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Diabetes mellitus impairs tendon-bone healing after rotator cuff repair

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Abstract

Introduction—Studies have demonstrated a significant decrease in skeletal mass, bone mineral density, and impaired fracture healing in the diabetic population. However, the effect of sustained hyperglycemia on tendon-to-bone healing is unknown.

Materials and methods—Forty-eight male, Lewis rats underwent unilateral detachment of the supraspinatus tendon followed by immediate anatomic repair with transosseous fixation. In the experimental group ($n = 24$), diabetes was induced preoperatively via intraperitoneal injection of streptozotocin (STZ, 65 mg/kg) and confirmed with both pre- and post-STZ injection intraperitoneal glucose tolerance tests (IPGTT). Animals were sacrificed at 1 and 2 weeks postoperatively for biomechanical, histomorphometric, and immunohistochemical analysis. Serum hemoglobin A1c (HbA1c) levels were measured at 2 weeks postoperatively. Statistical comparisons were performed using Student t tests with significance set at $P < .05$.

Results—IPGTT analysis demonstrated a significant impairment of glycemic control in the diabetic compared to control animals ($P < .05$). Mean HbA1c level at 2 weeks postoperatively was $10.6 \pm 2.7\%$ and $6.0 \pm 1.0\%$ for the diabetic and control groups, respectively ($P < .05$). Diabetic animals demonstrated significantly less fibrocartilage and organized collagen, and increased AGE deposition at the tendon-bone interface ($P < .05$). The healing enthesis of diabetic animals demonstrated a significantly reduced ultimate load-to-failure ($4.79 \pm 1.33\text{N}$ vs $1.60 \pm 1.67\text{N}$ and $13.63 \pm 2.33\text{N}$ vs $6.0 \pm 3.24\text{N}$ for control versus diabetic animals at 1 and 2 weeks, respectively) and stiffness compared to control animals ($P < .05$).

Discussion—Sustained hyperglycemia impairs tendon-bone healing after rotator cuff repair in this rodent model. These findings have significant clinical implications for the expected outcomes of soft tissue repair or reconstructive procedures in diabetic patients with poor glycemic control.

Level of Evidence—Basic Science Study.

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Keywords

Diabetes; rotator cuff repair; tendon-bone healing

Diabetes mellitus remains one of the most common and debilitating medical conditions, with prevalence in the United States estimated at approximately 17.5 million people.²⁴ Unfortunately, diabetes is associated with a myriad of systemic complications involving nearly every tissue in the body. Long-term effects of sustained hyperglycemia include peripheral neuropathy, kidney and gastrointestinal dysfunction, immunodeficiency, retinopathy, and tissue-repair disorders.^{24,57} The American Diabetic Association (ADA) estimates that the total economic burden of diabetes in 2007 was \$174 billion, including \$116 billion in medical care and \$58 billion in reduced national productivity.²⁴

Diabetes mellitus (DM) has been recognized to cause a wide range of musculoskeletal disorders, including tenosynovitis, joint stiffness, and tendon contracture.^{1,4–8,10,17,41,43,46,47,49,52,57} Commonly recognized musculoskeletal sequelae of diabetes include Dupuytren's contracture, carpal tunnel syndrome, flexor tenosynovitis, and adhesive capsulitis.^{1,4–8,10,17,41,43,46,47,49,52,57} It is believed that these complications result from alterations in the quantity and function of the structural macromolecules of the extracellular matrix in connective tissues.^{3,11–15,31,44,53,55} Both clinical and basic science studies have also revealed that the diabetic phenotype is associated with impaired fracture healing,^{9,21,25,26,28,33,34,40,42,60,61} reduced bone mass and bone mineral density,^{23,30,32,54} decreased collagen content and defects in cross-linking,^{28,31,34,53,59,64} a propensity for shoulder stiffness,^{5–7,10,17,43,46–48} as well as a significantly greater risk of infection and complications following open rotator cuff repair.²⁰

The impact of sustained hyperglycemia on tendon-to-bone healing, however, has not been well-characterized. Such information has critical implications for defining the appropriate nonoperative and surgical management of rotator cuff disease and ligament injuries in the diabetic population. The purpose of this study was to determine the effect of a diabetic phenotype on tendon-to-bone healing after rotator cuff repair in a rat model.¹⁸ While this model presents a “worst case scenario” of uncontrolled hyperglycemia in the acute postoperative period and does not necessarily reflect the commonly encountered insulin resistant, diabetic patient, it provides a reliable and reproducible model to examine our hypothesis that sustained hyperglycemia would be associated with inferior biomechanical and histological properties of the healing enthesis compared to matched, euglycemic controls.

