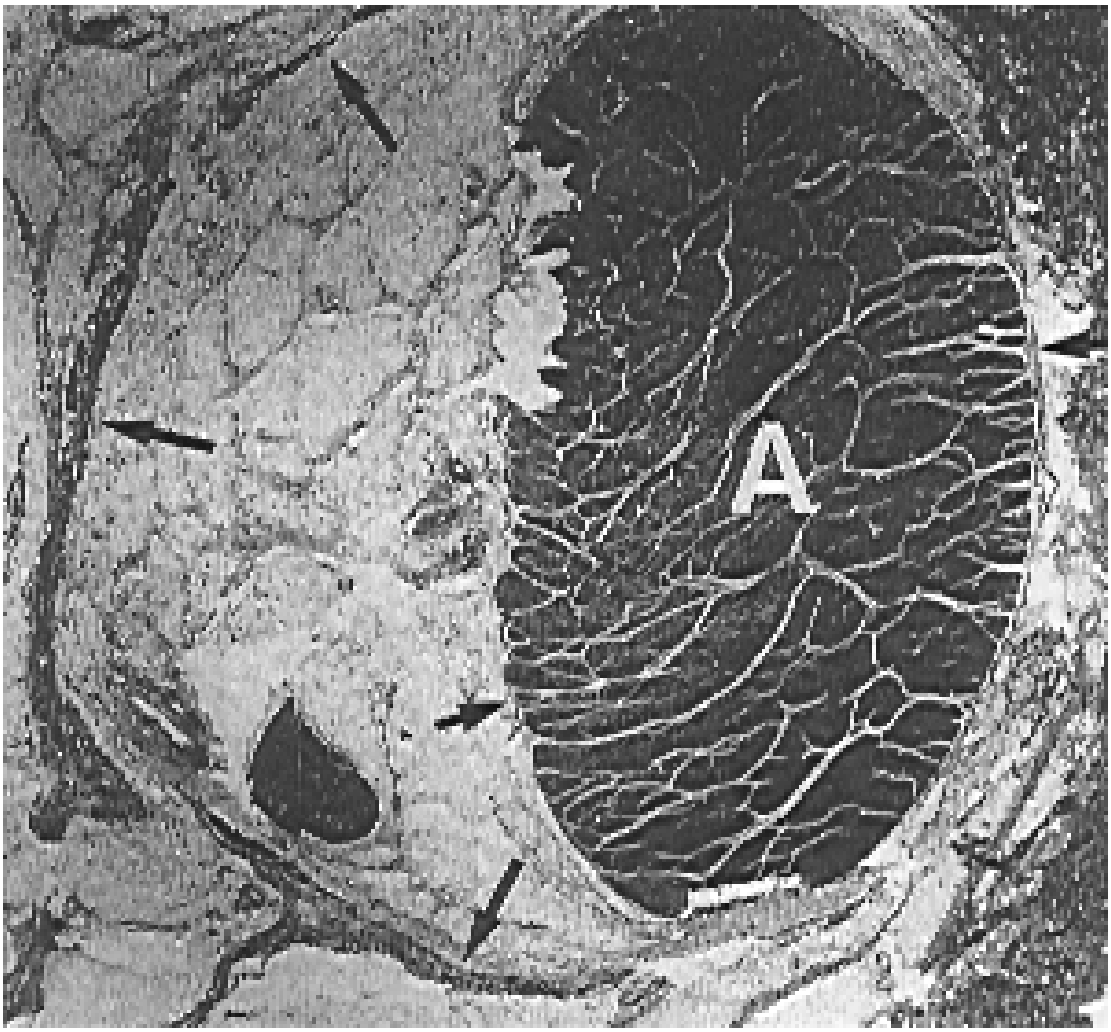
The human Achilles tendon

Circulatory and metabolic changes with exercise



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The figure on the front page: The Achilles tendon (A) is composed of fiber bundles of various sizes separated from each other by endotenon septa. The gliding membranes of the paratenon (small arrows) surround the tendon. The bigger arrows point to the crural fascia (from M. Kvist, Finland (88)).

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CONTENT

Fortes fortuna adjuvat

1 PREFACE

This thesis addresses the present knowledge on circulatory and metabolic changes around the human Achilles tendon during rest and exercise. The experimental work, on which the present thesis is based, was initiated in the period 1997-1999, during my appointment as research fellow at the Sports Medicine Research Unit, Department of Rheumatology H, Bispebjerg Hospital. The experimental work was carried out at this department and at the Department of Clinical Physiology, Bispebjerg Hospital.

First of all I wish to express my sincere gratitude to my two supervisors Michael Kjær, M.D. Professor, Sports Medicine Research Unit, BBH, and Jens Bülow, M.D., Ph.D., Department of Clinical Physiology, BBH for their valuable support during the studies.
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2 LIST OF ABBREVIATIONS

^{133}Xe:	The radioactive isotope of Xenon
^{99m}Technetium:	The radioactive isotope of Technetium microaggreated to albumin
AT:	The human Achilles tendon
AUC:	Area under the curve
B.f.:	Blood flow
C_a:	Arterial plasma concentration
C_d:	Dialysate concentration
C_i:	Interstitial concentration
COP:	Colloid osmotic pressure
C_p:	Perfusate concentration
$C_{v\ calc}$:	Calculated venous concentration
Eq:	Equation
MBq:	10^6 Becquerel (disintegrations per second)
N:	Newton
NIRS:	Near Infrared Spectroscopy
Nm:	Newton meter
NO:	Nitric Oxide
PET:	Positron-emission tomography
PGE_2:	Prostaglandin E_2
PS:	The permeability surface area
Q:	Plasma water flow
RL:	Relative loss
RR:	Relative recovery
SEM:	Standard error of mean
TXB_2:	Thromboxane B_2
W:	Watt
κ:	The elimination rate constant for the monoexponential washout of ^{133}Xenon
λ:	The partition coefficient tissue/blood

3 STRUCTURE, VASCULARISATION AND METABOLISM OF THE HUMAN ACHILLES TENDON

3.1 Introduction

Tendons are designed to transmit the force of muscle contraction to bone effecting limb movement. With changes in load it is essential for function that both muscles and tendons are capable of adapting to these changed with increases in strength and vascularisation. However often the adaptation of the tendon tissue is not sufficient and overuse of the tendon tissue resulting in pain and malfunction represent a major problem within sports and ergonomics (13;14;27;46;55;80;82;86;87;105;108;110;129). In spite of the high incidence of tendon overuse injuries only little is known about the aetiology of this problem.

3.2 Macroscopic structure of the human Achilles tendon

The human Achilles tendon (AT) is the continuation of the triceps surae muscle, which originates from the medial and lateral femoral condyles as the two heads of the gastrocnemius and continues as the gastrocnemius blends with the soleus distally (33;221). The AT inserts onto the middle third of the posterior tuberosity of the calcaneus. The AT is the largest and strongest tendon of the human body with the ability to withstand a tensile load of more than 600 kp (221;225). The mechanical property of the AT is dependent on the cross-sectional area, which varies from 0.8 to 1.4 cm^2 along the course of the tendon (152). In addition the cross-sectional area of the AT has been found to vary between individuals as a result of differences in activity level and type of exercise (49;190;211;230-232). The tendon consists of a posterior component arising from the aponeurosis covering the anterior (deep) side of the gastrocnemius muscles and an anterior component from an aponeurosis covering the posterior (superficial) side of the soleus muscle. The profile of the AT changes over the length of the tendon, being flat as it rises from the proximal aponeurosis, becoming more narrow and round in the midportion of the tendon and fanning out at the distal insertion on the calcaneus. The gastrocnemius portion of the tendon ranges from 110 mm to 260 mm in length, and the soleus portion from 30 mm to 110 mm (38). As the tendon descends from the aponeurosis, it twists so that the posterior gastrocnemius tendon fibres rotate antero-laterally and the anterior soleus fibres run postero-medially (37), however with some individual variations in the pattern of rotation (37).

The rotation of the tendon produces a region of concentrated stress where the two tendons meet (168) approximately 20 to 50

mm above the calcaneal insertion (19). This area corresponds with the region subjected to poor vascular supply (27;39;62;110;182) and having the highest incidence of tendon ruptures (27;89;108;147).

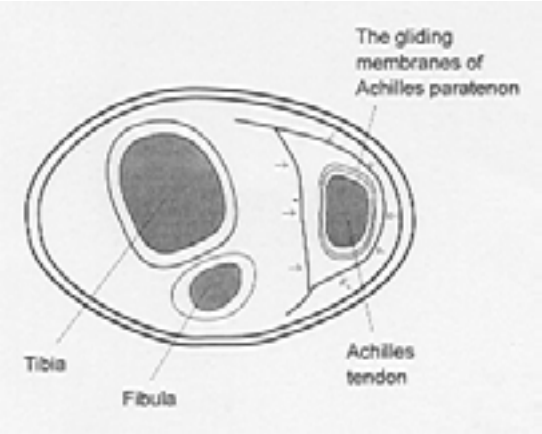


Figure 1. The gliding membranes of the human Achilles tendon. Arrows outline the crural fascia. Reprinted from (88).

The anterior portion of the tendon is attached to a richly vascularised adipose tissue, whereas the lateral and posterior surfaces are covered by a thin filmy, loose fibrillar tissue, the peritendinous sheet known as the paratenon (Figure 1 and Figure on the front-page). The paratenon surrounds the tendon and is able to stretch two to three centimeters with movement of the tendon, thereby allowing free movement of the tendon with minimal friction against the surrounding structures (112). In addition the paratenon carries the blood vessels, lymphatic vessels and nerves (112;125;226).

3.3 Functional loading of the muscle-tendon complex in the calf

From a functional standpoint, the gastrocnemius and soleus are important, strong muscles involved in plantar flexion of the foot. The gastrocnemius supplies the power for propulsion in walking, running, and jumping, whereas the soleus stabilises the leg on the foot through its proprioceptive function (144). During running, the Achilles tendon is often subjected to forces that are six to eight times body weight (52;103;106;189). The high tensile strength of the AT of more than 600 kp (221;225) ensures that total ruptures of the healthy human Achilles tendon are relatively rare despite subjected to these high loads (121;122;134;150).

3.4 Tendon properties

The Achilles tendon consists of fibrous connective tissues and has a complex structure of highly aligned matrix containing 70–80 % type I collagen to provide tensile strength, 10-30 % elastin yielding compliance and elasticity, proteoglycans as pulse dampeners, and lipids, whose presence in the tendon epitenon may reduce shear stress-induced friction (88). Exposed to load tendons have the ability to elongate as indicated on the stress-strain curve (17;24;152)(Figure 2).

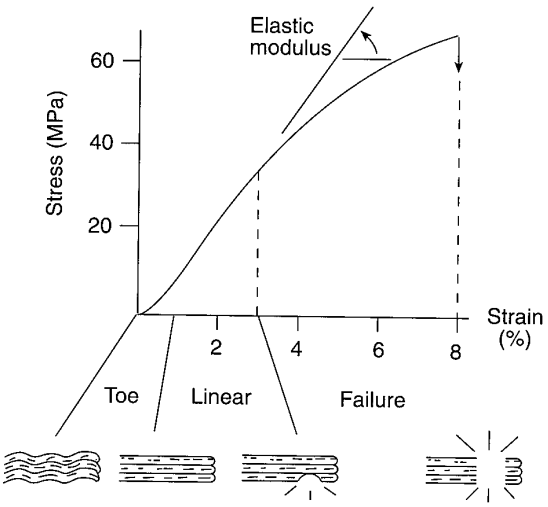


Figure 2. Example of a stress-strain curve for collagen fibers in a tendon. Reprinted from (24).

At low stresses (toe region), the crimp or waviness of the collagen fibers begins to disappear, and as the collagen fibers straighten, linearity on the stress-stain curve is present. It is in this range tendons are exposed to loading during sports and ordinary daily activities. Near the upper part of the linear loading region, some of the collagen fibers may exceed their load-bearing capacity and break. The tendon collagen starts to fail at 4 % to 8 % elongation, whereas the elastin can be stretched to up to 70 % of its length without rupture, and breaks at 150 % (152). If the load on the tendon at this point is removed only partial failures of the tendon will occur, which subsequently may induce an inflammation response.

3.5 Injury and rupture of the Achilles tendon

Often inflammation manifests initially as inflammation in the surrounding paratenon, peritendinitis (15;39). Thickening of the paratenon impairs the gliding function of the tissue, thereby intensifying the stimulus towards inflammation (48;59). If the stimulus is removed, peritendinitis is usually selflimited and can heal without consequence (59). However if the stimulus persists, such as when an athlete tries to work despite pain, scarring of the paratenon and structural disruption of the tendon can occur (59;134). Overuse injuries of the AT commonly occur in individuals who are physically active and who subject the tendon to repetitive forces beyond its ability to recover. This injury has been noted in all types of athletes, not just runners (29;39;226). Individuals who are regularly engaged in jumping activities subject the tendon to forces to a normal magnitude, but the forces are too frequently applied without a reasonable recovery time prior to subsequent training bouts, thus increasing the likelihood of overuse injuries.

Chronic Achilles tendon inflammation has mainly been considered to be an overuse injury due to excessive exercise or poor technique, but many other factors have also been recognised (46;106;170;187;195;226). These factors can be divided into:

1) factors related to the subject (intrinsic factors) such as increased age

(12;14;92;95;136), male gender (12;32;94), blood type (120), reduced tendon blood supply (27;39;107;109;144;207), biomechanical abnormalities (31;86;170), and previous injuries (94;96)

2) factors independent of the subject (extrinsic factors) such as shoes (31;43;119;167;202), exercise-induced hyperthermia (227) and training surfaces and errors (29;86;207).

However most of the studies on the aetiology of AT overuse are based on speculations or single observations rather than on scientific evidence, and controlled studies are lacking.

It is however, generally believed that reduced blood supply and inflammation leads to degeneration with reduction in tensile strength and terminal rupture of the tendon (7;39;135;144). In support of this theory ligation of the AT in rabbits resulting in a small reduction in resting blood supply to the tendon are found to produces degeneration of the tissue (29;159). Furthermore in the region of the Achilles tendon most prone to inflammation and ruptures (39;82;89;108;110;147) a zone of reduced number and relative area of blood vessels has been demonstrated (Figure 3)(27;62;110;182).

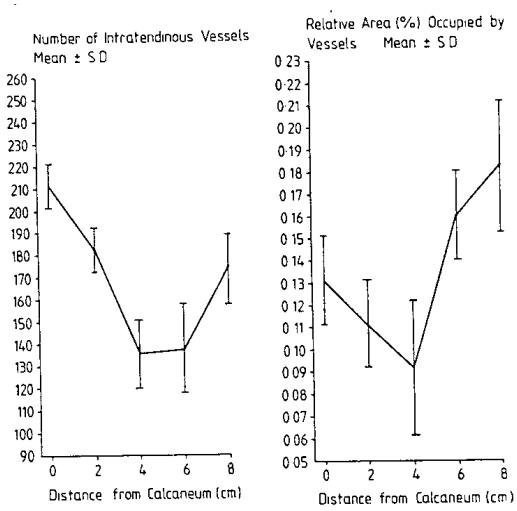


Figure 3. In the left panel the number of intratendinous vessels at various distances from the calcaneus is shown. Right panel is showing the relative area of intratendinous vessels at various levels. From (27).

All evidence in favour of hypoperfusion being an important aetiological factor in the development of AT inflammation and rupture is however indirect or circumstantial. Somewhat in contrast, the few in-vivo studies of blood flow during Achilles tendinosis show that the tendon blood flow on the injured side was increased almost two-fold compared with the healthy side (13;16).

