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Basic Science

The impact of type 2 diabetes on bone metabolism and growth after spinal fusion

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Abstract

BACKGROUND CONTEXT: Some clinical reports suggest diabetes may have a negative effect on spinal fusion outcomes, although no conclusive experimental research has been conducted to investigate the causality, impact, and inherent risks of this growing patient population.

PURPOSE: To analyze the hypothesis that type 2 diabetes (T2DM) inhibits the formation of a solid bony union after spinal fusion surgery by altering the local microenvironment at the fusion site through a reduction in growth factors critical for bone formation.

STUDY DESIGN/SETTING: In vivo rodent model of type 2 diabetes.

METHODS: Twenty control (Sprague Dawley, SD) and 30 diabetic (Zucker Diabetic Sprague Dawley, ZDSD) rats underwent posterolateral and laminar fusion surgery using a tailbone autograft implanted onto the L4/L5 transverse processes. A subset of animals was sacrificed 1-week postsurgery for growth factor analysis. Remaining rats were sacrificed 3-month postsurgery for fusion evaluation via manual palpation, micro-CT, and histology.

RESULTS: There was no significant difference in the manual palpation fusion rate between ZDSD rats and SD control rats. Growth factor assay of fusion site explants at early sacrifice demonstrated PDGF was upregulated in the ZDSD rats. TGFB, IGF, and VEGF were not statistically different between groups. Bone mineral density as determined by micro-CT was significantly lower in ZDSD rats compared to SD controls and was a significant function of HbA1c.

CONCLUSIONS: Data generated in this in vivo rat model of T2DM demonstrate that the metabolic dysregulation associated with the diabetic condition negatively impacts the quality and density of the formed fusion mass. Increased measures of diabetic status, as determined by blood glucose and HbA1c, were correlated with decreased quality of formed fusion, highlighting the importance of diabetic status monitoring and regulation to bone health, particularly during bone healing.

CLINICAL RELEVANCE: T2DM rats demonstrated increased rates of infection, metabolic dysregulation, and a reduction in spinal fusion consolidation. Clinicians should consider these negative effects during preoperative care and treatment of this growing patient population.

Keywords: Diabetes; Fusion; Growth factors; Lumbar spine; Micro-CT; Rat model

FDA device/drug disclosure: Not applicable.

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Introduction

Diabetes has reached epidemic proportions, affecting over 100 million people in the United States alone, the majority of which are noninsulin dependent, or type 2 diabetes mellitus (T2DM) [1]. An alarming 25% of US residents age 65 and older have been diagnosed with diabetes and approximately 50% are considered prediabetic [1]. These numbers are projected to increase as trends in glycemia and diabetes continue to rise with our aging population, sedentary lifestyle, and western diet [2]. Thus, health care professionals, including spine surgeons, will need to be prepared to effectively treat and manage this chronic and debilitating disease that has deleterious effects on numerous systems throughout the body.

Diabetes results in atherosclerotic macrovascular disease, as well as neuropathy, retinopathy, and nephropathy from microvascular disease. More recently, it has been recognized to adversely affect bone health [3]. Patients with both type 1 (T1DM) and type 2 diabetes mellitus (T2DM) have an increased risk of osteoporotic fractures, although patients with T1DM have significantly lower bone mineral density (BMD) values compared to normal subjects, whereas T2DM patients are reported as having normal or even slightly higher BMD values, suggesting a different mechanism of increased fragility [4]. Initially, this was attributed to trauma risk from impaired vision and peripheral neuropathy, but recent studies suggest that alterations in bone microstructure that are not detected by traditional dual-energy x-ray absorptiometry (DXA)-derived BMD may be causative [5,6]. Research into the underlying pathophysiology of these material and structural abnormalities has just begun and the precise mechanism by which T2DM impairs skeletal health remains largely unexplained [7].

T2DM is more frequent among older adults and therefore coexists with degenerative spinal conditions that may require spinal fusion procedures. Fusion in patients with diabetes has been associated with increased rates of pseudarthrosis, but a direct causal relationship between the two has not been established. Current literature on diabetes and spine fusion consists of retrospective studies that either have a limited heterogeneous sample size or do not control for the numerous confounding variables typical in diabetic patients, such as higher body mass index, hypertension, stroke, and cardiovascular disease, and making the exact contribution of diabetes difficult to quantify [8–12]. Therefore, the following study employed a rat model that reflects the human pathophysiology of diabetes to test the hypothesis that T2DM inhibits the formation of a solid bony union after spinal fusion surgery by altering the local microenvironment at the fusion site through a reduction in growth factors critical for bone formation.

Material and methods

Animals

The following study relied on the recently developed Zucker Diabetic Sprague-Dawley (ZDSD) rat model to investigate the effects of T2DM on spinal fusion. The ZDSD rat is a cross between the homozygous lean Zucker Diabetic Fatty rats (ZDFfa/+) and a substrain of the Sprague-Dawley rat that has been selectively bred for diet-induced obesity [13]. The result is a more translational model compared to the previously relied upon ZDFfa/fa rats, which become diabetic due to a leptin receptor mutation. Conversely, ZDSD rats gradually develop diabetes with age and can be induced with a high-fat diet, mimicking the pathogenesis of human T2DM [14].

