Achilles Tendon Tissue Turnover Before and Immediately After an Acute Rupture

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Background: An Achilles tendon rupture (ATR) is a frequent injury and results in the activation of tendon cells and collagen expression, but it is unknown to what extent turnover of the tendon matrix is altered before or after a rupture.

Purpose/Hypothesis: The purpose of this study was to characterize tendon tissue turnover before and immediately after an acute rupture in patients. It was hypothesized that a rupture would result in pronounced collagen synthesis in the early phase (first 2 weeks) after the injury.

Study Design: Cross-sectional study; Level of evidence, 3.

Methods: The study included patients (N = 18) eligible for surgery after an ATR. At the time of inclusion, the patients ingested deuterium oxide ($^{2}H_2O$) orally, and on the day of surgery (within 14 days of the injury), they received a 3-hour flood-primed infusion of an ^{15}N-proline tracer. During surgery, the patients had 1 biopsy specimen taken from the ruptured part of the Achilles tendon and 1 that was 3 to 5 cm proximal to the rupture as a control. The biopsy specimens were analyzed for carbon-14 (^{14}C) levels in the tissue to calculate long-term turnover (years), incorporation of ^{2}H-alanine (from $^{2}H_2O$) into the tissue to calculate the fractional synthesis rate (FSR) of proteins in the short term (days), and incorporation of ^{15}N-proline into the tissue to calculate the acute FSR (hours).

Results: Both the rupture and the control samples showed consistently lower levels of ^{14}C compared with the predicted level of ^{14}C in a healthy tendon, which indicated increased tendon turnover in a fraction (48% newly synthesized) of the Achilles tendon already for a prolonged period before the rupture. Over the first days after the rupture, the synthesis rate for collagen was relatively constant, and the average synthesis rate on the day of surgery (2-14 days after the rupture) was 0.025% per hour, irrespective of the length of time after a rupture and the site of sampling (rupture vs control). No differences were found in the FSR between the rupture and control samples in the days after the rupture.

Conclusion: Higher than normal tissue turnover in the Achilles tendon before a rupture indicated that changes in the tendon tissue preceded the injury. In addition, we observed no increase in tendon collagen tissue turnover in the first 2 weeks after an ATR. This favors the view that an increase in the formation of new tendon collagen is not an immediate phenomenon during the regeneration of ruptured tendons in patients.

Registration: NCT03931486 (ClinicalTrials.gov identifier).

Keywords: Achilles tendon; rupture; turnover; healing; etiology

An Achilles tendon rupture (ATR) is a relatively frequent injury (31-35/100,000 per year), with an increasing incidence rate in the past couple of decades.[14,24] An ATR may cause sick leave, permanent functional deficits, and marked prolonged symptoms for the majority of patients.[6,33,47] Its increasing trend and severe consequences underline the need for pathophysiological knowledge to develop treatment and prevention strategies.

The Achilles tendon is the strongest tendon in the body and can withstand heavy loads. However, it can rupture during routine movements.[32] This observation has suggested that a pathological weakness of the tendon is present before the rupture, which often occurs in combination with well-known risk factors such as male sex, genetic predisposition, treatment with fluoroquinolone, treatment with systemic corticosteroids, type 2 diabetes, and end-stage renal disease.[23,39,40,45,46] It has been previously demonstrated that a site-specific loss of larger collagen fibrils, as well as a reduced collagen amount and mechanical weakening in vitro, was present both in the core and periphery of the ATR site, and this was found despite the lack of any prerupture clinical symptoms in 90% of the

The American Journal of Sports Medicine
2023;51(9):2396–2403
DOI: 10.1177/03635465231177890

patients with an ATR.[17,29] However, a more direct mechanistic understanding of alterations in tendon collagen matrix turnover before a rupture, as well as the dynamic collagen response in the tendon tissue immediately after an ATR, has not been established.