3.6 Blood flow of the human Achilles tendon

Previously, tendons were believed to be inert and avascular structures, but in the beginning of this century, it became clear that tendons in general have a well-organised peri- and intratendinous network of blood vessels (4;166;229). In the case of the human AT, it was shown that it

receives vascular contributions from both its muscular and bony attachments sites, as well as along the length of the tendon through the surrounding paratenon (133;229)(Figure 4). Of the blood supply to the central third of the tendon, about 35 % originates from the paratenon (extrinsic vascular system) and 65 % from the musculo- and osteo-tendinous junctions (intrinsic system)(145). The vessels of the paratenon, sometimes referred to as the mesotenon (27;226), rise from branches of the posterior tibial and peroneal arteries and run transversely towards the tendon, branches several times and runs parallel to long axis of the tendon forming a uniform, meshlike vascular system along the length of the tendon (Figure 4)(45;110;168;182). The vessels of this network then penetrate into the tendon with a longitudinal appearance inside the tendon (182).

Apart from anatomical data (27;82;110) only little is known about the vascularisation of human tendons (55;108), and only very few data are provided on the blood flow in human tendons during exercise (13;14). From animal studies, blood supply to the tendons is known to vary substantially (ranging from 1 to 50 ml/100 g/min) depending on the species and the anatomical position of the tendon (162). This could indicate a close connection and balance between blood flow and function of tendons (16;79;111;131;145;160;205;222). In addition it has been shown that the blood flow of AT in animals is influenced by the physiological status, being increased after long-term exercise and after acute isometric and isotonic muscle contractions (16). One study by Fossgreen using ^{133}Xenon washout found the blood flow in the human AT to be 0.9 ± 0.6 ml/100 g tissue/min during rest (55), and more recently Åström *et al.* revealed that the blood flow in the human AT decreases during passive stretching and isometric contractions determined by Laser Doppler flowmetry (13;14). Thus, despite the fact that blood flow seems to be of great importance for the function and integrity of the human AT, the blood supply to the tendon has received remarkably little attention (13;14).

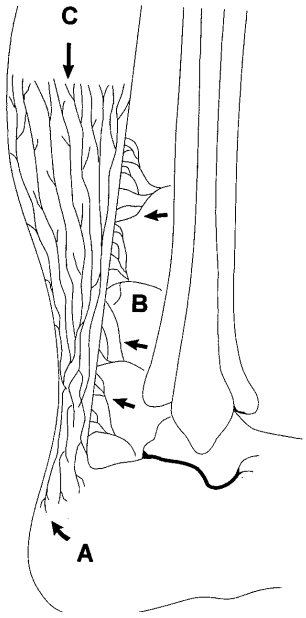


Figure 4. The blood vessels of the human AT, showing the supply from A) the osteotendinous junction, B) the "mesotenon", and C) the myotendinous junction (From (27))

3.7 Lymph drainage of the human Achilles tendon

The lymphatic system represents an accessory route by which fluids can flow from the interstitial space into the blood. In addition, the lymphatic system carries proteins and large particulate matter away from the tissue spaces, neither of which can be removed by absorption directly into the blood capillary. The lymphatic vessels in skeletal muscle consist entirely of lymphatic capillaries, lacking smooth muscle and as such are unable to contract spontaneously (186). Consequently, the lymph system relies on the arterial pulsation and muscle contractions for opening and closing of the lymphatic capillaries (134;186). Changes in blood flow through the leg tissues, activation of the muscle pump and physical activity in general are known to promote lymph flow (70;155;156;206).

3.8 Metabolism of the Achilles tendon

Ligaments and tendons have been found to be metabolic active (153;160;219), and influenced by external stimuli (11;47;81;104;113;163;179;204;209;220)

The metabolism and turnover of collagen in tendons is relative slow. It increases in response to injury and exercise, without any notable change in extracellular collagen (153), indicating the existence of a close balance between synthesis and breakdown. Although a low metabolic rate is thought to protect the tendon against being vulnerable towards injuries due to ischaemic stress, it at the same time results in a slow adaptive response to changes in loading, and a slow rate of recovery after activity as well as in relation to healing after injuries (226). The turnover time for tendon collagen has been shown to range from 50 to 100 days (38) as compared to 4 to 8 days for contractile proteins in muscles (60;61;208). The average oxygen consumption of tendons has been found to be 0.1 µl of oxygen per mg of dry mass of tendon per hour (160), providing tendons with a much lower oxygen consumption values compared to skeletal muscles (153). Kept in mind that only a minor percentage of the dry mass of a tendon consists of cells, as compared to a very high percentage in muscles, the difference in oxygen consumption is less pronounced when expressed in values per cell mass (88). The low metabolic rate of tendon tissue will contribute to the resistance of tendons to withstand loads and remain in tension over long time without developing ischaemia and necrosis (226). In addition, tendons have been shown to possess the enzyme chains for all the three main pathways of energy metabolism: the Krebs cycle, the anaerobic glycolysis, and the pentose phosphate shunt, enabling both aerobic and anaerobic metabolism (88), but the quantitative importance of the three systems is not known (211).

Histological studies of tissue from patients with chronic Achilles paratenonitis have demonstrated evidence of increased

collagen breakdown and anaerobic metabolic enzyme activity, simultaneously with a decrease in aerobic metabolic enzyme activity (109). Interestingly the metabolic pathway has also been found to become more anaerobic with age (53;95).

4 PURPOSE OF THE PRESENT THESIS

Vascularisation and metabolism of the human Achilles tendon seems to be of great importance for the development of tendon related problems. Most injuries in this area appear to result from impaired blood supply during exercise. If inflammation occurs and persists over prolonged time it might result in reduction of the strength of the tendon with total rupture as the terminal state. However, taken together, data are lacking regarding the effect of exercise on blood flow and metabolism of human tendons. This could be of great importance for the understanding of the pathogenesis of injuries and diseases in relation to tendons.

On this background it is therefore desirable to:

1. Establish a method for standardising static and dynamic loads of the human calf muscle.
2. Elucidate whether blood flow in the peritendinous area of the human Achilles tendon changes in response to various types of exercise.
3. Elucidate if the known zone with reduced number of blood vessels in the mid-portion of the human Achilles tendon has a functional impact on the regional blood flow in the peritendinous area of the human Achilles tendon during exercise.
4. Elucidate whether the blood flow in the peritendinous area of the human Achilles tendon is changed with age.
5. Establish a method for measuring metabolism of the area around the human Achilles tendon during rest and exercise.
6. Elucidate whether the peritendinous tissue of the human Achilles tendon is metabolically active during rest and exercise.
7. Investigate the release of vasoactive substances in the peritendinous area (such as products of the arachidonic acid metabolic pathway) in response to exercise.

In the present thesis methods for and data on the blood supply, the lymphatic drainage, the inflammatory response in and the metabolism of the human Achilles tendon during rest and exercise are provided.

5 METHODS

5.1 Xenon washout

Several techniques, such as thermodilution, eletromagnetic flow-meters, plethysmography, Positron-emission tomography (PET), microspheres, microdialysis, isotope-clearance and dye-indicator methods as well as Laser and Ultrasound Doppler Flowmetry have all been developed for determination of local blood flow (78;116;118;118;177;193;212).

However, most of the techniques have been inflicted with various technological limitations and inherent methodological errors, and the sensitivity and temporal resolutions of the techniques have in general been too poor for local tissue blood flow determination in humans (118;175). For in-vivo determination of local blood flow in adipose tissue the most suitable method has been found to be the isotope clearance technique (22;23;114;117;185;192-194;213-215).

The method of clearance or washout of the radioactive isotope ^{133}Xenon was originally described by Larsen and Lassen (114;117), who showed that the clearance rate of the inert gas ^{133}Xe from a tissue could be used to determine local blood flow. The Xenon washout method can be used in any tissue, if the tissue fulfill the following requirements (214):

1. homogenous in structure
2. homogeneously perfused
3. the washout of ^{133}Xe from the tissue is entirely perfusion limited

These requirements are to a large extent fulfilled in the adipose tissue (22;23). If it is assumed that no recirculation of tracer takes place, i.e. the tracer concentration in the inflowing blood is zero, and the tracer only leaves the tissue with the blood, then the changes in tracer concentration in the tissue with time can be described by a monoexponential equation:

(Equation 1) $C(t) = C_0 \cdot e^{-kt}$

where k is the fractional washout rate constant from the tissue compartment (98).

The method has been used to monitor blood flow in muscles, although with some difficulties, (114;117;213-215) as well as for determining blood flow in the adipose tissue (22;23;185;192-194). As the peritendinous tissues are known to contain adipose tissue the ^{133}Xenon washout method is a suitable method for determination of blood flow in this area.

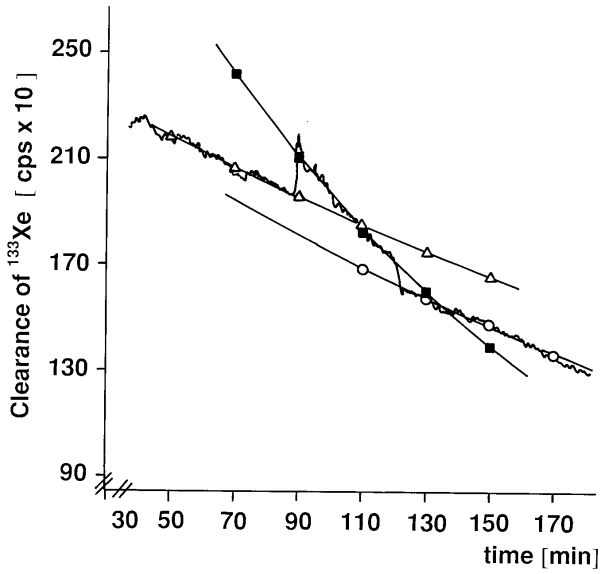


Figure 5. A typical example of a ^{133}Xe-clearance curve obtained after injection of ^{133}Xe in the peritendinous tissue of one subject (own data). The clearance-rate of ^{133}Xe from the tissue is measured during a resting period (0-90 min), a period of intermittent isometric exercise of the calf muscles (contraction 1.5 seconds/rest 1.5 seconds) (90-120 min), and a recovery period with the person resting again (120-180 min). For determining blood flow elimination rate constant κ for the three mono-exponential curves fitting the various ^{133}Xe wash-out curves were used. The various elimination rate constants in the present example are: Rest (Δ) κ = -4.4 • 10^{-5} (r = -0.98); Exercise (■) κ = -11.6 • 10^{-5} (r = -0.99); and Recovery (o) κ = -6.4 • 10^{-5} (r = -0.99) respectively, resulting in a calculated blood flow of 2.6 ml/100 g tissue/min (rest), 7.0 ml/100 g tissue/min (exercise), 3.8 ml/100 g tissue/min (recovery).

5.1.1 Practical application of Xenon washout in the present thesis

When used in the present thesis ^{133}Xe was dissolved in sterile isotonic saline solution in a concentration of ≈ 10 MBq/ml, and transferred anaerobically into a syringe, from which 0.1 ml was injected directly into the tissue ventral to the AT. Great care was taken not to inject any gas bubble. The injection was made with a fine needle (outer diameter 0.4 mm) from the medial side at a depth of 10-20 mm. The insertion of the muscle fibers of m. soleus in the AT was verified by ultrasound to ensure that none of the depots were positioned within muscle tissue. The needle was withdrawn from the tissue half a minute after the injection had been given to ensure that no leek appeared. The ^{133}Xe-washout was measured via portable scintillation detectors strapped to the skin above the ^{133}Xe-depots. The detectors were connected to a multichannel analyser system

(Oakfield Instruments, Oxford, UK). The initial counting-rate was ≈ $1{,}5 \cdot 10^3$ decays/sec. Counts were collected in 30-second periods. No measurements of ^{133}Xe-clearance were performed during the first 30 minutes after injection of the depot to reduce the risk of the insertion trauma to influence the calculated blood flow.

5.1.2 Calculations of blood flow

From the clearance rate of ^{133}Xe (Figure 5) and (Equation 1) it is possible to calculate the blood flow (B.f.) in ml/100 g tissue/min, when the tissue blood partition coefficient λ is known (98;151):

(Eq. 2) B. f. = -100 • λ • κ ml/100 g tissue/min, where λ is the partition coefficient tissue to blood ((μC/g tissue)/(μC/ml blood)), which is between 5 and 10 for adipose tissue (23). κ is the elimination rate constant for the monoexponential washout of ^{133}Xe (117).

5.2 Microdialysis

Microdialysis is a technique, based upon a concept of perfused hollow dialysis tubes, which allows for determination of interstitial concentrations of various substances in-vivo in animals and humans (9;40;216;218). Microdialysis was originally developed for the in-vivo determination of biomechanical processes in the extracellular compartments of the brain (20;217).

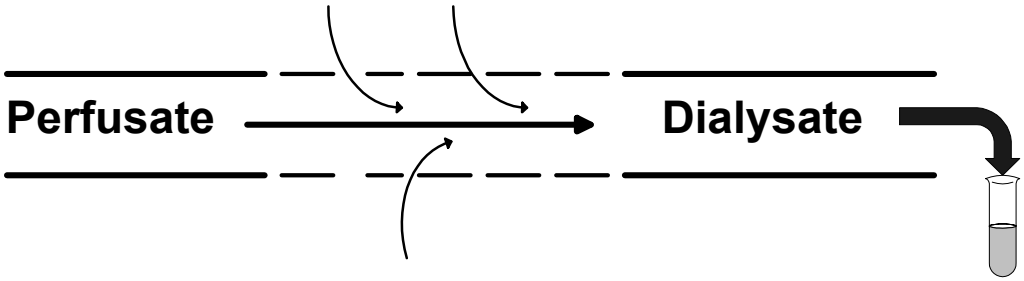


Figure 6. Drawing showing the principle of microdialysis. The microdialysis probe is being perfused with a fluid (the perfusate). Water-soluble substances diffuse over the membrane into the perfusate, and samples (the dialysate) can be collected for later analysis.

A thin dialysis tube is placed in the tissue of interest and a fluid resembling the interstitial fluid is being pumped through the tube (Figure 6). Water-soluble substances will be exchanged over the membrane in relation to the relative differences in concentration in the tissue and in the perfusing fluid (the perfusate). The concentration in the out-coming fluid (the dialysate) reflects the concentration in the tissue, making it possible to detect values of and changes in interstitial concentration of specific metabolites and mediators. In an ideal system, concentrations of substances in the dialysate equal interstitial concentrations.

However, since diffusion is dependent on the tortuousity of the surrounding water as well as the temperature, the properties of the membrane used and the perfusion rate, calibration is needed to estimate the interstitial concentration of a substance of interest accurately (126). Since regional differences may affect diffusion, each catheter should be calibrated in situ.

5.2.1 Relative recovery

By knowing the relative recovery (RR) expressed as:

(Equation 3) $RR = (C_d)/(C_i)$

the interstitial concentration can be calculated as:

(Equation 4) $C_i = C_d/RR$

Several different techniques, such as *the stop flow technique* (85), *the no net flux technique* (127), *the slow perfusion rate method (21)*, and *the internal reference technique* (181) have been developed for in situ determination of RR for microdialysis probes.

The internal reference technique has, compared to the other calibration techniques, the advantages of being substrate specific and with the ability to resample momentary changes in recovery as a result of intervention, and in addition is less time consuming.

With the internal reference technique the microdialysis probes are calibrated in situ by perfusing the probes with a fluid containing an indicator substance that resembles the diffusion of the substance of interest over the membrane, but which can be distinguished from the substance of interest during analysis of the dialysate (Figure 7)(181). It is assumed that the relative loss (RL) of the indicator substance from the perfusate to the interstitium equals the RR of the substance of interest from the interstitium to the dialysate (63;128;181). The indicator substance can either be the substance of interest labeled with radioactivity or a radioactive-labeled substance with the same diffusion characteristics as the substrate of interest. By using different radioactive labels (e.g. ^{3}H, ^{14}C, ^{32}P) it is potentially possible to monitor the diffusion of multiple molecules in the same microdialysis probe simultaneously.

