# Tendons from Non-diabetic Humans and Rats Harbor a Population of Insulin-producing, Pancreatic Beta Cell-like Cells

Authors

C. Lehner[1, 2, 3*], R. Gehwolf[2, 3*], A. Wagner[2, 3, 4], H. Resch[1, 2, 3], C. Hirzinger[1], P. Augat[5], D. Stephan[5], L. Aigner[3, 6], F. J. Rivera[3, 6], H.-C. Bauer[2, 3, 4], H. Tempfer[2, 3]

Affiliations

Affiliation addresses are listed at the end of the article

Key words
- tendon cells
- extrapancreatic insulin
- tenocyte characterization
- diabetic tendinopathy
- streptozotocin

## Abstract

Diabetes mellitus is a risk factor for various types of tendon disorders. The mechanisms underlying diabetes associated tendinopathies remain unclear, but typically, systemic factors related to high blood glucose levels are thought to be causally involved. We hypothesize that tendon immanent cells might be directly involved in diabetic tendinopathy. We therefore analyzed human and rat tendons by immunohistochemistry, laser capture microdissection, and single cell PCR for pancreatic β-cell associated markers. Moreover, we examined the short term effects of a single injection of streptozotocin, a toxin for GLUT2 expressing cells, in rats on insulin expression of tendon cells, and on the biomechanical properties of Achilles tendons. Tendon cells, both in the perivascular area and in the dense collagenous tissue express insulin and GLUT2 on both protein and mRNA levels. In addition, glucagon and PDX-1 are present in tendon cells. Intraperitoneal injection of streptozotocin caused a loss of insulin and insulin mRNA in rat Achilles tendons after only 5 days, accompanied by a 40% reduction of mechanical strength. In summary, a so far unrecognized, extrapancreatic, insulin-producing cell type, possibly playing a major role in the pathophysiology of diabetic tendinopathy is described. In view of these data, novel strategies in tendon repair may be considered. The potential of the described cells as a tool for treating diabetes needs to be addressed by further studies.

## Introduction

Patients suffering from diabetes, both type I and II, are often affected by various kinds of tendon disorders, such as tendinitis or tendon rupture, accompanied with impaired tendon healing [1–3]. This has recently been confirmed in animal experiments, showing that streptozotocin (STZ) induced diabetes type I significantly reduces tensile strength of rat Achilles tendons and rat patellar tendons after 10 weeks and 19 days, respectively [4,5]. Besides diabetes, obesity is recognized as a risk factor for tendinopathy, indicating the involvement of metabolic mechanisms in the pathophysiology of tendon disorders [6,7]. The mechanisms underlying diabetes- or obesity-associated tendinopathies are presently unclear, but the chronic exposure to systemic metabolic factors such as high blood-glucose levels in consequence of pancreatic β-cell deficiencies are thought to induce tendinopathies as a secondary event. We hypothesize that tendon-immanent cells might be directly involved in insulin-associated pathophysiology of tendinopathies. We therefore examined human and rat tendons for cells expressing pancreatic β-cell associated markers and their potential function.

## Materials and Methods

### Human materials, animals

All human materials were used with patients' oral informed consents; names of witnesses are held on file. The ethics committee of the Government of Salzburg approved this study and stated in approval # E699 from 2006 that oral informed consent is sufficient, if no extra material is dissected for the study, beyond medical indication. Only material accruing from medically indicated interventions was used.

All animal work was conducted according to relevant national and international guidelines. The animal experiments were approved by the ethics

received 27.12.2011
accepted 25.04.2012

Bibliography
DOI http://dx.doi.org/10.1055/s-0032-1312672
Horm Metab Res 2012; 44: 506–510

ISSN 0018-5043

Correspondence
*H. Tempfer*
Strubergasse 21
5020 Salzburg
Austria
Tel.: +43/662/442002 1257
Fax: +43/662/442002 1223
Herbert.Tempfer@pmu.ac.at

*These authors contributed equally to this work.

committee of the Austrian Ministry for Science and Research (approval # BMWF-66.012/0003-II/3b/2011).