The Achilles tendon tissue primarily consists of type I collagen ordered hierarchically in the direction of force, which provides significant strength to the Achilles tendon.[43] A previous study using the carbon-14 (^{14}C) bomb pulse method to determine life-long collagen turnover established that the collagen matrix of a healthy human Achilles tendon core is a dynamic structure during childhood and adolescence but permanent with very limited turnover in adults.[19] Further, using the same method, it was found that a fraction of the human tendon matrix in chronic tendinopathy had undergone continuous, elevated slow turnover for up to 10 to 15 years before the presentation of symptoms.[20] It is completely unknown whether a similar phenomenon is present in a human tendon with a rupture.

An acute tendon rupture induces increased cell activity as well as the upregulation of collagen expression both in animals[13,31,44] and in humans.[26] Further, it has been demonstrated that isolated primary fibroblasts derived from a healthy adult human tendon can be activated to form new tendon-like tissue in culture[2] and that even nonsurgical treatment of an ATR in adult humans can result in the regeneration and recovery of tendon function.[1] These observations suggest a marked capacity for cellular activation and matrix synthesis in the traumatized tendon, but it is unknown to what extent the matrix demonstrates immediate elevated turnover in relation to a tendon rupture in humans.

The present study aimed to describe tendon matrix turnover before and after an ATR in patients both in the immediate rupture region ("rupture") and in a neighboring macroscopically visually intact region of the same tendon ("control"). This was done by analyzing the level of ^{14}C in the tissue as a marker for long-term turnover (years), incorporation of ^{2}H-alanine (from orally administered deuterium oxide [$^{2}H_2O$]) into the tendon tissue to calculate the fractional synthesis rate (FSR) of proteins in the short term (days), and incorporation of ^{15}N-proline (intravenous infusion) into the tendon tissue to calculate the acute FSR (hours). It was hypothesized that an ATR would be preceded by increased tendon tissue turnover, which would be further increased in the early phase (first 2 weeks) after the injury.

METHODS

Patients

A total of 18 patients (6 female, 12 male; mean age, 44 ± 9 years; mean weight, 78 ± 12 kg) eligible for surgery after an ATR were included in this study from May 2019 to November 2020. The mean time from the rupture to surgery was 7 ± 3 days (range, 2-14 days). Patients with a diagnosis of an ATR in the emergency clinic at Hvidovre Hospital were referred for a consultation within 4 days after the rupture in the outpatient department. In the emergency clinic, a split plaster cast with the ankle in maximum plantarflexion was applied, and the patient was informed not to bear weight on the injured foot until the consultation in the outpatient department, where a trained physical therapist confirmed the diagnosis clinically as a part of standard care. The study was conducted parallel to an ongoing randomized controlled trial (RCT): "Detection of Bacterial DNA and Collagen Metabolism in Acutely Ruptured Achilles Tendons" (ClinicalTrials.gov: NCT03931486). Patients selected for operative treatment in the RCT and patients who declined to participate in the RCT but opted for operative treatment were eligible for the present study and were asked to participate if they met the inclusion and exclusion criteria. Inclusion criteria were patients (1) aged between 18 and 70 years, (2) seen in the outpatient clinic within 4 days after a rupture, and (3) able to speak and understand Danish. Exclusion criteria were (1) a rupture of the Achilles tendon at the insertion of the calcaneus or in the musculotendinous junction of the triceps surae, (2) a previous rupture of the same Achilles tendon, (3) previous surgery in the same region as the affected Achilles tendon, (4) clinical signs of an infection in the affected region, (5) current medical treatment for diabetes, and (6) a diagnosis of rheumatoid arthritis.

