Published in final edited form as:

Curr Osteoporos Rep. 2019 December ; 17(6): 455–464. doi:10.1007/s11914-019-00538-6.

Effects of diabetes on bone material properties

Sashank Lekkala[1], **Erik A. Taylor**[2], **Heather B. Hunt**[1], **Eve Donnelly**[1,3]

[1]Department of Materials Science and Engineering, Cornell University, Ithaca, NY

[2]Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY

[3]Research Division, Hospital for Special Surgery, New York, NY

Abstract

Purpose of Review—Individuals with type 1 and type 2 diabetes mellitus (T1DM, T2DM) have an increased risk of bone fracture compared to non-diabetic controls that is not explained by differences in BMD, BMI, or falls. Thus, bone tissue fracture resistance may be reduced in individuals with DM. The purpose of this review is to summarize work that analyzes the effects of T1DM and T2DM on bone tissue compositional and mechanical properties.

Recent Findings—Studies of clinical T2DM specimens revealed increased mineralization and AGE concentration and significant relationships between mechanical performance and composition of cancellous bone. Specifically, in femoral cancellous tissue, compressive stiffness and strength increased with mineral content; post-yield properties decreased with AGE concentration. In addition, cortical resistance to in vivo indentation (bone material strength index) was lower in patients with T2DM vs. age-matched non-diabetic controls, and this resistance decreased with worsening glycemic control. Recent studies on patients with T1DM and history of a prior fragility fracture found greater mineral content and concentrations of AGEs in iliac trabecular bone and correspondingly stiffer, harder bone at the nanosacle.

Summary—Recent observational data showed greater AGE and mineral content in surgically retrieved bone from patients with T2DM vs non-DM controls, consistent with reduced bone remodeling. Limited data on human T1DM bone tissue also showed higher mineral and AGE content in patients with prior fragility fractures compared to non-DM, non-fracture controls.

Contact information for corresponding author: Eve Donnelly, Ph.D., Associate Professor, Department of Materials Science and Engineering, Cornell University, 227 Bard Hall, Ithaca, NY 14853, Tel: 607-255-1067, Fax: 607-255-2365, eve.donnelly@cornell.edu.

Disclosures:
SL: None
EAT: None
HBH: None
ED: None

Conflict of Interest
Sashank Lekkala, Erik A. Taylor, Heather B. Hunt, and Eve Donnelly declare no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human participants or animals performed by any of the authors.

Keywords

Bone material properties; type 1 diabetes mellitus (T1DM); type 2 diabetes mellitus (T2DM); advanced glycation endproducts (AGEs); diabetic murine models; in vitro glycation

Introduction

Individuals with type 1 and type 2 diabetes mellitus (T1DM, T2DM) share a common complication of greater fracture risk relative to controls without DM [1,2]. In meta analyses, the risk of hip fracture is greater in individuals with T1DM (RR = 6.9 [1], 6.3 [2]) and in individuals with T2DM (RR = 1.4 [1], 1.7 [2]) both compared to controls without DM. Bone mineral density (BMD) is lower in individuals with T1DM compared with an age-matched control population [1]; however, the increased fracture risk in T1DM is not explained by the decreased BMD in this population [3]. On the other hand, individuals with T2DM have normal or even greater BMD compared to an age-matched control population [1], yet the increased fracture risk with T2DM persists after adjustment for BMD and potential confounders like BMI and falls [4,5]. Despite the increased risk of falls in individuals with DM due to several risk factors [6], falls do not completely account for the increased fracture risk [3,7]. Therefore, metabolic or biochemical changes associated with DM may alter aspects of the bone microstructure and tissue properties independently of the bone mass, though the precise mechanisms responsible for these changes may be T1DM- or T2DM-specific.

The mechanisms by which diabetes mellitus may degrade the fracture resistance of bone are complex, as addressed in several recent reviews [3,8–10]. In T1DM, pancreatic beta cell failure and insulin/IGF1 deficiency impair osteoblastic bone formation and inhibit accrual of peak bone mass during growth, and advanced glycation endproducts (AGEs) may directly and indirectly alter matrix properties [8,9]. In T2DM, a constellation of factors comprising hyperglycemia; oxidative stress; fat-derived inflammatory cytokines and adipokines; and AGEs collectively inhibit osteocyte function, alter bone turnover, and degrade collagen properties [9]. Facets of bone quality that may contribute to decreased fracture resistance in diabetic bone include altered bone microarchitecture and tissue material properties, which may arise from disease-induced changes in bone formation or remodeling, as well as direct alteration of collagen matrix properties by accumulation of AGEs. AGEs are the reaction products of reducing sugars with free amino groups in proteins and result in a diverse array of structures including crosslinking and non-crosslinking products. Crosslinking AGEs have been implicated in embrittling bone tissue in men with T2DM [11], rodent models of T2DM [12], and in *in vitro* ribosylation/glycosylation studies [13,14]. Non-crosslinking AGEs, like carboxymethyl lysine (CML), can also be deleterious to bone tissue through interactions with the receptor for AGEs (RAGE), which induces oxidative stress and inflammation [15].

The objective of this review is to summarize work that elucidates the material factors that may contribute to fragility in T1DM and T2DM. Many studies have evaluated alterations in BMD and bone microarchitecture in patients with DM (reviewed in [1,2,16,17]); here we

focus on investigations that have evaluated the changes in tissue-level compositional and mechanical properties associated with diabetic bone disease.

Type 1 Diabetes Mellitus

In individuals with T1DM, insulin/IGF1 deficiency impairs osteoblastic bone formation and inhibits accrual of peak bone mass during growth, resulting in characteristically low bone formation rates and low BMD [1,8]. Decreased osteoblast activity and survival have generally been observed in both humans and animals with T1DM; however, the activity of osteoclasts is not yet well characterized, with the limited studies available reporting either no changes or increases in bone resorption [18]. Low turnover in patients with T1DM is evident in several markers (recently reviewed in [19]): lower osteocalcin, a bone formation marker; lower C-terminal telopeptide (CTx), a bone resorption marker; and higher sclerostin, a potent inhibitor of the Wnt signaling pathway. Further, hyperglycemia; hypoinsulinemia; autoimmune inflammation; and low levels of insulin-like growth factor-1 (IGF-1), osteocalcin, and vitamin D observed in patients with T1DM may additionally contribute to bone fragility [20,21]. Finally, accumulation of AGEs may embrittle the matrix or alter bone turnover [13,14]. These mechanisms are yet to be confirmed by studies on human patients with T1DM.

