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INCREASING DURATION OF TYPE 1 DIABETES PERTURBS THE STRENGTH-STRUCTURE RELATIONSHIP AND INCREASES BRITTLENESS OF BONE

Jeffry S. Nyman^{1,2,3}, Jesse L. Even², Chan-Hee Jo⁴, Erik G. Herbert⁵, Matthew R. Murry², Gael E. Cockrell⁴, Elizabeth C. Wahl⁴, R. Clay Bunn⁴, Charles K. Lumpkin Jr.⁴, John L. Fowlkes⁴, and Kathryn M. Thrailkill⁴

¹ Department of Veterans Affairs, Tennessee Valley Healthcare System, Nashville, TN 27212

² Department of Orthopaedics & Rehabilitation, Vanderbilt University Medical Center, Nashville, TN 37232

³ Vanderbilt Center for Bone Biology, Vanderbilt University Medical Center, Nashville, TN 37232

⁴ Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, Little Rock, AR 72202

⁵ Department of Materials Science & Engineering, University of Tennessee, Knoxville, Knoxville, TN 37996

Abstract

Type 1 diabetes (T1DM) increases the likelihood of a fracture. Despite serious complications in the healing of fractures among those with diabetes, the underlying causes are not delineated for the effect of diabetes on the fracture resistance of bone. Therefore, in a mouse model of T1DM, we have investigated the possibility that a prolonged state of diabetes perturbs the relationship between bone strength and structure (i.e., affects tissue properties). At 10, 15, and 18 weeks following injection of streptozotocin to induce diabetes, diabetic male mice and age-matched controls were examined for measures of skeletal integrity. We assessed 1) the moment of inertia (I_{MIN}) of the cortical bone within diaphysis, trabecular bone architecture of the metaphysis, and mineralization density of the tissue (TMD) for each compartment of the femur by microcomputed tomography and 2) biomechanical properties by three point bending test (femur) and nanoindentation (tibia). In the metaphysis, a significant decrease in trabecular bone volume fraction and trabecular TMD was apparent after 10 weeks of diabetes. For cortical bone, type 1 diabetes was associated with decreased cortical TMD, I_{MIN} , rigidity, and peak moment as well as a lack of normal age-related increases in the biomechanical properties. However, there were only modest differences in material properties between diabetic and normal mice at both whole bone and tissue-levels. As the duration of diabetes increased, bone toughness decreased relative to control. If the sole effect of diabetes on bone strength was due to a reduction in bone size, then I_{MIN} would be the only significant variable explaining the variance in the maximum moment. However, general linear modeling found that the relationship between peak moment and I_{MIN} depended on whether the bone was from a diabetic mouse and the duration of diabetes. Thus, these

Correspondence: Jeffry S. Nyman, Vanderbilt Orthopaedic Institute, Medical Center East, South Tower, Suite 4200, Nashville, TN 37232, jeffry.s.nyman@vanderbilt.edu, (615) 936-6296.

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findings suggest that the elevated fracture risk among diabetics is impacted by complex changes in tissue properties that ultimately reduce the fracture resistance of bone.

Keywords

Diabetes; Bone Toughness; Bone Density; Biomechanics; Nanoindentation

1. INTRODUCTION

As is the case with aging and osteoporosis, type 1 diabetes mellitus (T1DM) increases the risk of experiencing a bone fracture [1–6]. The costs associated with these fractures should increase in the near future as the aging population grows [7] and as the prevalence of diabetes increases [8] including the incidence of T1DM [9]. Moreover, an estimated 30% of patients treated for hip fracture die within a year [10,11]. Despite the growing economic burden of bone fractures to society as well as the negative impact they have on morbidity and mortality, the causes for the disproportionate increase in fracture risk amongst those with diabetes is not well understood.

Outside of a direct effect of diabetes on bone, this increased fracture risk could be partially related to complications of diabetes, such as poor vision that could increase the propensity to fall [12,13], especially when diabetes is poorly controlled [14]. Another possibility is that diabetes causes low bone mineral density (BMD), a determinant of bone strength. Indeed, areal BMD as measured by dual energy X-ray absorptiometry (DXA) has been found to be lower in subjects with T1DM than in age-matched control subjects [15]. Moreover, in statistical models accounting for gender, race, menopausal status, and disease duration in a large cohort of patients, low BMD was associated with poor glycemic control [16].