Before providing written consent, the patients were informed in written form and orally regarding the design of the study and its risks in accordance with the Declaration of Helsinki. On the basis of this information, the patients had the opportunity to decide whether they would like to participate in the study. All patients were given the

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Submitted September 22, 2022; accepted March 28, 2023.
One or more of the authors has declared the following potential conflict of interest or source of funding: This study was partly supported by the BRIDGE – Translational Excellence Programme (bridge.ku.dk) at the Faculty of Health and Medical Sciences, University of Copenhagen, funded by the Novo Nordisk Foundation (grant agreement No. NNF20SA0064340). K.W.B. has received consulting fees and project support from Enovis. AOSSM checks author disclosures against the Open Payments Database (OPD). AOSSM has not conducted an independent investigation on the OPD and disclaims any liability or responsibility relating thereto.

option to be included in analysis of ^{14}C levels. The first 11 patients were additionally given the option to ingest $^{2}H_2O$ on the day of inclusion and have ^{15}N-proline infused on the day of surgery. One of these 11 patients declined the ingestion of $^{2}H_2O$ but accepted the ^{15}N-proline infusion. The inclusion flow is shown in Appendix Figure A1 (available in the online version of this article). After inclusion in the present study, patients continued to be treated with a split plaster cast with the ankle in maximum plantarflexion and no weightbearing until surgery.

Institutional review board approval was provided by the ethical review board of the Capital Region of Denmark (H-18010363) and by the Danish Data Protection Agency of the Capital Region of Denmark (2012-58-0004).

Experimental Design

The 18 included patients were recruited within 1 to 4 days after the rupture, and all underwent surgery within 14 days. At the time of inclusion, the first 11 patients were offered to ingest 150 mL of 70% $^{2}H_2O$ (one subject declined to ingest $^{2}H_2O$). In addition, 7 of the 10 patients had a blood sample taken before ingesting $^{2}H_2O$ as a baseline measurement, and the average of this measurement was used as a baseline value for all 10 patients. On the day of surgery, 11 patients had 2 venous catheters inserted (1 in the antecubital vein and 1 in the back of the hand on the contralateral side). A 3-hour flood-primed continuous infusion of ^{15}N-proline (Cambridge Isotope Laboratories, Inc.) was started with a bolus injection (3500 mg of unlabeled proline and 420 mg of ^{15}N-proline) through the antecubital vein. Immediately after the bolus was given, a continuous and individualized infusion (1.115 mg/kg/h) was started in a venous catheter on the contralateral side.

Blood samples were drawn from the antecubital vein before the bolus was given as well as 30, 60, and 120 minutes after and again when biopsy specimens were taken. Surgery began approximately 2.5 hours into the tracer infusion protocol, and the tissue was extracted between 188 and 282 minutes afterward. The 7 included patients who were not offered $^{2}H_2O$ or ^{15}N-proline followed the general preoperative protocol at the hospital.

Biopsy specimens were taken by experienced orthopaedic consultants (K.W.B. and P.H.) during surgery of the ATR. Open surgery was performed through a 5- to 8-cm incision as previously described.[16] Each patient had 2 tendon biopsy specimens taken (~5 mm long, ~2 mm wide, and ~2 mm deep): 1 biopsy specimen from one of the stump ends of the ruptured part of the Achilles tendon (rupture) and one biopsy specimen 3 to 5 cm proximal to the rupture site where the Achilles tendon appeared macroscopically healthy (control). The biopsy specimens were snap-frozen in liquid nitrogen and stored for later analysis of ^{14}C, ^{2}H-alanine (from $^{2}H_2O$), and ^{15}N-proline to investigate tendon turnover in the years before the rupture, in the days from the rupture to surgery, and in the hours leading up to surgery, respectively. The patients had additional biopsy specimens taken for bacterial detection. The results from these analyses are published by Cramer et al.[7]

^{14}C Analysis and Calculation

Samples were freeze-dried and taken for analysis at the Aarhus AMS Centre, Aarhus University. The samples were combusted with cupric oxide (CuO) in sealed combustion tubes at 950°C before accelerator mass spectrometry analysis. The radiocarbon dating results are reported in accordance with international conventions,[41] and the content of ^{14}C is expressed as percentage modern carbon (pMC). The ^{14}C content is based on the measured $^{14}C/^{13}C$ ratio, which has been normalized to the standard $\delta^{13}C$ value of –15‰ Vienna Pee Dee Belemnite ($\delta^{13}C$ calibration standard) to correct for natural isotopic fractionation.