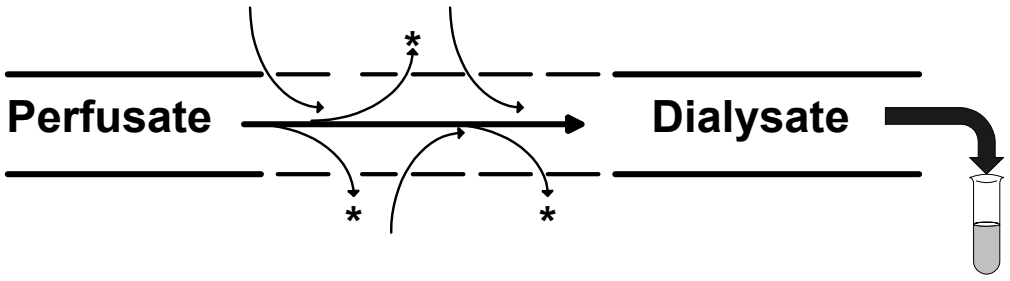


Figure 7. The internal reference technique for determination of in situ recovery determination of microdialysis probes. A radioactive compound (*) with the same diffusion characteristics as

the molecule of interest is added to the perfusate. The Relative Loss can be calculated based on the radioactivity left in the dialysate (Equation 5).

5.2.2 Calculation of tissue uptake and output

The interstitial concentrations (C_i) can be calculated using the internal reference calibration method (181). The relative recovery (RR) is calculated for each microdialysisfiber as:

(Equation 5) $RR = (C_p - C_d)/C_p$,

where C_p is disintegration/minute in the perfusate and C_d is disintegration/minute in the dialysate.

Venous concentrations can be calculated ($C_{v\ calc}$) based on Fick's law of diffusions for thin membranes (83):

(Equation 6) $J = -PS \bullet (C_1-C_2)$,

where J is the substrate flux, P is the membrane permeability of the substrate, S the membrane surface area, and C_1 and C_2 the concentrations on the two sides of the membrane with C_1 being higher than C_2. If this equation is integrated over the entire length of the capillary the following expression is obtained:

(Equation 7) $(C_v - C_i)/(C_a - C_i) = e^{(-PS/Q)}$

Using (Equation 7) the tissue uptake can be calculated as:

(Eq 8) $C_{v\ calc} = [(C_a - C_i) \bullet e^{(-PS/Q)}] + C_i$,

and the tissue output as:

(Eq 9)

$C_{v\ calc} = [(C_i - C_a) \bullet (1 - e^{(-PS/Q)})] + C_a$,

$C_{v\ calc}$ being calculated venous plasma water concentration, C_a arterial plasma water concentration, C_i intercellular water concentration, Q plasma water flow, and PS the permeability surface area product in ml/100 g tissue/min. By calculating plasma flow as:

(1 - haematocrit) • blood flow,

where the haematocrit is measured in the arterial blood, blood water flow is calculated by multiplying plasma flow by 0.94 (158). The PS product can be set to 3 ml/100 g tissue/min for glycerol and lactate and 2 ml/100 g tissue/min for glucose, since values in this range have been found for molecules of similar sizes (124). The PS product is assumed to be constant within the range of blood flow variations registered (158;193).

The tissue substrate net uptake (Eq 8) or net release (Eq 9) can then be calculated as the product of difference between $C_{v\ calc}$ and C_a and the blood water flow in which the metabolites are distributed.

The microdialysis technique has been used to determine changes in metabolic parameters during rest and physical exercise in both skeletal muscle (57;67;126;142;171;172;172) and subcutaneous adipose tissue (8;56;57;67;126;171;172;192;194).

Furthermore it has been possible to characterise the release of inflammatory mediators in response to substance-P infusion in skin (161).

5.3 Lymph drainage measurments

Lymph drainage can be determined by injecting ^{99m}Technetium labelled albumin or Dextran in the tissue of interest and following the clearance rate by a γ-camera (72;154). A change in the number of disintegrations within the tissue could derive from 1) a local breakdown of the radioactive labelled compound, 2) disintegration of the radioactive compound, or 3) removal of the radioactive compound by the lymphatic system, and as the latter by far exceeds the two other factors the clearance rate can be used to calculate the lymph drainage of the tissue during rest and exercise.

In the present thesis the lymph drainage of the tissue ventral to the human Achilles tendon was determined during exercise to elucidate whether changes in ^{133}Xenon clearance rate during calf muscle exercise was influensed by changes in lymph drainage or could be used as a measure of a "true" change in blood flow. For measurements of lymph drainage a 0,1 ml sample ≈ 74 MBq of ^{99m}Technetium labelled microaggregated albumin (TCK-17, CIS Bio International) was injected in the adipose tissue 50 mm proximal to the insertion of the Achilles tendon on the calcaneus corresponding to the position where Xenon was injected during the investigation of blood flow. Clearance of ^{99m}Technetium labelled albumin was followed by a γ-camera (Multispector or Orbitor, Siemens), and the lymph drainage from the tissue was calculated.

5.4 Pressure measurements

To measure the pressure in the peritendinous space, the subjects were positioned in a specially constructed experimental set up (Figure 8), with the trunk perpendicular to the seat and the knees extended. During the experiment one foot at a time was positioned on the vertical sheet with the axis of the sheet aligned with the axis of flexion in the ankle joint. Extension of the knees ensured that the calf muscles alone generated the recorded torque-moment, and that activity in the extensor muscles of the knee and thigh were excluded. The pressure in the tissue was measured using a pressure-measuring device (Dialogue 2000, Danica Biomedical) connected via a catheter filled with sterile isotonic saline to a cannula. By inserting the cannula into the tissue, pressure could be measured. Before measuring a few drops of saline were flushed through the catheter tip to verify good fluid transmission. The catheter was calibrated to zero hydrostatic pressure by levelling the measurement site and adjusting the pressure transducer level until the recorder read zero pressure. Care was taken that no air bubble was present from the transducer to the catheter tip. Marks were made on the skin at the sights of interest. Corresponding with each mark the following procedure was performed: from the medial side just ventral to the AT a

cannula (outer diameter 0.8 mm) was inserted at a depth of 10-20 mm. To control that the cannula was ready to register, the subject was asked to generate a minor torque in plantar direction, resulting in a change in interstitial pressure. To measure the resting tissue pressure the subjects were asked to relax (> 20 sec) with the cannula positioned in the tissue. The subject was subsequently told to generate a plantar flexor torque by which the force at the strain gauge corresponded to the load of interest. Interstitial pressure was determined when the torque had stabilised. The experiment was terminated by a recovery measurement with relaxed m. triceps surae.

5.5 Catheterization

Blood concentrations of various substances were measured and used for calculation of tissue uptake/output during microdialysis experiments. During local analgesia an arterial catheter (Ohmeda, Swindon, UK) was inserted in the radial artery of the non-dominant arm for blood sampling. The catheter was kept patent by constantly flushing with isotonic sodium chloride containing heparin (10 U/ml).

5.6 Experimental set-ups

To investigate the metabolism and vascularisation of the peritendinous area around the human Achilles tendon, two experimental set-ups were constructed in which the workload of the triceps surae muscle could be standardised and monitored during isometric (Figure 8) and dynamic contraction (Figure 10), respectively.

5.6.1 Set-up for standardised isometric contractions

In the experimental set-up for standardised isometric contractions of the triceps surae muscle (Figure 8) the subject is seated with the trunk perpendicular to the seat and both knees extended. The extension of the knees ensures that the calf muscle alone generates the torque moment registered, and that the extensor muscles of the knee and thigh are excluded. Both feet are positioned on a vertical sheet with the axis of the sheet aligned with the axis of plantar/dorsal flexion in the ankle joint. The torque moment developed by m. triceps surae of the two legs in plantar direction is registered by a pre-calibrated
(range: 0 - 2000 N) strain gauge (lever arm: 280 mm). The torque is amplified by a custom-built instrumental AC-amplifier and displayed on-line to the subject (Figure 9).

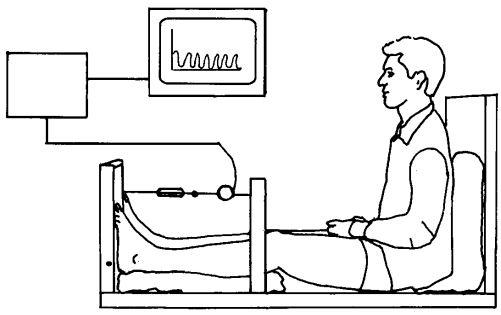


Figure 8. A schematically drawing of the experimental set up with a subject seated

and the generated torque displayed on-line to the subject.

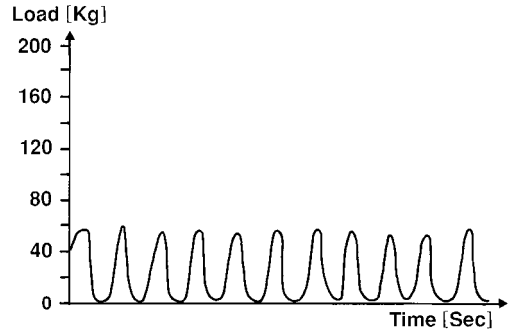


Figure 9. Graphic presentation of the display as seen by the subject. In the shown example the subject is told to generate a plantar flexor torque by which the force at the strain gauge corresponds to the body weight of the individual (60 kg). Intermittent contractions are performed in continuous for 1.5 seconds followed by a resting period of 1.5 seconds.

5.6.2 Set-up for standardised dynamic contractions

For investigation of physiological responses in the peritendinous area during dynamic contractions of the triceps surae muscle an experimental set-up was built in which the work load could be standardised (Figure 10). In this set-up the subject is seated with the trunk perpendicular to the seat and the foot of interest positioned on the vertical sheet with the axis of the sheet and the axis of plantar/dorsal flexion in the ankle joint aligned. By applying weight to the system, the load of the m. triceps surae in plantar flexion can be changed. A metronome controls the frequency of the dynamic contractions of the triceps surae.

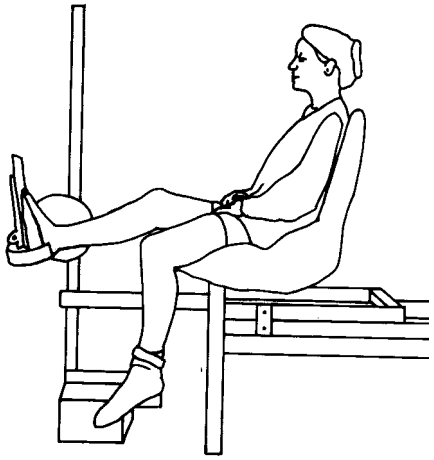


Figure 10. A schematically drawing of the experimental set up.

5.7 Statistical procedures

In the present thesis all data are presented as mean ± standard error of the mean (SEM) or if indicated as mean and range. Non-parametric ranking sum test for paired data (Wilcoxon) was used to detect significant differences between rest, exercise and recovery in the same area, leg, etc. A significance level of 0.05 (two tailed testing) was chosen a priori. Non-parametric ranking sum test for unpaired date (Mann-Whitney) was used to detect significant differences between legs, persons, etc. Again $p < 0.05$ (two tailed testing) was considered significant.

Friedman´s test was used to test whether significant changes over time occurred if more than two measurements were to be compared (184). When the primary test demonstrated a significant difference, such changes were located by the multiple comparison procedure (184).

6 BLOOD FLOW DURING DIFFERENT TYPES OF EXERCISE

6.1 Introduction

The present study investigated the influence of dynamic or intermittent static contractions of the calf muscle on blood flow in the peritendinous area of the human AT using the ^{133}Xenon wash-out method (Chapter 1.1). In healthy volunteers (Table 1) a depot of ^{133}Xe was injected just ventral to the AT 50 mm proximal to the upper medial portion of the tendon insertion on the calcaneus (Figure 4). The subjects were told to perform either dynamic (heel-lift or standardised dynamic contraction (Figure 10)) or intermittent static contractions (Figure 8) of the triceps surae muscle. The three specific work-protocols are described in details below.

6.2 Subjects and methods

Three groups of healthy volunteers with no previous history of Achilles tendon symptoms or injuries were included in this study (Table 1). All volunteers were involved in recreational endurance sport, and were non-smokers. The subjects were told not to do any kind of exercise 24 hours prior to the experiments, except for ordinary daily working activities. The study was approved by The Ethical Committee of Copenhagen (KF) 01-164/97 (heel-lift), (KF) 01-065/98 (isometric-load), (KF) 01-392/98 (dynamic-load).

	Sex	Age	Body weight	Training status hours/week
Dynamic exercise (heel-lift)	2w/8m	29 years (range, 23-39)	73 kg (range, 59-83)	5
Isometric contraction (Figure 8)	2w/4m	27 years (range, 23-31)	78 kg (range, 66-85)	4
Dynamic exercise (Figure 10)	1w/6m	26 years (range, 22-30)	74 kg (range, 58-87)	6

Table 1. The number of subjects in each of the three studies as well as subject data on age, weight and training status (including all exercise performed) given as means.

6.2.1 Dynamic exercise (heel-lift)

After injection of a depot of ^{133}Xe, the clearance-rate was measured during a 40-minute resting period with the subjects in supine and the ankle joint in a relaxed neutral position. The resting period was followed by the subject standing bare-footed on the floor doing heel-lifts (50 mm heel lift; 40 contractions/min; metronome-paced) for 40 minutes. By EMG it was verified that both the soleus and the gastrocnemii muscles were active during

the working period. The study was terminated by additional 40 minutes of resting.

6.2.2 Isometric contraction

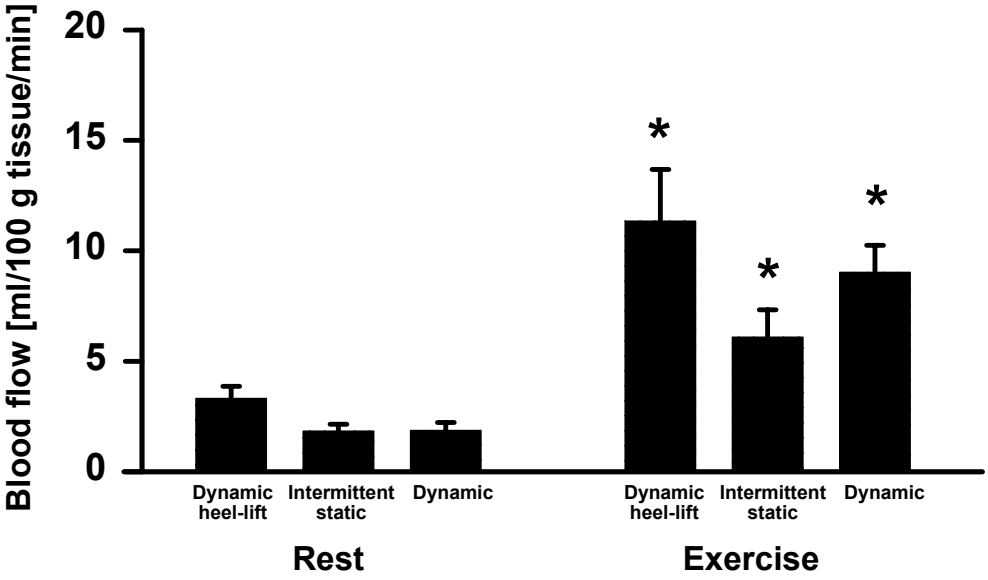
A depot of ^{133}Xe was injected in the peritendinous area on both right and left side. The Xenon clearance-rate was measured during a resting period of 90 minutes with the subjects being supine and the ankle joints in a relaxed neutral position. The resting period was followed by an exercising period during which the subjects were seated (Figure 8), and generating plantar flexor torque with a force at the strain gauge corresponding to their respective body weights. This torque was chosen to simulate the workload of the triceps muscles during normal walking (and heel-lift). Intermittent isometric contractions were performed continuously for 1.5 seconds followed by resting periods of 1.5 seconds (40 contractions/min; metronome-paced), for a total of 30 minutes. The study was terminated by an additional 60 min of rest.

6.2.3 Dynamic exercise

^{133}Xe was injected in the tissue ventral to the AT on the right side, and blood flow was measured as previously described during a resting period of 10 min with the subject in the seated position (Figure 10). The subjects then performed rhythmic dynamic plantar flexion at 3 Watt (45 contractions/min; metronome-paced) for a period of 5 min, before resting for additional 10 min.