Low BMD is not, however, the only factor that can decrease the resistance of bone to fracture. Elevated fracture risk among those with diabetes still persists even after adjusting for hip BMD [17], and the observed low BMD in T1DM does not completely explain the high fracture risk in this population [18]. Given the potential anabolic effects of insulin on bone [19] and the role of glucose in the formation of advanced glycation end-products (AGEs) [20], diabetes likely affects both bone structure and tissue integrity of bone. Bone structure is a known determinant of bone strength [21], and age-related increases in non-enzymatic collagen crosslinking have been associated with a decrease in the toughness of cortical bone (i.e., energy required to break a uniform specimen of cadaveric bone tissue) [22]. Thus, there is a supposition that diabetic bone is more fragile than non-diabetic bone for a given areal BMD, and thus diabetes affects the quality of bone, not just the amount and architecture of bone [4].

In the two studies assessing the mechanical properties of bone from humans with diabetes, material properties such as ultimate strength and fracture toughness of bone were not different between middle aged diabetics and elderly non-diabetics, suggesting that diabetes could deleteriously impact the fracture resistance of bone [23,24]. Nonetheless, without proper controls and limited samples, there is a paucity of information on the mechanism by which diabetes affects the fracture resistance of bone.

There are a number of studies using rodent models of T1DM documenting the effects of the diabetic condition (e.g., insulinopenia and hyperglycemia) induced by the injection of streptozotocin (STZ) on the biomechanical properties of bone (Table 1). Consistently, the loss of insulin production causes a reduction in the structural strength of rat long bones [25–29]. That is, less force or torque is required to break a bone from a rodent with diabetes than

from a normal rodent because the diabetic condition lowers the minimum or polar moment of inertia, a structural parameter characterizing the distribution of tissue about the axis of bending or twisting, respectively. Inconsistently, STZ-induced diabetes has been found to decrease the degree of mineralization of cortical bone in female Wistar rats [30] but not affect the mineralization density of the cortical tissue (Ct.TMD) in male Fischer 344 and Sprague-Dawley rats [29]. Likewise, STZ-induced diabetes has not always been found to affect material strength or toughness (Table 1). Interestingly, rat models of type 2 diabetes mellitus (T2DM), in which insulin resistance and hyperglycemia develop spontaneously or on a high fat diet, exhibit many of the same effects on bone as T1DM models (Table 1). Moreover, in these rat models of T2DM, the decrease in Ct.TMD did not necessarily translate into lower estimates of elastic modulus and material strength as assessed by three point bend testing [31–33].

Thus, there is still an open question of whether the effect of diabetes on fracture risk is primarily due to changes in bone structure or changes in the inherent material properties of bone tissue (i.e., bone quality), or both. In addition, to our knowledge, there is only one report [34] in the literature that investigated the effects of diabetes on the biomechanical properties of mouse bone. This is a rather significant lapse because genetically altered mouse models are more readily available than genetically altered rat models as research tools to identify important pathways in the pathogenesis of diabetes affecting the fracture resistance of bone. With this in mind, we have comprehensively examined the effects of T1DM on compositional, architectural, structural, and biomechanical properties of bone. We hypothesized that a prolonged state of T1DM not only affects bone structure and architecture, but perturbs the relationship between strength and structure (i.e., affects composition), reduces tissue-level hardness, and increases the brittleness of bone. Such effects would indicate that diabetic bone is less resistant to failure and at greater risk of fracture because of multiple deficiencies in structure and tissue quality.

2. MATERIALS AND METHODS

2.1. Model of Type 1 Diabetes (T1DM)

Male DBA/2J mice received STZ injections (40 mg per kg of body weight) for 5 days starting at 11 weeks of age. These T1DM mice were sacrificed at 21 weeks of age or 10 weeks of diabetes (n=11), 26 weeks of age or 15 weeks of diabetes (n=10), and 29 weeks of age or 18 weeks of diabetes (n=6). A few diabetic animals expired during the extended course of the study, as has been reported for the STZ-treated DBA/2J strain [35]; and, these mice were eliminated from the final analyses. Diabetes was confirmed by measurement of blood glucose using a glucometer (Accucheck Aviva meter, Roche). Placebo treated control (CON) mice were of the same genetic background and were age- and gender-matched at the time of sacrifice: 21 weeks (n=12), 26 weeks (n=10), and 29 weeks (n=13). The blood glucose levels at the time of sacrifice were higher in the diabetic than in the control mice as determined by two-sided t-test for each time point: 1) 501 ± 134 vs. 120 ± 22 mg/dl ($p < 0.001$), 2) 562 ± 52 vs. 136 ± 30 mg/dl ($p < 0.001$) at 15 weeks, and 3) 581 ± 18 mg/dl vs. 110 ± 18 mg/dl ($p < 0.001$), respectively. Following sacrifice, the femur and tibia were harvested and stored at -80°C until analyzed. All procedures were performed in accordance to a protocol approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