The amount of newly synthesized tendinous tissue, using the ^{14}C measurements, was calculated using the following:

$$\%\ newly\ synthesized\ tendinous\ tissue = \left(\frac{biopsy\ ^{14}C\ [pMc] - expected\ ^{14}C}{Atmospheric\ ^{14}C\ 2019\ [pMc] - expected\ ^{14}C}\right) * 100\%,$$

where *biopsy ^{14}C* is the ^{14}C level in the Achilles tendon at the time of surgery, *atmospheric ^{14}C 2019* is the atmospheric ^{14}C level in the Northern Hemisphere in 2019,[22] and *expected ^{14}C* is the ^{14}C level that we expect in a healthy tendon based on the year of birth and a 13-year moving-average model.[20]

Stable Isotope Tracer Analysis and Calculation

Tendon Tissue. To measure the enrichment of ^{2}H-alanine and ^{15}N-proline in the tendon samples, approximately 10 mg of the tendon tissue was homogenized in a 2-mL microvial with a screw cap (BioSpec) containing 5 lysing beads (Lysing Matrix D Bulk; MP Biomedicals), 1 silicon carbide bead (BioSpec), and 1 mL of homogenization buffer (0.02 M Tris [pH 7.4], 0.15 M NaCl, 2 mM EDTA, and 0.05% Triton X-100 (SIGMA-ALDRICH)). The samples were vigorously shaken 5 times on a FastPrep instrument (MP Biomedicals) at 5°C for 45 seconds at speed 5.5. Subsequently, the samples were incubated for 2 hours at 5°C and spun at 4°C at 1600*g* for 20 minutes, after which the supernatant was discarded. Then, 1 mL of 0.7 M KCl was added to the pellets, and the samples were homogenized and left standing overnight at 5°C. After centrifugation at 4°C at 1600*g* for 20 minutes, the supernatant was discarded, and the pellets were washed in 70% ethanol. The samples were spun once again at 4°C at 1600*g* for 20 minutes and hydrolyzed in 1 mL of 6 M HCl overnight at 110°C. After hydrolysis, the samples were purified using a cation exchange column with resin (AG 50W-X8 resin; Bio-Rad Laboratories), which had been prepared by washing with 3 × 2 mL of 1 M HCl, creating an acidic environment. The purified amino acids were eluted by adding 2 × 2 mL of 2 M NH_4OH. After this, samples were converted to *N*-acetyl-propyl derivatives and analyzed on a gas chromatography combustion isotope ratio mass spectrometer as described by Bornø et al.[3]

Blood Plasma. To measure the enrichment of ^{2}H-alanine and ^{15}N-proline in the plasma samples, free amino

acids were extracted. An internal standard in 500 μL of 50% acetic acid solution was added to 100 μL of plasma, and then, plasma was poured over cation exchange columns with resin, similar to the tendon tissue samples. The samples were then derivatized using a phenyl isothiocyanate derivatization agent, converting the samples into phenyl isothiocyanate derivatives. Then, 10 μL of the 100-μL derivatized samples were loaded and analyzed on an ultra-performance liquid chromatography system coupled to a triple quadrupole mass spectrometer (Thermo Fisher Scientific) as described by Bornø and van Hall.[4]

The acute FSR at the time of surgery was estimated using the single biopsy specimen approach with a flood-primed ^{15}N-proline infusion.[21] The FSR was determined by the following:

$$FSR[\%/h] = \left(\frac{\Delta E_{Protein}[APE]}{\hat{E}_{precursor}[MPE]*\Delta time[h]}\right)*100\%,$$

where $\Delta E_{protein}$ (atom percent excess [APE]) is the difference in enrichment between a standard proline baseline sample and enrichment in the tendon at the time of surgery, and $\hat{E}_{precursor}$ (mole percent excess [MPE]) is the weighted average plasma enrichment.