6.3 Results

The peritendinous flow was determined based on the clearance rate of ^{133}Xe. Resting blood flow ranged from 1.9 ± 0.3 ml/100 g tissue/min to 3.3 ± 0.5 ml/100 g tissue/min during the three studies (Figure 11). With all three types of exercise blood flow was found to increase significantly compared to resting values, but with some differences in the magnitude being less pronounced during intermittent static exercise (Figure 11).



Figur

e 11. Mean blood flow values during rest and exercise determined by ^{133}Xe washout in the peritendinous space 50 mm proximal to the insertion of the human Achilles tendon. Error bars indicate SEM. Resting blood flow values are calculated as a mean blood flow based on the resting flow in the resting periods before and after the working period.

* indicates a significant increase in blood flow during exercise vs. rest ($p < 0,05$).

6.4 Discussion

The main finding in this study is the demonstration of a marked increase in blood flow from rest to exercise (3.5 fold (heel-lift); 3.4 fold (isometric-load); 4.7 fold (dynamic-load)) in the peritendinous space round the human AT. To our knowledge, this is the first report of changes in the blood flow around the human Achilles tendon during exercise. This finding corresponds with the demonstration of an increased blood flow within the Achilles tendon during running in dogs using the radio-labelled microsphere technique (25).

Earlier studies have shown that the Achilles region 20-70 mm proximal to the calcaneal insertion receives approximately 40 % of its blood supply from the extrinsic vascular system through the mesotenon (160;180;188). In studies on rabbits in which blood flow was measured within the AT as well as in the peritendinous area, it was found that during prolonged dynamic exercise blood flow increased in both regions, and that the ratio between flow within and around the tendon was unaltered (15;16). In the present study we did not determine the flow within the tendon, but

only in the peritendineous area and obviously our finding of increased flow during exercise does not guarantee any increased flow within the tendon. However based on the above mentioned findings in rabbits our findings indicate that increased flow in the peritendinous region most likely results in increased blood supply to central parts of the Achilles tendon during exercise.

Somewhat in contrast with this view, Laser Doppler flowmetry have been used to estimate AT flow and it was found that during passive stretch and static contraction of the triceps surae the flow in the AT decreases compared to resting values (14). However, from the few raw data actually provided in that paper it is clear, that marked decreases in local blood flow during isometric contraction only was pronounced when a tourniquet was inflated around the exercising limb and thereby interrupting the vascular supply, that the findings were not uniform with large variation between individuals, and as Laser Doppler Flowmetry does not provide absolute flow values only relative changes is presented (14). As the time resolution of the ^{133}Xenon wash-out method does not allow for determination of short acute changes in blood flow, the overall increase in blood flow during intermittent static contraction obtained in the present study could be a result of an increased blood flow during the short rest periods (1.5 sec), and that this rise exceeded the decrease in blood flow during the static contraction phase, resulting in a net increase in flow. Such a flow pattern has been observed in skeletal muscle, where intense static contractions are known to cause a reduction in flow, whereas dynamic exercise results in markedly increased flow (58).

The obtained blood flow values at rest in the present study were around 2-3 ml/100 g tissue/min, corresponding to findings in resting muscle and adipose tissue (114;117), but somewhat higher than values (0.9 ml/100 g tissue/min) found within a resting Achilles tendon using a similar technique (55). However, the prerequisites for the ^{133}Xe-washout method, namely homogeneous tissue and homogeneous flow distribution within the tissue, are probably not fulfilled inside tendons, implying that the ^{133}Xe washout curves are not mono-exponential, making it difficult to find the “final slope” (Figure 5), and thus to determine true regional blood flow of the tendon.

In conclusion the present studies demonstrate that peritendinous blood flow increases during both dynamic and intermittent static contractions of the calf muscle. Whether the found increase in blood flow reflects increased metabolic/nutritive needs of the tendon or rather is a passive phenomenon associated with increased muscle flow is however not possible to conclude from the present study. The increase in ^{133}Xe-clearance during exercise could theoretically be due to increased lymph drainage, as exercise has been reported to increases lymph flow

across an exercising limb (34;155), due to a pumping effect within contraction muscle. To clarify this lymph drainage was measured in the peritendinous area during rest and intermittent static exercise (Figure 8) in three subjects (3m, 29-33 years) using ^{99m}Technetium labelled microaggregated albumin (154)(KF) 01-065/98. However, in the present study the lymph drainage of the region studied was found to be low and no significant difference during exercise in the clearance-rate of ^{99m}Technetium labelled albumin could be detected indicating that lymphatic drainage did not change in response to exercise, and that the demonstrated increased ^{133}Xe-disappearance rate during exercise reflects an enhanced blood flow in the peritendinous region of the AT.

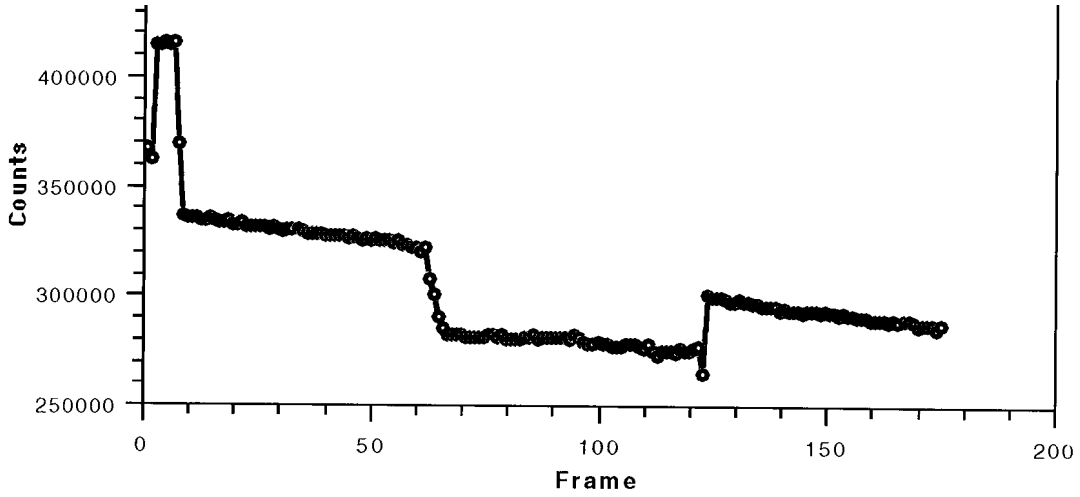


Figure 12. The graph is showing the clearance curve of ^{99m}Technetium labeled albumin in one of the subjects. ^{99m}Technetium-clearance was measured during 30 min of rest (frame 0-60), 30 min of intermittent static exercise (Figure 8)(frame 61-120) and 30 min of recovery (frame 121-180).

7 FLOW WITH INCREASING WORK INTENSITY

7.1 Specific protocol

To elaborate on the findings of increased blood flow in the peritendinous area of the Achilles tendon during exercise the present study used graded, dynamic plantar flexion exercise (Figure 10) at incremental work loads of 1-9 Watts (W) for periods of 5 min. The intention was to determine whether a positive correlation between blood flow in the peritendinous area and workload existed.

Sex	1w/6m
Age	26 years (range, 22-30)
Body weight	74 kg (range, 58-87)
Training status hours/week	6

Table 2: Subject data on sex, age, weight and training status (including all exercise performed) given as means.

7.2 Subjects and methods

Seven young, healthy individuals (Table 2) with no previous history of Achilles tendon symptoms or injuries participated in the study after informed consent as approved by the Ethical Committee of Copenhagen (KF) 01-392/98. The subjects were told not to do any kind of exercise 24 hours prior to the experiments, except for ordinary daily working activities. All subjects were non-smokers.

^{133}Xe was injected in the tissue just ventral to the Achilles tendon as described above (Chapter 5.1.1), and blood flow was measured by the ^{133}Xe-washout technique. After a resting period of 30 min in the seated position (Figure 10), baseline measurements for peritendinous flow were collected over a 5 min period. The subjects then began rhythmic dynamic plantar flexion exercise at 1 W (45 contractions/min; metronome-paced) on the ergometer for a period of 5 min, followed by a period of rest for 10 min. The same procedure was repeated for the 3, 5 and 7 W loads. In pilot studies, it was found that not all subjects could complete the 5 min duration at 9 W. Therefore, the final exercise bout consisted of a ramped bout starting at 5 W for 1 min, followed by 7 W for 2 min, and finally 9 W for 2 min.

7.3 Results

Blood flow in the peritendinous region of the AT increased significantly from 1.3 ± 0.2 ml/100 g tissue/min at rest to 14.1 ± 4 ml/100 g tissue/min (Figure 13) during the ramp bout to 9 Watts ($p < 0.05$). The increase in blood supply was most pronounced from rest to the light loads (1-3 W). With further increase in loading the increase in blood flow was only minor. During 9 Watt a relative large individual variation was found in blood flow.

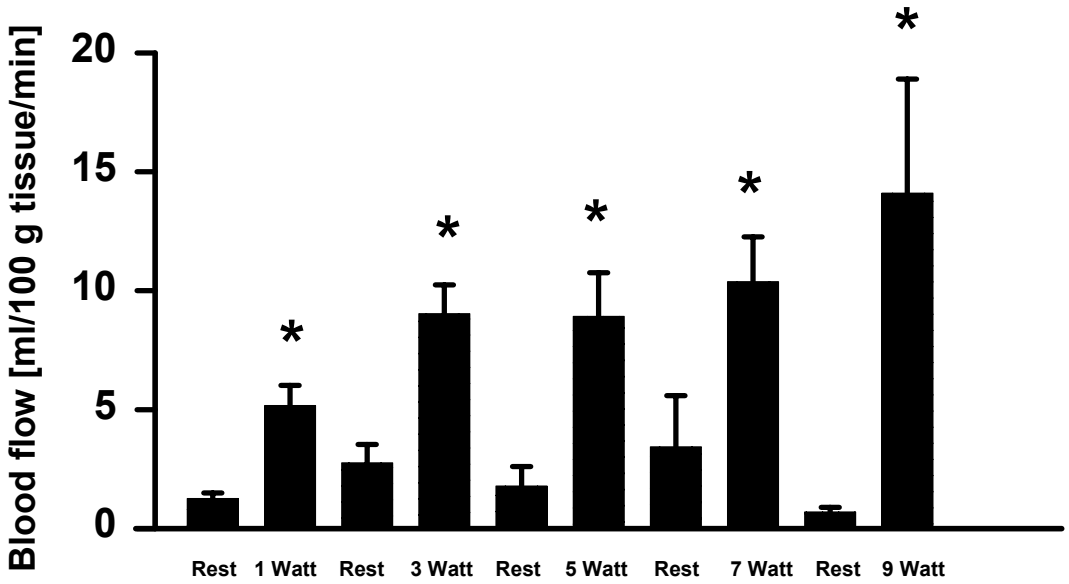


Figure 13. Mean blood flow values during rest and incremental dynamic exercise (1-9 Watt) determined by ^{133}Xe washout in the peritendinous space 50 mm proximal to the insertion of the human Achilles tendon. Error bars indicate SEM. * Indicates a significant increase in blood flow during exercise vs. rest ($p < 0,05$).

7.4 Discussion

The present study expands on the previous finding of a 3-4-fold increase in blood flow in the peritendinous region for the human Achilles tendon during moderate exercise (heel-lift), and demonstrates that peritendinous blood flow not only increases with exercise, but also can reach a level 10-fold above the resting value with intense muscular activity.

It is well known that oxidative metabolism and blood flow in skeletal muscle increases with intensity of muscle contraction (178), and it has been suggested that perfusion of tendons may represent a functional shunt serving as a flow reserve for the contracting muscle during exercise (30). However the present results illustrate that peritendinous blood flow increases with increasing loading of the calf muscle. Thus, the present data do not suggest that the peritendinous tissue flow of the Achilles tendon is restricted during hyperemia of skeletal muscle arguing against the notion that blood flow is shunted away from the tendon during muscular contraction. In addition, shunting blood from the tendons to the exercising muscles would not quantitatively result in a marked increase in blood flow calculated per gram exercising muscle tissue as the absolute blood flow to

muscles is much higher compared to tendons (m. triceps surea: 75 ml/100 g tissue/min vs. AT: 14 ml/100 g tissue/min) and as the amount of involved tissue is much larger for muscle compared to tendon (m. triceps surae: 1.3 l vs. AT 0.08 l (MRI-measurements))(Boushel *et al*, unpublished data).

The muscle mass exercising in the present study is however small which could influence the conclusion, if no contradiction exists between the supply and demand of the exercising muscles during exercise.

From the present data it is not possible to deduce whether the 10-fold increase in peritendinous flow reflects increased metabolic/nutritive needs of the tendon or rather is a passive phenomenon associated with increased muscle flow, it is also not known whether the metabolic activity in peritendinous tissue is markedly enhanced during loading of the calf muscle.

In muscles, substances such as NO (41;42;76;102;176), prostaglandins (26;35;42;93;99;197;198;228;233), potassium (64;173;174;178), bradykinin (197;198), and adenosine (26) released from the muscle itself or the vessel wall are known to cause vasodilation and as such regulating local blood flow. Whether the same metabolic substances regulate flow around the tendon is not known, and the role of locally released metabolic vasoactive substances in regulating tendon blood flow remains to be elucidated.

8 REGIONAL DIFFERENCES IN PERITENDINOUS BLOOD FLOW DURING EXERCISE

8.1 Specific protocol

The fact that the relative area of blood vessels 40 to 60 mm proximal to the calcaneal insertion of the AT is reduced (Figure 3)(7;27;110;182), and that this area corresponds with the area most prone to injuries (39;82;89;108;147;183) has led to the hypothesis of hypovascularisation being related to the aetiology of AT inflammation and overuse. However it is not known whether these regional differences results in a diversity in the distribution of blood during exercise. Therefore the specific aim of the present study was to investigate whether regional differences in peritendinous blood flow could be detected during rest and exercise. Thus, blood flow in the peritendinous area was measured in a region with a high number of blood vessels (20 mm proximal to the tendon insertion) and a region with relative few vessels (50 mm proximal to the tendon insertion) during rest and exercise (heel-lift) using the ^{133}Xenon clearance technique.

8.2 Subjects and methods

Ten healthy volunteers (Table 3) with no previous history of Achilles tendon symptoms or injuries participated in the study after informed consent as approved by the Ethical Committee of Copenhagen (KF) 01-164/97. The subjects were told not to do any kind of exercise 24 hours prior to the experiments, except for ordinary daily working activities. All subjects were non-smokers.

Sex	2w/8m
Age	29 years (range, 23-39)
Body weight	73 kg (range, 59-83)
Training status hours/week	5

Table 3. Subject data on sex, age, weight and training status (including all exercise performed) given as means.

To measure blood flow a depot of ^{133}Xe was placed in the tissue just ventral to the Achilles tendon 50 mm (right side) and 20 mm (left side) proximal to the upper medial portion of the Achilles tendon insertion on the calcaneus (Chapter 1.1).

8.3 Results

^{133}Xenon clearance was measured during rest, exercise and recovery period respectively. As no systematic difference was found between the calculated blood flow during rest and recovery neither at 20 mm nor at 50 mm proximal to the insertion a mean resting blood flow was calculated for each of the two regions based on (Eq. 2). No significant difference in the resting blood flow was found between the two regions. From rest to exercise a significant

increase in blood flow was found both in the region 20 mm and the region 50 mm proximal to the Achilles insertion ($p < 0,01$)(Figure 14).

During the working period, a significantly higher blood flow ($p < 0,05$) was found in the region 50 mm proximal to the Achilles insertion compared to the value at the more distal region (11,4 ± 2,3 ml/100 g tissue/min vs. 5,4 ± 1,0 ml/100 g tissue/min).