2.2. Micro-Computed Tomography (μCT) analysis

To assess the effect of diabetes on the trabecular architecture and cortical bone structure, each femur was immersed in phosphate buffered saline (PBS) and the long axis of the bone was aligned with the scanning axis of the $\mu\text{CT}40$ (Scanco Medical; Brüttisellen,

Switzerland). Cross-sectional images of the regions of interest were acquired with an isotropic voxel size of 12 μm , at energy settings of 55 kVp and 145 mA, with 500 projections per 180° rotation, and an integration time of 300 ms. The regions of interest were the cortex of each femur at the mid-point (2 mm in length) and the trabeculae within the distal metaphysis of the femur (0.24 mm 1.20 mm below the growth plate). To select the trabecular compartment, the Scanco auto-contouring script based on a published, dual threshold algorithm [36] was used to identify the endocortical surface. The parameters of the algorithm did not vary across image stacks, and visual inspections were routinely performed to confirm fidelity of the contours within the endosteum. Bone tissue was segmented from air or soft tissue such that each region of interest had unique thresholding parameters (trabecular: 597.7 mgHA/cm³ and cortical: 1009 mgHA/cm³) and image filtration parameters (Gaussian support of 2 and sigma of 0.8) that were held constant across the experimental groups. The density calibration was checked by weekly scans of a hydroxyapatite phantom and did not vary during the course of the study. A beam hardening correction developed by the manufacturer specifically for bone applications was used in all scans, and partial volume effects were suppressed by peeling the first voxel from all surfaces following segmentation of the bone [37].

The Scanco evaluation software was used to quantify structural parameters of the mid-shaft: mean cortical thickness (Ct.Th), average cross-sectional area (Ct.Ar), and the average moment of inertia with respect to the orientation of three-point bending test (I_{MIN}). Standard architectural characteristics of trabecular bone within the metaphysis were measured including: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and connectivity density (Conn.D). The average volumetric mineral density of the mineralized tissue in mgHA/cm³ was also measured for both the trabecular (Tb.TMD) and cortical (Ct.TMD) compartments.

2.3. Flexural testing

To determine the differences in biomechanical properties between the experimental groups, a pre-load (1 N) first held each hydrated femur in place on the lower support points of a three point bending fixture with the anterior side down (*i.e.*, bending about the medial-lateral plane). The span between the lower supports was 7 or 8 mm, and the load rate was 3.0 mm/min. Then, forces from a 100 N load cell (Honeywell; Morristown, NJ) and displacements from the LVDT (Dynamight 8841, Instron, Canton, OH) were recorded at 50 Hz during a monotonic load-to-failure test. As reported by Silva et al. [29], biomechanical properties included: the rigidity, the peak moment (force*span/4), and the normalized post-yield deflection (PYD). Yielding occurred when the secant stiffness was 15% less than the initial stiffness [38]; and the post-yield toughness was the area under the moment vs. normalized displacement (12*deflection/span²) curve from yielding to fracture divided by 2 Area (*i.e.*, accounting for the structural contribution to force). Material properties of modulus and strength of the cortex were also estimated using standard beam theory [39]. The previously described μCT scans provided the moment of inertia and the distance between the neutral axis of bending and the outermost point in the anterior-posterior direction (C_{min}).

2.4. Nanoindentation

Bone samples were: 1) embedded in plastic following dehydration in a series of ethanol concentrations [40]; 2) sectioned at the diaphysis using a diamond-embedded, circular saw (660, South Bay Technology, Inc., Torrance, CA), 3) ground through successive grits of silicon carbide paper (800 through 4000), then 4) polished on a synthetic cloth with alumina slurry (9 μm to 0.05 μm). Once the specimen of dry bone was mounted within the nanoindentation system (XP, MTS; Eden Prairie, MN), a Berkovich diamond tip (a pyramidal-shaped indenter with a center-line-to-face angle of 65.3 degrees) was driven into

the surface of the bone section using the following scheme: 1) load at a constant loading rate chosen to reach a maximum depth of 1 m in 30 s, 2) hold at $P_{\max}$ for 30 s (in order to minimize the effects of viscoelastic deformation), 3) unload at the same rate used in the first loading step to 10% of $P_{\max}$, 4) hold the indenter on the surface for 60 s in order to establish thermal drift, and 5) completely remove the indenter from the bone surface. From the resulting force-displacement curve, the elastic modulus (E) and hardness (H) of the tissue at the point of indentation (0.25 m resolution) was calculated following the method of Oliver and Pharr [41]. This requires an initial calibration procedure using fused silica in order to establish the relationship between depth of indent and contact area (A_c) of the tip. Twelve indents were attempted throughout the cortex of available tibia, and those specimens in which there were less than 8 successful indents were removed from the analysis (CON: $n = 12, 6, 9$ and T1DM: $n = 10, 11, 5$ for 10 wk., 15 wk., and 18 wk., respectively). The average value of the indents per specimen (vast majority of which included 12 indents) was used in the statistical analysis.