Human Studies

Trabecular bone from patients with T1DM and history of a prior fragility fracture had greater mineral content and concentrations of AGEs than age- and sex-matched controls [22]. Specifically, in a case-control study of iliac crest biopsies (n = 5/group), the concentration of the AGE, pentosidine (Pen) measured by HPLC and degree of mineralization measured by microradiography was higher in trabecular bone in patients with T1DM with a prior fracture vs. that of non-diabetic non-fracture controls [22]. However, no significant changes in Pen and degree of mineralization were observed between patients with T1DM without a history of fracture and non-diabetic non-fracture controls. Serum HbA1c was positively correlated with trabecular Pen, suggesting that AGE accumulation increases with worsening glycemic control in this cohort. Together, both high Pen content and mineralization may embrittle the bone matrix and contribute to the bone fragility observed in patients with T1DM. A key limitation of this study is that patients with any complications of DM—who are at a greater relative risk for fracture than those without complications [1]—were excluded from the study, which may have attenuated potential differences between T1DM and control groups. Studies of a larger cohort and greater range of disease severity are required to confirm these observations in a broad T1DM population.

The mechanical behavior of bone from patients with T1DM has been characterized in a small number of opportunistic studies, which have generally noted modest effects of T1DM. Cortical and trabecular bone at the iliac crest from patients with T1DM trended toward being stiffer and harder vs. non-diabetic controls at the nanoscale as assessed by nanoindentation, but were not different at the microscale as assessed by Vickers microindentation [22]. Ultimate stress and Young's modulus estimated by whole-bone three-point bending did not differ in patients with T1DM undergoing amputation of the 2^{nd} - 5^{th} metatarsal (mean age =

51 years) vs. aged controls from deceased donors (mean age = 72 years) [23]. Similarly, estimated elastic modulus, yield strength, and ultimate strength assessed by three-point bending of tibial explants did not differ in patients with T1DM (mean age = 51 years) vs. aged controls (mean age = 75 years) [24]. A limitation of these studies is that the microarchitecture, which may potentially reflect altered gait and vasculature in patients with T2DM, was not characterized; therefore, the outcomes of three-point bending were not adjusted for potential differences in bone volume fraction (BV/TV). These results suggest that changes in mechanical properties of human bone with T1DM may be similar to those of aging in a non-diabetic environment; however, this assertion awaits confirmation in future studies with age-matched controls across multiple anatomic sites.

Assessment of bone material properties at the tissue level in clinical samples of patients with T1DM is limited. Thus, an urgent need in the field is to confirm changes in tissue-level composition and mechanical properties observed in animal models of T1DM (see below) with studies in humans to improve our understanding of the mechanisms of clinical fracture in T1DM populations.

Animal Models

Studies of the effects of T1DM on bone material properties have predominantly been conducted in animal models and have demonstrated changes in mineral and collagen properties and mechanical behavior.

Streptozotocin-Induced Diabetes

T1DM can be induced in mice and rats with streptozotocin (STZ) injections, typically at 8-12 weeks of age, which causes necrosis of pancreatic beta cells thereby inducing permanent diabetic hyperglycemia. STZ-induced T1DM rodents present similar blood glucose (>300 mg/dl) and plasma insulin levels as T1DM syndrome in humans [25]. However, the effects of STZ vary in rats and mice (Table 1). This difference may be partly attributed to differences in timing of induction of T1DM relative to lifespan. Induction of T1DM at 8-10 weeks occurs before skeletal maturity in rats (15 to 17 weeks [26]), potentially causing more profound deficits in composition and mass accrual of bone, but near skeletal maturity in mice (10 to 12 weeks [26]), potentially modeling the effects of later onset of T1DM. Overall, bone tissue of STZ-induced T1DM rats showed decreased collagen maturity—assessed by the ratio of mature trivalent to immature divalent enzymatic crosslinks [27] —and increased AGE accumulation vs. vehicle-injected controls [28–32], whereas T1DM mice showed increased collagen maturity vs. vehicle-injected controls [33].

STZ injections may also have differential effects on mineralization and mineral properties in rats compared to mice. In rats, STZ injection decreased the mineral:matrix ratio, the ratio of mineral content to organic matrix content, assessed by FTIR imaging in femoral cortical and trabecular bone [29] but increased humeral ash content [31] vs. controls. In contrast, in mice, STZ injection did not alter the FTIR mineral:matrix ratio [33] or calcium content assessed with quantitative backscattered electron imaging (qBEI) [32] vs. controls. C-axis mineral crystal length observed by x-ray diffraction [34] was shorter consistent with higher carbonate:phosphate [29] in STZ-injected rats vs. controls. The variable reports on changes

in mineral properties with STZ-induced T1DM may arise from the use of different measurement techniques and different outcomes across studies, e.g., multiple mineral:matrix ratios [35,36]. Nevertheless, the STZ-injected rat model currently best reflects the changes in material properties observed in humans with T1DM (Table 1).

In STZ-induced T1DM, at the tissue level, lower nano- and microhardness were observed; while at the whole-bone level, the bones were had reduced strength and toughness in bending [30,31,34,37,38]. Reduced nanoindentation modulus, hardness and Vickers microhardness were observed in the femoral cortex of STZ-injected mice vs. vehicle-injected controls [37].

Combined, the impaired tissue-level and whole-bone mechanical properties with STZ-induced T1DM contributes to our understanding of the effects of T1DM on bone biomechanical performance and may partially explain the increased fracture risk with T1DM. Further studies are needed to quantitatively relate tissue-level compositional changes with mechanical properties at multiple levels of structural hierarchy.

OVE 26 Mouse Model

The OVE 26 mouse model demonstrates systemic changes of severe and progressive T1DM through overexpression of calmodulin, which regulates insulin secretion by pancreatic B-cells [39,40]. This model shows similar changes in collagen properties to STZ-injected mice. Enzymatic and non-enzymatic collagen crosslinking are increased in OVE 26 mouse femurs vs. wild type (WT) controls. Specifically, pyridinoline content measured by Raman spectroscopy, an outcome similar to collagen maturity measured by FTIR spectroscopy, was increased in OVE 26 mice vs. WT controls [39]. Additionally, the AGEs CML and Pen, measured by Raman spectroscopy, were increased in OVE 26 mice vs. WT controls [39]. The increase in AGE content, which may arise from increased serum glucose levels with T1DM, is associated with decreased remodeling and osteoclast activity and may explain the increase in enzymatic crosslinking. However, alterations in the mineral phase of OVE 26 mice suggest biological activity different from those that drove the observed change in the collagen phase. The Raman mineral:matrix ratio of bone tissue in OVE 26 mice was reduced vs. WT controls [39], which suggests impaired bone formation/osteoblast activity or increased remodeling/osteoclast activity.

In addition, tissue-level resistance to crack initiation and propagation is impaired in the OVE 26 mouse. Initiation toughness and propagation toughness, measured by whole-bone notched bending, were decreased, and indentation distance increase (IDI, difference in indentation distance into the bone between first and last cycles), measured by cyclic reference point indentation (RPI), was increased in OVE 26 mice vs. WT controls [39]. Furthermore, inverse correlations were observed between CML and Pen with initiation toughness and propagation toughness in both OVE 26 and WT mice [39]. Combined, the greater collagen crosslinking and reduced mineral content observed in the OVE 26 mouse may explain the observed decrease in resistance to fracture of the femur compared to non-diabetic controls.