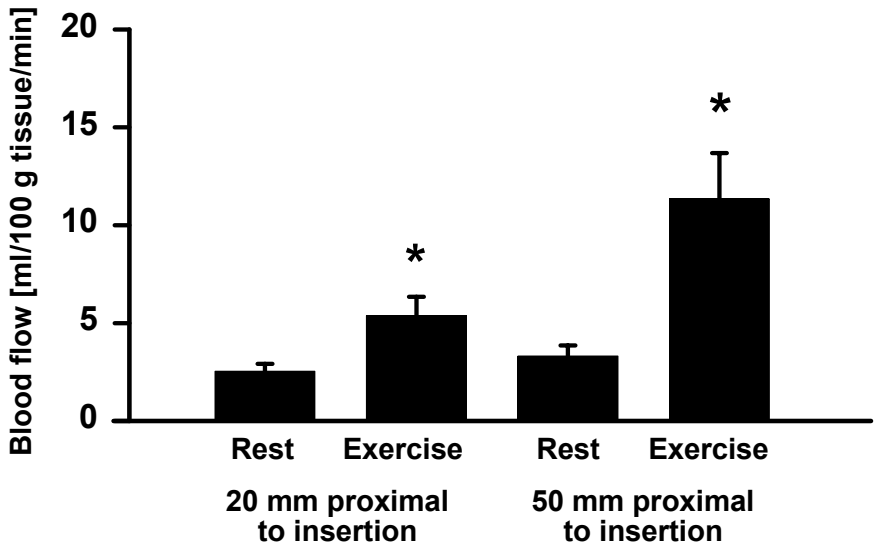


Figure 14. Mean blood flow values during rest and dynamic exercise (heel-lift) determined by ^{133}Xe wash-out in the peritendinous space 20 and 50 mm proximal to the insertion of the human Achilles tendon. Error bars indicate SEM. * Signifies a significant increase in blood flow during exercise vs. rest ($p < 0,05$).

8.4 Discussion

In the present study blood flow was determined in two specific locations in the peritendinous space ventral to the AT. During rest blood flow did not differ between the two areas, and as a result of exercise blood flow was found to increase significantly in both regions. However, interestingly, the rise in blood flow was more pronounced (3.5 fold vs. 2.2 fold) in the mid-portion of the Achilles peritendinous space (50 mm above tendon insertion) known to have relatively fewer blood vessels compared to the more distal region (20 mm above tendon insertion) having a high number of vessels.

The pattern in flow distribution determined in the present study has likewise been demonstrated by Åström *et al*., who showed that blood flow in the Achilles tendon is lower near the calcaneal insertion compared to other regions of the Achilles tendon (14). Those findings as well as the present data, argue against the area 40-60 mm proximal to the AT insertion having a reduced blood flow, as suggested based on the anatomical findings (7;27;110). It has been shown by others that there exists another area of diminished blood supply just above the bony insertion of the AT (3;182). This area is rarely the site of ruptures, making the link between the anatomy of the blood vessels and the possible sites of ruptures less promising. Based on these facts a direct relationship between vascularisation and rupture of the Achilles tendon is unlikely. In spite of these compelling data speeking against hypovascularisation being the reason for ruptures of and inflammation in the AT, a resent review paper still stresses hypovascularisation in the mid-portion of the AT as being *the aetiology* for Achilles tendon ruptures (144).

9 FLOW AND AGE

9.1 Specific protocol

Many clinical studies has been performed on young athletes and do not consider age an aetiological factor, although incidence of chronic Achilles tendinopathy has be found to increase with age (105;132;138). The explanation for this increase could be found among the profound effects of ageing on the locomotion system in terms of function and mechanical properties of the tissues, ranging from reduction in local tissue blood flow (82), changed vascularisation (149), increase in size and cross-linking of the collagen molecules resulting in reduced flexibility (84), to neural degeneration with loss of motor units and decreased production of hormones (66;123). The blood supply to the hypovascular zone in the midprotions for the AT previously described may be even further compromised by age (29)(39), but no difference could be detected between patients with previous ruptures and healthy controls (82). These findings have been supported by studies on post mortem tendon biopsies showing an age related increase in the incidence of asympomatic degenerative lesions similar to those encountered in tendon ruptures (92;95).

9.2 Subjects and methods

In the present protocol a group of healthy volunteers with no previous history of Achilles tendon symptoms or injuries were included (Table 4). All volunteers but one were involved in recreational endurance sport, and all were non-smokers. The subjects were told not to do any kind of exercise 24 hours prior to the experiments, except for ordinary daily working activities. The study were approved by The Ethical Committee of Copenhagen (KF) 01-164/97 (heel-lift) and (KF) 01-065/98 (isometric-load). The data of the young group correspond to the ones determined in the chapter on “Blood flow during different type of exercise”.

	Study	Sex	Age [years]	Body weight	Training status [h/week]	Traning experience [years]
Middle-aged (>45)	Dynamic exercise (heel-lift) and isometric contraction (Figure 8)	3w/3m	48 (41-56)	71 kg (57-77)	6	12 ± 4
Young group	Dynamic exercise (heel-lift)	2w/8m	29 (23-39)	73 kg (59-83)	5	9 ± 2
	Isometric contraction (Figure 8)	2w/4m	27 (23-31)	78 kg (66-85)	4	8 ± 3

Table 4. The number of subjects in the two groups as well as subject data on age, weight and training status (including all exercise performed) given as means ± SEM or range in brakes.

9.3 Results

During rest blood flow was found the be identical in the two groups ranging from 1.9 ± 0.3 ml/100 g tissue/min to 3.3 ± 0.5 ml/100 g tissue/min. With dynamic exercise (heel-lift) blood flow increased significantly in both groups reaching an almost identical level (young: 11.4 ± 2.3 ml/100 g tissue/min; middle-age: 11.6 ± 2.5 ml/100 g tissue/min). The same pattern was found during isometic exercies (young: 6.1 ±1.2 ml/100 g tissue/min; middle-age: 7.0 ± 0.3 ml/100 g tissue/min). No significant difference could be detected in blood flow between the two types of exercise.

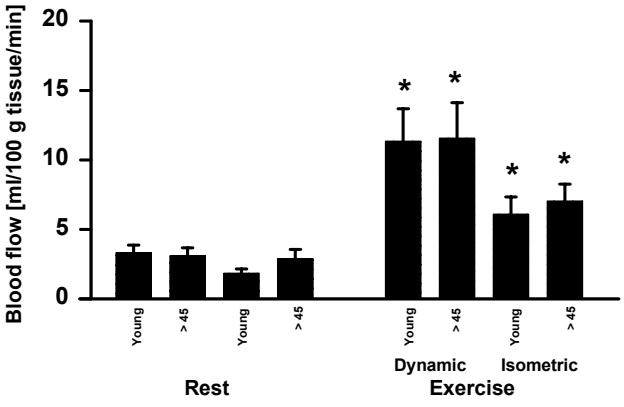


Figure 15. Mean blood flow values during rest and dynamic (heel-lift) and intermittent isometric (Figure 8) determined by ^{133}Xe washout in two age groups. Error bars indicate SEM. * indicates a significant increase in blood flow during exercise vs. rest ($p < 0,05$).

9.4 Discussion

In the present study no difference in blood flow between young and middle-aged subjects were found neither during rest nor during two types of exercise.

In contrast Åstrom et al. observed with Laser Doppler flowmetry that the blood flow in AT in healthy volunteers decreased with increasing age (14). In support of this Håstad *et al.* observed a decrease in resting blood flow after the 3rd decade of life in healthy men (82). In that study a correlation was found between increase in age and decrease in blood flow, although without linearity. A possible explanation for the discrepant between the present study and the studies showing decreased flow with age could be due to the middle-aged group in the present study not being old "enough" and being well trained (6 h training/week) and with a long training background (12 years). The middle-aged group however comprises a high percentage of the patients with peritendinitis (6;7;105;132), and as such the present findings does not support the hypothesis of hypovascularisation alone being linked to the aetiology of Achilles tendon inflammation. Instead some of the other factors influenced by age such as decreased water content and elasticity of the tendon tissue, reduced strength of the muscles and connective tissue may be responsible for the age-related increase in tendon inflammation (66;84;123). A simple explanation could be the training pattern of middle-aged people, as most middle-aged have experienced a period of low or no training (due to sedentary jobs, family, etc) before resuming regular exercise which could lead to reduced adaptation to changes in load.

In conclusion, no differences in blood flow in the peritendinous space of the human AT could be detected neither during rest nor during two types of exercise between a group of young (mean age 29 years) and a group of middle-aged people (mean age 48 years). In the future it would be interesting to test a group of elderly people (+70) as well as an untrained group of middle-aged to further investigate the role of age on tendon related blood flow.

10 PRESSURE

10.1 Specific protocol

In an attempt to measure in-vivo metabolism and inflammatory processes in relation to the AT, microdialysis was performed in the peritendinous area immediately ventral to the Achilles tendon in humans. However pilot studies in three subjects revealed that perfusion fluid was lost when microdialysis was performed during muscular contraction of the m. triceps surae (dialysate volume during rest: 91 % ± 1 % of expected volume; during exercise: 11 % ± 2 %; recovery after stop of exercise: 92 % ± 2 %). A possible explanation for this could be ultrafiltration, as previous studies in skeletal muscle using low perfusion rates had found a minor loss of the dialysate during rest (74;172). Alternatively, peritendinous tissue pressure decreases during muscular contraction and a mass transfer of fluid from the microdialysis catheter to the tissue occurs (5). To test the later hypothesis, the present study determined the pressure in the peritendinous space ventral to the human Achilles tendon at rest and during graded workloads. This was done during intermittent isometric contractions with the triceps surae muscles (Figure 8).

10.2 Subjects and methods

A group of eleven healthy volunteers (Table 5) with no previous history of Achilles tendon symptoms or injuries were included in the present study approved by The Ethical Committee of Copenhagen ((KF) 01-164/97). Subjects were told not to undertake any kind of exercise 24 hours prior to the experiment, except for ordinary daily working activities.

Sex	4w/7m
Age	28 years (range, 23-35)
Body weight	78 kg (range, 55-93)
Training status hours/week	6

Table 5. Subject data on sex, age, weight and training status (including all exercise performed) given as means.

10.2.1 Pressure

To measure tissue pressure in the peritendinous area during exercise of the calf muscle, marks were made on the skin on both legs 20, 40 and 50 mm proximal to the insertion of the AT on the calcaneus, respectively. Corresponding to each of the marks a cannula was introduced in the tissue as previously described (Chapter 5.4), and the subject was subsequently told to generate a plantar flexor torque by which the force at the strain gauge corresponded to 200 N. Interstitial pressure was determined when the torque had stabilised.

The experiment was terminated by a recovery measurement with relaxed m. triceps surea. The same procedure was performed with a plantar torque of 400 N and 600 N, respectively. In all cases the interstitial pressure returned to resting values during relaxation of the calf muscle.

10.3 Results

At rest no significant difference was found between the pressures measured in any of the three regions (Figure 16). Furthermore a nearly linear decrease in pressure was found with increase in torque in the three regions (Figure 16), with no significant differences between regions.

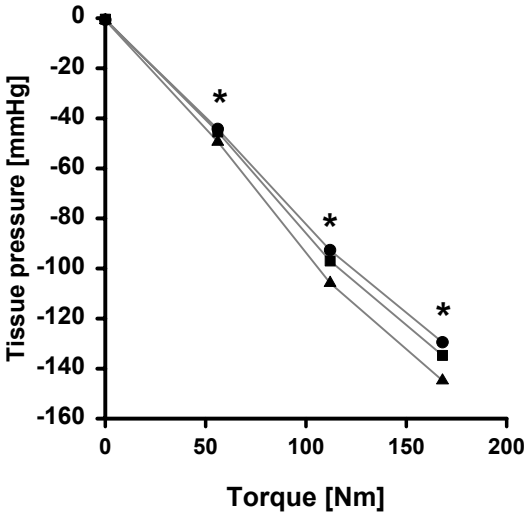


Figure 16. Mean changes in the tissue pressure in the peritendinous space 20 mm (▲), 40 mm (●) and 50 mm (■) proximal to the insertion of the human Achilles tendon. Pressure was recorded while the subjects generated a force of 200 N, 400 N and 600 N representing a torque of 56 Nm, 112 Nm and 168 Nm respectively. No significant difference in the tissue pressure in the peritendinous space was found between the three different regions. Tissue pressure increased significantly (indicated by *) in all three regions when increasing the plantar torque ($p < 0,05$).

10.4 Discussion

A marked decrease in peritendinous tissue pressure ventral to the Achilles tendon was found during intermittent static contractions of the triceps surae muscle in humans (Figure 16). The method has been evaluated for measuring negative intramuscular pressure and found suited for recording negative pressures over a wide range (36). In a previous study on pressure within the human AT pressure a similar decrease in pressure was found during contraction of the calf muscle (as low as –127 mmHg), however without a proportionality between the pressure in pursuance of the contraction (54). The negative intratendon and interstitial pressure, during contraction of the calf muscle is in contrast with changes in muscle tissue pressure, where exercise is found to cause a rise in intramuscular pressure in a variety of muscle groups (5;146;148;200;201). The fact that peritendinous and intratendon pressure decreased several fold during exercise could explain why collected dialysate volumes were lower than expected during exercise. The decreased pressure could be created as a result of the muscles contracting, expanding the dense structures

surrounding the Achilles tendon. The role of this marked negative pressure during exercise could be of importance for fluid shifts and microvascular flow during exercise, as well as promoting lymph drainage from the tissue. The lymphatic vessels in skeletal muscle are known to lack smooth muscle (186), and activation of the muscle pump and physical activity in general are known to promote lymph flow (70;155;156;206). In a similar way pressure changes could be of great importance for the lymph drainage of the peritendinous tissue.

A reduced tissue pressure could reduce vascular resistance, and as such be involved in the increase in blood flow in the peritendinous area around the human Achilles tendon previously shown during both dynamic and intermittent static exercise. In muscle it is well described that changes in intramuscular pressure influences blood flow through the region, and that chronic elevated intramuscular pressure is associated with decreased venous outflow (200). Whether the negative pressure measured within the peritendinous space has a similar impact on the flow within this region is however not known.

In summary the present study shows that the interstitial pressure decreased during exercise, corresponding to a decrease previously found within the AT (54). The decrease in pressure along the Achilles tendon was linear with increasing torque. The clinical consequence of this drop is however not known.

11 REQURIMENTS FOR MICRODIALYSIS IN THE PERITENDINOUS AREA OF THE HUMAN ACHILLES TENDON

As tissue pressure was found to decrease during exercise a colloid osmotic substances was added to the perfusion fluid in an attempt to counteracting fluid loss when performing microdialysis during muscle contraction (171).

11.1 Subjects and methods

In the same group of volunteers as used for the pressure study (Chapter 10) microdialysis was performed (Table 5). At least a two weeks period was allowed between the measurements to reduce the risk of a potential insertion trauma from the pressure measurements influencing the results.

11.1.1 Microdialysis measurements

One microdialysis catheter (CMA 60; membrane 30 x 0.62 mm, 20 000 molecular weight cut-off: CMA/Microdialysis AB, Stockholm, Sweden) was placed in the peritendinous space ventral to each Achilles tendon with the active part of the catheter covering the area from 20 to 50 mm above the insertion of the tendon on the calcaneus (equal to the area where the pressure measurements had been performed). One additional catheter (CMA 60; membrane 30 x 0.62 mm, 20 000 molecular weight cut-off: CMA/Microdialysis AB, Stockholm, Sweden) was placed in the m. gastrocnemius lateralis. The dialysis catheters were perfused, via a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden), at an infusion rate of 1 μl/min. The precision of the pump was verified by weighing samples collected from tubing attached to syringes in the pump.

A mean torque for the "exercising-cycle" consisting of a rest period (1.5 sec) and a contraction period (1.5 sec) was calculated using the area under the curve (AUC)(Figure 9). Based on the calculated mean torque and on the linear relationship between torque and tissue pressure (Figure 16) the average negative pressure generated during one "exercising-cycle" was calculated to be 25-30 mmHg. Based on these calculations the perfusion fluid (Ringer acetate solution) was supplemented with 0.1 g/ml of Dextran (71 Kda; D-1537, Sigma Chemical, St. Louis; USA). The colloid osmotic pressure (COP) of that perfusion fluid was calculated to be 27 mmHg.

Microdialysis was performed with the subjects resting supine for 60 minutes, followed by intermittent isometric contractions (1.5 seconds contraction/1.5 seconds rest) in plantar direction for 30 minutes with a total torque of 1 x body weight (Figure 8). The study was completed by an additional resting period of 60 min. The dialysate was collected in capped microvials (CMA Microdialysis,

Stockholm, Sweden) and the collected dialysate volume was determined immediately by weighing all samples on a high-precision weight.

