



## Detection of Bacterial DNA and Collagen Metabolism in Acutely Ruptured Achilles Tendons

**CLINICALTRIALS.GOV IDENTIFIER**  
NCT03931486

**RECRUITMENT STATUS**  
RECRUITING

**FIRST POSTED**  
APRIL 30, 2019

**LAST UPDATE POSTED**  
JULY 14, 2020

### STUDY DESCRIPTION

#### Brief Summary

The etiology and pathogenesis of acute Achilles tendon ruptures are complex and not fully understood. It is well known that they are associated with pre-existing pathological alterations, similar to the changes observed in tendinopathy. The present study investigates if bacteria and collagen metabolism play a role in the etiology of acute Achilles tendon rupture. During surgery, 20 patients will have taken two biopsies from the ruptured part of the tendon and two biopsies from the healthy tissue of the same tendon 2-4 cm proximal to the rupture, as a control.

**Condition or Disease:** Achilles Tendon Rupture

**Intervention/treatment:**

**Phase:** N/A

**DETAILED DESCRIPTION**

## Objectives

1. To investigate the collagen turnover in Achilles tendons prior to a rupture.
2. To investigate the relative collagen turnover in Achilles tendons the days immediately after a rupture.
3. To investigate the acute collagen protein synthesis rate in acutely ruptured Achilles tendons.
4. To investigate if bacterial DNA is present in samples from the degenerative tissue in acutely ruptured Achilles tendons compared with samples from the healthy tissue of the ruptured tendon.

## Background

Tendon ruptures are severe injuries that potentially lead to reduced function, reduced activity and sick leave. In younger, sports active individuals with strong tendons, an acute traumatic event is usually needed to trigger a rupture. With age the tendons are exposed to degenerative changes and can therefore rupture in everyday activities (spontaneously). The etiology and pathogenesis of spontaneous tendon ruptures are complex and not fully understood. It is well known that they are associated with pre-existing pathological alterations, similar to the changes observed in tendinopathy. Recently, Dakin et al. found that ruptured Achilles tendons show evidence of chronic (non-resolving) inflammation. The condition might be a result of collagenolytic injury followed by failed tendon healing. Yet, other theories are still highly debated.

Many predisposing factors have been proposed to contribute to the pathological alterations of tendons. Studies found that factors such as increasing age, dominant arm, history of trauma and specific acromial anatomy are associated with rotator cuff tear. It is suggested that spontaneous tendon ruptures, primary Achilles tendon, are related to systemic and injectable steroids, fluoroquinolone use and some rheumatic diseases. Furthermore, studies have shown possible associations between genetic factors and tendon injury. The mentioned factors may influence the initiation and development of the pathological alterations that weaken the tendons. However, it is certain there are other mechanisms and predisposing factors that need to be investigated.

Heinemeier et al. found that the bulk of the collagen matrix of healthy human Achilles tendon core is an essentially permanent structure that is laid down during height growth and has limited turnover in adults. The findings were based on the <sup>14</sup>C bomb pulse method, and unpublished data from the same re-search group (using this method) indicates that an abnormally high rate of collagen turnover precedes symptoms of tendon overuse (tendinopathy). It is of great interest to investigate if increased collagen turnover predisposes to tendon rupture.

Recently, Rolf et al. demonstrated the presence of bacterial DNA in 25% of samples from ruptured Achilles tendons. Polymerase chain reaction (PCR) was used to identify the highly conserved bacterial 16S rDNA gene in the tendons. This finding opens the question, if bacterial depositions play a role in the etiology of spontaneous tendon ruptures. In order to prevent tendon ruptures it is essential to improve our understanding of the etiology. A better understanding may also lead to development of new therapeutic options.

In this cross-sectional study, we aim to investigate if bacteria potentially are contributing to the etiology of acute Achilles tendon rupture. Additionally, we also investigate if tendon ruptures are preceded by an increased collagen turnover.

## Design of the study

The study is conducted as a cross-sectional study. Patients with acute rupture of the Achilles tendon are included. The study aims to include 20 patients with acute Achilles tendon rupture.

