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## Type 2 diabetes impairs tendon repair after injury in a rat model

Aisha S. Ahmed,<sup>1\*</sup> Nikos Schizas,<sup>2\*</sup> Jian Li,<sup>2</sup> Mahmood Ahmed,<sup>3</sup> C.-G. Östenson,<sup>2</sup> Paul Salo,<sup>4</sup> Carolyn Hewitt,<sup>4</sup> David A. Hart,<sup>4</sup> and Paul W. Ackermann<sup>2</sup>

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**Ahmed AS, Schizas N, Li J, Ahmed M, Östenson CG, Salo P, Hewitt C, Hart DA, Ackermann PW.** Type 2 diabetes impairs tendon repair after injury in a rat model. *J Appl Physiol* 113: 1784–1791, 2012. First published October 4, 2012; doi:10.1152/jappphysiol.00767.2012.—Type 2 diabetes adversely affects the properties of native connective tissue. The underlying mechanisms, however, by which diabetes alters connective tissue metabolism, especially tendon, are poorly defined. The aim of this study was to determine the effect of type 2 diabetes on the mechanical, histological, and molecular properties of the intact and healing Achilles tendon. The right Achilles tendon was transected in 11 male diabetic Goto-Kakizaki (GK) and 10 age- and sex-matched Wistar control rats, while the left Achilles tendon was left intact. At 2 wk postinjury the intact and injured tendons were assessed by biomechanical testing and histology. The gene expression of collagen I and III, biglycan, versican, MMP-13, and MMP-3 was measured by quantitative RT-PCR, and their protein distribution was studied by immunohistochemistry. Intact tendons exhibited only small differences between the groups. In injured tendons, however, a significantly smaller transverse area and lower stiffness was found in diabetic GK compared with Wistar control rats. This correlated with impaired structural organization of collagen fibers and a reduced expression of collagen I and III in the injured tendons of the diabetic GK compared with Wistar control. Moreover, MMP-3 gene expression was down-regulated in the injured diabetic GK tendons compared with injured Wistar controls. Our results indicate that in a rat model of diabetes tendon healing is impaired mainly due to altered expression of collagen and MMPs reflecting decreased degradation of matrix proteins and impaired tissue remodeling. Further our data suggest that therapeutic modulation of collagens or MMPs might be targets for new regenerative approaches in operated, injured, or maybe also degenerative tendon diseases in diabetes.

type 2 diabetes; collagen; matrix metalloproteinase; tendon

PATIENTS WITH TYPE 2 DIABETES are at greater risk to suffer the morbidity of various musculoskeletal disorders (8, 30). Painful tendinopathies, including shoulder tendinitis, limited hand mobility, tendon ruptures, and adhesive capsulitis, affect 30–60% of diabetic patients and cause considerable disability (33). However, the underlying biological mechanisms by which diabetes alters connective tissue matrix metabolism, especially tendon, are poorly defined (1).

Tendon structure consists of a highly organized fibrillar matrix, consisting of mainly type I collagen along with other collagens, proteoglycans, and glycoproteins. Previous studies indicate that the diabetic state adversely affects the properties

of native connective tissues (30). Type 2 diabetes is most common in obese patients. A recent systematic review demonstrated that obesity, a known risk factor for impaired glucose metabolism, is also an independent risk factor for tendon disorders (14). Preclinical studies indicated that elevated glucose levels impair collagen production and also intensify the presence of advanced glycation end products (AGE), resulting in abnormal ultrastructure of collagen fibrils (11, 28, 32). A common complication of type 2 diabetes is delayed or defective tissue healing, resulting from inadequate production of growth factors, compromised angiogenesis, and impaired formation of a collagen matrix (5).

Endopeptidases such as matrix metalloproteinases (MMPs) play a major role in the tendon matrix, being responsible for the degradation of collagen and proteoglycan in both healthy and pathological conditions (34). Recent studies show an upregulated expression of versican and collagen I, as well as MMPs, in human spontaneous tendon rupture. The tissue in the area of rupture undergoes marked rearrangement at the molecular level, which supports the role of MMPs in the pathophysiology of tendinopathy that precedes a spontaneous rupture (21).

The aim of the present study was to determine the effect of diabetes on the mechanical properties of the Achilles tendon and to study the expressional changes of genes involved in healing processes in diabetes, using a study model of 12-month-old Goto-Kakizaki (GK) rats. The GK rat is a nonobese type 2 diabetes model produced by selective breeding over many generations from nondiabetic Wistar rats with glucose intolerance as a selection index (15). GK rats have significantly elevated blood glucose levels as well as reduced nerve conduction velocity (NCV) confirming the presence of neuropathy, a common complication of diabetes as reported by us earlier (23). It was hypothesized that mild to moderate diabetes would hamper the connective tissue matrix molecule metabolism by adversely affecting the expression of proteolytic enzymes.