FSR Calculation Based on Oral ^2H_2O Administration

A blood sample was taken on the day of surgery to measure ^{2}H-alanine plasma enrichment. Assuming nonlinear precursor enrichment (see Appendix 2, available online, for a detailed description), the integrated FSR based on ^{2}H-alanine incorporation was calculated as follows:

$$FSR\,[\%/day] = \left[\frac{-\ln\left(\frac{\Delta E_{protein}[MPE]}{\hat{E}_{precursor}[MPE]}\right)}{\Delta t[days]}\right] \times 100,$$

where $\Delta E_{protein}$ (MPE) is the difference in enrichment between a tracer-naive tendon and enrichment in the tendon at the time of surgery, $\hat{E}_{precursor}$ (MPE) is the mean plasma precursor tracer enrichment over the labeling period, and Δt is the time (days) of the labeling period.

Statistical Analysis

Comparisons between the tendon tissue at the rupture site and the control site were performed using a paired t test. GraphPad Prism (Version 8.0; GraphPad Software) was used for all statistical analyses. Because of the low number of observations, we chose not to perform any statistical correlative analysis.

RESULTS

Long-term Collagen Synthesis Assessed Through ^{14}C Levels

There was no difference in the pMC between the rupture and control samples ($P > .05$) (Figure 1A). In the tendon tissue taken from both the rupture site and the control site, the pMC was consistently lower compared with the expected level in a healthy Achilles tendon. The expected level was found using a 13-year moving-average model showing the expected progression of ^{14}C levels in healthy tissue.[20] Further, assuming that turnover occurred over the previous couple of years, this lower ^{14}C level translates into 48% renewal of the tendon tissue at both the rupture site and the control site (Figure 1B).

Tendon Protein Synthesis From Rupture to Surgery Assessed Through ^{2}H Labeling

In Figure 2, we show ^{2}H-alanine enrichment in the tendon tissue at the time of surgery. It is important to note that the enrichment displayed cumulative incorporation over time, and as the time from inclusion to surgery varied, so did the period over which ^{2}H was incorporated into the tissue, and higher enrichment was seen as the time from ^2H_2O intake to surgery increased (Figure 2A). The FSR in the period from inclusion to surgery was, on average, 0.010% ± 0.003% per hour. The integrated FSR of tendon proteins from the time of inclusion to the time of surgery showed no difference between rupture and control samples ($P > .05$) (Figure 2B).

Acute Tendon Protein Synthesis on Day of Surgery Assessed Through Proline Tracer Infusion

The FSR on the day of surgery revealed values between 0.015% and 0.056% per hour, with a mean value of 0.025% ± 0.006% per hour (Figure 3A). There was no significant difference between the control site and the rupture site with regard to the 3-hour FSR on the day of surgery (Figure 3B).

DISCUSSION

The present study demonstrates that a fraction of the Achilles tendon had higher than normal tendon tissue turnover over a prolonged time. Furthermore, we found moderate tendon collagen tissue turnover in the immediate days after an ATR in patients, with no difference between the region of the rupture and an adjacent region in the same tendon.

We compared ^{14}C levels in ruptured Achilles tendons with the expected ^{14}C level in a healthy tendon. The expected value was based on a 13-year moving-average model that relied on the initial growth of the tendon in the first 13 years of life, as seen in healthy human tendons,[20] and we found that ruptured Achilles tendons had lower ^{14}C levels than expected levels in healthy Achilles tendons (Figure 1A). The ^{14}C level in the tissue should, at the time of formation, match the atmospheric ^{14}C level. Atmospheric ^{14}C levels have decreased since a peak from 1963 to 1964,[22] and the tissue formed since will therefore contain continuously lower levels of ^{14}C. Consequently,