2.5. Statistical Analysis

A two way analysis of variance (ANOVA) determined whether differences in each bone property existed between normal and the diabetic condition and among the three time points (duration of T1DM or age). In the event that normality and/or variance assumptions of the ANOVA were violated, the p-value was determined using a non-parametric analysis (Mann-Whitney) for each comparison (T1DM vs. CON within each duration or age group as well as 10 wk. vs. 15 wk. and vs. 10 wk. and 18 wk. within each treatment group). Statistical significance was based on the Holm-Sidak criteria for multiple comparisons versus a control (overall significance level = 0.05). If an aging trend was apparent in a given property, a post-hoc comparison was done between 26 wk. and 29 wk. control mice. In order to determine whether the strength-structure relationship was different between the control and diabetic femurs, we developed a multiple linear regression model in which group, duration, and moment of inertia were the explanatory variables (Table 3). Group (Gp) was a categorical variable: diabetic or not diabetic (CON); Duration (Wk) was also a categorical variable: 10, 15, or 18; and moment of inertia ($I_{\min}$) was a continuous variable given by μCT . Moreover, all potential interaction terms were included in the model if they were statistically significant ($p < 0.05$). To examine how the strength-structure relationship may change with duration of diabetes, post-hoc regression models analyzed the contribution of explanatory variables to peak moment within each time point (10, 15, 18 wk as well as the combination of 15 + 18 wk). Initially, the models included Gp, $I_{\min}$, and $Gp \times I_{\min}$. The term with the highest p-value was then removed and the regression analysis performed again. The adjusted R^2 values are reported for all models (Table 3 and Table 4). In all regression models, a y-intercept term was included even if its p-value was greater than 0.05. Lastly, we reported the partial eta squared (η^2) values for each term in the model. This is an index of the strength of association between an independent variable and peak moment excluding variance produced by other terms.

3. RESULTS

3.1. Diabetes reduces cortical bone structure, trabecular bone volume, and mineralization density

Ten weeks of the diabetic condition was sufficient to cause a reduction in cortical thickness, moment of inertia, and cross-sectional area of the femur diaphysis (Table 2). These changes did not get progressively worse with an increase in the duration of diabetes. By 15 weeks following STZ-induced hyperglycemia, the femurs were also 5% shorter than femurs from non-diabetic mice as measured by calipers (Table 2). The mice with diabetes also weighed less than the control mice at all time points (Table 2).

With regards to the trabecular bone of the metaphysis, the T1DM mice had lower bone volume fraction, which was due to a diabetes-related decrease in trabecular thickness and number. The reduction in trabecular bone volume occurred by 10 weeks of diabetes with no progressive decreases as the duration of diabetes increased. BV/TV also did not change with an increase in the age of the control mice (Table 2; $p=0.756$ for 26 wk. vs. 29 wk.), even though the trabeculae in the metaphysis of the 29 wk. old control mice were thicker than the trabeculae of the 21 wk. old control mice (Table 2; $p=0.877$ for 26 wk. vs. 29 wk.). On the other hand, there were fewer trabeculae at 29 wk. or 26 wk. of age than at 21 wk. of age (Table 2; $p=0.473$ for 26 wk. vs. 29 wk.), irrespective of the whether the mice were diabetic or normal. Diabetes did not have other effects on trabecular architecture, with no significant difference in connectivity density existing between CON and T1DM at any of the duration or age groups. Conn.D did decrease as the non-diabetic mice aged from 21 wk. to 29 wk. (Table 2), but with no difference between 26 wk. and 29 wk. old control mice. Connectivity density characterizes the lack of fenestrations and is related to trabecular bone strength.

In both the cortical and trabecular compartments, there was a reduction in mineralization density of the tissue with diabetes at all durations (Table 2). Tb.TMD was lower at 26 weeks and 29 weeks of age compared to the 21 week old mice, whether the mice were diabetic or non-diabetic. Diabetes did not affect these age-related trends in Tb.TMD. Ct.TMD was greater at 26 weeks and 29 weeks of age compared to the 21 week old mice, though these differences were only statistically significant within the diabetic mice.