Methods and materials

Study design

This study was approved by our Institutional Animal Care and Use Committee (IACUC) with assigned protocol #11-08-12R. Diabetes was induced via intraperitoneal injection of streptozotocin (STZ, 65 mg/kg) (Sigma, St. Louis, MO), a selective toxin of pancreatic β cells, in 24 male, Lewis rats (weight, 250–300 g) (Harlan, Indianapolis, IN). Induction of

diabetes was confirmed with both pre- and post-STZ injection intraperitoneal glucose tolerance tests (IPGTT), and only rats with a stable diabetic phenotype were included in the study. Control animals (n = 24) received an intraperitoneal injection of citrate buffer solution only. Twelve animals were sacrificed at 1 and 2 weeks postoperatively for biomechanical testing (n = 8/group) and histomorphometric/ immunohistochemical (n=4/group) analysis (Figure 1).

Induction of diabetes

Rats were anesthetized with isoflurane and diabetes was induced by an intraperitoneal injection of STZ. The STZ was dissolved in citrate buffer (pH 4.6) containing 75 mM citric acid, 150 mM NaOH, and 25 mM HCl. Four days after the injection, STZ-treated and control (untreated) rats were fasted for 6 hours and subjected to an intraperitoneal glucose tolerance test (IPGTT). Blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 minutes after an intraperitoneal injection of a 50% dextrose solution in sterile saline at 1.5-gm/kg body weight (Sigma). Area under the curve (AUC) was calculated for each animal's IPGTT curve to provide an index of the severity of hyperglycemia. Only rats with a sustained diabetic phenotype, defined by persistent fasting blood glucose levels equal or greater than 250mg/dL, were included in the experimental group.^{27,36,56} Blood glucose levels were measured every 3 days in all rats to ensure maintenance of a euglycemic and hyperglycemic state in the control and diabetic rats, respectively.

Measurement of glycosylated hemoglobin

Serum HbA1c levels were measured at sacrifice to confirm a diabetic or euglycemic phenotype and quantify the severity of sustained hyperglycemia. Measurement of glycosylated hemoglobin (HbA1c) is an established procedure for estimating the chronic (average) plasma glucose concentration present for the 120-day life span of the red blood cell.^{2,16,36,50,51,56,63} For euglycemic animals, the HbA1c level should be ~6%. In contrast, greater than 6% is abnormal and consistent with insulin resistance and/or diabetes.^{2,6,36,50,51,56,63} Blinded samples of blood harvested at the time of sacrifice were provided to an independent institution for analysis (Louisiana State University, Baton Rouge, LA). HbA1c was measured using standard affinity microchromatographic methodology (Helena Laboratories, Beaumont, TX).

Surgical procedure

Based on previous studies that have demonstrated anatomical similarities with the human shoulder, a rat model was selected to study rotator cuff tendon healing after surgical repair. Using a previously established model described by Carpenter et al, each animal underwent detachment and immediate repair of the right supraspinatus tendon using bone tunnel and suture fixation.¹⁸

The rats were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg). Anesthesia was maintained using 2% isoflurane (Baxter Inc, Deerfield, IL). All operations were performed using a sterile technique with the rat in the lateral decubitus position. A deltoid-splitting incision was made, and the acromioclavicular joint was divided, allowing visualization of the rotator cuff tendons. The supraspinatus tendon

was isolated and a modified Mason-Allen stitch was placed using 4-0 Ethibond (Johnson and Johnson, Piscataway, NJ) nonabsorbable suture (Figure 2). The tendon was then sharply detached from the greater tuberosity and the footprint gently decorticated with a scalpel blade to assure complete debridement of the native enthesis. Crossed bone tunnels were drilled at the anterior and posterior margins of the footprint and 2 mm lateral to the articular surface using a 22-gauge needle. Suture ends were then passed through the bone tunnels and firmly tied over the humeral metaphyseal cortex, anatomically repairing the supraspinatus tendon to its native footprint (Figure 2). The deltoid split and wound was subsequently closed in a standard, layered fashion with absorbable sutures. Buprenorphine (0.05 mg/kg) was administered subcutaneously for analgesia during the postoperative period. Ad libitum weightbearing and cage activity was allowed postoperatively.