The procedure for the patient:

1. At the day of inclusion, the patients will have taken a blood sample and ingest 150ml of deuterium oxide (D<sub>2</sub>O).
2. At the day of the surgery, the patient will meet 3 hours before the beginning of the surgery. A bolus of stable isotope (<sup>15</sup>N marked proline tracer) will be injected and afterwards continuously infused through the antecubital vein. Blood samples are taken before infusion starts, 30min after, 60min after, 120min after and when the biopsies are taken.
3. During the surgery, the patients will have taken:
  1. Two biopsies from the stump end of the ruptured tendon or ligament.
  2. Two control biopsies 2-4 cm proximal to the stump end of the ruptured Achilles tendon.

## Procedure for biopsies

All biopsies are taken by trained orthopedic surgeons. Every biopsy is taken during surgery in an operating theatre. Before the beginning of the surgery a sterile table is prepared containing three packs of sterile scalpels and forceps, and 8 containers for biopsies with the patient's social security (CPR), the type of analysis and a continuous number individually describing each biopsy.

The 8 containers will hold:

1. Biopsy from the stump end of the ruptured tendon for 16S rDNA analysis.
2. Biopsy from the stump end of the ruptured tendon for <sup>14</sup>C bomb pulse method
3. Biopsy from the stump end of the ruptured tendon for isotope analysis (deuterium oxide and <sup>15</sup>N marked proline).
4. Biopsy from the stump end of the ruptured tendon for mRNA analysis.
5. Biopsy from the ruptured tendon 2-4 cm proximal to the stump end for 16S rDNA analysis.
6. Biopsy from the ruptured tendon 2-4 cm proximal to the stump end for <sup>14</sup>C bomb pulse.
7. Biopsy from the ruptured tendon 2-4 cm proximal to the stump end for isotope analysis (deuterium oxide and <sup>15</sup>N marked proline).
8. Biopsy from the ruptured tendon 2-4 cm proximal to the stump end for mRNA analysis

Biopsies from ruptured tendons

Achilles tendon:

The biopsies are taken during open surgery. To prevent contamination of the biopsies the following procedure is followed:

1. Surgical rub and draping according to normal guidelines.
2. Skin incision and dissection according to normal guidelines.
3. As the ruptured tendon is identified, a new pack containing a sterile scalpel and a forceps is opened. The instruments are only used for taking the biopsy and does not get in contact with the surrounding tissue.
4. 2 biopsies, 10mm long, 1mm broad and 1mm deep, are taken from the degenerated tendon tissue at the rupture site.
5. The first biopsy is placed in a container (for bacterial detection). On a sterile board, the second biopsy is divided in three and placed in three different containers (one for <sup>14</sup>C bomb pulse, one for isotope analysis (both deuterium oxide and <sup>15</sup>N marked proline) and one for mRNA). These containers are frozen down to -80°C.
6. A second new pack containing a sterile scalpel and a forceps is opened and 2 biopsies 10 mm long, 1mm broad and 1 mm deep are taken 2-4 cm proximal to the rupture site.
7. The first biopsy is placed in a container (for bacterial detection) On a sterile board, the second biopsy is divided in three and placed in three different containers (one for <sup>14</sup>C bomb pulse, one for isotope analysis (both deuterium oxide and <sup>15</sup>N marked proline) and one for mRNA). These containers are frozen down to -80°C.

Contamination tests

PCR and sequencing - Both a negative and a positive control is included in the test. Numbers of cycles in positive samples will also be included in the interpretation. Exclusively environmental bacterial species never been related to human disease are likely to be regarded as contaminants.

Blood culture - During surgery, blood a sample is sent for culture to investigate if bacteria were presented in the blood.

Bacterial cultures - Bacterial culture tests are conducted on all patients. The investigated samples are taken with swabs from:

- Patients skin area over the surgical site.
- The blade of the used scalpel.

The swabs are collected in separate glass tubes and send to Department of Microbiology, Hvidovre Hospital. The swabs are tested on multiple culture media for multiple species.

Blinding of the analysis

For each included patient, the 2 containers with biopsies for bacterial detection will get a randomized continuous number. These numbers can, via a key document (paper form), reveal if the biopsy has been taken from the degenerative tissue or from the healthy tissue. The person responsible for the analyze of the bacterial DNA has no access to the key document.