### MATERIALS AND METHODS

#### Study Design

The study included eleven 12-month-old Goto-Kakizaki (GK) rats from the breeding colony at the Karolinska University Hospital and 10 age- and sex-matched control Wistar rats. All animals were housed at 21°C in a 12:12-h light/dark cycle with pellets and water ad libitum according to the Karolinska Institutet protocol. All animals were raised until the age of 1 yr before the study was initiated. All experiments were approved by the local Committee for Animal Research and Ethics and conducted in accordance with the Institute's protocols.

\* A. S. Ahmed and N. Schizas contributed equally to this study.

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All rats (Wistar and GK) were subjected to blunt complete Achilles tendon transection in the right hindleg and subsequently allowed to have free cage activity. Tendons dissected from Wistar rats served as external normal controls, with the left hindleg tendons serving as intact specimens for both rat strains. Thus four groups of tendons, i.e., Wistar intact, Wistar ruptured, GK intact, and GK ruptured, were studied.

### Surgery

The rats were anesthetized by an injection of a mixture of ¼ Midazolam (5 mg/ml, Pharma Hameln) and ¼ Hypnorm (Janssen Pharmaceutica) in sterile water (2 ml/kg body wt sc). The operations were performed under sterile conditions. The Achilles and plantaris tendons were exposed through a 1-cm midline posterior longitudinal incision. Both tendons were fully ruptured in the mid part, ~0.5 cm from the calcaneal insertion, using a blunt instrument. The tendons were left unsutured and the skin was closed with two stitches of 5–0 nonresorbable suture material (2 × 2, Ethilon II, Ethicon). Postoperatively the animals were returned to their cages and allowed free cage activity. Postoperatively, Temgesic was administered.

### Dissections

At 2 wk postrupture all animals were anesthetized with sodium pentobarbitone (60 mg/kg ip) and euthanized. The right (ruptured) and left (intact control) Achilles and plantaris tendons were removed along with the gastrocnemius muscle and the calcaneal bone. Dissected tendons were either wrapped in normal saline (0.9% NaCl) soaked gauzes and frozen at –70°C for later mechanical assessment, or separated from muscle and bone and bisected sagittally into medial and lateral segments. The medial segments were immediately frozen in liquid nitrogen and kept in –70°C until qRT-PCR assessment. It was ensured that only tissue from the rupture site (i.e., callus) was taken from the ruptured tendons. The lateral segments were immediately soaked in Zamboni's fixative consisting of 4% paraformaldehyde in 0.2 mol/l Sörensen phosphate buffer, pH 7.3, containing 0.2% picric acid at 4°C until staining. Six tendons from each group were randomly chosen for the mechanical assessment while the remaining tendons from each group were used for qRT-PCR and histological analysis.

### Mechanical Testing

The sagittal and transverse diameters of the callus, the distance between the torn ends as well as the full length of the tendon, were measured with a digital slide caliper as previously described (12). The cross-sectional area of the callus was calculated assuming an elliptical geometry. For clamping, the gastrocnemius muscle was carefully scraped off the proximal tendon by blunt dissection to produce a fan of tendon fibers, which were attached between fine sandpaper and fixed in a metal clamp. Distally, the calcaneus was fixed in a custom-made clamp, in neutral position, i.e., 30° dorsal flexion, relative to the direction of tensile loading. The clamps were mounted vertically in a materials testing machine (100R, DDL, Eden Prairie, MN) and tested with stretching at a constant speed of 0.1 mm/s in a monotonic phase until failure. The biomechanical parameters were

maximum force (N), stiffness (N/mm), stress (N/mm<sup>2</sup>), and energy uptake (J) until maximum force, which were calculated by the software of the testing machine after the linear portion of the elastic phase of the curve for stiffness calculation had been marked.

### Real-Time Quantitative PCR

Frozen tissues were homogenized by Mikro-dismembrator (B. Braun Biotech International) and dissolved in 2–3 vol of Trizol reagent (Invitrogen Life Technologies). RNA was then extracted and further purified using the RNeasy MiniKit (Qiagen) following the manufacturer's protocol (29).

RNA integrity was assessed using the Agilent 2100 Bioanalyser (Agilent Technologies). Samples were prepared according to the company protocol and loaded to the Eukaryote total RNA nanochip and processed. First-strand cDNA was synthesized from 1 µg of total RNA using the first-strand cDNA Synthesis Kit (Roche Germany). Quantification assays were performed to detect the relative mRNA expression of collagen I and III, biglycan, versican, MMP-13, and MMP-3 using techniques developed and optimized in our group as previously described (17, 29). Briefly, total RNA (1 µg) was reverse transcribed to generate single-stranded cDNA using Qiagen Omniscript RT kit (Qiagen Sciences, Germantown, MD). qPCR primers were synthesized and validated for the target molecules (Table 1). BIO-RAD iQ SYBR Green Supermix (12.5 µl) (BIO-RAD, Hercules, CA), molecular biology water (3.5 µl), as well as forward and reverse primer (0.75 µl each), formed the PCR reaction mixture, using 7.5 µl RT for each reaction. The iCycler Thermal Cycler (BIO-RAD) was utilized during amplification and detection, validated through inspection of the melting curve (dF/dT vs. temperature) for nonspecific peaks. Level of gene expression was normalized to 18S rRNA. iCycler iQ Optical System Software version 3.0a (BIO-RAD) was used to quantify results.