Histologic analysis

Histologic analysis of the healing enthesis was performed at 1 and 2 weeks postoperatively ($n = 4/\text{group}$). The tissue specimens were fixed in 10% neutral buffered formalin for 48 hours. After fixation, tissues were decalcified in Immunocal (Decal, Congers, NY) for 48 hours and washed in phosphate-buffered saline solution. The tissues were then dehydrated and embedded in paraffin. Five micrometer thick sections, including the repaired supraspinatus tendon and greater tuberosity were, cut in the coronal plane and stained with hematoxylin and eosin, safranin-O, and picrosirius red. The qualitative appearance of the repair site was evaluated in a blinded fashion. The greater tuberosity, repaired tendon-bone insertion, and midsubstance of the supraspinatus tendon were examined under light and polarized light microscopy (Eclipse E800; Nikon, Melville, NY). Digital images were then captured using a SPOT RT camera (Diagnostic Instruments, Sterling Heights, MI). Picrosirius red staining and examination with monochromatic polarized microscopy was used for semi-quantitative analysis of collagen organization at the healing enthesis. The sulphonic acid groups on the sirius red molecules bind to the amino groups in collagen in an oriented fashion, resulting in a 7-fold increase in collagen birefringence. By quantifying the birefringence of collagen under polarized light (based on brightness), time-dependent differences in collagen deposition and maturation in the healing tendon could be detected. Measurements were obtained by rotating the polarization plane until maximum brightness was obtained to control for variations in specimen orientation on the slide. To facilitate comparisons between groups, all tissues were embedded and cut in exactly the same orientation, and sections were cut to a uniform thickness. Digital images obtained under 100 $\times$ magnification underwent 8-bit digitization by Image J software (NIH, Bethesda, MD), with a resolution of 640 (horizontal) by 480 (vertical) pixels. The microscope fields were digitized using a computer-video system, yielding an image in which noncollagenous material was dark, (gray level 0) and collagenous material was depicted by gray scales from 1 to 255. The measurement of gray scale was performed at the tendon end adjacent to the insertion site using the Image J Software program (NIH). Five rectangular areas (2500 μm^2 each) were randomly selected, and gray scales were measured (mean $\pm$ SD). The light intensities were measured under exactly the same conditions of illumination for all specimens.

The area of new fibrocartilage formation at the tendon-bone interface was determined by outlining the area of metachromasia with safranin-O staining at 40× magnification. The total area of metachromasia (mean ± SD) for each specimen was measured using computerized imaging software (Image J; NIH).

Immunohistochemistry

For immunohistochemical analysis, serial sections were treated with 3% H₂O₂ to quench endogenous peroxidase activity, and nonspecific antibody binding was blocked with 5% goat serum. One percent BSA/PBS (Bovine Serum Albumin in Phosphate Buffered Saline) was used as a negative secondary reagent control. The primary antibody to advanced glycosylation endproducts (AGEs) (MP Biomedicals, Solon, OH) was applied to separate serial sections for 60 min at 37°C. Bound antibodies were visualized using a goat avidin-biotin peroxidase system with diaminobenzidine (D.A.B.; Dako Corp., Carpinteria, CA) as a substrate. Negative controls were processed in an identical manner except for incubation with bovine serum albumin rather than the primary antibody.

Semi-quantitative assessment of AGE deposition was performed at the healing tendon-bone interface. Sections were graded as 0, 1+, 2+, or 3+ in a blinded fashion by 2 observers (AB, AJF) based on intensity of staining. Distribution of staining in supraspinatus muscle belly, healing enthesis, and/or bone was also noted.

Biomechanical testing

Biomechanical testing of the repaired tendon-bone interface was performed at 1 and 2 weeks postoperatively (n = 8/group). On the day of testing, each shoulder was thawed at room temperature, and the humerus with attached supraspinatus was meticulously dissected under magnification. All dissections were performed in a blinded fashion with respect to treatment group. The dimensions of each supraspinatus tendon were measured using a digital micrometer. The specimen was then placed into a custom-designed uniaxial testing system. The tendon was secured in a screw grip using sandpaper and ethyl cyanoacrylate (Elmer's Products Inc, Columbus, OH). The humerus was secured into a custom-designed vice grip that prevented fracture through the humeral physis. The supraspinatus tendon was secured to a 45-N load cell attached to a linear bearing that allowed alignment of the tendon in the direction of its pull. The humeral jig was secured to the linear stage and grip-to-grip distance was standardized across all specimens. The specimen was pre-loaded to 0.10 N and then loaded to failure at a rate of 14 µm/sec, corresponding to approximately 0.4% strain. The maximum load-to-failure and failure location were recorded. Displacement was measured using a 1-µm resolution micrometer system attached to the linear stage. The linear region of the load-displacement curve was used to calculate the stiffness for each specimen.