11.2 Results

With the addition of Dextran-70 a 100 ± 4 % recovery of dialysate volume was achieved during exercise (Figure 17). However, a net gain of approximately 10% in the dialysate volume was found during both rest and recovery. The addition of Dextran-70 to the perfusate resulted in the gastrocnemius muscle in a net gain of fluid of 10% during rest and almost 20% during exercise (Figure 17).

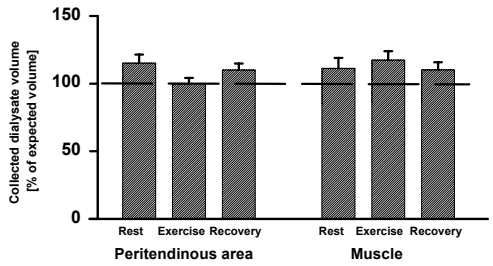


Figure 17. The amount of dialysate volume collected during microdialysis in the connective tissue ventral to the Achilles tendon and in the m. gastrocnemius lateralis are shown. The values are given as mean ± SEM of twenty-two samples (eleven subjects with two microdialysis probes each). Samples were collected during 60 min of rest, 30 min of exercise and additionally 90 min of rest. The microdialysis probes were perfused with flow rate 1 µl/min using a perfusion fluid containing 0.1 g/ml of Dextran-70. The expected dialysate volume is indicated as a dashed line.

11.3 Discussion

In the previous present study contration of the calf muscle was found to generate a negative pressure in the peritendinous area. The average negative pressure during an exercising period (Figure 9) was calculated to be 25-30 mmHg, and in an attempt to counteract this low negative pressure 0.1g Dextran-70 was added per ml of perfusate increasing the osmotic pressure of the perfusate with 27 mmHg. When using this perfusate during exercise dialysate volume was restored to 100 ± 4 % counteracting the loss of dialysate volume. This supports the hypothesis that the determined loss in dialysate volume during exercise is a result of a changes in tissue pressure.

In previous studies a similar imbalance between expected and collected volume has been demonstrated using low perfusion flow rates (75;172). This loss of dialysate could likewise be counteracted by addition of Dextran-70 (171).

The addition of Detran-70 to the perfusate resulted in an increase in collected dialysate volume at rest in the peritendinous area (110 ± 5 %). When performing microdialysis in the muscles with Detran-70 in the perfusate dialysate volume increased both during rest (110 ± 6 %) and exercise (117 ± 6 %)(Figure 17), which is most likely a result of the increased colloid osmotic pressure together

with a small increase in tissue pressure during exercise (2;5;146;148;199-201).

We chose in the present study to perfuse the microdialysis probes at a flow rate of 1 μl/min and with a membrane length of 30 mm, as in-vitro studies have demonstrated this combination to give the best relation between recovery (concentration) and volume (Langberg, unpublished data). However, the fluid loss could be markedly influence by changes in flow rates, membrane lengths and exercising intensities and as such altering the need for modifying the composition of the perfusate in order to counteract dialysate loss (171).

In summary the present study shows that microdialysis in human peritendinous space around the Achilles tendon during exercise requires the addition of a colloid osmotic active substance to the perfusate in order to counteract the negative tissue pressure generated during calf muscle contraction and that the addition of the colloid osmotic active substance results in a complete recovery of the dialysate volume.

12 INFLAMMATORY MEDIATORS

12.1 Background

It is general accepted that local factors, such as NO (41;42;76;102;176), prostaglandins (26;35;42;93;99;197;198;228;233), potassium (64;173;174;178), bradykinin (197;198), and adenosine (26) released during muscle work and ischeaemia, are involved in the local increase in blood flow (115). Among the most potent known mediators of vasodilatation are the prostaglandins (73;97). Prostaglandins are released from endothelial cells of the vascular wall during exercise (99;224;228;233), and has been argued to account for much of the local flow-induced vasodilatation (50;51;100-102).

Two of the products of the arachidonic acid metabolic pathway, prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2)(molecular weight 350 to 375 Da) are both well suited to be examined by the microdialysis technique. In the present study, microdialysis in combination with the ^{133}Xenon wash-out blood flow technique was used to determine changes in in-vivo inflammatory concentration in the peritendinous area just ventral to the human Achilles tendon at rest and during exercise.

12.2 Subjects and methods

Thirteen healthy volunteers (Table 6) were included in this study after obtaining written and oral acceptance. The study was approved by The Ethical Committee of Copenhagen ((KF) 01-065/98). All volunteers were involved in recreational endurance sports and had no previous history of Achilles tendon symptoms or injuries. None of the subjects took any medication.

Sex	5w/8m
Age	26 years (range, 23-31)
Body weight	75 kg (range, 62-85)
Training status hours/week	4

Table 6. Subject data on sex, age, weight and training status (including all exercise performed) given as means.

12.3 Experimental protocol

The subjects were told not to perform any kind of exercise 24 hours prior to the experiment, except for ordinary daily working activities (students or sedentary office jobs). All experiments were started at 09.00 h. During the experiment the subjects rested supine with the ankle joints in a relaxed neutral position (70-80°) at a room temperature of 25º C. One microdialysis catheters (CMA 60; CMA/Microdialysis AB; 20 kDa molecular cut off, 0.5 mm outer diameter; length 30

mm) was positioned on each side in the peritendinous area just ventral to the AT. The dialysis catheters were perfused, via a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden), at a rate of 1 µl/min with a Ringer acetate solution added 0.1 g/ml of Dextran (71 Kda; D-1537, Sigma Chemical, St. Louis, USA), and 5 nM [15-^{3}H(N)]-prostaglandin E_2 (specific activity: 3.7 GBq/mmol; NEN, Boston, USA) for recovery determination. After insertion of the microdialysis catheters and injection of ^{133}Xe for blood flow measurements the subjects rested for at least additional 30 min. before starting the experiment. This procedure ensured a time delay of at least 60 min. from insertion of the last microdialysis catheter to the first measurement and thus minimising the tissue response to the insertion trauma (Chapter 14). The experiment was initiated by a resting period of 90 minutes during which blood samples and microdialysis samples were collected for obtaining basal values. The resting period was followed by an exercise period. The subjects were told to generate a plantar flexor torque by which the force at the strain gauge corresponded to their respective body weights. Intermittent contractions were performed continuously for 1.5 seconds followed by resting periods of 1.5 seconds, for a total of 30 minutes (Figure 8). A metronome with a frequency of 40 Hz was used to control the length of the working/resting periods during exercise. The study was terminated by an additional recovery period of 60 min of rest. To obtain sufficient dialysate for analysis the samples from rest (n = 9), exercise (n = 3) and recovery (n = 6), respectively, were pooled for each leg separately in each subject. The samples from the right legs were used for determination of PGE_2 and from the left legs for determination of TXB_2. PGE_2 was analysed using a commercially available PGE_2 radioimmuno-assay kit (NEK-020, Du Pont, Boston, MA). Samples or standards, together with ^{125}I-PGE_2 as the tracer, were incubated with rabbit anti-PGE_2 antibodies overnight at 4° C. The samples were precipitated by polyethylene glycol, centrifuged, decanted and radioactivity in the pellet was determined in a gamma counter. TXB_2 was determined using a radioimmuno-assay (NEK-024, Du Pont, Bad Homburg, Germany).

12.4 Results

The interstitial concentrations (C_i) were calculated using the internal reference calibration method (181). The relative recovery (RR) of prostaglandin E_2 was 59 ± 4 % (rest), 77 ± 4 % (exercise), and 55 ± 6 % (recovery), with an significant higher recovery during exercise vs. rest ($p < 0.05$). Blood flow in the peritendinous area was found to increase 3 fold with exercise ($p < 0.05$) and returned to basal level in the recovery period.

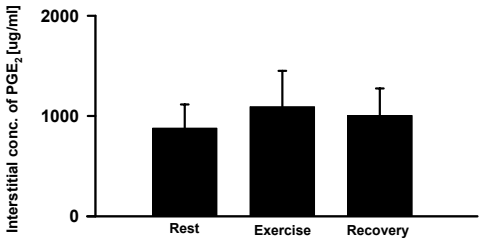


Figure 18. Interstitial concentrations of PGE_2 during rest, exercise and recovery. No significant differences were found.

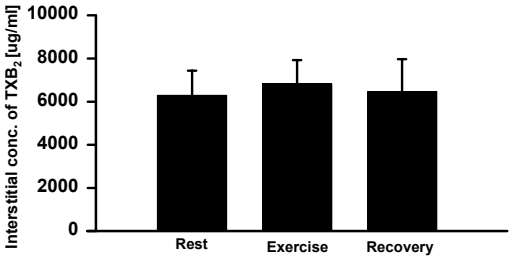


Figure 19. Interstitial concentrations of TXB_2 during rest, exercise and recovery. No significant differences were found.

No significant difference in the calculated interstitial concentration of prostaglandin E_2 (Figure 18) or thromboxane B_2 (Figure 19) was found in the tissue as a result of exercise. As no arterial concentration of neither PGE_2 nor TXB_2 were measured, it was not possible to calculate venous concentrations ($C_{v\ calc}$) of the inflammatory mediators, as well as no net release of prostaglandin E_2 and thromboxane B_2.

12.5 Discussion

In the present study the microdialysis technique was used to determine indicators of inflammatory activity in the peritendinous space of the human Achilles tendon. No significant increase in interstitial concentration of inflammatory mediators in response to exercise could be detected, but as blood flow during exercise was found to increase 3-fold the release of inflammatory mediators is most likely increased during exercise.

Inflammatory mediators have previously been measured in response to mechanical loading of human bone, where interstitial concentrations of PGE_2 were reported to increase (210). In that study however, no measurement of recovery for PGE_2 was performed neither at rest nor during mechanical loading, thus making it difficult to detect true quantitative changes in inflammatory mediators with exercise (210). In the present study, recovery was determined and found to increase with exercise. This indicates that any attempt to calculate the interstitial tissue concentration without taking recovery into account would overestimate changes occurring with onset of contraction in the peritendinous tissue.

In in-vitro experiments using osteoblast cell line from mice have demonstrated that mechanical strain increased PGE_2 release by two fold (143). Furthermore, in fibroblast cell line from human finger flexor tendons PGE_2 release increased with graded mechanical loading, and addition of indomethacin completely inhibited the exercise induced increase in PGE_2 (1). In the present study subjects did not subjectively experience any pain due to the

presence of the microdialysis catheters within the tissue either at rest or during exercise. In addition, isometric contractions were used during the exercising period giving as little movement of the contracting tissue in relation to the catheter as possible, and thus reducing an eventual trauma to a minimum. Despite this it cannot be excluded that local tissue reactions due to the placement of the catheters could influence the measurements. This topic is further elucidated in the chapter on insertion trauma (Chapter 14). Based on this, it is likely that the increased tissue release of inflammatory mediators represents inflammatory activity due to exercise rather than due to pain or local irritation by the catheter.

In conclusion, indicators of inflammatory activity are found being produced in the peritendinous region of the human AT in response to exercise.

13 METABOLISM

13.1 Specific protocol

It has been demonstrated that connective tissue of a tendon has a low basal metabolic rate (160), which has been suggested to contribute to a high tolerance towards low oxygen tension and resistance towards tissue injury associated with prolonged mechanical loading (226). However, it is at present unknown to what extent metabolism changes in and around tendons with physical activity. These processes could be important for understanding of the development of overuse inflammation and injury.

13.2 Subjects and methods

Six healthy volunteers were included in this study after obtaining written and oral acceptance (Table 7). The study was approved by The Ethical Committee of Copenhagen ((KF) 01-065/98). None of the subjects had any injuries, took any medication and all were non-smokers.

Sex	2w/4m
Age	27 years (range, 23-31)
Body weight	78 kg (range, 66-85)
Training status hours/week	4

Table 7. Subject data on sex, age, weight and training status (including all exercise performed) given as means.

The subjects were studied at rest, during 30 min of intermittent static plantar flexion of the ankle at workloads corresponding to individual body weight (Figure 8), and during 60 min of recovery, in a protocol similar to the one used in the previous study on inflammatory mediators. Microdialysis catheters (CMA 60; CMA/Microdialysis AB; 20 kDa molecular cut off, 0.5 mm outer diameter; length 30 mm) were placed under ultrasound guidance from the medial side as described previously (Chapter 12). The dialysis catheters were perfused, via a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden), at a rate of 1 µl/min with a Ringer acetate solution containing 3 mM glucose, 1 mM lactate and 0.1 g/ml of Dextran (71 Kda; D-1537, Sigma Chemical, St. Louis, USA). The in-vivo recovery of glucose and glycerol was determined by the internal reference method (181) by adding 11 nM D-[3-^{3}H]-glucose (specific activity: 6475 GBq/mmol; NEN, Boston, USA) and 5 nM [^{14}C(U)]-glycerol (specific activity: 7400 GBq/mmol; NEN, Boston, USA) to the perfusion solution. The recovery of [^{14}C(U)]-glycerol was used for calculation of interstitial lactate concentration, as glycerol and lactate are expected to have identical recovery due to equal size and molecular weight (191). Microdialysis was performed in both legs with simultaneous determination of blood flow by ^{133}Xe

washout in the same area, and blood sampling from the radial artery. Arterial blood samples were drawn every 5 min at both rest and during exercise.

Glucose, lactate and glycerol concentrations in the arterial plasma were determined by a Monarch Plus 750 (Instrumentation Laboratory, Lexington, USA). A CMA600 Microdialysis Analyser (CMA/Microdialysis AB, Solna, Sweden), determined the corresponding concentrations of glucose, lactate and glycerol in the dialysates.

13.3 Results

The relative recovery (RR) of glucose and glycerol was found to be significantly higher during exercise compared to rest ($p < 0.05$)(Table 8). For both substances recovery was found to return to values not significant different from resting values. Blood flow in the peritendinous area was found to increase 2-3 fold with exercise ($p < 0.05$) and returned to basal level in the recovery period.

The arterial plasma glucose concentrations (C_a) were found to decrease significantly during exercise ($p < 0.05$) and to be restored during the recovery phase (Table 9), whereas the interstitial glucose concentration (C_i) was maintained during exercise but decreased significantly in the recovery period ($p < 0.05$) (Table 9).

	Glucose	**Glycerol**
Rest	48% ± 5%	55% ± 5%
Exercise	70% ± 4% *	76% ± 5% *
Recovery	56% ± 5%	62% ± 6%

Table 8. Glucose and glycerol recovery determined by internal reference calibration. Values are mean ± SEM.
* indicates significant difference between rest and exercise.

		Rest	**Exercise**	**Recovery**
Glucose	C_i [mmol/l]	4.1 ± 0.5	3.8 ± 0.6	3.4 ± 0.5 **
	C_a [mmol/l]	5.2 ± 0.1	4.6 ± 0.2 *	5.0 ± 0.1
Lactate	C_i [mmol/l]	1.8 ± 0.2	1.9 ± 0.3	2.3 ± 0.3
	C_a [mmol/l]	0.6 ± 0.0	0.7 ± 0.1	0.6 ± 0.1
Glycerol	C_i [µmol/l]	171.9 ± 31.0	170.5 ± 16.9	187.9 ± 28.0
	C_a [µmol/l]	61.8 ± 8.8	77.5 ± 12.7 *	87.0 ± 7.5 **

Table 9. Interstitial (C_i) and plasma (C_a) concentrations of glucose, lactate and glycerol. * indicates significant difference between rest and exercise, and ** indicates significant difference between recovery and rest values ($p < 0.05$).

13.3.1 Glucose

The decrease in glucose C_a during exercise and in C_i during recovery did not result in any significant change in calculated glucose net uptake, which was found to be unchanged during exercise compared with resting values (Figure 20).

