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Determinants of Bone Strength and Quality in Diabetes Mellitus in Humans

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Abstract

There is growing evidence that the higher fracture rate observed in patients with type 2 diabetes mellitus (T2DM) is associated with normal, or even increased, areal bone mineral density (aBMD) by DXA. This has led to the hypothesis that patients with T2DM may have abnormalities in bone microarchitecture and/or material composition – i.e., key determinants of bone “quality.”

Consistent with this hypothesis, several studies using high-resolution peripheral quantitative computed tomography (HRpQCT) have demonstrated preserved indices of trabecular microarchitecture but increased cortical porosity in T2DM patients. In addition, a recent study using a novel *in vivo* microindentation device found an impairment in a measure of bone material properties (bone material strength index, BMSi) in postmenopausal women with longstanding T2DM; notably, the reduction in BMSi was associated with chronic glycemic control, suggesting that the skeleton should be included as another target organ subject to diabetic complications. The underlying pathogenesis of skeletal fragility in T2DM remains to be defined, although high levels of advanced glycation endproducts (AGEs) may play a role. In addition, T2DM is associated with reduced bone turnover, perhaps with an imbalance between bone resorption and bone formation. Although several studies have found increased serum sclerostin levels in patients with T2DM, the role of these increased levels in mediating the observed increases in cortical porosity or reduction in BMSi remains to be defined. Thus, although bone quality appears to be impaired in T2DM, the pathogenesis of these abnormalities and their relationship to the increased fracture risk observed in these patients needs further study.

Keywords

diabetes; bone strength; bone quality; HRpQCT

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Introduction

Because aging is the most significant risk factor for osteoporosis and fractures, skeletal health is an important consideration for older adults in general. However, aging is also a risk factor for a number of chronic diseases, including diabetes, and emerging evidence suggests that diabetes itself affects bone metabolism and exacerbates age-related reductions in bone quality; thus, diabetes is now considered an independent risk factor for fragility fractures [1, 2]. In this review, we summarize current evidence of altered bone “quality” in patients with diabetes mellitus, with a focus on *in vivo* assessment techniques.

Epidemiological data indicate that type 1 diabetes mellitus (T1DM) is associated with greater fracture risk than type 2 diabetes mellitus (T2DM) [3, 4]. Whereas patients with T1DM usually have modestly reduced bone mass and areal bone mineral density (aBMD) [3–5], T2DM patients typically have normal or increased values for these parameters [3], even when normalized for body mass index (BMI) [6]. Paradoxically, however, despite higher bone mass and aBMD, T2DM is associated with a 50–80% increased extremity fracture risk [7, 8], and a meta-analysis of 12 studies found a relative risk of 1.7 (95% CI: 1.3 to 2.2) for hip fracture [4]. Furthermore, large prospective studies in the United States [9] and Canada [10] have shown that patients with T2DM have a higher fracture risk for either a given femoral neck aBMD T-score and age or for a given FRAX probability (defined by the World Health Organization’s Fracture Risk Algorithm [FRAX®] score [11]). Collectively, these findings indicate that the higher bone mass in patients with T2DM does not sufficiently protect against fractures, suggesting that other factors are likely responsible for this increased fracture risk.

T1DM typically manifests during childhood or adolescents and results in insulin deficiency, which is commonly accompanied by low body weight. Since insulin has anabolic actions in bone [12], these patients tend to have lower bone formation rates [13] and suffer from suboptimal peak bone mass accrual during growth [5]. Although it is not known whether patients with T1DM have altered bone material properties, a recent study using high-resolution peripheral quantitative computed tomography (HRpQCT) found that patients with T1DM and microvascular disease had significant deficits in both cortical and trabecular bone microarchitectural parameters at the distal radius and tibia as compared to matched non-diabetic control subjects [14]. These data are consistent with abundant evidence suggesting that patients with T1DM are at significant risk for greater bone loss from diabetic complications such as vascular disease, retinopathy, nephropathy, and neuropathy [5]. These complications also increase the risk of falls [15]. Therefore, low bone mass and compromised bone microarchitecture in combination with an increased propensity for falls likely explains the higher fracture risk in patients with T1DM.