### Histology

Right (rupture) and left (intact control) tendons that were dissected from Wistar and diabetic GK rats and fixed in Zamboni's fixative (see *Dissection*) were sectioned using a Leitz 1720 cryostat (Ernst Leitz, Wetzlar, Germany) to a section thickness of 12 µm and mounted on SuperFrost/Plus slides. Three sections of each tendon at different depths (100 µm apart) were taken and stained with hematoxylin and eosin for histological assessment. Images were captured by a video camera (DEI 750; Optronics Engineering, Goleta, CA) attached to the microscope and stored in a computer.

Three sections of each tendon were stained with Sirius red according to the standard procedure as described earlier (20) and examined on the microscope, where the polarizer and analyzer were set according to the Kiernan protocol (22).

### Immunohistochemistry

Twelve-micrometer sections from right and left tendons from each group were immunostained with antiserum to collagen I, III, biglycan, versican, MMP-13, and MMP-3. Nonspecific binding was minimized by incubating the sections in 5% normal goat serum for 30 min. The sections were incubated overnight with antiserum to collagen I and

Table 1. Reverse and forward primers of genes selected for the study

Primer	Forward Primer	Reverse Primer	Origin
18 s	GTC CCC CAA CTT CTT A	CAC CTA CGG AAA CCT TGT TAC	X01117
Collagen I	CAG ACG GGA GTT TCA CCT C	GAC ATG TAG ACT CTT TGC GGC	J04464
Collagen III	CTG CCA TTG CTG GAG TTG	GCA GCC ATC CTC TAG AAC	AJ005395
Biglycan	GAT GAC TTC AAA GGC CTC CA	TCA GGC TCC CAT TCT CAA TC	NM_017087
Versican	CGA GAC TGG AGC TAC TGA TG	GCT TCC TCA GTT GGA GAC AGG	XM_215451
MMP-13	CGT TCA AGG AAT CCA GTC TC	CAT AAC TCC ACA CGT GGT TC	M60616
MMP-3	TGG ATT CTG CCA TTG AGA GAG C	AGA ACA AGA CTT CTC CCC GCA G	NM133523

collagen III (1:200, Abcam), biglycan, versican, MMP-3 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), and MMP-13 (1:100, Santa Cruz Biotechnology, Santa Cruz, CA). Sections were rinsed in PBS (3 × 5 min) and incubated for 30 min at room temperature with secondary antibodies: goat anti-rabbit or donkey anti-goat (1:250, Vector Laboratories, Burlingame, CA). The sections were then washed with PBS (3 × 5 min) and incubated with ABC reagent for 30 min at room temperature. This step was followed by application of diaminobenzidine (DAB) chromogen (Vector Laboratories, Burlingame, CA) and counterstaining with Hematoxylin QS (Vector Laboratories). Sections were dehydrated with 70, 95, and 99% ethanol. To demonstrate specificity of staining, primary antisera were either omitted or preincubated with the corresponding peptides and incubated.

#### Statistical Analysis

The significance of the differences between injured and intact tendon groups was analyzed by single-factor analysis of variance (ANOVA). For the mechanical results, nonparametric Mann-Whitney *U*-tests were used. The level of significance was set at  $P \leq 0.05$ .

## RESULTS

### Effects of Diabetes on Mechanical Properties

To assess the effect of diabetes on biomechanical properties, the transverse area of the callus, peak load, stiffness, energy at peak load, and stress were tested in intact and ruptured tendons at day 14 postrupture in diabetic GK rats and in age-matched Wistar controls.

The intact tendons of the diabetic GK animals and the Wistar animals were found not significantly different regarding biomechanical properties, except for stiffness, which was 19% lower in diabetic animals ( $P = 0.05$ ).

The injured tendons of the diabetic GK group exhibited 43% smaller transverse area of the callus ( $P = 0.01$ ) and 35% lower stiffness ( $P = 0.02$ ) compared with the injured Wistar control tendons. A 35% reduction in peak load was observed in the injured diabetic tendons compared with the injured Wistar controls, although this was not statistically significant ( $P = 0.14$ ). Stress and energy were not significantly different between the injured tendons of the two groups, diabetic GK vs. Wistar control. (Table 2).

### Effects of Diabetes on Matrix Molecule Gene Expression

Overall, RNA extracted from tendon was of good quality with RIN (RNA integrity number)  $7.3 \pm 0.5$  and  $7.4 \pm 0.6$  for intact Wistar and for diabetic GK rats, respectively, and  $7.5 \pm 0.5$  and  $8.0 \pm 0.4$  for injured Wistar and diabetic GK rats. At day 14 postrupture, quantitative PCR analysis showed that all genes of interest (collagen type I and III, biglycan, versican, MMP-13, and MMP-3) were expressed at measurable levels in intact and healing tendons of both Wistar and GK rats.