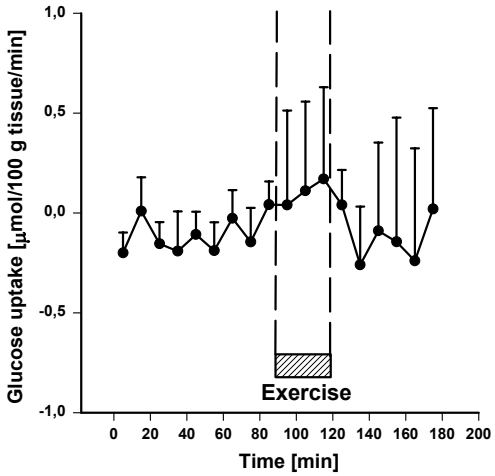


Figure 20. Tissue uptake of glucose in the peritendinous space of the human Achilles tendon during rest, intermittent exercise and subsequent recovery. Concentration were calculated for periods of 10 minutes, using mean blood concentrations determined with 5 minutes interval in the radial artery and interstitial concentrations measured by microdialysis every 10 minutes. No changes in tissue glucose uptake could be detected during the 30 minutes of intermittent static exercise with m. triceps surae.

13.3.2 Lactate

Both arterial plasma lactate concentration (C_a) and interstitial lactate concentration (C_i) were found to be stable throughout the whole experiment, with no significant changes during exercise (Table 9). The calculated net release of lactate from the tissue ventrally to the Achilles tendon increased significantly as a result of exercise ($p < 0.05$), and the net release continued to be significantly increased during recovery compared to the concentration determined prior to exercise ($p < 0.05$)(Figure 21).

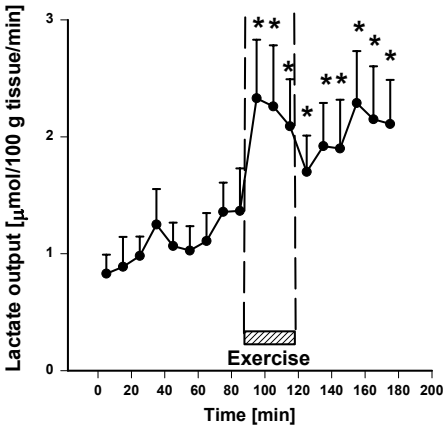


Figure 21. Tissue output of lactate from the peritendinous space of the human Achilles tendon during rest, intermittent exercise and subsequent recovery. Concentration were calculated for periods of 10 minutes, as for glucose. * indicate concentrations significant different from resting values ($p < 0.05$).

13.3.3 Glycerol

The arterial plasma glycerol concentration (C_a) was found to increase significantly during exercise (Table 9), and the concentration remained significantly elevated compared to resting level during recovery ($p < 0.05$)(Table 9). Interstitial glycerol concentration (C_i) was stable throughout the whole experiment (Table 9).

The calculated glycerol net release from the tissue increased significantly during exercise ($p < 0.05$)(Figure 22), and returned to resting level during recovery.

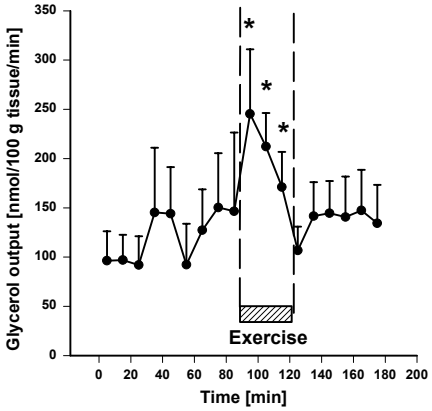


Figure 22. Tissue output of glycerol from the peritendinous space of the human Achilles tendon during rest, intermittent exercise and subsequent recovery. Concentration were calculated for periods of 10 minutes as for glucose and lactate.

* indicate concentrations significant different from resting values ($p < 0.05$).

13.4 Discussion

The microdialysis technique was used to determine interstitial concentrations of glycerol, glucose, and lactate as well as to calculate tissue substrate balance in the peritendinous region of the human Achilles tendon. Recovery of 48 - 62 % (range) at rest and 70 – 76 % during exercise were obtained for glycerol and glucose (Table 8), not significantly different from the recovery determined for PGE_2 during the previous study. The increase in recovery during exercise emphasises the importance of determine recovery before calculating interstitial concentrations. The explanation for this increase in recovery during exercise is not know but factors such as stirring of the interstitium due to pressure changes during exercise, possibly reducing diffusion barrier, as well as movement of the membrane within the tissue have been mentioned (164). However this remains to be elucidated.

The major finding of the present study is the demonstration of an increased net release of lactate and glycerol from the tissue during 30 min of intermittent static exercise. In addition to these metabolic changes blood flow was found to increase 3 fold in the peritendinous region. Histological examination of the peritendinous area indicates dominance of connective tissue together with adipose tissue (28;90;91). In support of this, the interstitial glycerol concentration at rest in the peritendinous space was 70 % of the values obtained in abdominal periumbilical subcutaneous adipose tissue (67;77;192), and 200 % of the values measured in the medial gastrocnemius muscle (67), and a significant tissue release of glycerol was demonstrated (Figure 22). Furthermore, during the first 10 min of exercise, glycerol release was increased indicating lipolytic activity during intermittent static leg calf muscle exercise, and was found to be of a similar magnitude as earlier demonstrated in adipose tissue during bicycling exercise (10;71;165;223). However the release of glycerol was found to decrease after the first initial increase in spite of maintenance

of the workload throughout the entire exercising period. This decrease in lipolytic activity over the exercising period could indicate an initial stimulation followed by a subsequent down regulation of local lipolysis during prolonged exercise and supports the view that the role of lipid in the peritendinous space does not contribute largely to the overall metabolic activity.

Interstitial glucose concentration in the peritendinous area was at rest lower compared with arterial values (Table 9). Despite this no tissue uptake could be demonstrated. The difference between interstitial and arterial values was in accordance with findings in skeletal muscle (142) and adipose tissue (192). During exercise no change in interstitial concentration of glucose was demonstrated despite a drop in plasma glucose (Table 9). This indicates that the peritendinous area does not contribute quantitatively to glucose disposal and that the major cause for the decline in plasma glucose is an increased glucose uptake in the contracting skeletal muscle, in the present case m. triceps surae (169).

For lactate the interstitial concentration was found to be higher than the corresponding arterial concentration, and lactate was released from the tissue during rest. Studies of microdialysis performed in the subcutaneous adipose tissue on the calf (185) and in the gastrocnemius muscle (67) support the present findings. Exercise resulted in increased lactate release from the peritendinous area (Figure 21) indicating increased anaerobic glycolytic metabolism despite a three-fold increase in blood flow. The fact that no increase in arterial lactate concentration could be determined indicates that overall lactate production was modest. Alternatively the exercise induced increase in lactate clearance of both the liver and muscles could be sufficient to maintain arterial lactate concentration at basal level (130;196). This is a very likely explanation since the decreasing glucose concentration will stimulate gluconeogenesis.

This study indicates that both lipid and carbohydrate metabolism is accelerated in the peritendinous region of the human Achilles tendon with dynamic loading.

In support of the present conclusion of the peritendinous tissue being metabolic active during exercise recent data have shown that the oxygen consumption determined by Near Infrared Spectroscopy (NIRS) within the AT is accelerated with dynamic loading of the calf muscle (Boushel *et al*, unpublished data).

14 INSERTION TRAUMA

14.1 Introduction

Despite the increasing use of microdialysis as a method for determination of interstitial concentrations of various substances only very few studies has dealt with the fact that the insertion of the microdialysis probe with a cannula most presumably produces a trauma (18;44;137;157;217). In the present study the inflammatory response to the insertion of the microdialysis probes was determined by measuring interstitial concentration of prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2) immediately after insertion of the probes and every 30 min during the following 4.5 hours.

14.2 Subjects and methods

The study was approved by The Ethical Committee of Copenhagen (KF). None of the subjects had any injuries, took any medication and all were non-smokers.

Sex	2w/2m
Age	29 years (range, 25-36)
Body weight	75 kg (range, 69-81)
Training status hours/week	4

Table 10. Subject data on sex, age, weight and training status (including all exercise performed) given as means.

The subjects were told not to perform any kind of exercise 24 hours prior to the experiment, except for ordinary daily working activities (students or sedentary office jobs). All experiments were started at 09.00 h. During the experiment the subjects rested for 4.5 hours in supine position. One microdialysis catheters (CMA 60; CMA/Microdialysis AB; 20 kDa molecular cut off, 0.5 mm outer diameter; length 30 mm) was positioned on each side in the peritendinous area just ventral to the AT. The dialysis catheters were perfused, via a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden), at a rate of 1 μl/min with a Ringer acetate solution added 0.1 g/ml of Dextran (71 Kda; D-1537, Sigma Chemical, St. Louis, USA), and 5 nM [15-^{3}H(N)]-prostaglandin E_2 (specific activity: 3.7 GBq/mmol; NEN, Boston, USA) for recovery determination. PGE_2 and TXB_2 were analysed using the same radioimmuno-assay kit as previously described (Chapter 12).

14.3 Results

The interstitial concentrations (C_i) of PGE_2 and TXB_2 were calculated using the internal reference calibration method (181). The interstitial concentration of both PGE2 and TXB2 was found to decline over time and reaching a stable level after 120 min (Figure 23).

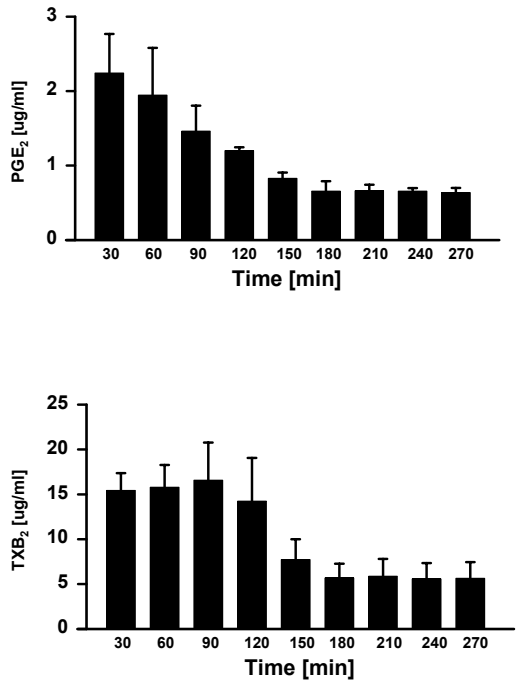


Figure 23. The tissue reaction upon insertion of the microdialysis catheter measured as interstitial concentration of prostaglandin E_2 (PGE_2)(upper panel) and thromboxane B_2 (TXB_2)(lower panel), in four subjects resting for 4.5 hours after insertion of a microdialysis just ventral to the Achilles tendon. The interstitial concentration of PGE_2 and TXB_2 was determined in 30-minutes periods with the first period starting immediately after insertion of the catheter.

14.4 Discussion

The results in the present study suggest that the insertion of the microdialysis catheters result in an immediate release of inflammatory mediators (PGE_2 and TXB_2), but that the response decreases over time and after two hours have returned to a "normal" constant level. This indicates that the reaction to the insertion trauma in the peritendinous area is short lasting, and based on the present data it is suggest that no sampling is done during the first 1½ to 2 hours after insertion of the microdialysis catheters. However one must assume that the reaction on the insertion vary from tissue and substance of interest.

15 PERITENDINOUS VS. TENDINOUS MEASUREMENTS

15.1 Introduction

Metabolism in the peritendinous area was determined by microdialysis to be accelerated with exercise. Whether this reflects a change in metabolism within the tendon itself is however not known. To test the relationship between concentrations of glucose and lactate around and within the tendon, microdialysis was performed in both areas simultaneously.

As microdialysis never previously has been performed within the tendon the experiment initally was performed done in anaesthetised resting pigs.

15.2 Subjects and methods

Eleven pigs (Landrace) were included in this study. The pigs participated in various scientific projects (eye-surgery and transplantation, minor gut-surgery, etc.) before entering this experiment, and were as such part of various protocols approved by the Ethical committee.

Sex	Age	Body weight
11w	12 weeks	26 kg (range, 16 - 37)

Table 11. Data on sex, age and weight of the pigs used.

15.3 Anaestaesia.

Due to the pigs participating in various studies before entering the present study and thus they were all anaesthetised before this experiment. Depending on the type of the previous study different anaestasia procedures were used. All the pigs were preanaesthetised with Midazolam 15 mg, before anaesthetised with 250 mg Ketalar and 250 mg Tiomebumal i.v. If eye-operated the pigs were continuously inhaling a mixture of 1 l O_2, 2 l NO_2 and 1 % Halothan. If gastrointestinal operated they were also given 1 g Chloralose and no Halothan. After the sampling the pigs were terminated with a Caliumchlorid or Pentobarbital injection.

15.4 Experimental protocol

During the experiment the pigs rested prone with the feet taped to the couch in a position were the Achilles tendon was stretched and exposed. On each side one microdialysis catheter was placed from the lateral side in the peritendinous space 5 mm ventral to the Achilles tendon with the active part of the membrane covering the area from 25 to 10 mm proximal to the Achilles tendon insertion on the calcaneus bone. The other microdialysis catheter was placed within the Achilles tendon. The positioning of the catheters was after the experiment checked by dissection. The microdialysis catheters were custom-made from single plasmaphoresis hollow fibres (0.4 mm in diameter, molecular weight cut-off 5 kDa; Alwall, GFE 11, Gambro Dialysatoren, Hechingen, Germany) glued to a gas-tight nylon inlet and outlet tubing (Portex Autoclavable Nylon Tubing, Portex Limited, Smiths Industries, Kent, England) and with an suture thread glued to the

membrane to improve the mechanical stability of the fibre. The fibres had a membrane of 15 mm available for diffusion. The catheters were perfused, via a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden), at a rate of 2 μl/min with a Ringer acetate solution containing 3 mM glucose and 1 mM lactate. A perfusion rate of 2 μl/min was chosen to increase the dialysate volume enabling determination of glucose and lactate concentrations using an YSI 2300 glucose/lactate analyzer (YSI Incorporated, Yellow Springs, Ohio, USA). The in-vivo recovery of glucose and lactate was determined by the internal reference method (181) by adding 11 nM D-[3-^{3}H]-glucose (specific activity: 6475 GBq/mmol; NEN, Boston, USA) and 5 nM [^{14}C(U)]-glycerol (specific activity: 7400 GBq/mmol; NEN, Boston, USA) to the perfusion solution. The recovery of [^{14}C(U)]-glycerol was used for calculation of interstitial lactate concentration, as glycerol and lactate are expected to have identical recovery due to equal size and molecular weight (191). Sampling of the dialysate was done every 20 min and begun 20 min after positioning of the microdialysis in order to reduce the risk of the insertion trauma influencing the results. The samples were immediately frozen to -70° C until analyses were done. The concentrations of glucose, lactate and glycerol in the dialysates were determined by an YSI 2300 glucose/lactate analyzer (YSI Incorporated, Yellow Springs, Ohio, USA).

15.5 Results

Microdialysis was performed in 11 anaesthetised pigs. The concentration of glucose was found to vary between 1.2–13.5 mmol and in the case of lactate between 0.7–12.0 mmol. The broad ranges of glucose and lactate concentration indicate that the procedures preceding the present study generated large variation in stress response.

15.5.1 Lactate

The relationship between the concentration of lactate in the peritendinous area and within the tendon was found to be significant ($r = 0.88$, $p < 0.05$) and with no significant difference in lactate concentration between the two regions (peritendon/tendon ratio: 1.04 ± 0.06, $p > 0.05$)(Figure 24).

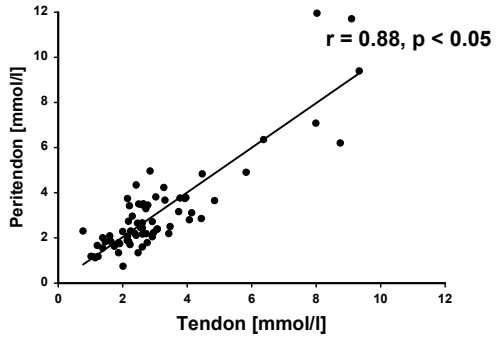


Figure 24. Relationship between concentration of lactate measured within the tendon and in the peritendinous area.