Study design

Approval for this study was obtained from the Institutional Animal Care and Use Committee (IACUC#005364) at Cedars-Sinai Medical Center, Los Angeles, California. Twenty control (Sprague Dawley, SD, Charles River, Wilmington, MA) and 30 diabetic (Zucker Diabetic Sprague Dawley, ZDSD, PreClinOmics, Indianapolis, IN) rats were obtained at 5 weeks of age. Nonfasting blood glucose (BG) and weight were monitored on ZDSD rats bi-weekly and monthly on SD controls. At 16 weeks of age, all ZDSD rats were switched from a standard diet (Purina 5008, LabDiet, St. Louis, MO) to a high-fat diet (5SCA, TestDiet, St. Louis, MO) for a period of 2 weeks to induce the diabetic condition. Glycated hemoglobin (HbA1c) was measured at surgery and sacrifice on all animals. At 20 weeks of age, all animals underwent posterolateral and laminar fusion surgery using a tailbone autograft implanted onto the L4/L5 transverse processes (TPs), as previously described [15]. Six controls and 10 ZDSD rats were sacrificed 1-week postsurgery for growth factor analysis. Remaining rats were sacrificed 3-month postsurgery (32 weeks of age) for fusion evaluation via manual palpation, micro-CT, Faxitron X-ray, and histology. Rats were housed in a vivarium and allowed to drink and feed ad libitum.

Surgical procedure

Rats were anesthetized (5% Isoflurane) and maintained under continuous anesthesia (2% Isoflurane). Posterolateral interlaminar fusion with tail vertebral bone grafting was performed at L4/L5 by the same surgeon blinded to the experimental group. The lumbar spine and tail were sterilized and prepped for surgery. The tail was amputated via caudectomy and incision was closed by suture. Six tail vertebrae were extracted and soft tissues removed, four were morselized using a rongeur, and two were ground using a dental bone mill for use as autograft. The morselized and milled bone was mixed together. While the bone graft was prepared, a surgical incision was made above the spinous processes of L4 with further dissection by blunt exposure followed by thorough cleaning of the transverse processes (TPs), facets, and laminar surfaces, and removal of the L4 spinous process. A high-speed burr was used to decorticate the TPs, facets, and lamina just until punctate bleeding was observed. Prepared autograft was implanted and tightly packed into the

posterolateral gutter spanning the TPs and across the posterior laminar space. The wound was sutured closed in two layers. Postoperatively, buprenorphine and lactated ringers were administered subcutaneously, antibiotics were not used. Rats were individually caged after surgery. Health status was evaluated daily throughout the follow-up period. Rats were allowed to drink and feed ad libitum and sacrificed at 12 weeks postoperatively via CO_2. Lumbar spine segments were harvested en bloc for fusion evaluation.

Fusion assessment—manual palpation

Gross explanted motion segments were visualized and subjected to manual palpation performed by two researchers blinded to experimental groups. Manual palpation included bending in sagittal and coronal planes. No motion at the segment was determined as fusion success, whereas any motion was deemed fusion failure.

Fusion assessment—high-resolution radiographs and micro-CT

Radiographic fusion was assessed via high-resolution radiographic images (LX-60, Faxitron X-Ray, LLC Lincolnshire, IL) taken at monthly intervals postoperatively under inhalation anesthesia.

Ex vivo micro-computed tomography (micro-CT, vivaCT 40, Scanco USA Inc.) was performed on explanted spines to determine the microarchitectural properties of the fusion mass. The fused segment was contoured and sectioned from soft tissue using a binary thresholding procedure. Direct three-dimensional morphometry was used to determine: volume of mineralized bone tissue (BV, mm^3), bone volume fraction (BV/TV), BMD(HA/cm^3), trabecular thickness, number, and connectivity density, based on calibration with a commercially available micro-CT phantom containing hydroxyapatite (HA).

Protein extraction and growth factor quantification

Spinal fusion sites were explanted on days 7 postsurgery, cleared from any surrounding soft tissue and stored at −80° C. For protein extraction, each sample was homogenized with scissors in RIPA lysis buffer supplemented with Halt protease inhibitor cocktail (Thermo Scientific, USA). After 15 minutes of incubation on ice, samples were centrifuged at 13,000 g for 15 minutes, purified, and the protein containing supernatant was collected. A BCA protein assay (Thermo Fisher Scientific, USA) was performed to determine the amount of total protein. Samples were then diluted to achieve an equal total protein of 9 mg/mL per sample. Growth factor analysis was performed using Quantikine ELISA kits for Mouse/Rat PDGF-BB, Mouse/Rat IGF-I, Rat VEGF, and Mouse/Rat/Porcine/Canine TGF-beta 1 (R&D systems, USA). Samples were diluted as follows: 1:10 for PDGF-AB, 1:200 for IGF-I, 1:100 for VEGF, and 1:2 for TGF-beta 1.