Human tendon and periosteum samples (biceps tendon, semitendinosus tendon, palmaris longus tendon) were obtained with patients' informed consents (4 males aged 21, 24, 33, 62 years; 5 females aged 38, 45, 62, 68 years). The samples were immediately transferred to a solution of 4% paraformaldehyde (PFA) in PBS.

Five female, 3 months old Lewis rats were fed with a high glucose diet (Ssniff EF R/M glucose-rich, Ssniff, Soest, Germany) for 1 month. Twelve female, 3 months old Lewis rats received a single intraperitoneal injection of streptozotocin (Zanosar®, TEVA Pharmaceuticals, USA) (80 mg/kg), and another 12 animals served as control. After 5 days, the animals were killed by $CO_2$ inhalation. Daily water uptake was determined for each animal at day 0 and day 5, blood glucose was measured immediately after killing the animals, using a blood glucose meter (Wellion, HOME Diagnostics, Ft. Lauderdale, USA). Achilles tendons were dissected and analyzed by qRT-PCR, immunohistochemistry, Western blot, and biomechanical testing.

### Immunohistochemistry

PFA fixed tissue samples were dehydrated in a graded ethanol series and further processed for paraffin embedding. Six µm thick sections were cut on a microtome (Microm HM 355, Germany) and immunohistochemically stained with antibodies specific for insulin, GLUT2, PDX-1, and SYP. In brief, sections were rehydrated in a descending xylene/ethanol series and endogenous peroxidase activity was quenched with methanol containing 3% hydrogen peroxide for 30 min at room temperature. Following blocking of unspecific binding sites in BSA-PBS-Tween containing diluent, incubation with primary antibody (insulin: ProteinTech Group/# 15848-1-AP; GLUT2: Millipore, Vienna, Austria #07-1402; PDX-1: ProteinTech Group # 10951-1-AP; Synaptophysin: Santa Cruz # sc-7569; β-actin: Millipore, Vienna, Austria MAB1501R; secondary Ab: anti rb IgG-Peroxidase, Sigma-Aldrich, Vienna, Austria # A9169) was performed at 4 °C overnight in a humidified chamber. After treatment with Power Vision poly HRP-anti-rabbit IgG (ImmunoLogic, The Netherlands) for 30 min at room temperature, the sections were incubated with DAB (3,3′-diaminobenzidine tetrahydrochloride), ready to use tablets (Sigma-Aldrich, Vienna, Austria), counterstained with NovocastraTM hematoxylin dye (Leica, Vienna, Austria) for 4 min, dehydrated, and mounted in Eukitt (Sigma-Aldrich, Vienna, Austria).

### Western blot

Lysates from rat and human tendons were prepared as described previously [8]. Protein contents were quantified by using a BCA protein assay kit (Pierce, Rockford, USA). Equal amounts of protein were subjected to SDS-PAGE using a 10 or 12.5% gel. After blotting, the PVDF membrane was incubated in 5% nonfat dried milk. Immunodetection was performed using primary antibodies recognizing insulin, GLUT2, β-actin and a secondary HRP-labeled goat anti-rabbit/mouse antibody (Sigma Aldrich, Vienna, Austria). Bands were visualized using the SuperSignal West Pico Chemiluminescent Substrate from Pierce (Thermo Scientific, Vienna, Austria).