3.2. Diabetes primarily reduces structural strength and long-term diabetes makes bone brittle

As might be expected from the lower moment of inertia (I_{MIN}), the diabetic bones had lower rigidity and peak moment (structural strength) than did the non-diabetic bones (Table 2). These changes occurred by week 10 following the induction of diabetes, and the diabetic condition arrested the age-related increase in structural rigidity and strength. For the present sample size, there were not however any significant differences in the estimated bending modulus or yield strength between control and diabetic bones (Fig. 1). The inability to observe a difference is surprising since the diaphysis of the mice with insulin deficiency had lower Ct.TMD than that of the normal mice. Lastly, as diabetes progressed from 10 wk. to 18 wk., the femur became more brittle. This was evident in the reduction of post-yield deflection (Table 2) and the post-yield toughness that occurred in the diabetic bone after 18 wk. of T1DM (Fig. 1).

3.3. Diabetes modestly affected the tissue-level properties as determined by nanoindentation

Biomechanical tests of whole bones do not provide a measurement of material properties that are entirely independent of bone structure and microscopic porosity [42]. Therefore, to determine the effect of diabetes on tissue-level properties (independent of size), the tibia were prepared for nanoindentation. At the tissue level, the diabetic condition affected the modest age-related increase in nanoindentation modulus (Fig. 2; $p=0.001$ for 26 wk. vs. 29 wk.) and hardness (Fig. 2; $p=0.126$ for 26 wk. vs. 29 wk.) such that these properties were less in bone tissue from diabetic mice than in normal tissue at the 18 wk. time point (Fig. 2). There was also a difference between the two groups in hardness at the 15 wk. time point.

3.4. Diabetes perturbed the strength-structure relationship

Another way to understand whether diabetes affects material behavior is to examine the relationship between structural strength (i.e., peak moment) and the moment of inertia. If the sole effect of diabetes on bone strength is due to a reduction in bone size, then the moment of inertia would be the only significant variable explaining the variance in the peak moment.

In order to determine whether this strength-structure relationship was different between the CON and T1DM femurs, we developed a statistical model in which Group (Gp) (diabetic or not diabetic) and time point (Wk) were used as categorical variables (10, 15, or 18), and moment of inertia (I_{MIN}) was used as a continuous variable. The multiple linear regression indicated that nearly 72.5% of the variance in peak moment was explained when the diabetic condition and the duration of diabetes along with the moment of inertia were the explanatory variables (Table 3). In contrast, a linear regression analysis of all data points revealed I_{MIN} and y-intercept term alone explained 45.1% of the variance in peak moment (y-intercept: $p=0.038$ and I_{MIN} : $p<0.0001$).

Significant interactions indicated that the contribution of moment of inertia to peak bending moment depended on whether the bone was diabetic or not diabetic as well as the duration of the diabetic state. To further explore the strength-structure relationship, sequential multiple regression models were performed within each time point (Table 4). At 10 weeks of diabetes (21 weeks of age), the explanation of the variance in peak moment did not require indicating whether the bone was from a diabetic or normal mouse (Table 4). However, at 15 weeks of diabetes, the contribution of I_{MIN} to peak moment depended on whether the bone was from a T1DM mouse. This was not strictly true for 18 week time point in that the interaction term (Gp x I_{MIN}) had a p -value = 0.06. With the removal of the interaction term, the presence of diabetes (Gp) was not a significant explanatory variable (Table 4). Since the number of specimens per time point within each Gp was limited, we performed the multiple regression analysis on the combined 15 wk and 18 wk data. In doing so, the presence of diabetes was a significant factor in explaining the variance of peak moment (graphically depicted in Fig. 3).

4. DISCUSSION

While type 1 diabetes mellitus is typically manageable with insulin, diet, and exercise, many complications are still associated with this diabetic condition. A bone fracture is one such complication that is not readily anticipated and can severely hinder quality of life. Moreover, fracture healing in diabetic patients may be suboptimal because diabetes delays the regenerative processes of connective tissues, including bone [43]. Despite the need to protect persons with diabetes from fractures, there is a paucity of mechanistic information on how insulinopenia and hyperglycemia affect the fracture resistance of bone. The present study provides three possibilities for the effect of diabetes on bone: 1) deterioration of bone structure/architecture, 2) reduction in the capacity of the bone tissue to dissipate energy (i.e., toughness), and 3) perturbation in the relationship between whole bone strength and bone structure.