Statistical analysis

Statistical analysis was performed using SigmaStat (Systat Software Inc, Chicago, IL) with $P > .05$ defined as significant. Mean serum HbA1c levels, quantitative histomorphometry, AUC, load-to-failure, and stiffness were compared between diabetic and nondiabetic groups using Student *t* test with appropriate post-hoc corrections. Results were reported as mean

values $\pm$ standard error of the mean. The study was powered to detect significant differences in load-to-failure and stiffness between diabetic and control specimens.

Results

Induction of diabetes

Induction of diabetes with streptozotocin (STZ) was both effective and sustained. Area under the curve (AUC) analysis of intraperitoneal glucose tolerance tests (IPGTT) provided a quantitative index of the severity of hyperglycemia. Mean AUC was significantly greater in the diabetic ($21,510 \pm 2826$) compared to control animals ($9,826 \pm 1366$) ($P = .0001$) (Figure 3, A). Furthermore, the AUC in experimental animals pre- and post-STZ injection demonstrated a significant impairment in glycemic control ($10,278 \pm 410$ and $22,421 \pm 3162$, respectively) ($P = .001$). Mean HbA1c level at 2 weeks postoperatively was significantly greater in the experimental compared to control group and consistent with the diabetic phenotype ($10.6 \pm 2.7\%$ vs $6.0 \pm 1.0\%$, respectively) ($P = .0001$) (Figure 3, B).

Gross and histological findings

All rotator cuff repairs were grossly intact at the time of sacrifice. No failed repairs or proximal humeral physeal fractures were encountered; however, the healing enthesis of the diabetic animals was qualitatively different from those of control specimens. The supraspinatus tendon was friable, atrophic, and demonstrated a yellowish discoloration compared to the more robust, healthy tissue observed in control animals (Figure 4). Cross-sectional area of the healing enthesis was 2.37 ± 1.25 and $4.41 \pm 0.57 \text{ mm}^2$ for diabetic and control specimens, respectively.

Quantitative histomorphometry revealed that the diabetic animals had significantly reduced fibrocartilage at the healing enthesis compared to control animals at both 1 and 2 weeks postoperatively ($17254 \pm 14957 \mu\text{m}^2$ vs $61724 \pm 10493 \mu\text{m}^2$ and $25025 \pm 14705 \mu\text{m}^2$ vs $61000 \pm 9175 \mu\text{m}^2$ for 1 and 2 weeks, respectively) ($P < .05$) (Figure 5). Furthermore, evaluation of collagen birefringence with polarized light microscopy revealed significantly less organized collagen at the tendon-bone interface in the diabetic group compared to control animals at both 1 and 2 weeks postoperatively (13.81 ± 2.28 vs 94.30 ± 13.13 and 30.75 ± 4.97 vs 70.82 ± 11.04 for 1 and 2 weeks, respectively) ($P < .05$) (Figure 6).

Immunohistochemistry was performed to evaluate for differences in the quantity and distribution of advanced glycosylation endproducts (AGEs) in the supraspinatus muscle belly and healing enthesis of diabetic and control animals. Significantly greater advanced glycosylation endproducts (AGE) were noted in both the supraspinatus muscle and its repaired enthesis of diabetic compared to control animals ($P < .05$) (Figure 7).

Biomechanical testing

All specimens in both the experimental and euglycemic control group failed at the healing enthesis. The tendon-bone complex of diabetic animals demonstrated a significantly reduced mean load-to-failure compared to control animals at both 1 and 2 weeks postoperatively ($4.79 \pm 1.33\text{N}$ vs $1.60 \pm 1.67\text{N}$ and $13.63 \pm 2.33\text{N}$ vs $6.0 \pm 3.24\text{N}$, respectively) ($P < .05$).

