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REVIEW ARTICLE

Assessment of bone turnover and bone quality in type 2 diabetic bone disease: current concepts and future directions

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Substantial evidence exists that in addition to the well-known complications of diabetes, increased fracture risk is an important morbidity. This risk is probably due to altered bone properties in diabetes. Circulating biochemical markers of bone turnover have been found to be decreased in type 2 diabetes (T2D) and may be predictive of fractures independently of bone mineral density (BMD). Serum sclerostin levels have been found to be increased in T2D and appear to be predictive of fracture risk independent of BMD. Bone imaging technologies, including trabecular bone score (TBS) and quantitative CT testing have revealed differences in diabetic bone as compared to non-diabetic individuals. Specifically, high resolution peripheral quantitative CT (HRpQCT) imaging has demonstrated increased cortical porosity in diabetic postmenopausal women. Other factors such as bone marrow fat saturation and advanced glycation endproduct (AGE) accumulation might also relate to bone cell function and fracture risk in diabetes. These data have increased our understanding of how T2D adversely impacts both bone metabolism and fracture risk.

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INTRODUCTION

Type 2 diabetes mellitus (T2D) is an exceedingly common chronic metabolic disorder that has an enormous impact on public health. Currently, diabetes affects over 387 million adults worldwide and is projected to reach 592 million by 2035.[1] Until recently, the list of target organs affected by T2D did not include the skeleton. Yet it is now well-established that T2D is an independent risk factor for fractures, which is not attributable to increased body mass index (BMI) nor other classical osteoporosis risk factors.[2] New data from epidemiologic and pathophysiologic reports, as well as from studies employing state-of the-art investigational tools, have recently increased our understanding of how T2D adversely impacts both bone metabolism and fracture risk.

Epidemiological data indicate that older adults with T2D have a higher risk of fractures, with a 50%–80% increased extremity fracture risk.[3–4] A meta-analysis of 12 studies reported a relative risk of 1.7 (95% confidence interval: 1.3–2.2) for hip fracture in both men and women with T2D.[5] The risk of all clinical fractures was also increased, with a summary relative risk of 1.2 (95% confidence interval: 1.0–1.5).[5] Other studies have reported similar results,[6] with a direct association between the duration of diabetes and increased fracture risk.[7] Given this increased fracture risk, it is perhaps surprising that bone mineral density (BMD) is generally higher in those with T2D compared with those without.[8] In a meta-analysis, Vestergaard et al[8] reported an increased Z-score of +0.41 at the spine and +0.27 at the hip associated with T2D. In addition, a large prospective study has shown that patients with T2D have a higher fracture risk for a given femoral neck BMD T-score.[9] Although dual-energy x-ray absorptiometry (DXA) is the gold standard method for the quantitative assessment of BMD, providing areal BMD of the hip, spine, radius, and total body (including body composition), it has limitations in patients with complex metabolic bone diseases such as chronic kidney disease or diabetic bone disease. It has

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therefore been suggested that fragility fractures in T2D may result from diabetes-related alterations in skeletal properties not captured by DXA.[2] Similarly, patients with T2D have a higher fracture risk for a given FRAX probability (https://www.shef.ac.uk/FRAX/).[9] The FRAX algorithm provides the 10-year probability of major osteoporotic fractures per individual tested. A recent study has demonstrated that diabetes mellitus does not modify the effects of risk factors incorporated into FRAX.[10] Nevertheless, the study has shown that diabetes has stronger effects on hip fracture risk in younger than older individuals which warrants special consideration for diabetic fracture prevention in clinical practise.

The paradox of higher BMD in association with increased fractures might be attributed to more frequent trauma, as diabetes is associated with an increased frequency of falls.[11] However, in studies of diabetes and fracture that controlled for fall frequency, diabetes still remained independently associated with increased fracture risk.[3–4] Thiazolidinediones use might also be considered as an explanation, since it has been proposed that these agents divert mesenchymal stem cells from the osteogenic to the adipocytic lineage and are associated with bone loss and increased fracture risk, particularly in women.[12] However, thiazolidinedione use cannot fully account for the increased risk of fracture observed with diabetes, since most studies included substantial observation time prior to the widespread use of these medications. Rather, it appears that other bone properties, which are undetectable by DXA, are probably contributing to fracture risk in diabetes.