The deleterious effect of diabetes on bone structure is well established in rodent models of the disease (Table 1). As for humans, there is some evidence that diabetics may have smaller bones. Using peripheral quantitative CT (pQCT), Saha et al. [44] found that the radius and tibia of T1DM adolescents, especially boys, had a smaller cross-sectional area (CSA) than did these bones of appropriately matched non-diabetic adolescents. However, another pQCT study by Bechtold et al. [45] indicated that as T1DM adolescents reached 14 yr. and 15 yr. of age, their cortical CSA normalized, becoming equivalent to the cortical CSA of non-diabetics at the same age. In recent a report, the deletion of insulin receptor signaling in the osteoblasts of mice resulted in reduced postnatal bone accrual [46], further supporting the notion that systemic insulin deficiency thus the lack of insulin signaling in osteoblasts can affect bone formation and architecture, thereby increasing risk of fracture.

Given a sufficient duration and intensity of diabetes, the present study suggests that diabetic bone becomes more brittle or less tough. A decrease in bone toughness certainly occurs with

aging in humans [47], and diabetes with hyperglycemia likely accelerates the accumulation of AGEs in bone tissue, thereby prematurely causing brittleness. As animals age, AGEs accumulate in a variety of connective tissues [48,49] including bone [50–52] even though bone undergoes turnover. Also known as non-enzymatic, glycation-mediated (NEG) collagen crosslinks, AGE concentration in bone was greater for STZ-induced T1DM rats than for normal rats [29,51,53]. While an increase in NEG crosslinks remains to be established as a contributor to bone brittleness in the mouse model of type 1 diabetes, these crosslinks are thought to affect bone quality, leading to an increase in fracture risk [54]. While more work is needed to strongly establish the causal relationship between AGEs and bone toughness with respect to diabetes and aging, reduced fracture risk among diabetics is quite possibly a problem of inadequate bone toughness as this study suggests.

For the first time, we show a difference in the strength-structure relationship between normal and diabetic bone. As an engineering principle, the distribution of tissue about the neutral axis of bending contributes to the stiffness and peak force experienced by a bone under loading. Differences in this direct relationship between two groups indicate that compositional differences exist in the tissue properties between the groups. In the present study, the slope of the strength-structure relationship was only statistically significant when the regression was performed on combined data from the control and diabetic mice at 21 wk. of age (Table 4 and Fig. 3A). As the diabetic condition progresses from 10 wk. to 18 wk, the slope and the y-intercept of the relationship (Fig. 3D) can be different between T1DM (thin regression line) and CON (solid regression line). This suggests a complex change in the compositional nature of the tissue. Given the observed reduction in Ct.TMD for diabetic bone and the potential for time-dependent alterations to the organic matrix of diabetic bone, this change in the strength-structure relationship for T1DM bone is likely due to a reduced mineralization density interacting with overly-crosslinked collagen fibrils. That is, the contribution of the diabetes-related decrease in mineralization density to material strength is offset by the diabetes-related increase in non-enzymatic collagen crosslinking that may actually strengthen the organic matrix. This would explain why diabetes did not affect the whole bone bending modulus and strength (Fig. 1) despite the fact that diabetic bone had a lower Ct.TMD than did control bone (Table 2).

There are several conditions of the present mouse model of T1DM that may have indirect effects on the reported differences in bone properties. The diabetic mice weigh less than control mice, and an indirect consequence via mechanical adaptation of this difference could be a reduction in bone size and structural strength. However, insulin has recently been shown to have direct anabolic effects on bone accrual [46,55]. Furthermore, a difference in body mass would not necessarily explain the difference in the strength-structure relationship between T1DM and CON as the drop in body mass stabilizes in diabetic mice by 10 weeks following injection of STZ (Table 2). The long-term metabolic effects of diabetes prevented some mice from surviving 18 weeks of diabetes as observed by others for DBA/2J mice [56], so metabolic decompensation could indirectly affect either strength-structure relationship or bone toughness. De-confounding such indirect effects would require complex nutritional and/or genetically modified models in which the effects of blood glucose, insulin production and action, weight loss, lypolysis, acidosis, etc. are independently controlled.