**Collagen type I and III expression.** In the intact tendons no differences in collagen type I and III gene expressions were observed between the GK and Wistar animals. In the injured tendons, however, the diabetic GK group exhibited 35% and 55% lower collagen I ( $P = 0.07$ ) and III ( $P = 0.008$ ) mRNA levels, respectively, compared with the corresponding Wistar injured tendons.

Healing, i.e., injured compared with intact tendons, showed a 96% increase in collagen I ( $P = 0.001$ ) and fivefold increase in collagen III ( $P = 0.003$ ) expression in the Wistar animals. In the diabetic GK rats meanwhile, healing tendons demonstrated

Table 2. The biomechanical parameters tested in the intact and healing tendon of Wistar and diabetic GK rats

	Intact	Ruptured	%Difference	<i>P</i> Value
Transverse area, mm <sup>2</sup>				
Wistar	6.9 ± 0.8	15.6 ± 1.7*	124.56	0.01
Diabetic GK	6.4 ± 0.9	8.9 ± 0.9#	40.07	0.10
%Difference	8.60	43.0		
<i>P</i> value	0.52	0.01		
Peak load, N				
Wistar	70 ± 6	51.9 ± 7	-25.86	0.10
Diabetic GK	66.3 ± 11	33.9 ± 6	-48.82	0.05
%Difference	5.30	34.60		
<i>P</i> value	0.75	0.14		
Stiffness, N/mm				
Wistar	35.9 ± 1.7	16.2 ± 1.1*	-54.97	0.01
Diabetic GK	29 ± 2.7	10.4 ± 1.8#	-64.03	0.03
%Difference	19.20	35.4		
<i>P</i> value	0.05	0.02		
Energy at peak load, J				
Wistar	84 ± 16	108 ± 22	28.36	0.27
Diabetic GK	115 ± 32	69 ± 15	-40.46	0.26
%Difference	-37.6	36.20		
<i>P</i> value	0.33	0.36		
Stress, N/mm <sup>2</sup>				
Wistar	10.7	3.3*	-68.61	0.01
Diabetic GK	11.2	3.8#	-65.62	0.01
%Difference	-4.8	-14.8		
<i>P</i> value	0.63	0.27		

Results are expressed as means ± SE with  $n = 4$  in the Wistar group and  $n = 5$  in the diabetic Goto-Kakizaki (GK) group. Parameters tested were the transverse area of the mid part of the tendon, peak load, stiffness, energy at peak load and stress. \* $P \leq 0.05$  compared with Wistar intact and # $P \leq 0.05$  compared with diabetic GK intact group (Mann-Whitney *U*-tests).

no significant increases in collagen I expression, but a mean threefold increase in collagen III ( $P = 0.003$ ) mRNA levels (Fig. 1, A and B).

**Biglycan and versican expression.** No significant differences in biglycan or versican gene expression were observed between the diabetic GK animals and Wistar controls either in the intact or in the injured tendons, nor were any significant changes noted in mRNA level during healing. Although the data indicated a 44% decrease in versican gene expression in the injured diabetic GK animals compared with intact GK tendons, the differences proved to be nonsignificant ( $P = 0.06$ ) (Fig. 1, C and D), but a strong trend.

**MMP-13 and MMP-3 expression.** In intact tendons no differences in MMP-13 or MMP-3 gene expression were observed between diabetic GK animals and the Wistar controls. However, in the injured tendons, 42% lower MMP-3 mRNA levels ( $P = 0.05$ ) were noted in diabetic GK animals compared with the Wistar controls. In the Wistar animals, no differences in MMP-13 and -3 mRNA levels were observed between injured and intact tendons. On the other hand, the diabetic GK animals exhibited a 191% increase in MMP-13 ( $P = 0.04$ ) and a 62% decrease in MMP-3 ( $P = 0.02$ ) mRNA levels in the injured compared with intact tendons (Fig. 1, E and F).

### Effect of Diabetes on Histological Aspects of Tendon Healing

Overall, the histological examination supported the biomechanical and gene analysis findings, by showing minor differences between intact tendons from Wistar and GK rats and