15.5.2 Glucose

For glucose the relationsship was significant, but however not very strong ($r = 0.70$, $p < 0.05$), and with a systematic lower concentration in the peritendinous area (peritendon/tendon ratio: 0.81 ± 0.04, $p < 0.05$)(Figure 25).

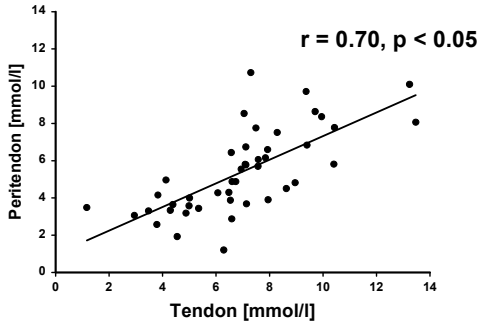


Figure 25. Relationship between concentration of glucose measured within the tendon and in the peritendinous area.

15.6 Discussion

In the present study the relative relationships between the concentrations of glucose and lactate within and around the AT, respectively, were determined.

The data show a relative strong relationship between the lactate concentration measured within and around the AT as 77 % of the variation in the lactate concentration within the AT can be explained by variation in peritendinous concentration ($r^2 = 0,77$)(65). This indicates that at least in the resting situation determination of interstitial concentration of lactate by microdialysis shows parallel results in the tendon and peritendon. However, given the variability it cannot be recommended in individual experiments to extrapolate from one region to the other, but only one calculated mean values in a relative large group. No significant difference in concentration was found between the two regions (peritendon/tendon ratio: 1.04 ± 0.06). In contrast no strong relationship was found for glucose concentration ($r^2 = 0,49$), thus making it difficult to estimate tendon concentration of glucose from measurements made in the peritendinous area (peritendon/tendon ratio: 0.81 ± 0.04).

The difference in the glucose concentration around and within the tendon could be a result of a difference in metabolism, and one explanation could be that the peritendinous area is known to contain adipose tissue, and as such has a higher potential uptake of glucose compared to the AT.

Although the present data represents a broad range of glucose and lactate concentrations (1.2–13.5 mM and 0.7–12.0 mM, respectively) the present model does

not allow for determination of relationships during exercise and thus metabolism of the AT during loading of the calf muscle was not investigated in the present study. One possibility for direct measurement of tendon concentrations of various substances could eventually be to use the micro-biopsy technique where small samples of tissue is taken out from the tendon and analysed (139-141). This technique however lacks the opportunity of the microdialysis technique with continuous measurement over time and during interventions. Alternatively unpublished data by Alfredson *et al.* (personal communication) suggest that within the near future it will become possible to perform microdialysis within the human AT, thus enabling direct measurements of the tendon metabolism. Such developments of the microdialysis method could have great potentials in the future, but due the dense structure of the tendon tissue diffusion of substances within the tendons could be restricted and as such limit the use and require a lot of modifications.

16 CONCLUSION AND FUTURE STUDIES

In the present thesis, methods for and data on the blood supply of, the inflammatory response in and the metabolism of the human Achilles tendon during rest and exercise are provided.

16.1 Blood flow

With the Xenon washout method it was shown that blood flow increases from rest to exercise, and that the increase is dependent on the type of exercise. As expected, the increase in blood flow during intermittent static exercise (Figure 8) was less pronounced (3 fold) compared to dynamic exercise (heel-lift: 4 fold; standardised dynamic ex: 10 fold)(Figure 26).

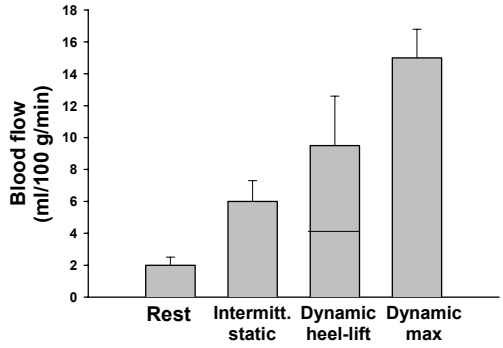


Figure 26. Blood flow in the peritendinous area measured with the Xenon washout method during rest and various types of exercises. During the experiment with dynamic heel-lift blood flow was determined 20 mm and 50 mm proximal to the AT insertion. The horizontal line within the column of dynamic heel-lift indicates the blood flow 20 mm proximal to the insertion and the full column being the flow 50 proximal to the insertion.

In contrast to anatomical findings (27;62;110;182) the rise in blood flow was less pronounced close to the insertion of the AT (20 mm: 2 fold) compared to a somewhat higher increase at a more proximal location (50 mm: 4 fold)(Figure 26). These data argues against the notion of the area 40–60 mm proximal to the insertion of the AT having a relative restricted blood supply (27;62;110;182), and illustrate that physiological measurements disagree with predictions made on the basis on anatomical observations. However it is from the present data not possible to conclude on whether the increase in blood flow to the tendon during exercise is sufficient, but when comparing the blood flow in the adipose tissue around the AT with blood flow in adipose tissues in other regions, it appears that the blood flow during rest is quite similar and that the increase in blood flow is quantitatively identical (Figure 27).

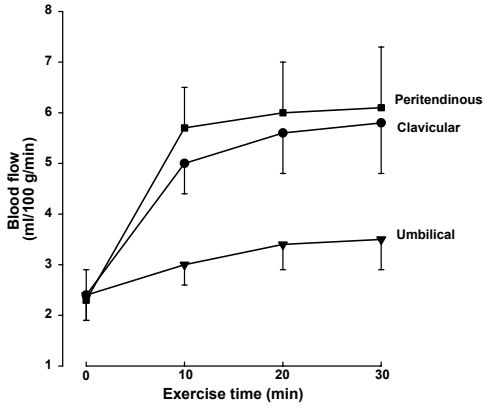


Figure 27. Blood flow during rest and exercise in the peritendinous area of the

human AT compared with blood flow in the umbilical and clavicular adipose tissue (Stallknecht and Langberg, unpublished data).

With the methods and experimental set-ups developed during the present thesis it will in the future be possible to investigate whether blood flow is perturbed in people with acute as well as chronic AT inflammation.

16.2 Tissue pressure

The finding in the present thesis of a very low negative tissue pressure in the peritendinous area during exercise could be involved in and maybe essential for the increase in blood flow during exercise. During dynamic exercise pressure changes could be involved in the metabolism of the tendon tissue and the transport of substances from the tissue to the lymph system. The role of the negative tissue pressure on blood flow could be further investigated by applying a external negative pressure and measure blood flow in the region. To elucidate whether pressure changes in general appears around tendons during exercise simular experiments as the one performed in the present thesis could be performed on the patella tendon and the supraspinatus tendon.

The role of this low negative pressure is however not know, as well as it is not know whether the ability to generate tissue pressure changes is changed with age, inflammation or immobilisation of the peritendinous area.

16.3 Metabolism

The metabolism of the peritendinous tissue was determined by the use of the microdialysis technique. With exercise, the net release of lactate as well as of glycerol from the peritendinous space of the Achilles tendon increased two folds ($p < 0.05$). This indicates that both lipid and carbohydrate metabolism is accelerated in the peritendinous region of the human Achilles tendon with loading of the calf muscle. The increase in release of glycerol from the peritendinous tissue found during the first 10 min of exercise corresponds with releases from other types of adipose tissues in the body (Figure 28).

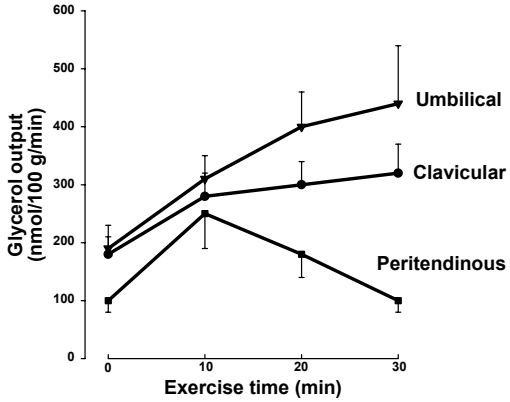


Figure 28. The output of glycerol from the peritendinous area compared to outputs from the umbilical and clavicular adipose tissue (Stallknecht and Langberg, unpublished data).

However after the first initial increase the release of glycerol was found to decrease in spite of maintenance of the workload throughout the entire exercising period. This decrease in lipolytic activity over the exercising period could indicate an initial

stimulation followed by a subsequent local down regulation of lipolysis during continued exercise and support the view that the role of lipid in the peritendinous space is not a substrate store in contrast to the umbilical and clavicular adipose tissue. In the future the microdialysis technique can be used to elucidate whether metabolism of the tendon is changed in response to repeated or prolonged exercise, resulting in a overall breakdown of the adipose tissue in the peritendinous region probably important for the support and function of the tendon and thus loss of this adipose tissue would result in a higher risk of injury.

16.4 Inflammatory mediators

With the use of the microdialysis technique it was as well demonstrated that inflammation mediators are released from the tissue following the insertion of the microdialysis catheters. The concentration of inflammatory mediators declined over time and reached a constant (basal) level after 120 min. During exercise the level of PGE_2 and TXB_2 was not significant changed, but as peritendinous blood flow was enhanced by 2-3 fold during contraction of the calf muscle the release of these substances from the tissue during exercise is properly increased.

The role of prostaglandins in the peritendinous area is however not known. Prostaglandins are known to be involved in the inflammatory response after trauma, as well as being responsible for local increases in blood flow and stimulation of bone metabolism (50;51;100-102;210). With the use of NSAID pilotstudies have indicated that it is possible to block the release of prostaglandins and thus investigate the role of prostaglandins on local blood flow stimulation and collagen turnover. As NSAID is often first choice in the treatment of AT disorders the possible benefits might be linked to such mechanism as reduction of the hyperaemia known to accompany inflammation of the AT (13;16). With the microdialysis technique it is possible to introduce molecules or substances (e.g. NSAID) into the tissue and measure the direct effect of the compound on the tissue.

It also remains to be elucidated whether the concentration of inflammatory mediators is increased in the peritendinous area during acute and chronic inflammation of the AT, and as such could be expected to account for the symptoms experienced during AT overuse. During chronic inflammation substances such as bradykinin could be involved in the generation of pain and hyperaemia (68;69;198;203), and with a size of 1.5 kDa bradykinin could easily be measured by microdialysis.

16.5 Collagen metabolism

During the last decade, several studies have been performed looking at collagen synthesis and breakdown in the human body in response to immobilisation and exercise (11;47;81;104;163;179;204;209;220). Due

to the lack of methods, collagen turnover have however only been measured in the blood making assumptions on collagen turnover within specific tissues impossible. Pilot studies using microdialysis probes with membranes having very high molecular cut-off values have showed that it is possible to measure local collagen metabolism by determining local concentrations of indicator molecules of collagen turnover. Such methods will have tremendous perspectives in the future as they will allow for investigation of the specific mechanisms by which tendons detect and convert mechanical loading into physical properties, as well as to interpret which growth factors are being involved in the adaptation of tendons to changes in loading. Hopefully it will by the use of such methods be possible in the future to determine and treat tendon overuse injuries by introducing relevant concentrations of growth factors in the tissue, thus enhancing the adaptive response by increasing the rate of collagen synthesis.

17 SUMMARY – ENGLISH

Overuse injury of connective tissue – especially in and around tendons – in relation to physical loading (work or leisure) represents a major problem in terms of aetiology and treatment. Although the incidence of overuse injuries especially related to the Achilles tendon, the patella tendon and the rotator cuff in the shoulder is high, the knowledge regarding structural, vascular and metabolic changes in overused or injured human tendons is limited.

Microtrauma, inflammation and reduced vascularisation have been suggested to be crucial for the development of overuse injuries, but studies confirming these hypotheses are lacking.

In the present Ph.D. thesis methods for investigation of blood flow, metabolism and inflammation of the peritendinous tissue ventral to the human Achilles tendon during rest and exercise have been established. By the use of the developed methods it is shown that blood flow measured by ^{133}Xenon washout technique in the peritendinous area of the human Achilles tendon is significantly increased during exercise of the calf muscle (10 fold, exercise vs. rest), and that blood flow is enhanced during dynamic as well intermittent static exercise calf muscle contraction. From these studies it was also demonstrated, that the increased Xenon washout was due to a rise in blood flow, rather than reflecting changes in lymph drainage of the region. In addition a developed dynamic ergometer model was used and blood flow in the peritendinous area was found to rise 10 fold with intense exercise compared to values obtained at rest.

Somewhat surprisingly, it was demonstrated that the pressure in the peritendinous region decreased markedly during exercise and that negative pressures up to 150 mmHg were demonstrated during static contraction to the calf muscle. The role of this negative tissue pressure is however not known.

With the use of the microdialysis technique, peritendinous tissue metabolism has been investigated, and increases of both lipolysis and glycolysis during mechanical loading has been demonstrated. It was observed that the activation of lipolysis was only transiently elevated during continuous exercise. This indicates that although a more general stimulation of adipose tissue in the body is brought forth with exercise, other factors may down regulated lipolysis locally.

Likewise using the microdialysis technique the concentration of inflammatory mediators (PGE_2 and TXB_2) in the peritendinous area during rest and exercise was determined. No increase in interstitial concentration of neither PGE_2 nor TXB_2 could be demonstrated in response to exercise, but taking into account that blood flow in the tissue increased 3 fold during

exercise, it is likely that inflammatory mediators are released from the tissue in response to loading of the calf muscle.

In the present thesis new models and methods (static and dynamic ergometers, microdialysis) have been developed allowing for in-vivo determination of tissue concentrations, release rates of inflammatory mediators and metabolites and blood flow. In the future these methods will allow for investigation of basic physiological questions as well as addressing clinically related problems.

18 RESUME – DANSK

Skader på bindevævsstrukturer, som følge af belastning ved repetitive bevægelser i forbindelse med idræt, udgør et stort problem af såvel ætiologisk, behandlings- og forebyggelsesmæssig karakter. Det anslås, at op mod 50 % af alle idrætsskader skyldes overbelastning af bindevæv, som eksempelvis senestrukturer.

Overbelastningsskader i achillessenen vides at være associeret til en kraftig øgning i træningsmængde samt til tilstedeværelsen af anatomiske fejlstillinger i underekstremiteten. Imidlertid savnes der viden om de strukturelle og metaboliske ændringer, der optræder i væv, der udsættes for overbelastning. Man formoder, at mikrotraumer, inflammation og ændret vaskularisering er af afgørende betydning for udviklingen af overbelastningsskader, men der mangler studier, der bekræfter dette.

I forbindelse med nærværende ph.d. afhandling er der etableret metoder til studier af blodgennemstrømning, metabolisme og inflammatorisk aktivitet i det peritendinøse achillessenevæv hos mennesker under og efter fysisk aktivitet. Ved anvendelse af de udviklede og beskrevne metoder har det været muligt at påvise at blodgennemstrømningen omkring achillessenen målt ved hjælp af ^{133}Xenon udvaskning stiger betydeligt under muskelarbejde (10 fold arbejds- vs. hvileværdier), og at denne stigning i blodgennemstrømningen sker ved såvel intermittent statisk som dynamisk lægmuskel arbejde. Ved hjælp af et udviklet fodledsergometer har der kunnet påvises en positiv korrelation mellem arbejdsintensitet og blodgennemstrømning i det peritendinøse væv.

Noget overraskende kunne der påvises et betydeligt undertryk i vævet foran achillessenen i forbindelse med statisk kontraktion af lægmusklen. Betydningen af dette undertryk kendes imidlertid ikke.

Under anvendelse af mikrodialyse-teknikken har det endvidere været mulig at påvise en vævsfrigørelse af såvel glycerol som laktat fra det peritendinøse achillesenevæv i forbindelse med kontraktion af lægmuskulaturen, ligesom en øget frisætning af den inflammatoriske mediator og potente vasodilator prostaglandin E_2 blevet demonstreret.

Der er således etableret modeller til belysning af ændrede forhold ved overbelastningsskader, ligesom de udviklede teknikker i fremtiden vil tillade studier af såvel basalfysiologiske som kliniske former for intervention.

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