Type 2 Diabetes Mellitus

In contrast to T1DM, which is characterized by a lack of insulin production, T2DM develops in response to insulin resistance. T2DM is characterized by hyperglycemia and hyperinsulinemia, which may differentially alter bone material properties. Hyperglycemia disrupts bone remodeling via osteoblasts and osteoclasts. In addition, excess glucose can alter bone tissue material properties through the accumulation of AGEs and downstream effects of AGE-RAGE interactions. On the other hand, insulin is an anabolic agent, and hyperinsulinemia may help explain the greater BMD observed in people with T2DM [41]. Moreover, insulin signaling helps regulate osteoblastic proliferation and supports osteoclastogenesis [42]. The extent to which bone remodeling and material properties are affected by the simultaneous effects of hyperglycemia, AGE accumulation, and hyperinsulinemia, is not yet known.

Several recent studies of clinical specimens have given insight into the properties of human tissues from patients with T2DM. Additionally, in vitro glycation studies provide a basis for hypothesized changes to bone material properties in vivo. Finally, studies of bone metabolism, mass and structural properties in several animal models of T2DM, are reviewed elsewhere [26]; here we focus on the changes in material properties observed in these models.

Human Studies

Two recent studies related compositional changes with T2DM to mechanical properties in the proximal femur of subjects undergoing total hip arthroplasty. In the first study, cortical tissue from the T2DM group trended towards having greater total fluoresent AGE (fAGE) content (+21.3%, $p = 0.09$) and exhibited greater indentation distance and IDI measured by cyclic RPI of vs. the non-DM control group. However, the total fAGE content and most monotonic compression properties (with the exception of yield stress) did not differ between groups in the cancellous tissue [43]. In the second study, cancellous tissue in the T2DM group had greater Pen concentration and lower pyridinoline concentration assessed by HPLC, greater sugar:matrix and mineral:matrix assessed by FTIR, no difference in total fAGE content, and greater compressive stiffness and strength normalized by bone volume fraction vs. the non-DM control group [11]. Moreover, statistical models from the latter study demonstrated that T2DM has both beneficial and adverse effects on the apparent-level mechanical behavior of cancellous bone. Patients with T2DM had numerically higher BV/TV (+24%, NS, $p = 0.13$), which had a large positive effect on bone strength, stiffness, and toughness vs. controls. In contrast, after accounting for the effects of BV/TV, bone tissue from patients with T2DM exhibited adverse effects of Pen, total fAGEs, and mineral maturity on post-yield toughness. Individuals with T2DM that have average or greater changes in tissue composition related to T2DM (e.g., AGE accumulation or mineral maturity) but do not have the protective effect of greater BV/TV are at a greater risk of bone embrittlement compared to those with or without T2DM that do not have this set of deleterious tissue changes. Individuals with T2DM that have average or greater changes in tissue composition related to T2DM (e.g., AGE accumulation or mineral maturity) but do not have the protective effect of greater BV/TV are at a greater risk of bone embrittlement

compared to those with or without T2DM that do not have this conglomeration of deleterious tissue changes. Additionally, Pen content measured in tibial explants of men undergoing total knee replacement was higher in the DM group (9 patients with T2DM and 1 patient with T1DM) vs. non-diabetic controls [44]. Therefore, these studies provide evidence that T2DM is associated with accumulation of AGEs and that these compositional changes adversely affect bone tissue properties.

Additionally, alterations in mineral properties have also been observed with T2DM in similar clinical specimens. A higher mean calcium concentration and a narrower distribution of mineralization were observed in trabecular bone of the femoral neck in subjects with T2DM vs. non-diabetic controls [45]. These changes are consistent with the greater FTIR mineral:matrix observed in a similar patient population [11], and reduced bone remodeling with T2DM [46], which enables progression of secondary mineralization and results in a more mineralized tissue with a more homogeneous mineral distribution. Together, these studies indicate altered mineralization and increased AGE accumulation in T2DM, which may contribute to the inferior fracture resistance observed clinically at the whole-bone level. Furthermore, additional studies are needed to relate changes in tissue-level compositional and mechanical properties to disease severity and duration through long-term assessment of HbA1c in populations with a wide range of glycemic control.

In addition to the prior studies, which required ex vivo analysis of mechanical properties and composition, one key study measured the resistance of the tibial cortex to impact indentation [Bone Material Strength Index (BMSi)] in vivo in individuals with and without T2DM [47]. BMSi was lower in patients with T2DM vs. non-DM controls [17,47,48]; decreased with longer duration of diabetes [48]; and inversely correlated with 10-year HbA1c [47]. Because the relationship between BMSi and clinical fracture risk or other measures of fracture resistance is not yet well established [49], further assessment of fracture properties of bone in the T2DM population is required to interpret these data and inform estimation of fracture risk.

In Vitro Glycation Models

In vitro glycation or ribosylation simulates exposure to high blood glucose in T2DM. These models, in which bone specimens are incubated in a solution containing glucose or ribose for durations of ~7 days (equivalent to 2-3 decades of aging) [14,50,51], can be used to understand the mechanisms through which glycation affects the compositional and mechanical properties of bone.

In vitro glycation increases the AGE content of bone specimens compared to non-glycated controls [14,50,51]. Glycation increased non-enzymatic crosslinks assessed by FTIR, HPLC, and a fluorometric assay in the human femoral cortex vs. non-glycated controls [52]. Similarly, ribation of bovine metatarsi increased Pen, measured by HPLC, but did not change mineralization, measured by qBEI, vs. non-glycated controls [53]. These results indicate that in vitro glycation modifies the collagen crosslinking profile without altering mineralization. One limitation of this model is that it cannot capture the effects of metabolism and related dynamic changes in AGE accumulation due to remodeling in vivo.

Because T2DM alters both bone mineral and matrix properties [11,43], the in vitro models are useful for understanding the effects of increased glycation associated with T2DM on the collagen properties in bone but cannot capture not all changes in bone tissue properties with T2DM.

AGE accumulation degrades post-yield properties and increases bone stiffness. Post-yield strain energy and damage fraction assessed by unconfined compression testing on femoral cancellous bone and post-yield strain measured by three-point bending of bovine metatarsi were reduced in the ribosylated group vs. non-glycated controls [14,53]. Stress at equilibrium and equilibrium modulus measured by stress relaxation tests on demineralized specimens from the mid-diaphysis of human tibiae were higher in glycated specimens vs. non-glycated controls, suggesting stiffening and residual stress accumulation in the matrix because of glycation [50]. Although these results inform the relationship between AGE accumulation and mechanical properties *in vitro*, this relationship has been recently reported in human T2DM [11,44] but remains an area of active investigation.

Animal Models

Diet-Induced Obesity

A high fat diet (HFD) induces mild T2DM in C57BL/6 mice. Although this model does not produce overt diabetes, it enables examination of changes in bone material properties due to prediabetes and impaired glucose tolerance (Table 2).