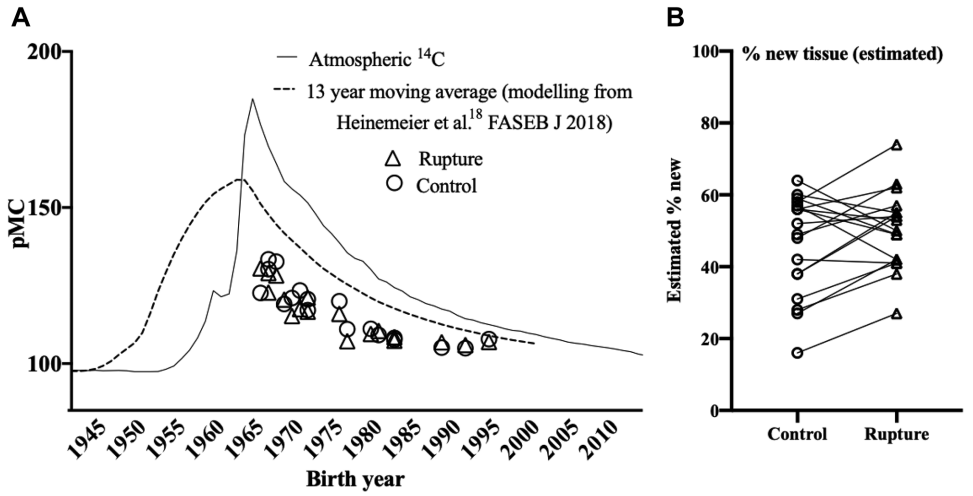

Figure 1. (A) Percentage modern carbon (pMC) in the atmosphere (full line), a healthy Achilles tendon using a 13-year moving-average model (in which dynamic turnover occurred in the first 13 years of a person's life) (dotted line),[20] and samples from the rupture (triangle) and control (circle) sites in patients with an Achilles tendon rupture. (B) An estimate of newly synthesized carbon-containing tendinous tissue in biopsy specimens from both the rupture site and the control site over the previous couple of years. No difference in the pMC between the rupture and control samples ($P > .05$) was found.

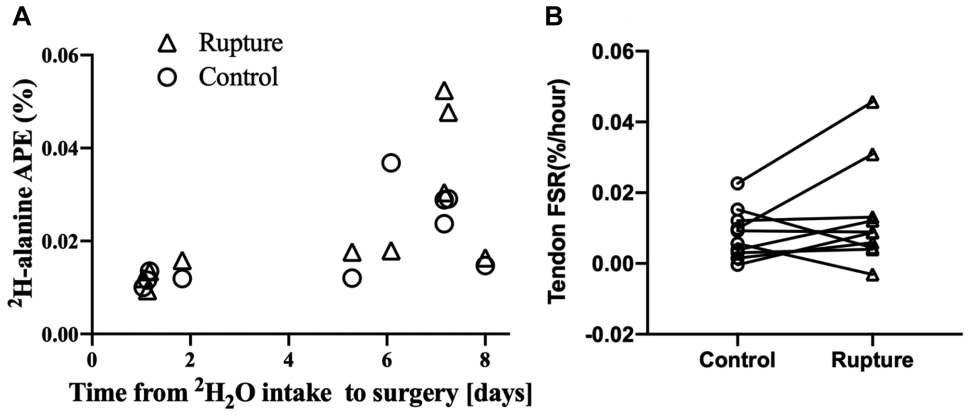

Figure 2. Patients ingested ^2H_2O at the time of inclusion, and surgery followed between 1 and 8 days afterward. (A) ^2H enrichment (atom percentage excess [APE]) in alanine in the tendon tissue measured at the time of surgery. The *x*-axis shows the time that passed from ^2H_2O ingestion to surgery, during which the cumulative incorporation of ^2H-alanine was determined. (B) The integrated fractional synthesis rate (FSR).

tissue with no turnover, like the lens of an eye, will have a ^{14}C level found in the atmosphere at the time of birth,[27] while tissue with high turnover, like muscle tissue, will have a ^{14}C level matching the atmospheric ^{14}C level at the time of sampling.[19] Thus, the finding that the ^{14}C level in a ruptured Achilles tendon was lower compared with the expected level in a healthy tendon shows that tissue turnover in ruptured tendons is higher than in healthy tendons, with an estimated average of 48% (±2% standard error of the mean [SEM]) newly formed tissue. In the current study, it cannot be concluded that the renewed fraction of collagen is caused by injuries sustained earlier in life and the result of a late phase of a prolonged disease process or is caused by habitually elevated turnover of the tendon tissue in certain patients (eg, because of genetics), who thus are more prone to tendon ruptures.[5] It is interesting that in human chronic tendinopathic tendons, ^{14}C levels are similar to those in the present study, with a fraction (~47%) of the tendon having undergone renewal in the time up to the presentation of the symptoms of tendinopathy.[17] Although a tendon rupture and tendinopathy may share some similarities in the early phase of the disease, such as elevated inflammation[8] and higher signaling for collagen synthesis,[36] there are also distinct differences between the 2 conditions[28] (eg, in regard to cell gene expression,[25] inflammation in the later state,[34] and tissue morphology[35]), suggesting that tendinopathy and a rupture represent 2 very different disorders.