In conclusion, T1DM was associated with complex changes in mouse bone. Early on in the disease process, there was diminished cortical structure and trabecular micro-architecture translating into weakened diabetic bone relative to the non-diabetic bone. As the duration of the diabetic condition increases, further change in these parameters did not occur, suggesting a limit to which insulin-deficiency affects the bone architecture. On the other hand, progression of the diabetic state continuously associates with the post-yield toughness of bone, which may reflect accumulation of AGEs in skeletal tissues. Taken together, the

outcomes presented in these studies suggests that the elevated fracture risk among diabetics is impacted by progressive and complex alterations in tissue properties that over time reduce bone toughness and increase the risk to fracture.

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Figure 1.

Effect of duration of diabetes on estimated material properties of whole bone. Graphs are mean standard deviation with indicating a difference between control and diabetes within duration ($p < 0.05$) and # indicating a difference between 10 wk. and 15 wk. or between 10 wk. and 18 wk. within group ($p < 0.05$). Diabetes prevented the age-related increase in modulus (A), in yield strength (B), and in peak strength. As the duration of diabetes increased, the bone became brittle (D).

Figure 2.
Effect of diabetes on tissue-level properties. At the longest duration, diabetes had modest effects on both the nanoindentation modulus (A) and hardness (B) of bone.

Figure 3.

Differences in the strength-structure relationship of bone. At the 10 wk. time point, peak moment was directly related to moment of inertia when combining both groups (A). At the 15 wk., this direct relationship was only statistically significant for T1DM, not CON (B); but, at 18 wk., peak moment was directly related to moment of inertia for each group (C). When combining the 15 wk. and 18 wk. time points, the slopes of this direct relationship were different between T1DM and CON (D) indicating different changes to the compositional properties were occurring with the progression of diabetes relative to normal aging. R^2 values are given, all regression lines have a $p < 0.05$, and dashed indicates a regression through an individual group whereas as solid indicates regression through both groups.

Table 1

Summary of the recent literature showing percent differences in cortical bone properties, as determined by three point bend testing and DXA or μ CT analysis of the femur, between diabetic and non-diabetic rodents. Negative value indicates the property decreased with diabetes.

Model	Strain of rat	Age at start of diabetes (wk)	Gender	Duration of diabetes or age (wk)	Peak force or moment (%)	Peak strength (%)	Energy-to-failure (%)	Ct.TMD or aBMD (%)	Ref
T1DM	Fischer 344	13-14	Male	12	-21	-7.9	N.S. ^a	N.S.	[29]
T1DM	Sprague-Dawley	13-14	Male	12	-28	N.S.	N.S.	N.S.	[29]
T1DM ^d	Sprague-Dawley	11	Male	5	-26	N.R. ^b	-16	-9.1	[28]
T1DM	Wistar	39-42	Female	12	N.R.	-16 ^c	N.S.	-16	[30]
T1DM	Sprague-Dawley	10	Male	7	-37	N.R.	-34	N.R.	[27]
T2DM	Zucker diabetic fatty	21	Male	12	-30	N.S.	-36	-2.8	[32]
T2DM	Zucker diabetic Sprague-Dawley	15-21	Male	12-18	-19	N.S.	N.S.	-1.6	[32]
T2DM	Goto-Kakizaki vs. Wistar		Male	26	-22	N.R.	-30	-24	[33]
T2DM	Zucker diabetic fatty	7	Male	13	-19	N.S.	N.R.	-5	[31]
T2DM	WBN/Kob vs Wistar	48-52	Male	22-26	-28	N.R.	-46	N.S.	[57]

^aN.S.: Not statistically significant;

^bN.R.: Not reported;

^cMoment of inertia was calculated assuming elliptical cross-section, not derived by the μ CT analysis; and

^dAlloxan induced the diabetic condition whereas all other T1DM studies used streptozotocin.

Table 2

Structural and biomechanical properties (mean standard deviation) of cortical bone and architectural properties of trabecular bone for each experimental group that include different durations of T1DM and age-matched controls.