(Figure 8). Stiffness of the construct was also reduced significantly in diabetic compared to control animals at both 1 and 2 weeks postoperatively ($1.56 \pm 1.46\text{N/mm}$ versus $4.00 \pm 2.05\text{N/mm}$ and $6.19 \pm 1.49\text{N/mm}$ and $9.26 \pm 2.28\text{N/mm}$ for 1 and 2 weeks, respectively) ($P < .05$).

Discussion

The purpose of this study was to determine the effect of a diabetic phenotype on tendon-to-bone healing after rotator cuff repair in a rat model. We found that sustained hyperglycemia negatively influenced both the qualitative appearance of the repair, as well as all quantitative histological and biomechanical parameters of healing at the enthesis. Significantly reduced collagen fiber organization and fibrocartilage formation were observed at the tendon-bone interface in diabetic compared to euglycemic animals. These differences were accompanied by a corresponding reduction in the ultimate strength and stiffness of the repair construct. These findings have significant clinical implications for the expected outcomes of all soft tissue repair or reconstructive procedures in diabetic patients with poor glycemic control.

Diabetes mellitus has been shown to alter the properties of bone and impair fracture healing.^{9,21,25,26,28,33,34,40,42,60,61} Using a rat femur fracture model, both Macey et al and Funk et al found that diabetic rats exhibited inferior healing compared to euglycemic animals and had significantly reduced failure torque, failure stress, and material stiffness of the healing fracture.^{26,42} Decreased total collagen content and defects in collagen cross-linking have been observed in the fracture callus of rats with streptozotocin-induced diabetes compared with the callus in controls.^{23,42} Histological studies demonstrate a delay in the appearance and maturation of chondrocytes in the fracture callus of diabetic rats compared to controls after 2–3 weeks of healing.^{28,33,42} In support of these findings, diabetes has been strongly associated with reduced bone mass and delayed fracture healing in humans.^{21,40}

Tendon structure and healing after injury is affected by diabetes as well.^{1,4,8,29,37,38,44,52,53} Ramirez and Raskin described diabetic foot tendinopathy as a syndrome of hardened, stiff plantar tendons in diabetic patients.⁵² Batista et al found that 89% of diabetic patients had disorganized tendon fibers and 76% had calcification within the Achilles tendon.⁸ Grant et al reported the consistent clinical observation of extreme shortening of the Achilles tendon-gastrocnemius-soleus complex with advanced diabetic neuropathy.²⁹ Others have similarly reported structural changes in diabetic tendon, with one study showing diabetic tendon preparations to be 13% shorter than controls in a canine model.³⁷ Chbinou and Frenette investigated the affect of diabetes on tendon healing using a collagenase-induced, Achilles tendonitis rat model.¹⁹ Relative to nondiabetic and insulin-treated diabetic animals, the number of accumulated neutrophils and ED1+ and ED2+ macrophages in diabetic rats decreased by 46%, 43%, and 52%, respectively. The density of newly formed blood vessels also decreased by 35% and 29% in the paratenon and the core of tendon in diabetic animals at days 3 and 7 after injury, and the concentration of proliferative cells decreased by 34% in the paratenon at day 7 after injury in diabetic rats compared to control animals. Their results suggested significant impairment of the inflammatory, angiogenic, and proliferative processes in the diabetic state that could adversely affect tendon healing or remodeling after injury.¹⁹ Other in vitro and in vivo studies have corroborated these findings. Genetically

diabetic mice demonstrate a major reduction of cell proliferation and an aberrant control of apoptotic death at *day 7* during skin repair.²² Furthermore, fibroblasts from nonhealing ulcers in diabetic patients have a reduced proliferative capacity in culture and have abnormal morphological features such as multiple lamellar and vesicular bodies with no microtubular structures.⁴¹

Our study provides novel information on the effect of the diabetic metabolic condition on tendon-to-bone healing. Using a rat model of rotator cuff repair, the results demonstrate that sustained hyperglycemia is associated with a clear impairment of healing at the enthesis, manifest by inferior histological characteristics and decreased load-to-failure and stiffness of the repair construct compared to euglycemic animals. The fundamental cellular and molecular components of the tendon-bone healing process, which are disrupted by diabetes, remain to be defined. However, advanced glycosylation endproducts (AGEs) that result from nonenzymatic modification of tissue proteins by excess physiologic sugars *in vivo* have been shown to play a central role in the pathogenesis of diabetic complications.^{3,11–16,31,44,53,55} They can alter signal transduction pathways, reduce levels of important cytokines, and directly alter protein function in target tissues during the healing process. AGEs can induce abnormal covalent, intermolecular bonds of collagen in the matrix and interfere with cell-matrix interactions as well.^{3,11–16,31,44,53,55} Our immunohistochemical analysis demonstrated significant accumulation of AGEs in both the supraspinatus muscle belly and healing enthesis of diabetic animals, while they were virtually absent in control animals. We hypothesize that these products of advanced protein glycosylation are critical in disrupting the normal tendon-to-bone healing process.