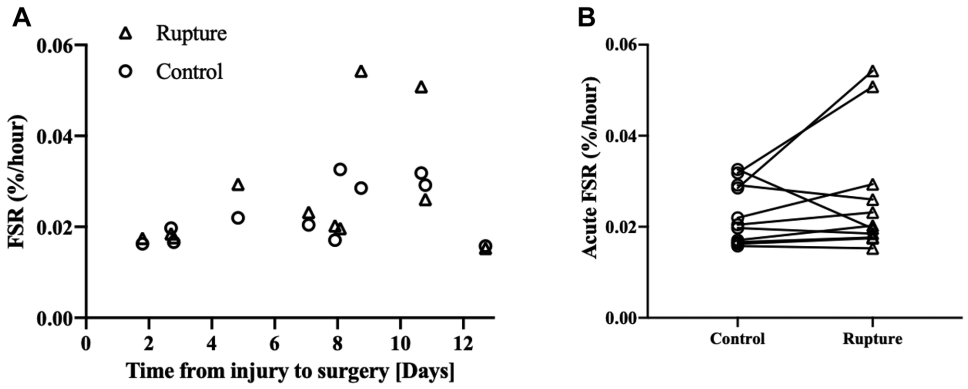

Figure 3. (A) The fractional synthesis rate (FSR) on the day of surgery in relation to the time between the injury and surgery. (B) The 3-hour FSR on the day of surgery determined from biopsy specimens from both the rupture site and the control site.

In the current study, samples representative of the entire tissue from ruptured Achilles tendons were analyzed for ^{14}C levels. Therefore, the results from the study do not answer whether increased tissue turnover was caused by (1) the increased synthesis of cross-linked collagen (the weightbearing collagen matrix) before a rupture or (2) the fact that the tendon tissue before a rupture had a larger percentage of substances with fast turnover, such as glycosaminoglycans (GAGs), compared with healthy Achilles tendon tissue. It has been shown that the percentage of GAGs is larger in tendinopathic tissue compared with healthy tendons.[37,48] This was also found in the study by Heinemeier et al,[20] who showed that removing GAGs in tendons with tendinopathy resulted in higher ^{14}C levels, indicating lower turnover during life. However, they also showed that even after isolating cross-linked collagen (by removing GAGs) in tendinopathic samples, the ^{14}C level was still lower than in healthy tendons, indicating increased turnover during life. Hence, they concluded that cross-linked collagen consisted of both old and relatively new collagen, indicating a continuous, slow renewal of the collagen matrix in adults. Based on these findings, it is likely that the amount of newly synthesized tendon tissue in the ruptured Achilles tendon is a combination of a higher percentage of GAGs and increased turnover in the collagen matrix before the rupture.

To investigate whether tissue turnover was elevated not only at the time of surgery but also in the days from the rupture to surgery, the integrated FSR in the initial healing phase was estimated by $^{2}H_2O$. Although the exact value of the FSR calculated from ^{2}H incorporation has some percentage of error, as there was no direct baseline value obtained directly from tendons before the administration of heavy water, it is clear that the calculated FSR was not overly high during the initial phase after a tendon rupture. In addition, the FSR calculated acutely during surgery was not very high. In fact, all previous studies on the FSR of human patellar tendons in resting conditions revealed values of 0.04% to 0.06% per hour,[10,12,15,30,38] which are slightly higher or similar to what was observed in the present study. This suggests that there is no marked, accelerated new collagen formation at least during the first week after a tendon rupture in humans. Further, this observation also supports our finding that a proportion of newly synthesized proteins estimated with the ^{14}C method cannot be explained by any marked elevation in collagen synthesis in the very first few days after an injury.