### Measurement of insulin secretion

Achilles tendons from 3 female Lewis rats were collected, cut into small pieces under sterile conditions, followed by a 4-h digestion in DMEM (Gibco/Invitrogen (Lofer, Austria) supplemented with 30 mg/ml collagenase II (Gibco, Lofer, Austria) at 37 °C, 95% humidity and 5% $CO_2$. The resulting suspension of single cells and vessel fragments was centrifuged; the pellet was washed 3 times with PBS to dispose insulin from residual blood. Insulin secretion was measured as described by Lumelsky et al. [9] using static incubations in Krebs-Ringer with bicarbonate buffer as follows: 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.1 mM $MgCl_2$, 25 mM $NaHCO_3$, and 0.1% bovine serum albumin at 37 °C, either with 5 mM glucose or 30 mM for 10 min. Secreted insulin was assayed using insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden) according to the manufacturer's protocol. This experiment was repeated 3 times.

### RNA isolation and quantitative reverse transcription PCR (qRT-PCR)

Total RNA of rat Achilles tendons was isolated using PureZol reagent (BioRad, Vienna, Austria) according to manufacturer's instructions. RNA was quantified photometrically and analyzed on an agarose gel. One µg of each RNA sample was transcribed into cDNA using the High Capacity RNA-to-cDNA master mix (Applied Biosystems, Vienna, Austria). Quantification of gene expression of insulin, scleraxis, and nestin was done with TaqMan Gene Expression Assays (**Table 1**) and TaqMan Gene Expression master mix (Applied Biosystems, Vienna, Austria) according to manufacturer's instructions. As reference genes HPRT1 and/or GAPDH were used and relative gene expression was calculated according to Pfaffl [10].

### Laser capture microdissection and single cell RT-PCR

A contact-free Leica AS LMD (Leica Microsystems, Vienna, Austria) was used. Tissue sections of human biceps tendons (3 µm) mounted on PEN membrane slides (Leica Microsystems, Vienna, Austria) were immunostained for insulin (as described above) and cells positive and negative for insulin were cut under bright-field microscopy. Cells were harvested directly into a mineral oil containing RNase-free PCR-tube cap. For cDNA synthesis, high capacity RNA-to-cDNA master mix (Applied Biosystems, Vienna, Austria) was directly added to the cells and reverse transcription was performed according to manufacturer's instructions. For PCR amplification TaqMan Gene Expression Assays for insulin, scleraxis, nestin, and GAPDH as control gene (**Table 1**) and the TaqMan Gene Expression master mix were used according to

**Table 1** TaqMan gene expression assays.

| Gene of interest | TaqMan assay ID | | Acc. No. | |
|---|---|---|---|---|
| HPRT1 | Hs01003267_m1 | Rn01527840_m1 | NM_000194.2 | NM_012583.2 |
| GAPDH | Hs99999905_m1 | – | NM_002046.3 | – |
| INS1 | Hs02741908_m1 | Rn02121433_g1 | NM_001185097.1 | NM_019129.3 |
| Scleraxis | Hs03054634_g1 | Rn01504576_m1 | NM_001080514.1 | NM_001130508 |
| Nestin | Hs00707120_s1 | Rn00564394_m1 | NM_006617.1 | NM_012987.1 |