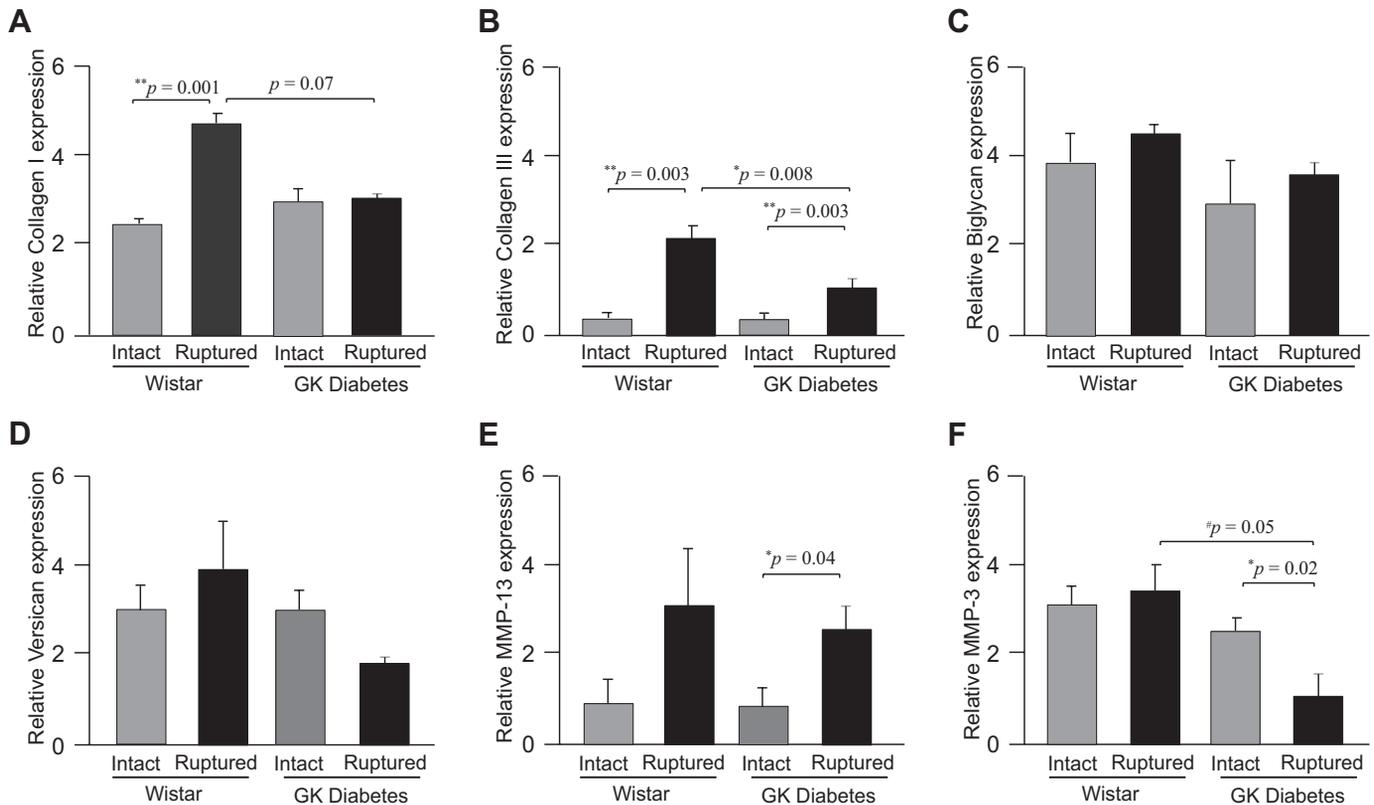


Fig. 1. Relative expression of collagen I (A), collagen III (B), biglycan (C), versican (D), MMP-13 (E), and MMP-3 (F) mRNA in the intact and ruptured tendon of Wistar and diabetic Goto-Kakizaki (GK) rats at day 14 postrupture. Results are expressed as means  $\pm$  SE with  $n = 4$  in Wistar groups and  $n = 5$  in diabetic GK groups. \* $P \leq 0.05$ , \*\* $P \leq 0.005$  compared with intact Wistar and # $P \leq 0.05$ , ### $P \leq 0.005$  compared with intact GK group (single-factor analysis of variance).

more obvious differences between the corresponding injured groups.

#### Hematoxylin and Eosin

Intact tendons from GK rats exhibited slightly lesser transverse area, but no other evident difference in the structural organization of collagen fibers compared with the intact Wistar tendons.

The histological analysis of tendons at day 14 postrupture indicated a lower regenerating activity in the diabetic GK animals compared with the Wistar tendons. This was illustrated by a much smaller transverse area, poor structural organization with fewer longitudinally oriented collagen fibers along the functional loading axis, and decreased vascularity in the diabetic GK tendons (Fig. 2).

#### Sirius Red

Polarized light examinations of intact tendons showed no apparent differences in structural organization of collagen fibers between the two rat strains. At day 14 postrupture, the injured Wistar tendons exhibited apparent short thin pale green fibrils, arranged in a longitudinal direction at the ruptured area, denoting regenerating collagen III-like (LI) structures (31). In the injured GK tendons, however, very few fibers of pale green color were seen; most fibers were of yellowish red color and arranged irregularly, denoting ruptured collagen I structures. Overall, the highest density of collagen III-LI fibers was present at the rupture site of the Wistar animals (Fig. 2).

#### Effects of Diabetes on Matrix Protein Expression

Overall, the immunohistochemical analysis of the matrix proteins confirmed the differences in gene expression observed between the groups assessed. However, some smaller alterations in protein occurrence compared with gene expression were noted.

**Collagen I and III.** In intact tendons of the diabetic GK and Wistar rats, no apparent differences in collagen I or III immunoreactivity were detected. Collagen I and III positivity was localized to tenocytes, in the proper tendon matrix and surrounding connective tissues. In the injured tendons, sparser collagen I and III occurrence was noted in the diabetic GK tendons compared with the Wistar tendons (Fig. 3).