PTH AND BIOCHEMICAL MARKERS OF BONE TURNOVER IN T2D

Decreased bone remodeling in T2D has been demonstrated by a number of lines of evidence. Levels of parathyroid hormone (PTH) tend to be 20%–50% lower in T2D subjects than in controls, even in the setting of reduced eGFR, suggesting a state of reduced PTH secretion in T2D.[13–15] Circulating biochemical markers of bone formation, including P1NP, osteocalcin (OCN)[14–15] and bone specific alkaline phosphatase[16] have been found to be decreased in T2D. These decreases in formation measures are associated with reductions in the bone resorption marker serum CTx.[13–16] The decrease in bone remodeling in T2D appears to be predictive of fracture risk regardless of BMD. In a study of 255 T2D women and 240 controls, T2D women with the combination of the lowest PTH and OCN levels had nearly a fivefold increased risk of vertebral fractures independent of lumbar spine BMD.[15]

DYNAMIC HISTOMORPHOMETRY IN T2D

Lower bone formation in T2D on biopsy was reported in one study, but the numbers were very small ($n = 6$ T2D patients; 2 female), and the results were confounded by selecting for low BMD and a problematical control group.[17] In a more recent pilot study, low-bone formation was observed in six T2D postmenopausal women and six postmenopausal age-matched non-diabetic controls, where tetracycline double-labeled iliac crest bone biopsies showed virtually no uptake of label in diabetic subjects (Figure 1), with reduced mineralizing surface, osteoid surface, and osteoblast surface.[18] Interestingly, corresponding reductions in bone resorption indices were not present, perhaps suggesting a disproportionate reduction in bone formation in diabetes as compared with bone resorption.

OTHER BONE MARKERS IN T2D

Insulin-like growth factor (IGF)-1, an anabolic factor which stimulates osteoblast proliferation, has been inversely associated with the risk and number of vertebral fractures in diabetic women independent of BMD.[14–19] Another marker which might reflect bone formation is that of circulating osteogenic precursor cells,[20] which have been reported to be decreased in patients with T2D (Figure 2).

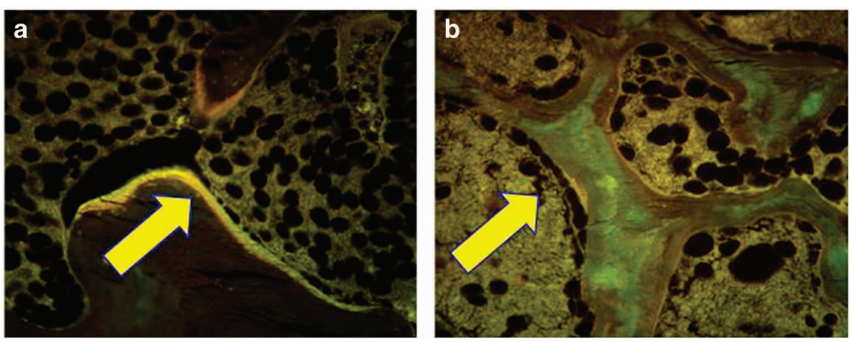

Figure 1. Histomorphometric changes in bone formation. (**a** and **b**) Tetracycline double-labeled bone biopsies in a 57-year-old Caucasian female control (**a**) and a 58-year-old T2D Caucasian woman (**b**) bone formation is decreased in T2D with reduced mineralizing surface. The arrows highlight tetracycline uptake in the control subject and the absence of uptake in the diabetic subject. Adapted with permission from ref. 18.

Circulating osteogenic precursor cells can be detected in the peripheral blood by flow cytometry using antibodies specific for the osteoblast matrix protein OCN.[21] Peripheral blood mononuclear cells that were positive for OCN were lower in postmenopausal women with T2D as compared with non-diabetic controls.[18] Moreover, within the decreased pool of overall OCN+ cells, the T2D subjects had an increased subpopulation of immature OCN+ cells, that is, cells that also had early markers CD146 and CD34, subpopulations which diminish when osteoblasts mature.[20] An additional novel bone marker in T2D may be sphingosine 1-phosphate (S1P), a lipid mediator which increases osteoclastogenesis by increasing RANKL.[22] S1P was found to be increased in T2D women ($n = 482$) as compared with controls and was associated with vertebral fractures. Interestingly, this marker suggests an elevation in bone resorption in T2D, in contrast to the reports of decreased s-CTx levels.[13–16] It is possible that s-CTx underestimates the level of bone resorption in diabetes because enzymatic cross-linking of bone collagen by lysyl oxidase is reduced in diabetes,[23–24] such that less cross-linked telopeptides might be released in diabetes during bone resorption.