The explanation for the higher fracture risk and yet normal or increased aBMD in patients with T2DM is seemingly more complicated. Although the known risk factors for age-related fractures also contribute to fracture risk in patients with T2DM, there appear to be specific fracture risk factors that are either more prevalent in patients with T2DM (e.g., reduced muscle quality, poor balance, and falls [16, 17]) or T2DM-specific (e.g., poor glycemic control, T2DM duration, and diabetic complications [1, 2]). In addition, several of the

diabetic medications may exacerbate fracture risk in T2DM. For example, long-term use of oral glitazones (e.g., thiazolidinediones [TZDs]) doubles female fracture risk [18]. Also, in a population-based study of Olmsted County, Minnesota, residents with T2DM [19], use of insulin was associated with increased fracture risk, whereas biguanide (e.g., metformin) use and fractures were not related. However, it is also possible that the association of diabetes medications with fracture risk may be driven, at least in part, by the fact that patients on these medications may have worse glycemic control. This possibility is supported by *in vitro* studies showing that insulin and metformin stimulate osteoblast proliferation and differentiation [12, 20]. In addition, there is conflicting evidence regarding the relationship of glycemic control to fracture risk in T2DM patients [21, 22]. For example, while evidence exists that poor glycemic control (i.e., a glycated hemoglobin level $\geq 7.5\%$) is associated with higher fracture risk in T2DM patients [23], this relationship has not been established unequivocally [24]. Thus, further work is needed to test this important question more rigorously. Moreover, although both the duration of T2DM and the presence of multiple diabetic complications have been associated with fracture risk [4, 7, 8, 19], these risk factors collectively do not explain the increased fracture risk observed in T2DM patients [25].

The normal or higher DXA-derived aBMD [3] and yet increased fracture risk for a given femoral neck aBMD T-score and age in patients with T2DM compared to non-diabetic controls [9, 10] has led to the suggestion that fragility fractures in T2DM may result from diabetes-related alterations in skeletal properties not captured by DXA [1, 2]. In addition, it is possible that despite their higher BMD cross-sectionally, subsets of T2DM patients may undergo rapid bone loss [26–28], which may be independent risk factor for fracture regardless of baseline BMD [29, 30]. Further work is needed to better identify these potential “rapid losers”.

Although widely available and commonly used clinically, DXA has neither the required sensitivity nor specificity for optimal fracture risk assessment [31] and is unable to assess critical aspects of bone’s complex design. However, a recently developed analytical tool called the trabecular bone score (TBS) has been shown to capture vertebral trabecular bone “texture” from lumbar spine DXA images [32]. To date, multiple studies have reported negative associations between lumbar spine TBS (lower values reflect worse vertebral bone structure) and fracture risk in both patients with T1DM and T2DM as compared to non-diabetic controls [33–35]. Thus, lumbar spine TBS may serve as an indicator of vertebral skeletal deterioration in patients with diabetes mellitus. Further, it is also possible that bone macrostructure (i.e., geometry) is altered by diabetes. For example, studies using central and peripheral QCT have shown that patients with T2DM have similar or modestly reduced bone sizes (i.e., cross-sectional areas) and load to strength ratios [36, 37] as compared to non-diabetic controls. In addition, patients with T2DM may have abnormalities in bone microarchitecture and/or material composition; i.e., determinants of bone “quality” [38, 39].

Alterations in bone microarchitecture

To test whether bone microarchitecture is altered in patients with T2DM, several studies have used HRpQCT that allows for 3-D assessment of bone macro- and microarchitecture (e.g., cortical porosity and trabecular connectivity), essentially providing a non-invasive

“virtual bone biopsy” of the distal radius and tibia. In the first study to utilize HRpQCT in patients with T2DM, Burghardt et al. [40] found that 19 postmenopausal women with T2DM had preserved trabecular microarchitecture but significantly compromised cortical microarchitecture (i.e., higher cortical porosity, by 124% at the distal radius) as compared to 19 non-diabetic postmenopausal control subjects (Table 1 and Figure 1). By contrast, a later study by Shu et al. [41] reported no significant differences in HRpQCT-derived trabecular or cortical bone parameters at the distal radius or tibia in postmenopausal women with T2DM versus non-diabetic postmenopausal control subjects. However, this study may have been underpowered as only 14 subjects per group underwent HRpQCT scanning and cortical porosity was not reported [41]. More recently, Farr et al. [42] found that radial cortical porosity tended to be higher (by 32%) in 30 postmenopausal women with T2DM as compared to 30 age-matched non-diabetic postmenopausal control subjects after adjustment for BMI, although this difference did not reach statistical significance. Nonetheless, these findings were consistent with the initial study by Burghardt and colleagues [40]. Further, Patsch et al. [43] reported significantly higher cortical porosity (by 58%) at the distal radius in 20 T2DM fracture patients as compared to 20 T2DM patients without fracture. This same group, however, reported no difference in cortical porosity between patients with T2DM and non-diabetic subjects, although with only 20 subjects per group this study may have been underpowered to detect significant differences.