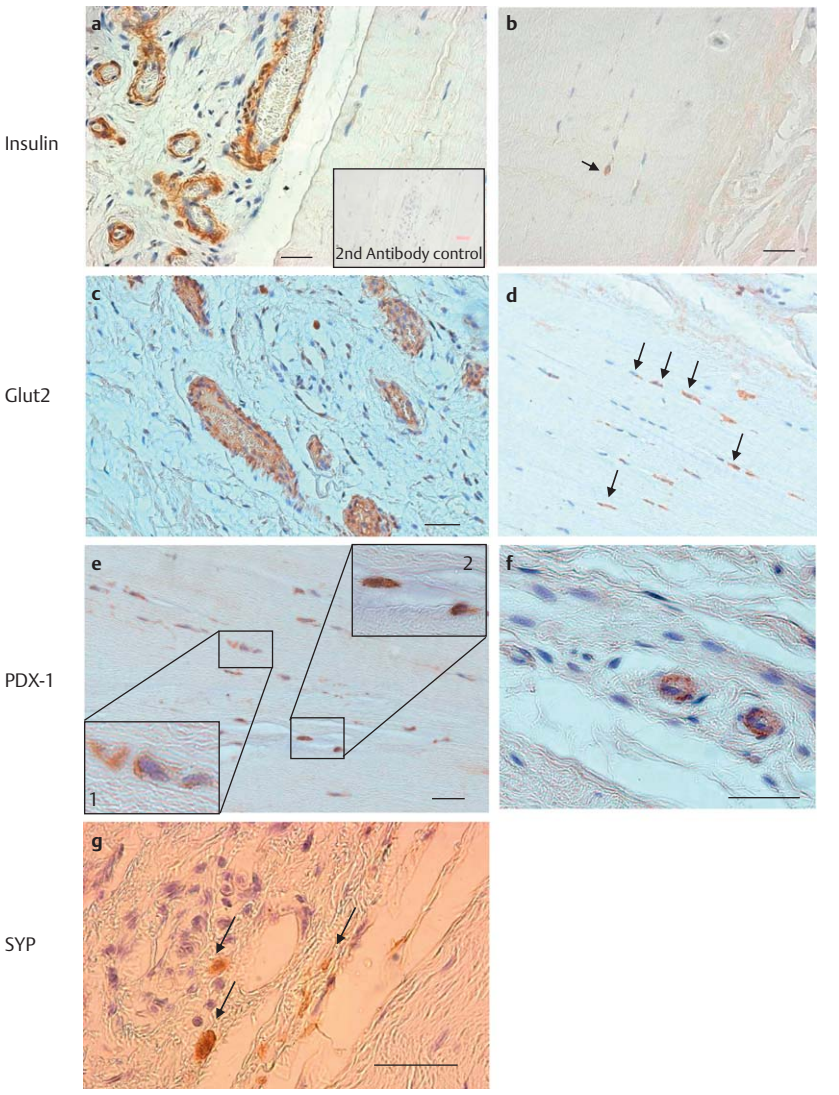


**Fig. 1** Immunohistochemical analysis of 6 µm sections of a human biceps tendon stained with antibodies directed against insulin **a**, **b**, GLUT2 **c**, **d**, and PDX-1 **e**, **f** shows immunoreactive cells in the perivascular area **a**, **c**, **f**. Also in the dense, collagenous part of the tissue, some cells express insulin (**b**, arrows), GLUT 2 (**d**, arrows), and PDX1 **e**. PDX-1 localizes both in the cytoplasm (**e**, detail 1) and in the nucleus (**e**, detail 2). Few cells in the perivascular area are immunoreactive to an antibody specific for synaptophysin (SYP) (**g**, black arrows). Scale bars: 200 µm.

manufacturer's instructions. Amplified PCR products were analyzed by agarose gel electrophoresis.

### Biomechanical testing

Achilles tendon-calcaneal bone complexes were isolated, 2 K-wires were inserted, one in the frontal plane of the calcaneal bone, the other in sagittal plane, in order to allow correct positioning. The calcaneal bone was positioned and embedded in a custom-made device by filling it up with Technovit (Heraeus Kulzer, Germany). The tendon attachment area was not embedded. The tendon was fixed in a screw grip using sandpaper (grain size 100) and superglue. Specimens were tested on a universal material testing machine (Zwick, Ulm, Germany) at 15 ° loading angle at 0.1 mm/min until failure after a preload of 0.5 N had been applied. Force (N) was measured with a load cell of 200 N (accuracy class 1, Gassmann und Theiss) and recorded by a corresponding software (testXpert 1, Zwick, Ulm, Germany). Only intratendinously ruptured tendons were included in the study.

### Statistical analysis

All values are given as means with SD. For evaluating qPCR results, student's *t*-test was applied. Data on maximum tensile load, water uptake, and blood glucose levels were analyzed by One Way ANOVA; results with a significant ANOVA were corrected for multiple comparisons using the Tukey HSD test. All p-values < 0.05 are considered significant.