HFD increases non-enzymatic crosslinking of collagen. Pen measured by Raman spectroscopy and total fAGEs were greater in cortical regions of the femur and tibia, respectively, in obese mice vs. lean controls [54,55]. HFD did not change the bone mineralization, crystallinity, and carbonate substitution at the femoral mid-diaphyseal cortex [54]. In addition, the carbonate:phosphate ratio in trabecular bone from the femoral distal epiphysis was lower in the obese group than in lean controls [54]. These results suggest that AGE accumulation in the bone matrix begins in a prediabetic state, while the mineral properties are only subtly altered during this period.

In general, HFD impairs structural and material performance in this model. Femurs of obese mice were weaker and stiffer vs. lean controls [54–56]. Fracture toughness assessed by notched whole-bone testing, as well as ultimate strength, yield strength, and Young's modulus estimated from whole-bone tests, were lower in obese mice vs. lean controls [55,57]. However, Young's modulus determined by finite element analysis of the femur was higher in obese mice vs. lean controls, and the Pen content positively correlated with Young's modulus, indicating that AGE accumulation increases bone stiffness [54]. Although the effects of HFD on the tissue modulus varied across studies, altered bone material composition in HFD mice was associated with decreased bone fracture toughness.

Single Gene Mutation Models

Single gene models are spontaneous models that enable examination of the effects of an individual gene mutation on bone material properties. The Zucker Diabetic Fatty (ZDF) rat model, has a leptin receptor deficiency that leads to overt diabetes ~9-10 weeks of age, in

males only [58]. The yellow Kuo Kondo (KK/Ay) mouse develops severe obesity and insulin resistance by eight weeks [59]. The ob/ob mouse is a model of severe obesity resulting from a spontaneous inactivating mutation in the leptin gene, whereas the db/db mouse is a model of severe T2DM resulting from an autosomal recessive mutation of the db gene [26]. The material properties of bone in single-gene mutation models are somewhat dependent on the mutation (Table 2).

At the whole-bone level, the femora and tibia of ZDF rats were weaker and more compliant in bending, and the L4 vertebrae were weaker and more compliant in compression, compared to lean controls [60,61]. Vickers microhardness did not differ in the tibial cortex of ZDF rats vs. lean controls [62]. ZDF rats had wider bone mineral density distributions (BMDDs) measured by qBEI in metaphyseal bone vs. controls, suggesting altered endochondral ossification compared to non-diabetic rats [63].

Likewise, ultimate bending load was lower in the tibiae of KK/Ay mice vs. C57BL/6 controls [64]. In addition, tissue from KK/Ay mice have an increased proportion of mature collagen crosslinks and mineral content. Mean FTIR collagen maturity was greater in femora of KK/Ay mice vs. black homozygous a/a controls [65]. Additionally, whole-femur mineral matrix ratio, was greater in KK/Ay mice vs. a/a controls [65]. These results suggest decreased bone turnover in KK-Ay mice, which is supported by decreased serum osteocalcin levels in adult KK/Ay mice [66].

Similarly, the femora of ob/ob and db/db mice were weaker in bending, and db/db mice had a lower estimated elastic modulus vs. C57BL/6 WT controls [67–69]. At the tissue level, the reduced modulus determined by nanoindentation of the femoral cortex was lower in db/db mice vs. WT controls [68].

Overall, single gene mutation models of T2DM have impaired mechanical performance at the tissue and whole-bone level. However, there are no studies to conclusively relate these changes to changes in bone composition. Studies assessing both compositional and mechanical properties are required to elucidate the mechanisms through which T2DM increases bone fragility.

Polygenic models

Polygenic animal models are spontaneous models that can mimic the complex genetic alterations and subsequent changes in bone material properties in patients with T2DM. These include Zucker Diabetic Sprague Dawley (ZDSD) rats, created by breeding heterozygous ZDF rats with diet-induced obese rats [60], which develop diabetes at an older age than ZDF rats. WBN/Kob rats are non-obese rats produced by selective inbreeding of Wistar rats and develop hyperglycemia by about 12 months of age [12]. The TallyHo mouse is an obese model of early onset T2DM developing hyperglycemia around 12 weeks of age [70]. Models for which, to our knowledge, there are no data on compositional properties but for which the structural properties have been reviewed recently [26], have been omitted in the current discussion.

Overall, polygenic models of T2DM show increased mineralization similar to human studies but collagen composition is not consistent across models (Table 2), indicating that increased mineralization could be a consistent trend across all patient groups of T2DM while collagen properties may depend on the pathogenesis.

ZDSD rats and TallyHo mice showed higher Raman Mineral:matrix ratio vs. CD(SD) and SWR/J [71] controls respectively [72,73]. Pen concentration, measured by HPLC, was similar between ZDSD rats and TallyHo mice vs. respective controls [73,74] but was greater in WBN/Kob rats vs. Wistar controls [12]. But, the distribution of collagen D-spacing, assessed by AFM in cortical regions of the tibia was altered in ZDSD rats vs. CD(SD) controls [72]. Additionally, mineral crystallinity assessed by Raman spectroscopy was greater in the femoral cortex [73] but did not differ at the tibial cortex [72] of ZDSD rats vs. CD(SD) controls. Anatomic site and duration of HFD (6-7 weeks vs. 2 weeks) may contribute to the discrepancy.

Polygenic models show improved or deteriorated structural performance at whole-bone level depending on the model. But, surprisingly the fracture toughness parameters were comparable to the controls in these models.

Femora of ZDSD rats and WBN/Kob were weaker in bending vs. respective controls [12,60,75,76]. The study on WBN/Kob rats showed that the ratio of Pen to total enzymatic crosslinks was significantly associated with all the measured mechanical properties[12]. These results suggest that load-bearing properties of bone depend on both enzymatic and non-enzymatic crosslinks. Crack initiation toughness and propagation toughness measured by notched whole-bone bending did not vary with age for CD(SD) rats but decreased with duration of diabetes in the ZDSD rats [73]. Aditionally, in cortical bone from ZDSD rats creep indentation distance assessed by RPI was lower [72]; and indentation distance increase was lower [72] and higher [75] vs. CD(SD) controls, a difference potentially attributable to the differing indentation forces (5 N vs. 10 N) used. The altered material properties in tissue from ZDSD rats could be attributable to the increased tissue mineralization [72,73].

Structural properties measured by three-point bending of the femur were comparable to superior in TallyHo mice vs. SWR/J controls, consistent with increased cortical thickness [74,77] and greater tissue mineral content [74]. In addition, two differences in tissue-level mechanical properties have been noted : (1) lower post-yield displacement in TallyHo, indicating reduced ductility, consistent with greater mineralization [74,77] yet (2) first-cycle and total indentation distance from RPI were greater in TallyHo tibiae vs. SWR/J controls, suggesting less tissue-level resistance to indentation. Further studies assessing the outcomes of reference point indentation at different loads and anatomical regions are required to understand the significance of this result.