**Biglycan and versican.** A scarce versican staining was observed in the intact tendons of the diabetic GK animals compared with intact Wistar tendons, while prominent biglycan staining was seen in tendons from both rat strains. In contrast, in the healing tendons the diabetic GK rats exhibited weaker biglycan staining and fewer biglycan positive cells compared with the Wistar tendons, whereas no apparent difference in versican staining was observed between these two groups. In both intact and healing tendons in groups, versican and biglycan immunostaining was seen localized in the tendon proper matrix, the paratenon, and in the surrounding connective tissues (Fig. 3).

**MMP-13 and MMP-3.** Intact tendons from both rat strains exhibited an equally strong MMP-13 and MMP-3 immuno-

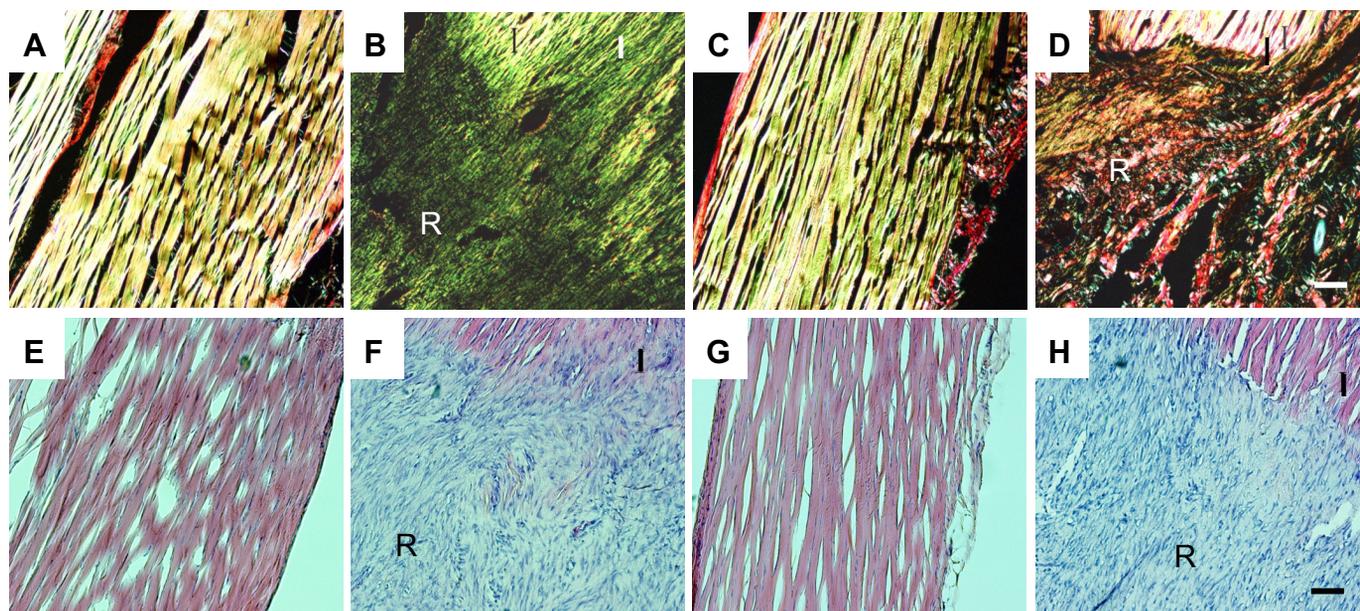


Fig. 2. Photomicrographs of the transverse area of the Achilles tendon (A–D, Sirius red stained) and synovium (E–H, hematoxylin and eosin stained) on day 14 of experiment. Representative images are from Wistar intact (A, E) with typical appearance of the tendon; Wistar ruptured (B, F), Diabetic GK intact (C, G), and diabetic GK ruptured tendon (D, H). Original magnification is 20 $\times$  and bar is 100  $\mu$ m. I, intact part of the tendon; R, ruptured area.

staining, localized in tenocytes, matrix, and surrounding connective tissues. In the healing tendons, MMP-13 was more abundant in the diabetic GK tendons, while MMP-3 appeared equally distributed between the two strains. Increased MMP-13 immunoreactivity in the healing diabetic GK tendons was observed around blood vessels and cells in the callus. MMP-3 positive cells were also observed in the callus (Fig. 3).

## DISCUSSION

Our present study clearly demonstrates that type 2 diabetes impairs tendon repair in the GK rat model. Moreover, we observed a markedly deranged expressional pattern of major matrix proteins: collagen types I, III, and matrix metalloproteinase MMP-13 in the tendon of diabetic GK rats. To our knowledge, this is the first study to analyze the expression level changes of major matrix molecules and proteases in the diabetic GK tendon.

In the Wistar rats, biomechanical studies indicated a significant increase in the transverse area of the callus and the peak load values at the tendon healing site. The difference in peak load values between the injured and intact tendons was not significantly different, indicating normal tendon healing. In the GK rats, the transverse area of the callus was not increased and the peak loads were significantly reduced compared with intact tendons, probably reflecting impaired healing (13). Our results also demonstrated that the stiffness was significantly lower both in the intact and healing tendons of diabetic GK rats compared with those from the non-diabetic Wistar rats, corroborating previous studies (4). One can speculate that the observed reduced stiffness may lead to degenerative changes in tendons during diabetes, resulting in increased risk for developing tendinopathy.