Direct human data on protein synthesis in the early healing phase after an ATR are currently lacking, and our findings in the present study indicate that no rapid increase in protein synthesis occurs immediately after a rupture. Although a few patients operated on up to 14 days after a rupture revealed a somewhat higher FSR, too few patients (n = 11) have been included to draw a conclusion about a potential progression of the FSR in the early healing phase. When interpreting protein synthesis in the early healing phase, the immobilization of the patient should be considered. An earlier study investigating protein synthesis in the patellar tendon found a substantial decline in the FSR after 2 weeks of immobilization.[9] If the Achilles tendon responds similarly to immobilization, patients operated on approximately 2 weeks after a rupture might have their FSR underestimated because of the immobilization period. However, a study that used a biopsy specimen from controlled trauma in healthy tendons demonstrated the widespread upregulation of tendon cell activity and their matrix protein expression[18] as well as collagen synthesis.[12] This occurred also in situations in which patients were immobilized for 3 weeks, overruling the effect of immobilization itself completely.[18] Thus, even though immobilization lowered collagen synthesis, it is likely that any significant upregulation of the FSR due to a rupture would have overruled this.

Knowledge of the healing phase after a rupture is relevant from a clinical perspective. Svedman et al[42] suggested that surgery within 48 hours after an ATR resulted in a better outcome than surgery performed later than 72 hours. They hypothesized that this finding was because of the biology of tendon healing but did not determine

any further results in their study. Several previous studies have demonstrated an immediate response of tendon cell activity and matrix protein expression.[11,13,18,26,44] Therefore, it is very likely that a matrix-synthesizing process is begun right after a rupture and that this will lead to a rise in collagen synthesis. The present study showed that a marked rise in collagen synthesis did not seem to be an immediate phenomenon within the first 2 weeks after an ATR in patients.

One of the drawbacks of the present study is that we did not make any direct comparisons with healthy Achilles tendon tissue in the same patients, and we were only able to compare the ruptured area with an adjacent area (3-5 cm from the rupture) within the same tendon that appeared macroscopically healthy at inspection. By doing so, no differences were found in any of the investigated parameters for collagen turnover either before or immediately after the injury between the tissue obtained at the rupture site and the control site (Figures 1-3). Although we do not have any control biopsy specimens from a completely healthy tendon within the same patient, we nevertheless believe that the characterization of the control site as an adjacent healthier region of the tendon provides significant information on immediate changes in collagen protein synthesis in the tissue. The study was also limited because of the comparison with a prediction model based on earlier studies; this can potentially increase the risk of bias. Finally, the generalizability is reduced because the patients included in the present study were primarily recruited from an RCT. However, because the inclusion and exclusion criteria of the present study and the RCT were similar and relatively broad, the limitation is considered modest.

CONCLUSION

In the present study, we found higher tissue turnover in the Achilles tendon before a rupture than that expected in a healthy Achilles tendon, suggesting potential pathological tissue changes in the tendon for years preceding the injury. Furthermore, the results did not seem to show a marked increase in tendon collagen tissue turnover in the immediate days after an ATR, either in the rupture region or in an adjacent region of the same tendon, suggesting a more delayed formation of new collagen during the regeneration of ruptured tendons in patients.

ACKNOWLEDGMENT

The authors acknowledge Maria Swennergreen Hansen, Julie Jenlar, and colleagues from the Arthroscopic Center, Hvidovre Hospital, for helping with the inclusion of patients in the study. They also thank the Department of Orthopedic Surgery, Hvidovre Hospital; the Ambulatory Surgical Department, Hvidovre Hospital; and the Department of Clinical Research, Hvidovre Hospital, for assisting with logistics and laboratory facilities.

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