In summary, polygenic models show increased mineralization, altered collagen crosslinking and inferior-to-superior structural properties, and a subset show deterioration of intrinsic resistance to fracture with duration of disease (Table 2).

Summary and Conclusions

Our understanding of mechanisms through which T1DM affects bone material properties is limited. Limited data on bone tissue from humans with T1DM showed higher mineral and AGE content and mechanical properties that may be similar to that of aging bone in a non-diabetic environment. In addition, tissue pentosidine content positively correlated with serum HbA1c, suggesting that AGE accumulation increases with worsening glycemic control. Further studies in larger cohorts are needed to quantitatively relate 1) glycemic control with compositional changes and 2) tissue-level compositional changes with mechanical properties across multiple levels of structural hierarchy.

Several mechanisms have been proposed to understand the effect of T2DM on bone tissue fragility [9,10,21], but the contribution of each mechanism to clinical fracture risk remains unknown. Recent studies on clinical specimens from humans with T2DM suggest that AGE accumulation and decreased bone remodeling [46] are important considerations in understanding the fragility of diabetic bone. Several mechanical measurements were made to investigate the fragility of bone tissue in human patients with T2DM; however, only a few studies directly investigated the association between mechanical properties and bone compositional properties. The sole study to date to characterize the composition and mechanical performance of cortical tissue found no relationship between AGEs and cyclic RPI outcomes [43]. On the other hand, significant relationships between composition and mechanical performance of cancellous bone have been reported in two studies. Specifically, AGEs were inversely correlated with post-yield properties [11,43], and mineral content was positively correlated with compressive stiffness and strength in cancellous tissue from the proximal femur [11]. These studies highlight the complex effects of T2DM on the mineral and matrix components of bone tissue, especially with regard to AGE accumulation, and offer insight into how micro-scale material property changes may affect macro-scale mechanical integrity in a compartment-specific way.

Translating rodent studies to clinical outcomes remains challenging. No model holistically captures the changes in the material properties of bone observed in human studies associated with diabetes. For example, among T1DM rodent models, STZ-induced T1DM rats potentially reflect observed changes in the human T1DM studies. Among T2DM rodent models, obese mice and WBN/Kob rats reflect alterations in collagen properties, whereas ZDSD rats and TallyHo mice reflect changes in mineral composition observed in human studies. Interestingly, most of the rodent models of T2DM develop higher mineral content compared to controls, suggesting that bone turnover is consistently reduced regardless of the model-specific pathogenesis of T2DM. One major drawback in current rodent models is that most develop a reduced BMD, whereas in humans a normal to high BMD is observed. Additionally, a rodent model is needed to simulate conditions similar to T2DM in an older population, as no current model recapitulates all the characteristics of T2DM. Nevertheless, these models enable examination of the relationship of glycemic control and duration of disease with structural and material properties. Thus, these models can provide insight into the mechanisms of increased bone fragility in the human diabetic population.

Acknowledgments

Funding for this work:

SL: None

EAT: None

HBH: None

ED: NIH K01 AR064314

Funding for this work was provided in part by NIH K01 AR064314 to ED.

References

Papers of particular interest, published recently, have been highlighted as: • Of importance •• Of major importance

1. Vestergaard P Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes - A meta-analysis. Osteoporos Int. 2007;18:427–44. [PubMed: 17068657]
2. Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol. 2007;166:495–505. [PubMed: 17575306]
3. Shah VN, Carpenter RD, Ferguson VL, Schwartz AV. Bone health in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes. 2018;25:231–6. [PubMed: 29794498]
4. Schwartz AV, Vittinghoff E, Bauer DC, Hillier TA, Strotmeyer ES, Ensrud KE, et al. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. JAMA - J Am Med Assoc. 2011;305:2184–92.
5. Bonds DE, Larson JC, Schwartz AV, Strotmeyer ES, Robbins J, Rodriguez BL, et al. Risk of fracture in women with type 2 diabetes: The women's health initiative observational study. J Clin Endocrinol Metab. 2006;91:3404–10. [PubMed: 16804043]
6. Starup-Linde J, Frost M, Vestergaard P, Abrahamsen B. Epidemiology of Fractures in Diabetes. Calcif Tissue Int. Elsevier Inc.; 2017;100:109–21.
7. Schwartz AV Epidemiology of fractures in type 2 diabetes. Bone. 2016;82:2–8. [PubMed: 26027505]
8. Hough FS, Pierroz DD, Cooper C, Ferrari SL, IOF CSA Bone and Diabetes Working Group _. MECHANISMS IN ENDOCRINOLOGY: Mechanisms and evaluation of bone fragility in type 1 diabetes mellitus. Eur J Endocrinol. 2016;174:R127–38. [PubMed: 26537861]
9. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL. Mechanisms of diabetes mellitus-induced bone fragility Nat Rev Endocrinol. Nature Publishing Group; 2017;13:208–19. [PubMed: 27658727]
10. Shanbhogue VV, Mitchell DM, Rosen CJ, Bouxsein ML. Type 2 diabetes and the skeleton: New insights into sweet bones. Lancet Diabetes Endocrinol. 2016;4:159–73. [PubMed: 26365605]
11 ••. Hunt H, Torres A, Palomino P, Marty E, Saiyed R, Cohn M, et al. Altered tissue composition, microarchitecture, and mechanical performance in cancellous bone from men with type 2 diabetes mellitus. J Bone Miner Res. 2019.This study relating compositional and mechanical properties found increased pentosidine and mineralization in men with T2DM, and showed that high concentrations of AGEs can increase fragility, especially for T2DM patients with low BV/TV.
12. Saito M, Fujii K, Mori Y, Marumo K. Role of collagen enzymatic and glycation induced cross-links as a determinant of bone quality in spontaneously diabetic WBN/Kob rats. Osteoporos Int. 2006;17:1514–23. [PubMed: 16770520]
13. Vashishth D, Gibson GJ, Khoury JI, Schaffler MB, Kimura J, Fyhrie DP. Influence of nonenzymatic glycation on biomechanical properties of cortical bone. Bone. 2001;28:195–201. [PubMed: 11182378]

14. Tang SY, Zeenath U, Vashishth D. Effects of non-enzymatic glycation on cancellous bone fragility. Bone. 2007;40:1144–51. [PubMed: 17257914]

15. Yamagishi S-I. Role of advanced glycation end products (AGEs) and receptor for AGEs (RAGE) in vascular damage in diabetes. EXG. 2011;46:217–24.