A further potential limitation of studies using HRpQCT is that this technique can only assess bone microarchitecture *in vivo* in humans at peripheral skeletal sites. However, Liu and colleagues [44] have shown associations between HRpQCT-derived bone microarchitectural properties at the distal radius and tibia and biomechanical properties (assessed by QCT) at central skeletal sites such as the hip and spine. These data suggest that measures of bone microarchitecture at peripheral skeletal sites may have clinical utility in identifying subsets of patients (e.g., patients with diabetes mellitus) who may be at risk for fragility fractures at both appendicular and axial skeletal sites, although this possibility needs further assessment..

Finally, another concern of the studies that have used HRpQCT to assess bone microarchitecture in patients with diabetes mellitus is that they consisted predominantly of white subjects. Nevertheless, the findings of these previous studies are consistent with a recent HRpQCT analysis of African-American participants in the Study of Women Across the Nation (SWAN) cohort by Yu and colleagues [45] showing that 22 African-American women with T2DM had increased radial cortical porosity (by 26%) as compared to 78 non-diabetic African-American women. These investigators found no significant associations between cortical parameters and duration of T2DM or a measure of insulin resistance (HOMA-IR). However, among the T2DM subjects, higher fasting glucose levels were associated with lower cortical volumetric BMD ($r = -0.54$, $p = 0.018$). Therefore, current collective evidence from cross-sectional studies in women suggests that T2DM may have detrimental effects on cortical, but not trabecular, bone morphology, and in particular may be associated with abnormally high cortical porosity. Notwithstanding, it should be emphasized that all of the studies that have assessed bone microarchitecture using HRpQCT in patients with T2DM thus far were confined to women and were limited by small sample

sizes. Thus, a definitive study with adequate power comparing bone microarchitecture (and specifically, cortical porosity) in women and men with T2DM and fragility fractures versus non-fracture T2DM controls is clearly warranted.

Alterations in bone material properties

In addition to bone microarchitecture, the material composition of bone is another important component of bone quality [38, 39]. Historically, however, the ability to directly measure bone material properties in humans was not practical because of the invasive nature of the direct measures needed to quantify these properties *in vivo*. Thus, there was a critical need to develop technology for non-invasively measuring *in vivo* bone material properties in humans for use in both research and clinical settings. Hansma and colleagues sought to fill this void by developing a bone microindentation device; this required the design, extensive validation, and iterative improvement of a series of instruments [46–50]. One of the first of these instruments to be utilized in human subjects for the measurement of indices of bone material properties *in vivo* was the “reference point indentation (RPI) instrument”; other versions were called the “bone diagnostic instrument” [46–48] and the “tissue diagnostic instrument” [49]. Using this technology, small studies have reported that *in vivo* indices of bone material properties may be worse in both hip fracture [51] and atypical femoral fracture [52] patients as compared to non-fracture control subjects. While application of this technology represented a noteworthy advance, the RPI procedure still required complete displacement of the periosteum from the bone surface at the site of measurement, which made routine clinical assessments of bone material properties in humans using the RPI prototype problematic.

To address this issue, Hansma and colleagues developed another bone microindentation instrument – called the OsteoProbe® (Active Life Scientific Inc., Santa Barbara, CA, USA), which is a small hand-held device that provides an index of bone material properties, later coined the “bone material strength index” or BMSi. Notably, this instrument does not require the need for a reference probe or displacement of the periosteum, and thus is ideal for providing *in vivo* measurements of bone material properties in humans [50, 53]. Although microindentation is a widely accepted technology for assessing material aspects of bone quality, further information is needed to determine the extent to which this technique captures traditional bone mechanical properties (e.g., bone elastic modulus, strength, toughness, and pre- versus post-yield parameters). Figure 2 shows the device positioned over the midshaft of the anterior tibia, the optimal testing site because it provides a viable flat surface, away from tendons, ligaments, blood vessels and nerves, for performing measurements of cortical bone material properties. Details regarding the measurement protocol and how the OsteoProbe® provides accurate and precise *in vivo* measurements of BMSi have been described in detail previously [42, 50]. Beyond the minimal discomfort associated with the local anesthesia, the procedure is well tolerated. However, it is likely contraindicated in patients who have skin lesions over the indentation site, excessive edema, infection, or those on anticoagulant therapy.