Histologic analysis

Two spines from each experimental group were reserved for histologic analysis. Fusion segments were fixed in 10% buffered neutral formalin, decalcified, and processed for standard paraffin embedding. Serial 5 μm sections were cut along the sagittal plane in the center of the fusion mass and stained with hematoxylin and eosin (H&E) and Masson's Trichrome. Photomicrographs were obtained (Leica model EZ4D stereomicroscope Leico Microsystems GmbH, Wetzlar, Germany) and evaluated for endochondral ossification.

Statistical analysis

Data were analyzed using SAS software (SAS, 9.3 Copyright (c) 2002 to 2010 by SAS Institute Inc., Cary, NC). Fishers Exact test (Freq procedure, "Exact") was applied to frequency data of manual palpation fusion (fused=1, not fused=0, dependent variable) to test for differences between experimental groups (ZDSD vs. SD). Manual palpation fusion as a function of BG and HbA1c along with rat strain group were modeled and tested via Logistic Regression. Analysis of variance (GLM; MIXED) was applied to dependent to test for overall rat strain group effect (ZDSD vs. SD). A repeated measures ANOVA (GLM, MIXED) was used to assess the relationships among blood measures of diabetes (BG and HbA1c) over time as a function of rat strain. Tukey test was utilized to determine significance for intergroup and pairwise comparisons. Interrelationships among BG, HbA1c, and rat weight were evaluated with Pearson correlation. Regression was employed to evaluate and compare the slopes between strains (ZDSD vs. SD) of BMD as a function of HbA1c ANOVA (GLM) and Logistic Regression was applied for multiple variable analyses to evaluate the influence of covariates.

Results

Overall mortality rate was 16% (8/50). Seven of the eight deaths occurred in the diabetic ZDSD group within the first week postsurgery and were presumed due to poor wound healing and postsurgical site infection, although postmortem diagnosis was not performed, and one died due to diabetic complications at 7 weeks postoperatively (ZDSD: 8/20 (40%) vs. SD: 0/14 (0%), $p<.01$). All data are presented for long-term cohort (ZDSD: n=12 and SD: n=14, Table 1), except for growth factor data which is reported for short-term cohort of rats (ZDSD: n=10 and SD: n=6, Table 2). Patterns of weight gain were significantly different between the two rat strains with less weight gain over the follow-up period in the ZDSD rats (average beta change in weight, 5.2±2.3) compared to SD control rats (11.9±2.1), $p<.0001$.

Diabetic status (BG and HbA1c)

Intake (baseline) blood glucose (BG) was not significantly different between ZDSD (129.2±10.6 mg/dL)

Table 1
Physical and biomechanical parameters for ZDSD diabetic rats vs. SD control rats (long-term cohort)

Measurement	ZDSD n=12	SD n=14	p Value
Characteristics of rats			
Intake weight (g) (prior to dietary manipulation)	184.2 ± 25.5	190.5 ± 8.8	NS
At surgery weight (g) (1 mo after controlled diet)	498.1 ± 56.5	607.8 ± 54.9	<.0001
Sacrifice weight (g)	461.0 ± 82.2	651.0 ± 63.2	<.0001
Intake blood glucose (BG, mg/dL) (prior to dietary manipulation)	129.2 ± 10.6	134.4 ± 14.9	NS
At surgery BG (mg/dL) (1 mo after controlled diet)	331.5 ± 180.6	179.8 ± 14.0	<.0001
Sacrifice BG (mg/dL)	438.9 ± 171.7	223.1 ± 143.9	<.001
BG>=250 mg/dL % (#/total)	66.7% (8/12)	21.4% (3/14)	<.05
Sacrifice HbA1c, >=6% (#/total)	66.7% (7/11)	0 (0/14)	<.001
Micro-CT			
BMD (HA/cm^3)	620.7 ± 27.4	640.5 ± 16.0	<.05
Connectivity density (mm^3)	5.5 ± 1.3	5.0 ± 0.9	NS
Bone volume fraction (BV/TV)	0.7 ± 0.1	0.6 ± 0.1	NS
Bone volume (BV, mm^3)	1029.0 ± 176.4	1045.2 ± 235.0	NS
Total volume (TV, mm^3)	1563.8 ± 280.0	1664.1±328.3	NS
Structural model index (SMI)	−5.3 ± 1.9	−3.8±1.5	<.05
Trabecular number (Tb.N)	1.9±0.2	1.9 ± 0.2	NS
Trabecular thickness (Tb.Th)	0.5 ± 0.1	0.5±0.1	NS
Trabecular separation (Tb.Sp)	0.7 ± 0.1	0.7 ± 0.1	NS

NS, not significant.
Mean±SD.
Values given as mean±SD across rat strain groups (ZDSD vs. SD).
SAS-t-test procedure, Satterthwaite utilized for unequal variance.

and SD control rats (134.4±14.9 mg/dL). BG was significantly higher at both the surgical and sacrifice time points in the ZDSD rats compared to SD control rats, p<0.0001, Figure 1, Table 1. Average HbA1c was correspondingly and significantly increased for ZDSD compared to SD controls at the sacrifice time point (7.78± 2.7% vs. 4.5±0.4 %, p<0.01, Figure 2). The ZDSD rat strain did not decisively yield a diabetic condition. Eight of the 12 (66.7%) ZDSD rats demonstrated HBA1c values that are indicative of diabetes (HBA1c value greater than 6%) at sacrifice. None (0/14) of the SD control rats had HBA1c levels above 6%.