16. Keenan HA, Maddaloni E. Bone Microarchitecture in Type 1 Diabetes: It Is Complicated Curr Osteoporos Rep. Springer US; 2016;14:351–8. [PubMed: 27704394]

17. Nilsson AG, Sundh D, Johansson L, Nilsson M, Mellström D, Rudäng R, et al. Type 2 Diabetes Mellitus Is Associated With Better Bone Microarchitecture But Lower Bone Material Strength and Poorer Physical Function in Elderly Women: A Population-Based Study. J Bone Miner Res. 2017;32:1062–71. [PubMed: 27943408]

18. Kalaitzoglou E, Popescu I, Bunn RC, Fowlkes JL, Thrailkill KM. Effects of Type 1 Diabetes on Osteoblasts, Osteocytes, and Osteoclasts Curr Osteoporos Rep. Springer US; 2016;14:310–9. [PubMed: 27704393]

19 •. Hygum K, Starup-Linde J, Harsløf T, Vestergaard P, Langdahl BL. Diabetes mellitus, a state of low bone turnover-a systematic review and meta-analysis. Eur J Endocrinol. 2017;176:R137–57. [PubMed: 28049653] This extensive review of biochemical markers of bone formation and resorption in diabetes and found that low bone turnover is observed in both T1DM and T2DM conditions.

20. Zhukouskaya VV, Eller-Vainicher C, Shepelkevich AP, Dydyshko Y, Cairoli E, Chiodini I. Bone health in type 1 diabetes: Focus on evaluation and treatment in clinical practice J Endocrinol Invest. Springer International Publishing; 2015;38:941–50. [PubMed: 25863666]

21. Hamann C, Kirschner S, Günther KP, Hofbauer LC. Bone, sweet bone - Osteoporotic fractures in diabetes mellitus Nat Rev Endocrinol. Nature Publishing Group; 2012;8:297–305. [PubMed: 22249517]

22 ••. Farlay D, Armas LAG, Gineyts E, Akhter MP, Recker RR, Boivin G. Nonenzymatic Glycation and Degree of Mineralization Are Higher in Bone from Fractured Patients with Type 1 Diabetes Mellitus. J Bone Miner Res. 2016;31:190–5 [PubMed: 26234180] This is the only study to our knowledge to have assessed compositional properties in patients with T1DM and found increased AGE accumulation in patients with T1DM.

23. Fleischli JG, Laughlin TJ, Lavery LA, Shah B, Lanctot D, Agrawal CM, et al. The effects of diabetes mellitus on the material properties of human metatarsal bones. J Foot Ankle Surg. 1998;37:195–8. [PubMed: 9638543]

24. Fleischli JG, Laughlin TJ, Athanasiou K, Lanctot DR, Lavery L, Wang X, et al. Effect of Diabetes Mellitus on the Material Properties of the Distal Tibia. J Am Pod Med Assoc. 2014;96:91–5.

25. Lenzen S The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51:216–26. [PubMed: 18087688]

26 ••. Fajardo RJ, Karim L, Calley VI, Bouxsein ML. A review of rodent models of type 2 diabetic skeletal fragility. J Bone Miner Res. 2014;29:1025–40. [PubMed: 24585709] This extensive review discusses the effect of T2DM on the skeletal phenotype of different rodent models.

27. Paschalis EP, Verdelis K, Doty SB, Boskey AL, Mendelsohn R, Yamauchi M. Spectroscopic characterization of collagen cross-links in bone. J Bone Miner Res. 2001;16:1821–8. [PubMed: 11585346]

28. Oliveira Limirio PHJ, Da Rocha HA, De Morais RB, Hiraki KRN, Balbi APC, Soares PBF, et al. Influence of hyperbaric oxygen on biomechanics and structural bone matrix in type 1 diabetes mellitus rats. PLoS One. 2018; 13.

29. Bozkurt O, Bilgin MD, Evis Z, Pleshko N, Severcan F. Early Alterations in Bone Characteristics of Type i Diabetic Rat Femur: A Fourier Transform Infrared (FT-IR) Imaging Study. Appl Spectrosc. 2016;70:2005–15. [PubMed: 27680083]

30. Donmez BO, Unal M, Ozdemir S, Ozturk N, Oguz N, Akkus O. Effects of losartan treatment on the physicochemical properties of diabetic rat bone. J Bone Miner Metab. 2017;35:161–70. [PubMed: 27038987]

31. Silva MJ, Brodt MD, Lynch MA, McKenzie JA, Tanouye KM, Nyman JS, et al. Type 1 diabetes in young rats leads to progressive trabecular bone loss, cessation of cortical bone growth, and

diminished whole bone strength and fatigue life. J Bone Miner Res. 2009;24:1618–27. [PubMed: 19338453]

32. Mansur SA, Mieczkowska A, Bouvard B, Flatt PR, Chappard D, Irwin N, et al. Stable Incretin Mimetics Counter Rapid Deterioration of Bone Quality in Type 1 Diabetes Mellitus. J Cell Physiol. 2015;230:3009–18. [PubMed: 26016732]
33. Mieczkowska A, Mansur SA, Irwin N, Flatt PR, Chappard D, Mabilleau G. Alteration of the bone tissue material properties in type 1 diabetes mellitus: A Fourier transform infrared microspectroscopy study. Bone. 2015;76:31–9. [PubMed: 25813583]
34. Facchini DM, Yuen VG, Battell ML, McNeill JH, Grynpas MD. The effects of vanadium treatment on bone in diabetic and non-diabetic rats. Bone. 2006;38:368–77. [PubMed: 16256449]
35. Makowski AJ, Patil CA, Mahadevan-Jansen A, Nyman JS. Polarization control of Raman spectroscopy optimizes the assessment of bone tissue. J Biomed Opt. International Society for Optics and Photonics; 2013;18:055005.
36. Taylor EA, Lloyd AA, Salazar-Lara C, Donnelly E. Raman and Fourier Transform Infrared (FT-IR) Mineral to Matrix Ratios Correlate with Physical Chemical Properties of Model Compounds and Native Bone Tissue. Appl Spectrosc. 2017;71:2404–10. [PubMed: 28485618]
37. Nyman JS, Even JL, Jo CH, Herbert EG, Murry MR, Cockrell GE, et al. Increasing duration of type 1 diabetes perturbs the strength-structure relationship and increases brittleness of bone. Bone. 2011;48:733–40. [PubMed: 21185416]
38. Zhang H, Gan L, Zhu X, Wang J, Han L, Cheng P, et al. Moderate-intensity 4 mT static magnetic fields prevent bone architectural deterioration and strength reduction by stimulating bone formation in streptozotocin-treated diabetic rats. Bone. 2018;107:36–44. [PubMed: 29111170]
39. Rubin MR, Paschalis EP, Poundarik A, Sroga GE, McMahon DJ, Gamsjaeger S, et al. Correction: Advanced glycation endproducts and bone material properties in type 1 diabetic mice. PLoS One. 2016;11:1–14.
40. Epstein PN, Overbeek PA, Means AR. Calmodulin-induced early-onset diabetes in transgenic mice. Cell. 1989;58:1067–73. [PubMed: 2673540]
41. Thrailkill KM, Lumpkin CK, Bunn RC, Kemp SF, Fowlkes JL. Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues. Am J Physiol Endocrinol Metab. 2005;289:E735–45. [PubMed: 16215165]
42. Lecka-Czernik B Safety of Antidiabetic Therapies on Bone. Clin Rev Bone Miner Metab. 2013;11:49–58. [PubMed: 23543908]
43 ••. Karim L, Moulton J, Van Vliet M, Velie K, Robbins A, Malekipour F, et al. Bone microarchitecture, biomechanical properties, and advanced glycation end-products in the proximal femur of adults with type 2 diabetes Bone. Elsevier; 2018; 114:32–9. [PubMed: 29857063] This is the first study to examine compositional and mechanical properties of bone from humans with T2DM and found reduced resistance to creep indentation in cortical bone from patients with T2DM.
44. Bucknell A, King KB, Oren TW, Botolin S, Williams A. Arthroplasty in veterans: Analysis of cartilage, bone, serum, and synovial fluid reveals differences and similarities in osteoarthritis with and without comorbid diabetes. J Rehabil Res Dev. 2012;48:1195.
45. Pritchard JM, Papaioannou A, Tomowich C, Giangregorio LM, Atkinson SA, Beattie KA, et al. Bone mineralization is elevated and less heterogeneous in adults with type 2 diabetes and osteoarthritis compared to controls with osteoarthritis alone Bone. Elsevier Inc.; 2013;54:76–82. [PubMed: 23356988]
46. Manavalan JS, Cremers S, Dempster DW, Zhou H, Dworakowski E, Kode A, et al. Circulating Osteogenic Precursor Cells in Type 2 Diabetes Mellitus. J Clin Endocrinol Metab. 2012;97:3240–50. [PubMed: 22740707]
47 ••. Farr JN, Drake MT, Amin S, Melton LJ, McCready LK, Khosla S. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. J Bone Miner Res. 2014;29:787–95. [PubMed: 24123088] This comprehensive assessment of geometric and microarchitectural properties in women with T2DM demonstrated that bone material strength index decreased with 10-year HbA1c.