## Results

Immunohistochemical analysis of human biceps tendon samples reveals a population of perivascular cells to be immunoreactive with antibodies against insulin (**Fig. 1a**), GLUT 2 (**Fig. 1c**) and PDX-1 (**Fig. 1f**). Also cells in the dense, collagenous part of the tissue express these proteins (**Fig. 1b, d, e**). Neither insulin, nor GLUT 2, or PDX-1 could be detected in periosteum by immunohistochemistry. Mainly perivascular cells are immunoreactive to the synaptophysin antibody, less than 1 % of total tendon cells are stained (**Fig. 1g**). The observed GLUT2 protein expression was confirmed by Western blot, showing a protein size of about 53 kD in both rat and human tendon (**Fig. 2a**). RT-PCR analysis of insulin expressing cells harvested by laser capture microdissection shows that these cells express insulin and glucagon as well as the tendon progenitor associated markers scleraxis and nestin (**Fig. 2b**). Single cell RT-PCR analysis of tendon perivascular cells confirms that insulin is coexpressed

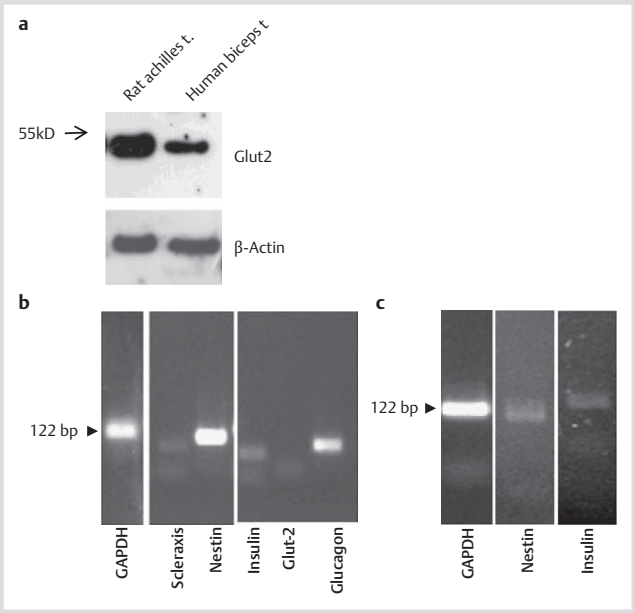


**Fig. 2** Western blot analysis of rat Achilles tendons and a human biceps tendon (male, 62 years) shows the expression of GLUT2 **a**. RT-PCR analysis of insulin-positive human biceps tendon cells harvested by Laser capture Microdissection shows that these cells express nestin, scleraxis, insulin, and glucagon **b**. Single cell RT-PCR of manually isolated tendon perivascular cells confirms that nestin and insulin are coexpressed **c**.

with nestin (**Fig. 2c**). Freshly isolated cells from rat tendons respond to glucose stimulation by insulin secretion. Within 10 min, cells in a suspension of $10^7$ tendon cells secrete 0.14 pg insulin (**Fig. 3a**).

Rats treated with 80 mg/kg streptozotocin develop signs of diabetes within 5 days: water uptake increases 3-fold, blood glucose levels increase from 124 mg/dl to 442 mg/dl. Western blot analysis of the Achilles tendons of these animals 5 days after the injection shows a complete loss of hexameric insulin (36 kD) (**Fig. 3b1**). This observation is supported by qRT-PCR (**Fig. 3b**). In the pancreas of these animals, the amount of insulin mRNA relative to HPRT is significantly reduced by 21 % (**Fig. 3b2**).

The amount of insulin mRNA in the Achilles tendons of rats fed with high glucose diet was found to be 16.2-fold increased (±15 %, $p < 0.01$, $n = 5$) after 4 weeks. The maximum tensile load of Achilles tendon of STZ treated animals after 5 days is 39 % lower than in the control animals ($p = 0.01$) (**Fig. 3c**).