Manual palpation

Manual palpation fusion rates were 75% (9/12; CI: 27%−73%) for ZDSD rats compared to 85.7% (12/14; CI: 57%−98%) for control SD rats, NS, Figure 3. Manual palpation rates were not significantly correlated to BG or HbA1c values.

Table 2
Growth factors (short-term cohort)

Measurement	ZDSD (n=10)	SD (n=6)	p Value
Weight at Intake	197.9 ± 10.9	202.2 ± 9.6	NS
Weight at presurgery	508.1 ± 26.8	598.9 ± 55.1	<.01
Weight at surgery	489.3 ± 33.7	610.9 ± 74.2	<.01
Weight at sacrifice	469.6 ± 33.2	603.2 ± 78.8	<.01
Intake blood glucose (BG, mg/dL) (prior to dietary manipulation)	133.5 ± 11.4	133.6 ± 16.5	NS
At surgery BG (mg/dL) (1 mo after controlled diet)	329.4 ± 166.1	170.2 ± 31.4	<.05
Sacrifice BG (mg/dL)	409.2 ± 180.9	167.4 ± 51.1	<.01
BG>=250 (mg/dL)% (#/total)	80% (8/10)	0% (0/6)	<.01
Growth factors			
IGF (ng/mg)	5.4 ± 1.3	5.9 ± 1.8	NS
PDGF (ng/mg)	14.9 ± 9.9	5.8 ± 2.8	<.05
TGFβ (ng/mg)	25.9 ± 23.4	10.7 ± 8.0	=.08
VEGF (ng/mg)	948.1 ± 679.6	541.6 ± 108.9	NS

NS, not significant.
Mean±SD, or % (#/total in group).
Values given as mean±SD across rat strain groups (ZDSD vs. SD).
SAS-t-test procedure, Satterthwaite utilized for unequal variance.

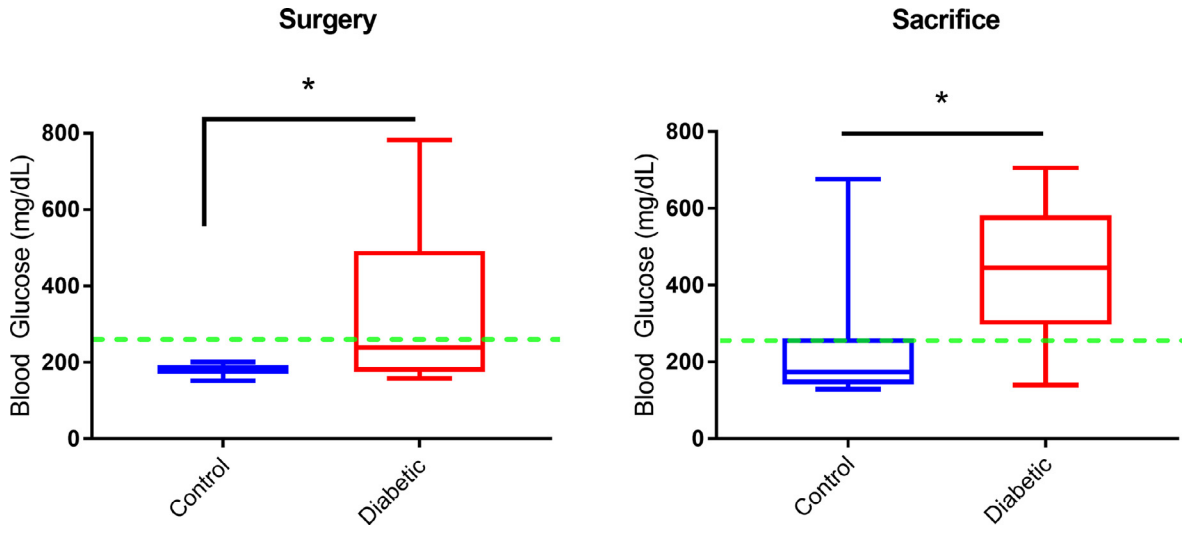

Fig. 1. Blood glucose levels at both surgery (left) and sacrifice (right) were significantly higher in diabetic rats compared to controls, p<.0001. The dotted line indicates the cutoff for diabetic status (BG>250 mmol/dL).