48. Furst JR, Bandeira LC, Fan WW, Agarwal S, Nishiyama KK, Mcmahon DJ, et al. Advanced glycation endproducts and bone material strength in type 2 diabetes. J Clin Endocrinol Metab. 2016;101:2502–10. [PubMed: 27115060]
49. Arnold M, Zhao S, Ma S, Giuliani F, Hansen U, Cobb JP, et al. Microindentation – a tool for measuring cortical bone stiffness? Bone Jt Res. 2017;6:542–9.
50. Poundarik AA, Wu PC, Evis Z, Sroga GE, Ural A, Rubin M, et al. A direct role of collagen glycation in bone fracture. J Mech Behav Biomed Mater. 2015;50:82–92.
51. Karim L, Tang SY, Sroga GE, Vashishth D. Differences in non-enzymatic glycation and collagen cross-links between human cortical and cancellous bone. Osteoporos Int. 2013;24:2441–7. [PubMed: 23471564]
52. Schmidt FN, Zimmermann EA, Campbell GM, Sroga GE, Püschel K, Amling M, et al. Assessment of collagen quality associated with non-enzymatic cross-links in human bone using Fourier-transform infrared imaging. Bone. 2017;97:243–51. [PubMed: 28109917]
53. Willett TL, Sutty S, Gaspar A, Avery N, Grynpas M. In vitro non-enzymatic ribation reduces post-yield strain accommodation in cortical bone Bone. Elsevier Inc; 2013;52:611–22.
54. Marin C, Papantonakis G, Sels K, Van Lenthe GH, Falgayrac G, Vangoitsenhoven R, et al. Unraveling the compromised biomechanical performance of type 2 diabetes- and Rouxen-Y gastric bypass bone by linking mechanical-structural and physico-chemical properties Sci Rep. Springer US; 2018;8:1–12.
55. Ionova-Martin SS, Wade JM, Tang S, Shahnazari M, Ager JW, Lane NE, et al. Changes in cortical bone response to high-fat diet from adolescence to adulthood in mice. Osteoporos Int. 2011;22:2283–93. [PubMed: 20941479]
56. Kerckhofs G, Durand M, Vangoitsenhoven R, Marin C, Van Der Schueren B, Carmeliet G, et al. Changes in bone macro-and microstructure in diabetic obese mice revealed by high resolution microfocus X-ray computed tomography Sci Rep. Nature Publishing Group; 2016;6:1–13. [PubMed: 28442746]
57. Ionova-Martin SS, Do SH, Barth HD, Szadkowska M, Porter AE, Ager JW, et al. Reduced size-independent mechanical properties of cortical bone in high-fat diet-induced obesity Bone. Elsevier Inc.; 2010;46:217–25. [PubMed: 19853069]
58. Clark JB, Palmer CJ, Shaw WN. The diabetic Zucker fatty rat. Proc Soc Exp Biol Med. 1983;173:68–75. [PubMed: 6344096]
59. Fu C, Zhang X, Ye F, Yang J. High Insulin Levels in KK-Ay Diabetic Mice Cause Increased Cortical Bone Mass and Impaired Trabecular Micro-Structure. Int J Mol Sci. 2015;16:8213–26. [PubMed: 25872143]
60. Reinwald S, Peterson RG, Allen MR, Burr DB. Skeletal changes associated with the onset of type 2 diabetes in the ZDF and ZDSD rodent models. Am J Physiol Endocrinol Metab. 2009;296:E765–74. [PubMed: 19158319]
61. Prisby RD, Swift JM, Bloomfield SA, Hogan HA, Delp MD. Altered bone mass, geometry and mechanical properties during the development and progression of type 2 diabetes in the Zucker diabetic fatty rat. J Endocrinol. 2008;199:379–88. [PubMed: 18755885]
62. Pereira M, Gohin S, Lund N, Hvid A, Smitham PJ, Oddy MJ, et al. Sclerostin does not play a major role in the pathogenesis of skeletal complications in type 2 diabetes mellitus. Osteoporos Int. Osteoporosis International; 2017;28:309–20. [PubMed: 27468901]
63. Hamann C, Goettsch C, Mettelsiefen J, Henkenjohann V, Rauner M, Hempel U, et al. Delayed bone regeneration and low bone mass in a rat model of insulin-resistant type 2 diabetes mellitus is due to impaired osteoblast function. Am J Physiol Metab. 2011;301:E1220–8.
64. Xu F, Dong Y, Huang X, Li M, Qin L, Ren Y, et al. Decreased osteoclastogenesis, osteoblastogenesis and low bone mass in a mouse model of type 2 diabetes. Mol Med Rep. 2014;10:1935–41. [PubMed: 25109926]
65. Hunt HB, Pearl JC, Diaz DR, King KB, Donnelly E. Bone Tissue Collagen Maturity and Mineral Content Increase With Sustained Hyperglycemia in the KK-Ay Murine Model of Type 2 Diabetes. JBone Miner Res. 2018;33:921–9. [PubMed: 29281127]
66. Takagi S, Miura T, Yamashita T, Ando N, Nakao H, Ishihara E, et al. Characteristics of Diabetic Osteopenia in KK-Ay Diabetic Mice. Biol Pharm Bull. 2012;35:438–43. [PubMed: 22382334]