Our results have important clinical implications. Based on our results, the risk of structural failure after soft tissue repair or reconstructive procedures in diabetic patients with poor glycemic control may be higher than in euglycemic patients. Appropriately counseling patients regarding the modest expectations for structural healing in this setting is important. Furthermore, preoperative counseling and strict glycemic control in the perioperative period may be more important than intraoperative, technical factors to achieving successful tendon-to-bone healing after rotator cuff repair or ACL reconstruction. We recommend that patients with diabetes or insulin resistance be routinely screened with HbA1c levels preoperatively, and those with evidence of poor glycemic control (ie, A1c level >7%) seek counsel from an endocrinologist to optimize their oral hypoglycemic and/or insulin regimen. Achieving strict glycemic control may not only improve the biologic milieu for structural healing, but also reduce the risk of infection, stiffness, or other complications after rotator cuff repair. Chen et al compared the results of open repair for full-thickness rotator cuff tears in 30 diabetic patients with those of a matched, nondiabetic population.²⁰ Complications occurred in 5 diabetic patients (17%) with 2 failures (7%) and 3 infections (10%), as compared with 1 failure (3%) and no infections in the control group. Additionally, a statistically significant decrease in range-of-motion was observed throughout a mean follow-up of 34 months.²⁰ A higher incidence of shoulder stiffness has been reported in insulin-dependent diabetic patients, with shoulder stiffness developing in as many as 36% of diabetic patients as compared to 3% of the general population.^{5–7,10,17,43,46–48} This has been shown to be not merely a consequence of the general disability caused by diabetic sequelae but a result of

pathological changes at the biochemical level from hyperglycemia that result in irreversible cross-linking of collagen molecules.^{46–49}

Future studies will focus on defining the underlying cellular and molecular changes that occur during tendon-bone healing in diabetic animals. Understanding these fundamental mechanisms and the role of AGEs will help to develop effective therapeutic interventions that limit or even eliminate the detrimental musculoskeletal sequelae of sustained hyperglycemia. Preliminary studies with pharmacological inhibitors of AGE formation and AGE-specific receptors in diabetic animals have proven to be effective in the prevention of retinopathy, nephropathy, and neuropathy.^{39,45,58,62} Furthermore, the efficacy of both systemic and local insulin therapy in the perioperative period to control excess sugar accumulation at the healing tendon-bone interface is currently being investigated. Prospective clinical trials in euglycemic and diabetic patients are also necessary to correlate the metabolic state with both structural and functional outcomes after rotator cuff surgery.

Our study is not without limitations. The rat model of streptozotocin-induced diabetes is the most widely used experimental model in the study of diabetes.^{23,28,35,42,59,60} Nonetheless, STZ-induced diabetes in animals is not representative of the most commonly encountered insulin resistant, diabetic patient that is encountered in the orthopaedic clinic. Streptozotocin poisons the pancreatic islet β cells directly, creating a phenotype more analogous to type I diabetes than the more common insulin-resistant, type II diabetes in humans. In this regard, we recognize that our pilot study examines a “worst case” scenario of acute postoperative hyperglycemia. Furthermore, a STZ-induced diabetic model does not accurately reproduce many of the chronic sequelae of longstanding disease. We also evaluated animals at only 1 and 2 weeks postoperatively in order to maintain good general health of the animals, thus further limiting our insight into the chronic effects of longstanding diabetes. Longer timepoints may have provided additional information regarding the effect of chronic hyperglycemia on the tendon and healing enthesis. It is also important to note that the STZ induced a number metabolic alterations throughout the body that led to a less robust condition compared to euglycemic animals. These generalized effects may have been of tantamount importance to the acute hyperglycemia in explaining our findings. Our study may also have benefited from the additional measurement of changes in inflammatory markers and cell populations to help define the underlying mechanism of disrupted tendonbone healing.