The extent to which bone material properties are impaired in fracture patients generally, and whether patients with diabetes, and in particular T2DM patients, have altered bone material

properties as compared to non-diabetic persons are issues of great interest [1, 54]. In a recent study using the OsteoProbe[®], Farr et al. [42] found that compared to controls, postmenopausal women with T2DM had significantly lower BMSi: unadjusted (−11.7%; $P < 0.001$; Figure 3A) and following adjustment for BMI (−10.5%; $P < 0.001$; Figure 3B), pointing to impaired bone material properties as a potential cause of the increased fracture risk observed in these patients. In addition, the mean glycated hemoglobin level over the previous 10 years was inversely correlated with BMSi ($r = -0.41$; $P = 0.026$) in women with T2DM. Taken together, these findings represented the first demonstration, using a direct *in vivo* measure of bone material properties, of compromised bone quality in T2DM and highlighted potential detrimental effects of prolonged hyperglycemia on bone. From these observations, it can be inferred that the skeleton may need to be recognized as yet another important target tissue subject to diabetic complications. Whether patients with T2DM who suffer fragility fractures have further reductions in bone material properties (and even higher cortical porosity) as compared to T2DM patients with no fracture history is not known, but future studies are needed to test the pivotal hypothesis that compromised bone quality, not deficits in aBMD, is the underlying basis for fragility fractures in patients with T2DM. Further, as noted earlier, because it is not known whether patients with T1DM have altered bone material properties, this will also be important to resolve. Thus, to better understand these issues, carefully designed studies are needed which evaluate biomechanically relevant components of bone structure and quality in women and men simultaneously, using identical methodology to better evaluate and personalize fracture risk prevention in patients with diabetes mellitus.

Pathogenesis of Skeletal Fragility in Diabetes – Advanced Glycation Endproducts (AGEs)

It is also important to better define the cellular mechanisms responsible for compromised bone quality in diabetes, although high levels of advanced glycation endproducts (AGEs) are likely implicated [55]. AGEs form from glucose exposure and increase in concentration with increased glucose exposure. In the setting of diabetes, higher concentrations of AGEs are involved in undesirable chemical modifications of proteins in multiple tissues [56]. While enzymatic processes produce pyridinium crosslinks, which are necessary for the mechanical integrity of collagen, non-enzymatic glycation via AGEs may create undesired crosslinks that modify the extracellular matrix in bone and potentially decrease bone formation [57–59]. *In vitro*, AGEs impair bone formation by interfering with osteoblast differentiation, attachment to bone surfaces, function, and survival [60–62].

While many different AGEs have been identified in human tissues [56], the most studied is pentosidine because it can be directly measured in clinical samples such as blood and urine (e.g., using immunoassays) as well as in bone tissue using high-performance liquid chromatograph (HPLC) methods [63]. Although data on levels of AGEs in human bone tissue are largely derived from cadaveric specimens, several studies have measured levels of AGEs in blood and urine using commercially available immunoassays. For example, serum and urine pentosidine levels have been shown to correlate with clinical fractures in T2DM patients [64, 65]. Further, N^ε-carboxy-methyl-lysine (CML) is another dominant component

of total AGEs [66] that has been shown to be associated with osteoporosis [67] and hip fracture [68]. However, a concern with these immunoassays is potentially poor assay validity due to numerous factors in blood and urine that interfere with immunoassay standardization [69]. As such, there is a critical need for the development of assays that specifically and accurately assess levels of AGEs in clinical samples. In addition, further work is needed to determine the extent to which circulating levels of AGEs reflect those *in vivo* in human bone tissue.

Pathogenesis of Skeletal Fragility in Diabetes – Bone Turnover & Sclerostin Levels

Multiple studies in humans have found that serum markers of bone formation (e.g., amino-terminal propeptide of type I collagen [PINP] and osteocalcin [OCN]) and bone resorption (e.g., cross-linked C-telopeptide of type I collagen [CTX]) are reduced in diabetic patients relative to non-diabetic controls [13, 41, 42, 70–76]. Higher circulating levels of sclerostin in patients with diabetes [77–79] may be responsible for low bone formation, as sclerostin is a potent inhibitor of Wnt signaling in bone [80]. Therefore, perhaps similar to what may occur with prolonged, high-dose bisphosphonate therapy [81], long-standing low bone turnover in diabetes may result in defective microdamage repair and increased bone microcrack accumulation [82] – creating a “vicious cycle” that contributes to increased fracture risk.