67. Ealey KN, Fonseca D, Archer MC, Ward WE. Bone abnormalities in adolescentleptin-deficient mice. Regul Pept. 2006;136:9–13. [PubMed: 16764953]

68. Williams GA, Callon KE, Watson M, Costa JL, Ding Y, Dickinson M, et al. Skeletal phenotype of the leptin receptor-deficient db/db mouse. J Bone Miner Res. 2011;26:1698–709. [PubMed: 21328476]

69. Huang L, You YK, Zhu TY, Zheng LZ, Huang XR, Chen HY, et al. Validity of leptin receptor-deficiency (db/db) type 2 diabetes mellitus mice as a model of secondary osteoporosis Sci Rep. Nature Publishing Group; 2016;6:1–7. [PubMed: 28442746]

70. Kim JH, Sen Ś, Avery CS, Simpson E, Chandler P, Nishina PM, et al. Genetic Analysis of a New Mouse Model for Non-Insulin-Dependent Diabetes. Genomics. 2001;74:273–86. [PubMed: 11414755]

71. Kutscher CL, Miller M, Schmalbach NL. Renal deficiency associated with diabetes insipidus in the SWR/J mouse. Physiol Behav. 1975;14:815–8. [PubMed: 1187837]

72. Hammond MA, Gallant MA, Burr DB, Wallace JM. Nanoscale changes in collagen are reflected in physical and mechanical properties of bone at the microscale in diabetic rats Bone. Elsevier Inc.; 2013;60:26–32. [PubMed: 24269519]

73. Creecy A, Uppuganti S, Merkel AR, O'Neal D, Makowski AJ, Granke M, et al. Changes in the Fracture Resistance of Bone with the Progression of Type 2 Diabetes in the ZDSD Rat Calcif Tissue Int. Springer US; 2016;99:289–301. [PubMed: 27209312]

74. Creecy A, Uppuganti S, Unal M, Clay Bunn R, Voziyan P, Nyman JS. Low bone toughness in the TallyHO model of juvenile type 2 diabetes does not worsen with age Bone. Elsevier Inc.; 2018;110:204–14. [PubMed: 29438824]

75. Gallant MA, Brown DM, Organ JM, Allen MR, Burr DB. Reference-point indentation correlates with bone toughness assessed using whole-bone traditional mechanical testing Bone. Elsevier Inc.; 2013;53:301–5. [PubMed: 23274349]

76. Igarashi C, Maruyama T, Ezawa I, Takei I, Saruta T. WBN/Kob rat: a new model of spontaneous diabetes, osteopenia and systemic hemosiderin deposition. Bone Miner. 1994;27:133–44. [PubMed: 7711521]

77. Devlin MJ, Van Vliet M, Motyl K, Karim L, Brooks DJ, Louis L, et al. Early-onset type 2 diabetes impairs skeletal acquisition in the male TALLYHO/JngJ mouse. Endocrinology. 2014;155:3806–16. [PubMed: 25051433]

Table 1.

A symbolic summary of the effects of T1DM on bone material properties in humans and polygenic rodent models.

Each arrow represents the result of one study with compositional, material, or structural outcomes indicated as increased (↑), decreased (↓), and unchanged (↔) vs. non-diabetic controls. Material properties reported here were both directly assessed and estimated from whole-bone tests. Abbreviations XST = mineral crystallinity; C:P = Carbonate:Phosphate ; XLR = collagen maturity; Pen = Pentosidine concentration; E = elastic modulus; σ_y = yield stress; σ_{ult} = ultimate stress; K= fracture toughness; P_{max} = maximum load; IDI = Indentation distance increase.

	Mineral composition			**Collagen composition**				**Material properties**						**Structural properties**	
	Mineral content	**XST**	**C:P**	**XLR**	**Enzymatic Crosslinks**	**Pen**	**fAGEs**	**E**	**σ_y**	**σ_{ult}**	**Toughness**	**K**	**IDI**	**P_{max}**	**Stiffness**
Humans [22]	↑					↑									
STZ rats [28–31,34,38]	↓↓/↔/↑	↓/↔	↓/↔	↓↓↓	↑/↔	↑		↓↓/↔↔↔	↓/↔	↓↓↓/↔↔	↓↓/↔			↓↓↓	↓↓/↔
STZ mice [32,33,37]	↔	↔	↔	↑↑				↔	↔	↔	↓/↔			↓	↓
OVE 26 mice [39]	↓	↔			↑	↑						↓	↑		

Table 2

A symbolic summary of the effects of T2DM on bone material properties in humans and rodent models.

Each arrow represents the result of one study with compositional, material, or structural outcomes indicated as increased (↑), decreased (↓), and unchanged (↔) vs. non-diabetic controls. Material properties reported here were both directly assessed and estimated from whole-bone tests. Abbreviations: XST = mineral crystallinity; C:P = Carbonate:Phosphate ; XLR = collagen maturity; Pen = Pentosidine concentration; E = elastic modulus; σ_y = yield stress; σ_{ult} = ultimate stress; K= fracture toughness; P_{max} = maximum load.

	Mineral composition			Collagen composition				Material properties					Structural properties	
	Mineral content	XST	C:P	XLR	Enzymatic Crosslinks	fAGEs	Pen	E	σ_y	σ_{ult}	Toughness	K	P_{max}	Stiffness
Human [11,43–45]	↑↑	↔	↔	↔	↓/↔↔	↑/↔↔	↑↑	↑/↔	↓/↑	↑/↔	↔↔			
Obese C57BL/6 mice [54–57]	↔	↔	↓/↔			↑	↑	↓/↑	↓↓/↔	↓↓		↓↓/↔	↓↓/↔	↓↓↓
ZDF rats [60,61,63]	↓/↔↔							↓/↔↔↔	↔	↓/↔↔↔	↔		↓↓↓↓/↔↔	↓↓↓↓/↔↔
KK/Ay mice [64,65]	↑	↔	↔	↑	↔		↔						↓	
Ob/ob mice [67]											↓		↓	↓
Db/db mice [67,69]								↓			↓		↓/↔	↓
ZDSD rats [60,72,73,75]	↑↑	↑/↔	↔↔		↔		↔	↓/↔	↔	↓↓/↔↔	↓↓↓/↔	↔	↓	↓
WBN/Kob rats [12,76]					↓		↑	↓					↓	↓
TallyHo mice [74,77]	↑	↔	↓	↑	↔	↔↔	↔	↑/↔↔		↑↑/↔	↓	↔	↑↑/↔↔	↑/↔↔