Conclusion

Diabetes mellitus impairs tendon-bone healing after rotator cuff repair in this rodent model. Sustained hyperglycemia negatively influenced all histological and biomechanical parameters of healing at the repaired enthesis. These findings have significant clinical implications for the expected outcomes of soft tissue repair or reconstructive procedures in diabetic patients with poor glycemic control. Local and systemic control of hyperglycemia in the perioperative period may be of paramount importance to intraoperative, technical factors in achieving a healed rotator cuff repair in diabetic patients.

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Figure 1.

Study design. *SS*, supraspinatus; *STZ*, streptozotocin; *IPGTT*, Intraperitoneal Glucose Tolerance Test; *IHC*, immunohistochemistry, HBA1c – hemoglobin A1c.

Figure 2.

Acute rotator cuff repair model. (A) The acromioclavicular joint is divided and the deltoid is split in-line with its fibers to allow atraumatic exposure and isolation of the supraspinatus tendon. (B) A modified Mason-Allen stitch is placed with a 4-0 Ethibond suture in the distal supraspinatus tendon. (C) The tendon is divided directly off the greater tuberosity and the footprint is defined and gently debrided of any residual debris. (D) The sutures are passed via the crossed transosseous bone tunnels and tied over the humeral metaphysis to achieve an anatomic rotator cuff repair.

Figure 3.

Induction of diabetes. **(A)** Area under the curve (AUC) analysis of intraperitoneal glucose tolerance tests (IPGTT) provided a quantitative index of the severity of hyperglycemia. Mean AUC was significantly greater in the diabetic ($21,510 \pm 2826$) compared to control animals ($9,826 \pm 1366$) ($*P = .0001$). **(B)** Mean HbA1c level at 2 weeks postoperatively was significantly greater in the experimental compared to control group and consistent with the diabetic phenotype ($10.6 \pm 2.7\%$ vs $6.0 \pm 1.0\%$, respectively) ($*P = .0001$).

Figure 4. Gross appearance of tendon. At the time of sacrifice, the supraspinatus tendon (*red arrow*) was friable, atrophic, and demonstrated a yellowish discoloration in diabetic animals (**A**) compared to a more robust, healthy tendon tissue observed in control animals (**B**).

Figure 5.

Fibrocartilage formation. (A) Control enthesis (2 weeks, 40× magnification). (B) Diabetic enthesis (2 weeks, 40× magnification). (C) Quantitative histomorphometry revealed that the diabetic animals had significantly reduced fibrocartilage at the healing enthesis compared to control animals at both 1 and 2 weeks postoperatively ($17254 \pm 14957 \mu\text{m}^2$ versus $61724 \pm 10493 \mu\text{m}^2$ and $25025 \pm 14705 \mu\text{m}^2$ vs $61000 \pm 9175 \mu\text{m}^2$ for 1 and 2 weeks, respectively) (* $P < .05$).

Figure 6.

Collagen birefringence. (A) Control enthesis (2 weeks, 100× magnification). (B) Diabetic enthesis (2 weeks, 40× magnification). (C) Evaluation of collagen birefringence with polarized light microscopy revealed significantly less organized collagen at the tendon-bone interface in the diabetic group compared to control animals at both 1 and 2 weeks postoperatively (13.81 ± 2.28 vs 94.30 ± 13.13 and 30.75 ± 4.97 vs 70.82 ± 11.04 for 1 and 2 weeks, respectively) (* $P < .05$).

Figure 7. Advanced glycosylation endproducts

Immunohistochemistry was performed to evaluate for differences in the quantity and distribution of advanced glycosylation endproducts (AGEs) in the supraspinatus muscle belly and healing enthesis of (A) control (2 weeks, 100× magnification) and (B) diabetic animals (2 weeks, 100× magnification). Significantly greater advanced glycosylation endproducts (AGE) were noted in both the supraspinatus muscle and its repaired enthesis of diabetic compared to control animals ($P < .05$).

Figure 8.

Ultimate load-to-failure. The tendon-bone complex of diabetic animals demonstrated a significantly reduced mean load-to-failure compared to control animals at both 1 and 2 weeks postoperatively ($4.79 \pm 1.33\text{N}$ vs $1.60 \pm 1.67\text{N}$ and $13.63 \pm 2.33\text{N}$ vs $6.0 \pm 3.24\text{N}$ respectively) (* $P < .05$).