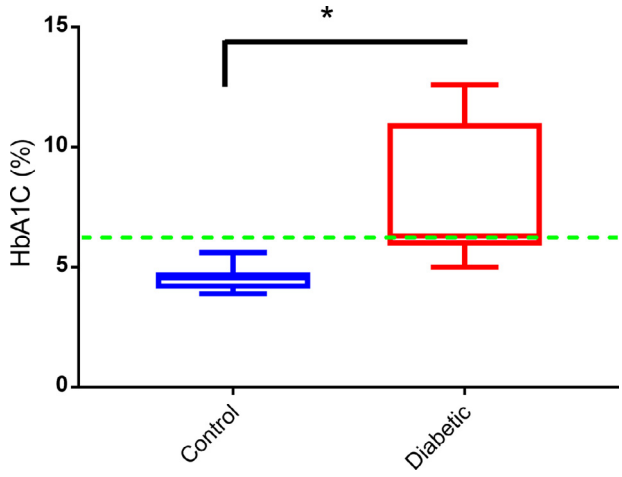

Fig. 2. HbA1c levels were significantly higher in diabetic rats than control at sacrifice, p<.0001. The dotted line indicates the cutoff for diabetic status (HbA1c>6%).

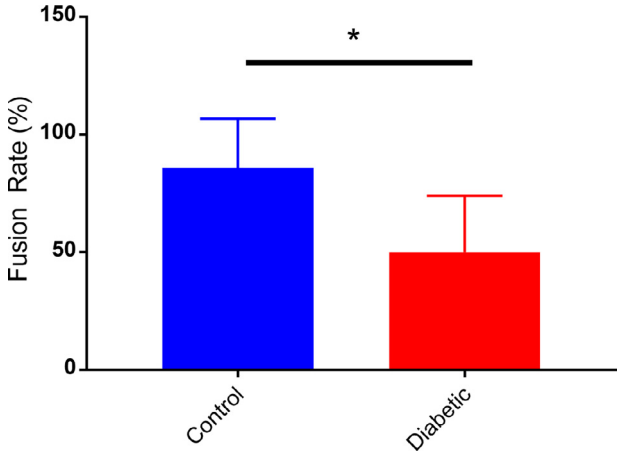

Fig. 3. Manual palpation results plotted with 95% confidence intervals, demonstrating no significant decrease in fusion rate in the ZDSD rats.

Micro-CT measures

Micro-computed tomographic scans confirmed bone volume in specimens that were fused. Mean BMD (HA/cm^3) as determined by micro-CT was significantly lower in ZDSD rats (620.7±27.4 HA/cm^3) compared to SD controls (640.5±16.0 HA/cm^3), and was significantly negatively correlated to HbA1c when data were pooled among all rats (R^2=0.49, r=−0.70, p<.0001), and in both the ZDSD diabetic rats (R^2=0.56, r=−0.75, p<.01) and SD controls (R^2=0.36, r=−0.60, p<.05) when analyzed separately, Figure 4. The structure model index (SMI, was significantly lower ZDSD (−5.3±1.9) than SD (−3.8±1.5). Trabecular connectivity density was not significantly different between ZDSD and control SD rats, but was significantly positively correlated to HbA1c values when data were pooled (R^2=0.26, r=0.51, p<.01), and when separately analyzed (ZDSD: R^2=0.56, r=0.74, p<.01; SD controls: R^2=0.31, r=0.56, p<.05, Figure 5). Average bone volume (BV), bone volume fraction (BV/TV), trabecular number, thickness, and separation were not significantly different between groups. Trabecular number and BV were significantly intra-correlated, but only in the SD control group (R^2=0.46, r=0.68, p<.01).

Growth factor analysis

Eighty percent (8/10) of the ZDSD rats sacrificed early (1 week postoperatively) for growth factor analysis demonstrated an overt diabetic status (BG>250 mg/dL), whereas none (0/6) of the SD control rats had diabetes (p<.01). Growth factor analysis of the fusion bed demonstrated significantly increased levels of PDGF for ZDSD rats (14.94± 9.87 pg/mL; CI: 7.88−22.0) compared to SD

