doppler er et følsomt redskab i diagnostik af springerknæ. Måling af farvefraktion og modstandsindeks synes at være anvendelige i diagnostik og i sygdomsgradering.


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