## Discussion

Insulin production is typically associated with pancreatic β-cells. Nevertheless, various reports described extrapancreatic insulin-producing tissues such as brain and thymus in nondiabetic individuals, as reviewed by Kojima et al. [11]. In the present work, we describe a so far unrecognized cell type in human and rat tendons expressing the pancreatic β-cell associated markers insulin, glucagon, PDX-1, and GLUT2. We identify 2 populations of insulin expressing cells: i) perivascular cells, and ii) spindle shaped "tenocytes" within the dense collagenous tendon tissue. Rats treated with STZ show an acute reduction in insulin levels in the tendons along with a deficiency of mechanical strength.

Apparently, at least a subpopulation of these cells is functional in terms of their capacity to release insulin upon glucose stimulation. In the cytoplasm of tendon cells we detect synaptophysin immunoreactivity; synaptophysin was shown to be associated with storage granules in pancreatic islet cells [12]. We could not detect the expression of C-peptide in tendon cells by immunohistochemistry, which may be due to the low expression level. Insulin mRNA expression is about 60-fold higher in pancreas than in tendon (**Fig. 3b**).

Taking into consideration that both insulin and insulin mRNA are detectable in tendon cells and that tendon insulin mRNA levels are influenced by dietary parameters, de novo synthesis of insulin by tendon cells is very likely.

The existence of a population of insulin-producing cells in human and rat tendons is surprising, since tendons are commonly considered to be of mesenchymal origin. Indeed, we and others have shown that tendon-derived stem cells have mesenchymal stem cell like properties, such as their capacity of differentiating into adipocytes, osteoblasts, and chondrocytes [13–15]. The present data, that is, the coexpression of the stem/progenitor cell markers nestin and scleraxis with insulin and glucagon, suggest a premature state of at least a subpopulation of the herein described insulin-producing tendon cells.

Possibly, insulin is involved in guiding the differentiation process leading to mature tendon cells and/or participates in maintaining this differentiated status. Along this line, it has been shown that insulin induces differentiation of mesenchymal stem cells towards a tendon cell like phenotype [16].

Insulin was shown to exert PDX-1-mediated protective effects on pancreatic β-cells at physiological concentrations [17]. Moreover, insulin protects human tendon cells from dexamethasone induced damage in vitro [18]. One may speculate that protective effects of tendon-derived insulin play a role for tendon cells in vivo, considering that tendon is a poorly vascularized tissue. However, the biological function of insulin-producing tendon cells under nonpathological conditions remains a matter of speculation and needs to be addressed by further experiments.

Understanding the role of insulin and insulin-producing tendon cells in tendon development and regeneration may pave the way for new strategies in tendon repair. So far, tendon cells have been poorly characterized, only few markers, such as scleraxis or tenomodulin, are known [15,19]. Interestingly, previous reports described chronic effects of STZ on tendon strength, ascribing their observations to elevated blood glucose levels [4,5]. Here, we observe an acute significant reduction in tendon strength already 5 days after STZ treatment. The short time period suggests that STZ directly affected tendon cells and caused impairment of the tendon's mechanical strength. Light microscopic analysis of sections from STZ treated tendons did not show any apparent structural alterations (not shown).

In addition to their potential involvement in tendinopathies, insulin-producing cells derived from tendon might have a therapeutic potential in diabetes mellitus. This, however, requires further and more detailed investigations.