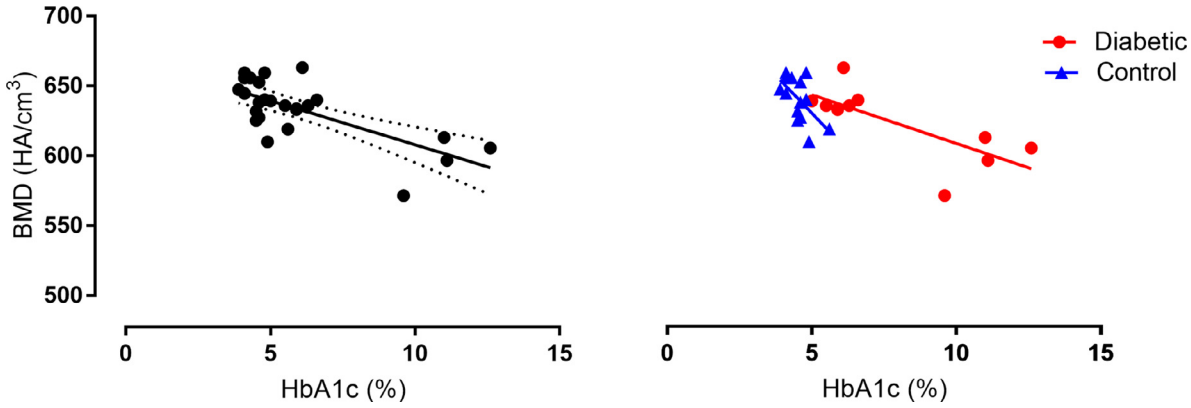

Fig. 4. Regression analysis demonstrates a significant negative correlation between BMD and HBA1C levels overall (left), and separately (right) for the ZDSD (r=−0.75, R^2=0.56, slope=−6.97, p<.01) and SD control (r=−0.59, R^2=0.35, slope=−22.14) rats, p<.05, with a significant difference in slopes between the two strains (ZDSD vs. SD control), p<.05.

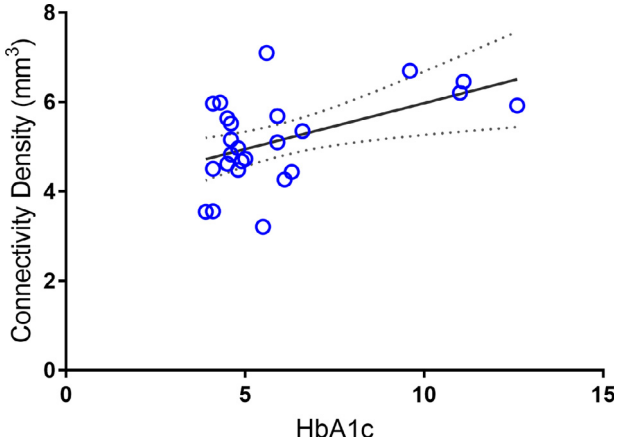

Fig. 5. Connectivity density was significantly correlated to HbA1c levels, R^2=0.26, slope=0.31, p<.01.

controls (5.80±2.86 pg/mL; CI: 2.80−8.80), p<.05, Figure 6. ZDSD rats also demonstrated a marginal increase in TGF-β (25.89±23.42 pg/mL; CI: 9.13−42.64) compared to SD controls (10.73± 8.02 pg/mL; CI: 2.31−19.15), p=.08. VEGF and IGF did not demonstrate any differences between the two groups (ZDSD vs. SD for VEGF: 948.1± 679.6 vs. 541.6± 108.9, p=.09 and IGF: 5.37±1.34 vs. 5.86±1.84, NS). None of the growth factors analyzed were correlated to diabetes status as determined by HbA1c, yet BG was significantly correlated to TGF-β (R^2=0.43, r=−0.66, p<.05) and marginally correlated to PDGF (r=−0.54, p=.10), Figure 7.

Histology

Histologic assessment of the two specimens reserved for histology showed a greater amount of fibrocartilage and newly formed bone surrounding the transverse processes in the SD control specimen. Comparatively, the diabetic sample had more fibrous tissues surrounding the bony fragments and less bone in the interspace between the two transverse processes.

Discussion

This study demonstrates that type 2 diabetes mellitus reduces the formation of a solid bony union after spinal fusion surgery in a rat model that closely mimics the condition of T2DM in humans. Specifically, our results indicate that diabetes reduces the quality of the formed fusion mass. BMD was significantly reduced in the diabetic group and was significantly negatively correlated to HbA1c levels in both the diabetic and control groups, highlighting the importance of blood glucose control during bone healing. In addition, a greater infection rate was observed in the ZDSD rat compared to SD controls, demonstrating T2DM as a pertinent risk factor for post-operative surgical site infection as seen clinically [16,17].

In contrast to our hypothesis and literature demonstrating lower levels of PDGF-BB in wounds of diabetic patients and animal models [18], we detected increased PDGF levels at the fusion site of diabetic rats compared to non-diabetic controls. This result may be due to differences in the type of injury, model system, time of tissue harvest, or the subtype of PDGF investigated. For example, in a skin wound model PDGF levels were reduced in streptozotocin-induced diabetic Sprague Dawley rats on day 5 post injury, but elevated at a later time point [19]. In homozygous genetically diabetic (db/db) mice, PDGF-A levels were elevated until day three, but reduced compared to normal mice after five days post-injury, whereas PDGF-BB was not altered between diabetic mice and controls [20]. Furthermore, the increased PDGF levels in combination with a poor healing outcome in ZDSD rats might be a result of growth factor resistance, caused by a shift in cell signaling [21−23]. For example, Tchaikovski et al. demonstrated that activation of VEGFR-1−related signaling pathways and desensitization of VEGFR-1 responses resulted in resistance to VEGF-A stimulation in human individuals with DM, which correlated with impaired chemotactic response of monocytes and impaired collateral growth [23]. Further research is needed to elucidate the cellular mechanisms resulting in the observed elevated PDGF levels in poorly-