Stress, which reflects the biomechanical quality of tissues, was found not to differ between the diabetic GK tendons and the nondiabetic Wistar tendons either in intact or in healing

tendons. At this proliferative phase of healing, the major components of the callus are collagen III fibers; thus the major differences in mechanical properties can be accounted for by the production of collagen III. The most striking differences between the tendons from the two strains of rat at 2 wk postrupture were the low collagen III gene and protein expression in the healing diabetic GK tendons as indicated by our histological and biochemical analysis. This correlated well with the decreased healing transverse area and peak load in the diabetic GK tendons. The disturbed collagen III production in the ruptured diabetic GK tendons likely reflects that synthesis of the main component of the callus in the proliferative healing phase is hindered (3). Taken together, these findings confirm that mild to moderate diabetes hampers early proliferative tendon repair and are consistent with previous clinical and preclinical data on wound healing and impaired diabetic fracture, but are novel regarding tendinous tissue.

The impaired production of collagen III in the callus of diabetic GK tendons could presumably be related to accompanying neuropathy and microangiopathy. We have previously shown that collagen synthesis at the tendon injury site is dependent on an adequate circulation and neuronal supply (6, 10). In fact, the expression levels for one vital neuronal mediator involved in tendon healing, substance P (SP), which is also an important factor for stimulating collagen III production, is known to be reduced in diabetes (7, 9). Interestingly, it has recently been demonstrated that SP regulates the release of matrix metalloproteinases by activating its receptor on mast cells (26). Thus one plausible explanation for the deficient collagen I and III synthesis observed in the diabetic GK tendons could pertain to a dysregulated metalloproteinase production. The protein expression of MMP-13 was higher in healing tendons of diabetic GK tendons compared with those of the Wistar tendons, although these differences were not observed in the corresponding gene expression data. Con-