Data registration during inclusion

- Patient ID
- Social security number
- Phone number
- Age
- Weight
- Date of examination
- Date and time of insult
- Corticosteroid injection (number and time)
- History of infection

---

## STUDY DESIGN

<b>Study Type:</b>	Observational	<b>Actual Study Start Date:</b>	May 2019
<b>Estimated Enrollment :</b>	20 participants	<b>Estimated Primary Completion Date:</b>	February 2021
<b>Intervention Model :</b>	N/A	<b>Estimated Study Completion Date:</b>	July 2022
<b>Masking:</b>	N/A		
<b>Primary Purpose:</b>	N/A		
<b>Official Title:</b>	Detection of Bacterial DNA and Collagen Metabolism in Acutely Ruptured Achilles Tendons		

## OUTCOME MEASURES

Primary Outcome Measures: 1. 14C concentration in acutely ruptured Achilles tendons [ Time Frame: The outcome measure will be assessed at time of surgery ]  
14C concentration in biopsies from the ruptured part of the tendons and in the control biopsies proximal to the rupture are compared to the known historical values of atmospheric 14C to estimate the rate of collagen turnover. The values for turnover in the biopsies will be compared to the known rates of turnover in healthy tendons.  
2. Heavy water (D2O) enrichment in acutely ruptured Achilles tendons [ Time Frame: The outcome measure will be assessed at time of surgery ]  
2H isotope enrichment in the biopsies from the ruptured part of the tendons and in the control biopsies proximal to the rupture.  
3. Fractional synthesis rate of protein at time of surgery in acutely ruptured Achilles tendons. [ Time Frame: The outcome measure will be assessed at time of surgery ]  
The enrichment of the 15N marked proline tracer measured in the biopsies from the ruptured part of the tendons and in the control biopsies and compared to the enrichment of 15N marked proline in the blood. Based on that, the fractional synthesis rate of protein at the time of surgery is calculated.  
4. Presence of bacteria in acutely ruptured Achilles tendons. [ Time Frame: The outcome measure will be assessed at time of surgery ]  
Presence of bacterial DNA in the biopsies from the ruptured part of the tendons and in the control biopsies proximal to the rupture detected by 16s rDNA PCR

Biospecimen Retention:

Samples With DNA

Biopsies from acutely ruptured Achilles tendons will be taken, Two biopsies from the ruptured part of the tendon and two biopsies from the healthy tissue of the same tendon proximal to the rupture, as a control.

---

## ELIGIBILITY CRITERIA

**Ages Eligible for Study:** 18 to 70 Years (Adult, Older Adult)

**Sexes Eligible for Study:** All

**Accepts Healthy Volunteers:** No

### Criteria

Inclusion Criteria:

- Age 18-70 years
- Appointment in the Outpatients Department within 4 days after injury.
- Total Acute Achilles tendon rupture
- The patient must be able to speak and understand Danish.
- The patient must be able to give informed consent.

Exclusion Criteria:

- Rupture of the Achilles tendon either at the insertion on the calcaneus or at musculotendinous junction of the triceps surae.
- Previous rupture of the same Achilles tendon
- Undergone any surgery in the same region as the affected Achilles tendon.
- In medical treatment of diabetes.
- Present infection in the affected region.
- Contra-indication for surgery: severe arthrosclerosis with no palpable pulse in the foot, broken skin in the Achilles region of the injured leg.
- Inability to lie in prone position on the operating table.
- Rheumatoid arthritis or any other inflammatory disorder of the joints.
- Terminal illness or severe medical illness. ASA score higher than or equal to 3.

---

## CONTACTS AND LOCATIONS

### Contacts

Contact: Allan Cramer +4560174294 [allancramer94@gmail.com](mailto:allancramer94@gmail.com)

### Locations

Denmark Department of Orthopedic Surgery, Copenhagen University Hospital, Hvidovre

Hvidovre

### Sponsors and Collaborators

Copenhagen University Hospital, Hvidovre

Bispebjerg Hospital

### Investigator

Study Chair : Kristoffer W Barfod, MD      Copenhagen University Hospital, Hvidovre

---

**MORE INFORMATION**

**Responsible Party :** Copenhagen University Hospital, Hvidovre

**ClinicalTrials.gov Identifier :** NCT03931486

**Other Study ID Numbers :** H-18010363

**First Posted :** April 30, 2019

**Last Update Posted :** July 14, 2020

**Last Verified :** July 2020

**Individual Participant Data (IPD) Sharing Statement:**

**Plan to Share IPD:** Undecided

**Studies a U.S. FDA-regulated Drug Product:** No

**Studies a U.S. FDA-regulated Device Product:** No

**Additional relevant MeSH terms :** *Rupture*