The reason(s) for low bone resorption levels in patients with diabetes is unknown, although this may partly result from coupling between bone formation and resorption. As noted earlier, diabetic medications such as insulin and TZDs have been shown to affect bone turnover [83, 84] and thus may play an important role in the pathogenesis of skeletal fragility in diabetes mellitus. Moreover, recent studies indicate that enzymatic cross-linking of bone collagen by lysyl oxidase is reduced in diabetes [85, 86]; as such, assays such as CTx, which measure cross-linked telopeptides released during bone resorption, may underestimate the level of bone resorption in diabetes. Consistent with this, reduced bone formation and an imbalance between bone formation and resorption is suggested by the few available histomorphometric studies in patients with diabetes [13, 70], which demonstrated suppressed dynamic indices of bone formation without corresponding reductions in bone resorption indices. Therefore, the current collective evidence indicates that diabetes is associated with low bone turnover, with perhaps a disproportionate reduction in bone formation relative to bone resorption.

Future Directions

Although it has been demonstrated that bone material properties are compromised in patients with T2DM and correlate inversely with chronic glycemic control as assessed cross-sectionally [42], whether bone material properties deteriorate over time to a greater extent in diabetic versus non-diabetic subjects remains unknown and is an important issue to resolve. In addition, whether glycemic control and AGEs in circulation or bone tissue itself are associated with changes in bone material properties over time in patients with diabetes and/or non-diabetic subjects is plausible but untested experimentally. There are also sparse

longitudinal data on changes in cortical porosity in patients with diabetes. Further, as noted earlier, several studies [77–79] have reported that patients with diabetes have increased circulating sclerostin levels which may impair intra-cortical bone formation [87]. However, whether these increased levels are related to the increased cortical porosity or altered bone material properties in these patients is unknown. Finally, in both patients with T1DM and T2DM, altered bone turnover may cause deterioration not only in bone material properties, but also in cortical porosity; however, this remains to be tested. Thus, there is a critical need to better define the longitudinal changes in bone material properties and cortical porosity *in vivo* in diabetic patients and establish the biochemical (e.g., AGEs, sclerostin, bone turnover makers, etc.) determinants of these changes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Patients with diabetes have increased fracture risk despite normal BMD
- Several studies have found increased cortical porosity in type 2 diabetes patients
- Bone material properties may also be compromised in type 2 diabetes patients
- Thus, abnormal bone “quality” may explain the increased fracture risk in diabetes

Figure 1. HRpQCT images of the distal radius from Control (top) and T2DM (bottom) subjects: distal-most slices (A, E); proximal-most slices (B, F). 3D visualization of the mineralized bone structure (C, G) and of cortical bone (transparent gray) and cortical porosity (solid green) (D, H). Reproduced from Burghardt et al. [40], with permission.

Figure 2.

Image of the OsteoProbe® (Active Life Scientific Inc., Santa Barbara, CA), a hand-held microindentation instrument designed for *in vivo* measurements of the material properties of bone (i.e., bone material strength index [BMSi]) in humans at the midshaft of the non-dominant anterior tibia. Reproduced from Farr et al. [42], with permission.

Figure 3. Unadjusted (A) and BMI-adjusted (B) comparisons of BMSi between patients with T2DM and age-matched, non-diabetic controls. Values are shown as mean $\pm$ SE. *** $P < 0.001$. Adapted from Farr et al. [42].

Table 1

Selected trabecular and cortical microarchitectural HRpQCT parameters in control and T2DM subjects (adapted from Burghardt et al. [40]).

	Radius			Tibia		
	Control	T2DM	%	Control	T2DM	%
Trabecular						
Tb.N. (mm ⁻¹)	1.77 ± 0.32	18.4 ± 0.30	3.4%	1.75 ± 0.41	1.73 ± 0.39	-0.9%
Tb.Th. (µm)	66.0 ± 10.4	65.4 ± 7.4	-0.9%	67.8 ± 13.3	77.2 ± 12.7	13.8% ^a
Tb.Sp. (µm)	513 ± 100	492 ± 91	-4.0%	534 ± 136	532 ± 160	-0.5%
Cortical						
Ct.PoV (mm ³)	9.11 ± 4.7	22.9 ± 32.6	151.1% ^a	55.8 ± 21.7	121 ± 213	117.5%
Ct.Po (%)	1.92 ± 0.90	4.29 ± 5.51	123.9% ^a	5.53 ± 2.00	7.53 ± 6.12	36.2%

Data are mean ± SD. Bold font indicates statistically significant percentage differences. Tb.N., Trabecular Number; Tb.Th., Trabecular Thickness; Tb.Sp., Trabecular Separation; Ct.PoV, Cortical Pore Volume; Ct.Po, Cortical Porosity;

^ap < 0.05.