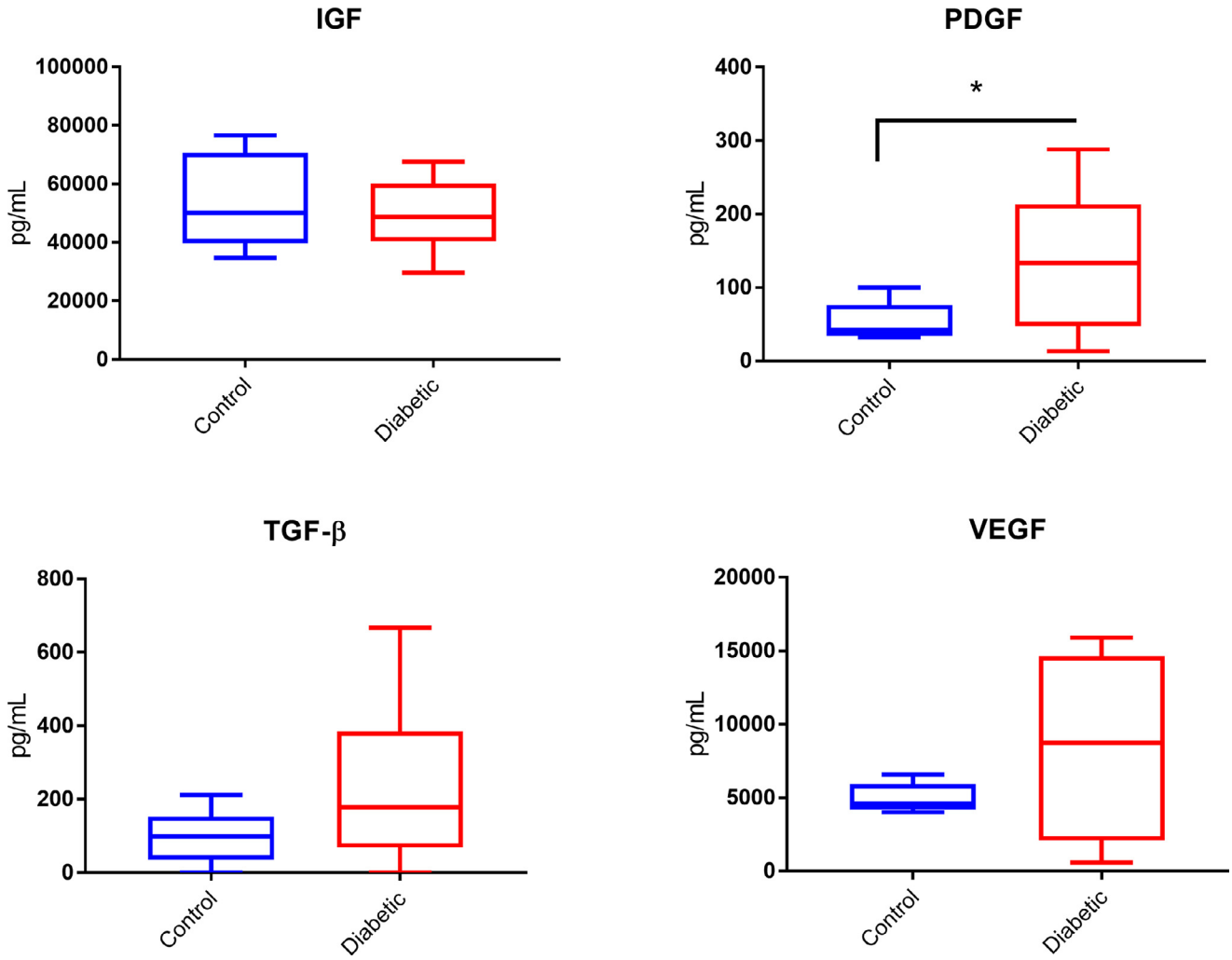

Fig. 6. Growth factor analysis at 1 week postoperatively demonstrated higher levels of average PDGF, TGF-β, and VEGF in the ZDSD rats, but only PDGF was significantly difference between the two strains ($p<.05$).