## Acknowledgements

This work was supported by the research fund of the Paracelsus Medical University Salzburg (grants PMU-FFF E-09/09/051-BAH, PMU-FFF R-10/02/012-HIR, PMU-FFF 05/02/008), the Hermann Straniak Foundation (Sarnen, Switzerland), and by a grant of the Lorenz Boehler Foundation (Vienna, AUstria). LA and FJR are supported by the State of Salzburg, the Bavarian State Ministry

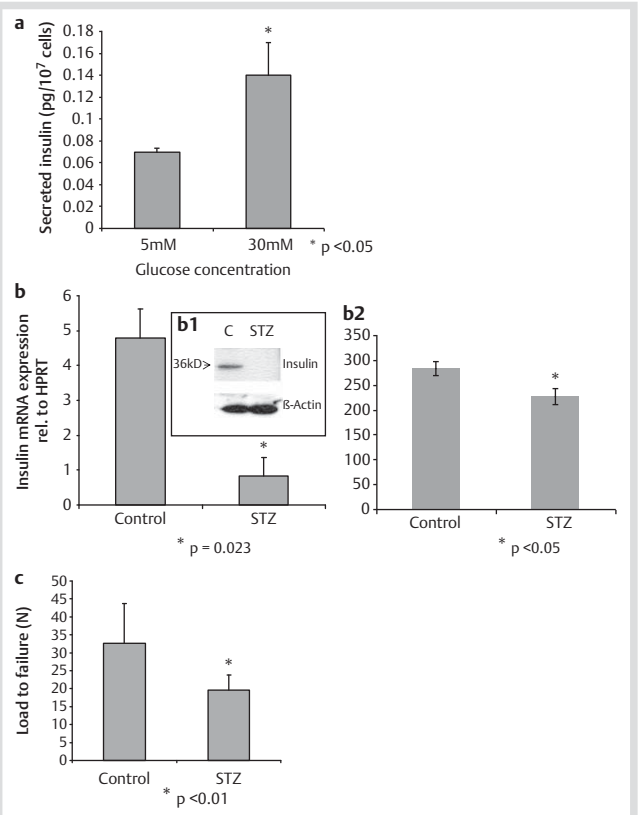


**Fig. 3** Enzymatically released rat Achilles tendon cells respond to glucose stimulation by increased insulin secretion. The insulin released to the cell supernatant is quantified by ELISA assay **a**. Both qPCR **b** and Western blot analysis **b1** demonstrate downregulation of both insulin mRNA and insulin in rat Achilles tendons 5 days after injection of 80 mg/kg streptozotocin. Pancreas levels of insulin mRNA were reduced by 20 % **b2**. Note that in control animals insulin mRNA expression is 60-fold higher in pancreas than in tendon. The maximum tensile load of these tendons is reduced by 39 % after 5 days compared to untreated animals **c**.

of Sciences, Research and the Arts (ForNeuroCell grant), the German Federal Ministry of Education and Research (BMBF grant 01GN0978), the PMU research fund PMU-FFF, the Propter Homines Foundation, and the FWF Special Research Program (SFB) F44 "Cell Signaling in Chronic CNS Disorders". The authors wish to thank Sabine Spiegl-Kreinecker, Dept. Neurosurgery, Wagner-Jauregg Hospital (Linz, Austria) for her support in LMD.

**Affiliations**
[1] University Hospital of Salzburg, Department of Trauma Surgery and Sports Injuries, Salzburg, Austria
[2] Paracelsus Medical University, Institute for Tendon and Bone Regeneration, Salzburg, Austria
[3] Paracelsus Medical University, Spinal Cord Injury and Tissue Regeneration Center, Salzburg, Austria
[4] University of Salzburg, Department of Organismic Biology, Salzburg, Austria
[5] Trauma Center Murnau, Institute for Biomechanics, Murnau, Germany
[6] Paracelsus Medical University, Institute of Molecular Regenerative Medicine, Salzburg, Austria