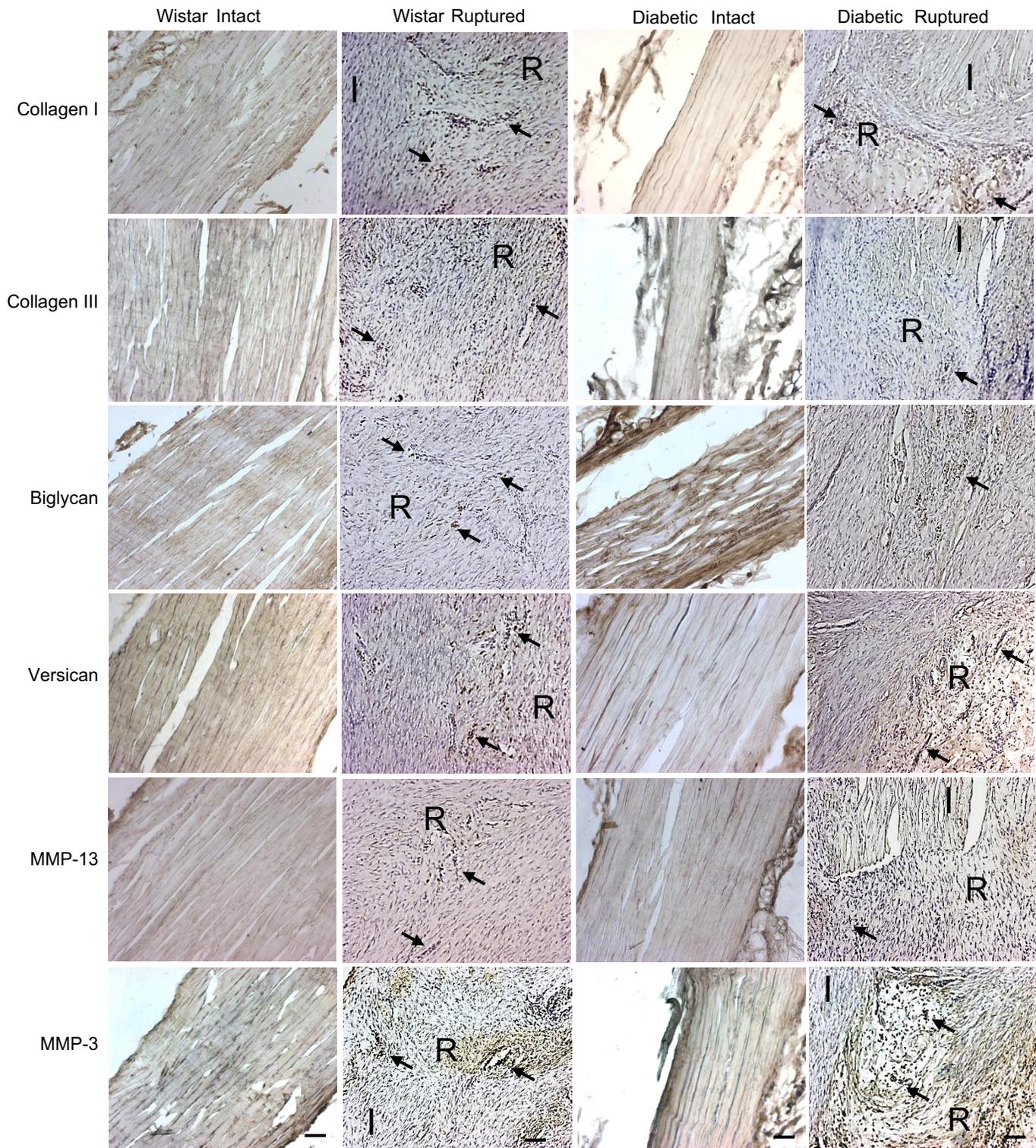


Fig. 3. Photomicrographs of intact and diabetes tendon of Wistar and diabetic GK rats at *day 14* postrupture stained with antibodies against collagen I (A), collagen III (B), biglycan (C), versican (D), MMP-13 (E), and MMP-3 (F). Original magnification is 20 $\times$  and bar is 100  $\mu$ m. I, intact part of the tendon; R, ruptured area.

versely, MMP-3 gene expression was lower in diabetic GK healing tendons compared with that of the Wistar tendons, although this was not confirmed by the immunohistochemical analysis. The discrepancy between the gene and protein expression results could be explained by transcriptional or post-

translational regulation, potentially influenced by high levels of glucose in diabetes (24).

As regards the growth factors such as IGF-1, it has been shown to enhance collagen synthesis in both animals as well as in humans (16, 25). Both clinical and experimental studies

have demonstrated reduced levels of IGF-1 in diabetes. Thus patients with type 1 diabetes seem to have low IGF-1 levels (19). Recently, we have demonstrated significant reduction of IGF-1 in serum and bone tissues of GK rats, and these changes were correlated with skeletal changes like endosteal erosions and osteopenia (2). It is reasonable to speculate that reduced synthesis of IGF-1 in GK-rats with type 2 diabetes may interfere with tissue repair.

The tendon is commonly affected by injury, fatigue, and inflammation in the surrounding structures, all of which may lead to rupture. After such injuries, the tissue undergoes a process of reorganization of the matrix molecules to regenerate the afflicted area (35). Both MMP-3 and MMP-13 are known to be involved in tissue remodeling and may degrade collagen fibrils. MMP-3 is found to activate other MMPs such as MMP-1, MMP-7, and MMP-9. Furthermore, in other pathological situations such as rheumatoid arthritis and osteoarthritis MMP-3 and -13 are highly overexpressed (18, 27). Based on the previous and present findings, we speculate that aberrant tissue remodeling involving dysregulated MMP-3 and MMP-13 activity is a possible pathomechanism connecting diabetic tendinopathy with different musculoskeletal disorders.

A major prerequisite for the translation of findings from preclinical studies is that the model employed mimics the characteristic pathophysiology of the disease observed in the clinical scenario. The animal model employed in the present study is a well characterized model of mild to moderate type 2 diabetes (15). Our findings, however, may be applicable to a larger patient population and warrant, e.g., glucose tolerance testing of patients with tendon-related (musculoskeletal syndromes) problems without other causative factors. The present study demonstrated the effect of diabetes on proliferative healing only at week 2 postinjury. In upcoming studies it would be interesting to examine the time-dependent effects of diabetes on the early inflammatory as well as late remodeling phases of connective tissue healing.

In summary, our present study demonstrated that the tendons assessed are affected by type 2 diabetes. Following injury, diabetic GK tendons exhibited biomechanical deficits reflected by reduced transverse area and peak load as well as deficient collagen III expression, suggesting impaired proliferative healing. The hampered tendon remodeling may partly be related to the observed metalloproteinase dysregulation in this model compared with the normal Wistar tendons. An improved understanding of the overall mechanisms through which diabetes alters connective tissue metabolism should lead to better preventive and therapeutic interventions in common musculoskeletal syndromes as well. The data from this study provide new insights into diabetes and its potential relationship with tendinopathy. These findings also have clear clinical implications for the expected outcomes of soft tissue repair or reconstructive procedures in patients with poor glycemic control.

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

#### AUTHOR CONTRIBUTIONS

Author contributions: A.S.A., N.S., J.L., and C.H. performed experiments; A.S.A., N.S., M.A., P.S., C.H., and P.W.A. analyzed data; A.S.A., N.S., J.L., M.A., C.-G.x., P.S., C.H., D.A.H., and P.W.A. interpreted results of experiments; A.S.A. and C.H. prepared figures; A.S.A. and P.W.A. drafted manuscript; A.S.A., N.S., M.A., C.-G.x., P.S., D.A.H., and P.W.A. edited and revised manuscript; A.S.A., N.S., J.L., M.A., C.-G.x., P.S., C.H., D.A.H., and P.W.A. approved final version of manuscript; J.L., M.A., and P.W.A. conception and design of research.

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