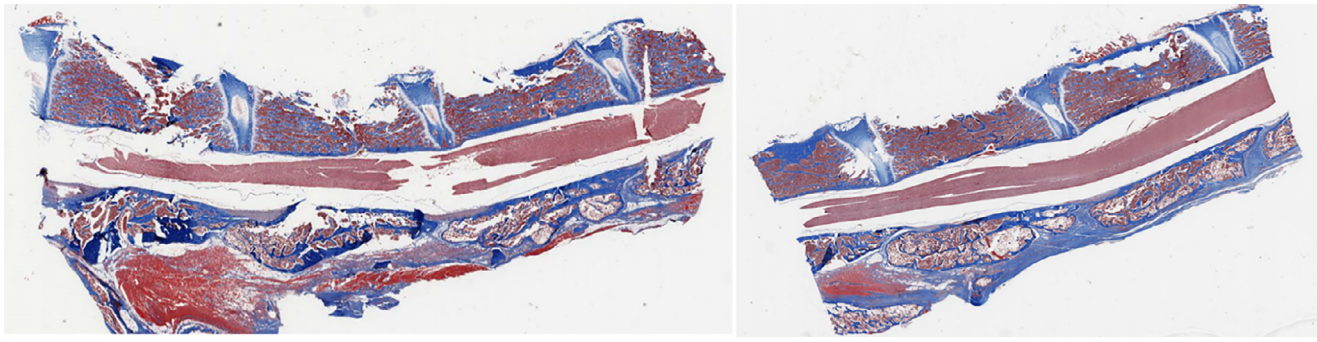

Fig. 7. Control (left) and diabetic (right) histologic images stained with Mason's Trichrome.

regenerating ZDSD rats. However, it is likely that the observed dysregulation of growth factor levels among diabetic ultimately affected cell signaling, inflammatory gene regulation, and/or extracellular matrix composition, which in turn led to a reduction in spinal fusion consolidation.

Clinical and experimental studies on diabetes mellitus have demonstrated alterations in bone and mineral metabolism [3]. Both T1DM and T2DM patients have an increased risk for osteoporotic fractures compared to controls. The increase in fragility of bone in T1DM is through a reduction

in BMD, whereas T2DM patients present normal or even slightly higher values of BMD [24]. Based on these differences and the fact that T1DM is characterized by lack of insulin and T2DM by hyperinsulinemia, it has been theorized that insulin itself has an anabolic effect on bone [25]. In support of this theory, Koerner et al. demonstrated increased rates of fusion through the application of a local time-released insulin implant applied to the fusion bed in a nondiabetic rat posterolateral lumbar spinal fusion model [26]. They found a significant increase in IGF-I in the fusion bed of the insulin treatment group compared to normal rats, suggesting that deficiencies of IGF-I could contribute to skeletal abnormalities observed in T1DM. In our study using a T2DM rat model, we did not observe any measurable differences in IGF between diabetic and nondiabetic rats. Other researchers have investigated whether hyperglycemia has an adverse effect on bone metabolism. Keat and Khan exposed blood- and bone marrow-derived endothelial progenitor cells and mesenchymal progenitor cells to either normal or high glucose levels and assayed for cellular activity and molecular changes [27]. They found that hyperglycemia skewed the differentiation of bone marrow-derived mesenchymal precursor cells (MPCs) toward the adipocyte lineage while suppressing other mesenchymal lineages, including chondrocytes and osteocytes. Hyperglycemia may also exert its detrimental effects on bone cells through increased oxidative stress [28] and elevated levels of advanced glycation end products (AGEs) in the bone matrix, such as pentosidine [6]. Unlike normal crosslinks in collagen that are responsible for bone toughness and scaffolding properties, AGE crosslinks lead to brittle bone that is less able to deform before fracturing [29]. In addition, AGEs may reduce bone formation by interfering with osteoblast development and function [30–32].

In the present study, trabecular connectivity density was not different between diabetics and controls, but was positively correlated to HbA1c. While increased connectivity density is typically a measure of bone health [33], previous studies have shown increased connectivity in both diabetic [34,35] and osteopenic subjects [36]. This increase in connections is presumably due to an increase in resorptive activity that leads to greater porosity and brittle bone. These changes in skeletal microarchitecture are a critical determinant of bone strength and may in part explain the paradoxical increase in fracture with normal or elevated BMD seen in diabetic patients.

The ZDSD rat model used in this study has increasingly become a common animal model of diet-induced T2DM, as it better replicates the cascade of metabolic dysregulation typical in late onset T2DM [14]. Previous work investigating the effects of T2DM on the musculoskeletal system in the ZDSD rat model have demonstrated reduced BMD, strength, and fracture toughness compared to controls [34,35,37]. In addition, Fields et al. reported an increase in disc degeneration in T2DM using a similar polygenic rat model [38]. The present study is the first to analyze the effect of T2DM on the unique healing process that occurs during spine fusion [39,40] using this well-validated model. Based on our experience, we found several limitations with the ZDSD rat that are worth noting. First, there was a relatively high rate of postsurgical deaths, which is likely due to their poor wound healing [41]. In addition, not all ZDSD rats developed diabetes and there was a considerable amount of variation among their BG and HbA1c values. Finally, the ZDSD rats lost body weight with the progression of diabetes, which presented another confounding variable.

In summary, this is the first basic science study to quantify specific changes in bone healing after spinal fusion in a translational model of T2DM that allows control for other surgical and patient-related risk factors. The information generated in this study demonstrates the complexity of this disease and the need for further clinical research to optimize the treatment and improve the quality of care for this growing patient population.

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