## References

1 *Ilan DI, Tejwani N, Keschner M, Leibman M.* Quadriceps tendon rupture. J Am Acad Orthop Surg 2003; 11: 192–200
2 *Clement ND, Hallett A, MacDonald D, Howie C, McBirnie J.* Does diabetes affect outcome after arthroscopic repair of the rotator cuff? J Bone Joint Surg Br 2010; 92: 1112–1117
3 *Bedi A, Fox AJ, Harris PE, Deng XH, Ying L, Warren RF, Rodeo SA.* Diabetes mellitus impairs tendon-bone healing after rotator cuff repair. J Shoulder Elbow Surg 2010; 19: 978–988
4 *de Oliveira RR, de Lira KD, Silveira PV, Coutinho MP, Medeiros MN, Teixeira MF, de Moraes SR.* Mechanical properties of achilles tendon in rats induced to experimental diabetes. Ann Biomed Eng 2011; 39: 1528–1534
5 *Fox AJ, Bedi A, Deng XH, Ying L, Harris PE, Warren RF, Rodeo SA.* Diabetes mellitus alters the mechanical properties of the native tendon in an experimental rat model. J Orthop Res 2011; 29: 880–885
6 *Wearing SC, Hennig EM, Byrne NM, Steele JR, Hills AP.* Musculoskeletal disorders associated with obesity: a biomechanical perspective. Obes Rev 2006; 7: 239–250
7 *Gaida JE, Ashe MC, Bass SL, Cook JL.* Is adiposity an under-recognized risk factor for tendinopathy? A systematic review. Arthritis Rheum 2009; 61: 840–849
8 *Traweger A, Lehner C, Farkas A, Krizbai IA, Tempfer H, Klement E, Guenther B, Bauer HC, Bauer H.* Nuclear Zonula occludens-2 alters gene expression and junctional stability in epithelial and endothelial cells. Differentiation 2008; 76: 99–106
9 *Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R.* Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 2001; 292: 1389–1394
10 *Pafflˆ M.* A new mathematical method for relative quantification of real-time RT-PCR. Nucleic Acids Res 2001; 29: 2002–2007
11 *Kojima H, Fujimmiya M, Terashima T, Kimura H, Chan L.* Extrapancreatic proinsulin/insulin-expressing cells in diabetes mellitus: Is istory repeating itself? Endocr J 2006; 53: 715–722
12 *Kalina M, Lukinius A, Grimelius L, Höög A, Falkmer S.* Ultrastructural localization of synaptophysin to the secretory granules of normal glucagon and insulin cells in human islets of Langerhans. Ultrastruct Pathol 1991; 15: 215–219
13 *Salingcarnboriboon R, Yoshitake H, Tsuji K, Obinata M, Amagasa T, Nifuji A, Noda M.* Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property. Exp Cell Res 2007; 287: 289–300
14 *Bi Y, Ehirchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, Li L, Leet AI, Seo BM, Zhang L, Shi S, Young MF.* Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 2007; 13: 1219–1227
15 *Tempfer H, Wagner A, Gehwolf R, Lehner C, Tauber M, Resch H, Bauer HC.* Perivascular cells of the supraspinatus tendon express both tendon- and stem cell-related markers. Histochem Cell Biol 2009; 131: 733–741
16 *Mazzocca AD, McCarthy MB, Chowaniec D, Cote MP, Judson CH, Apostolakos J, Solovyova O, Beitzel K, Arciero RA.* Bone marrow-derived mesenchymal stem cells obtained during arthroscopic rotator cuff repair Surgery show potential for tendon cell differentiation after treatment with insulin. Arthroscopy 2011; 1459–1471
17 *Johnson JD, Bernal-Mizrachi E, Alejandro EU, Han Z, Kalynyak TB, Li H, Beith JL, Gross J, Warnock GL, Townsend RR, Permutt MA, Polonsky KS.* Insulin protects islets from apoptosis via Pdx1 and specific changes in the human islet proteome. Proc Natl Acad Sci USA 2006; 19: 19575–19580
18 *Poulsen RC, Carr AJ, Hulley PA.* Protection against glucocorticoid-induced damage in human tenocytes by modulation of ERK, Akt, and forkhead signaling. Endocrinology 2011; 152: 503–514
19 *Schweitzer R, Chyung JH, Murtaugh LC, Brent AE, Rosen V, Olson EN, Lassar A, Tabin CJ.* Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. Development 2011; 28: 3855–3866