Property	Unit	10 wks. (21 wks. of age)		15 wks. (26 wks. of age)		18 weeks (29 wks. of age)	
		CON	T1DM	CON	T1DM	CON	T1DM
Body mass	(g)	27.4 ± 1.8	21.1 3.6*	30.6 ± 2.3 [#]	23.3 ± 2.4*	28.4 ± 4.4	22.8 ± 2.8*
Femur diaphysis							
Length	(mm)	14.6 ± 0.4	14.5 ± 0.6	14.2 ± 0.2	13.6 ± 0.6*, [#]	14.2 ± 0.4 [#]	13.4 ± 0.6*, [#]
Ct.Th	(mm)	0.235 ± 0.016	0.194 ± 0.021*	0.223 ± 0.009	0.196 ± 0.019*	0.242 ± 0.019	0.183 ± 0.020*
I _{MIN}	(mm ⁴)	0.072 ± 0.009	0.056 ± 0.011*	0.065 ± 0.007	0.054 ± 0.008*	0.079 ± 0.012	0.054 ± 0.004*
Ct.Ar	(mm ²)	0.829 ± 0.097	0.666 ± 0.098*	0.729 ± 0.037 [#]	0.617 ± 0.069*	0.842 ± 0.105	0.600 ± 0.069*
Ct.TMD	(mgHA/cm ³)	1540 ± 31	1498 ± 16*	1549 ± 13	1531 ± 16*, [#]	1556 ± 13	1532 ± 13*, [#]
Rigidity	(kN/mm ²)	0.985 ± 0.061	0.781 ± 0.151*	1.137 ± 0.085 [#]	0.893 ± 0.162*	1.241 ± 0.139 [#]	0.909 ± 0.210*
Peak moment	(N-mm)	31.2 ± 3.4	25.6 ± 3.8*	38.0 ± 2.6 [#]	29.9 ± 6.4*	42.1 ± 5.6 [#]	28.0 ± 9.0*
PYD	(1/mm)	0.032 ± 0.013	0.028 ± 0.014	0.025 ± 0.012	0.018 ± 0.015	0.023 ± 0.013	0.009 ± 0.008*, [#]
Femur metaphysis							
BV/TV	(%)	9.1 ± 1.5	6.2 ± 1.4*	8.6 ± 1.3	6.4 ± 2.4*	8.8 ± 3.1	4.9 ± 3.2*
Tb.Th	(mm)	0.047 ± 0.002	0.036 ± 0.004*	0.049 ± 0.002 [#]	0.041 ± 0.004*	0.051 ± 0.007 [#]	0.036 ± 0.004*
Tb.N	(1/mm)	4.00 ± 0.27	3.70 ± 0.16*	3.58 ± 0.23 [#]	3.39 ± 0.38 [#]	3.48 ± 0.31 [#]	3.15 ± 0.53*, [#]
Conn.D	(1/mm ³)	71.5 ± 19.7	58.4 ± 17.9	55.4 ± 15.4	55.8 ± 24.9	47.5 ± 16.5 [#]	37.1 ± 31.2
Tb.TMD	(mgHA/cm ³)	1079 ± 24	983 ± 19*	986 ± 24 [#]	907 ± 39*, [#]	1007 ± 42 [#]	914 ± 50*, [#]

* p<0.05 for CON vs. T1DM within each duration group

[#] p<0.05 for 10 wk. vs. 15 wk. or 10 wk. vs. 18 wk. within each diabetes group

Generated from a multiple linear regression analysis of the strength-structure relationship, the p-value and *partial eta squared value* are given for the primary study variables, including interaction terms, that best explained the variance (adjusted R²) in peak moment.

Table 3

Property	Intercept	Gp	Wk	I _{MIN}	Gp x I _{MIN}	Gp x Wk	Wk x I _{MIN}	Gp x Wk x I _{MIN}	R ² _{adj} (%)
Peak moment	0.098	0.001	0.016	<0.001	0.002	0.029	0.012	0.026	72.5
	0.055	0.209	0.156	0.306	0.178	0.135	0.166	0.139	

Gp is control or diabetic; Wk is the time point; and I_{MIN} is the moment of inertia as determined by μ CT.

Table 4

From a post-hoc analysis of the strength-structure relationship within each time point or combination of time points, p-values are given for the 2 primary study variables chosen to explain the variance (adjusted R^2) in peak moment.

Property	Within week	Intercept	Gp	I _{MIN}	Gp x I _{MIN}	R ² _{adj} (%)
Peak moment	10	0.002	0.748	0.023	0.987 ^a	48.4
		0.001	0.110	0.019	N.I.	51.1
		0.002	N.I.	<0.001	N.I.	46.7
	15	0.004	0.009	0.185 ^b	0.019	60.2
		0.269	0.048	0.004	0.060	71.5
	18	0.363	0.363	0.006	N.I.	65.9
	15+18	0.707	N.I.	<0.001	N.I.	66.1
		0.142	0.034	<0.0001	0.078	64.8
		0.008	0.033	<0.001	N.I.	62.6

Gp is control or diabetic; Wk is the time point; and I_{MIN} is the moment of inertia as determined by μ CT.

^aThe explanatory variable (excluding intercept) with the highest p-value greater than 0.05 was not included (N.I.) in subsequent regression model.

^bAn explanatory variable was kept in the model even though $p > 0.05$ when its interaction with another variable was significant.