SCLEROSTIN IN T2D

Sclerostin, an osteocyte product, is a negative regulator of bone formation which competes with the anabolic Wnt β-catenin pathway by binding to LRP5 or 6.[25] In healthy adults, sclerostin levels are increased by factors including age, BMI, inactivity, bone mineral content, and possibly fractures.[25] It was first reported in 2012 that sclerostin levels were higher in 74 T2D women and men versus 50 non-diabetic controls and that higher levels correlated with age, male gender and BMD.[26] This observation was corroborated by another report in which sclerostin levels were found to be twofold higher in T2D than in controls or T1D, after adjusting for age and BMI.[27] A correlation between Wnt disruption and decreased osteoblast activity was further observed in 40 T2D postmenopausal women who, as compared with controls, had decreased β-catenin levels which correlated with lower BAP.[16] In the largest diabetes sclerostin study, higher sclerostin levels in 321 men and women with T2D were associated with an increased risk of vertebral fractures independent of lumbar spine BMD.[28] Interestingly, diabetic postmenopausal women with fragility fractures were shown to demonstrate significantly higher serum sclerostin levels than diabetic postmenopausal women without fragility fractures.[29] While BMI, renal function, glycemic control, and diabetes medication (including insulin) were comparable between women with and without fragility fractures, diabetes duration was significantly longer in those that had sustained fractures. It could be posited from these data that the higher sclerostin levels in T2D reflect the presence of more deeply embedded osteocytes in older bone that has accumulated more microscopic damage. Thus prolonged low-bone turnover in diabetes, as a result of Wnt inhibition, may lead to defective microdamage repair and increased bone microcrack accumulation in a manner reminiscent of high-dose bisphosphonate therapy,[30] thus contributing to greater bone fragility. Stressing the interplay between bone health and vascular health and considering diabetic fractures to be true diabetic complications, it is also noteworthy that in diabetics higher serum sclerostin levels appear to be associated with higher amounts of vascular calcifications.[31]

AGES AND BONE REMODELING

Decreased bone formation might occur in part because of increased advanced glycation endproducts (AGEs) in bone collagen. AGEs are a diverse group of compounds that are generated through the non-enzymatic glycation or glycoxidation of proteins, lipids, and nucleic acids[32] with the best-studied being carboxymethyl-lysine and pentosidine.[33–36] These compounds are markedly increased in patients with diabetes,[35] forming non-enzymatic cross-links within and across collagen fibers.[37–38] AGEs interfere with normal osteoblast function[39] and attachment to the collagen matrix,[40] as well as impair osteoblast development.[41–42] AGEs also decrease bone resorption by altering the structural integrity of bone matrix proteins and inhibiting the osteoclastic differentiation process.[43] This might have long-lasting skeletal effects that are similar to the "hyperglycemic metabolic memory" that has been described with AGE accumulation in other tissues.[44] In the Diabetes Control and Complications Trial (DCCT), accumulation of AGEs in skin collagen of type 1 diabetes patients predicted complications decades later, regardless of subsequent improvements in glycemic control.[45] In the bone matrix, accumulation of AGEs leads to more biomechanically brittle bone that has lost its toughness and is less able to deform before fracturing.[30] Urinary pentosidine, the best-studied AGE, was associated with a 42% increase in clinical fracture incidence in T2D.[38] Although the relationship between *in vivo* bone and circulating levels of AGEs has not been fully elucidated, AGEs are likely related to both low-bone formation and increased bone fragility in T2D.

ASSESSMENT OF FRACTURE RISK IN T2D: FROM CURRENT CLINICAL PRACTICE TO NOVEL IMAGING BIOMARKERS

Irrespective of diabetes history, in clinical practice fracture risk is routinely assessed by measuring BMD and by

determining the presence of clinical risk factors including age, sex, BMI, smoking and drinking habits, history of fragility fractures, parental fractures, rheumatoid arthritis, and known secondary osteoporosis. These factors are part of the aforementioned, questionnaire-based fracture risk assessment tool (FRAX).[46]

BMD can be measured by DXA and quantitative computed tomography (QCT) at central and peripheral sites.[47] Although DXA can predict fracture risk in diabetic patients to a certain extent, experts have suggested the introduction of a "diabetic correction factor" for T-scores because given T-scores were shown to be associated with a higher risk of fracture in older adults with T2D than in those without DM.[48] Similar concepts have been suggested for FRAX scores in elderly subjects with T2D.[48] The use of trabecular bone score (TBS) has been also suggested to improve the diagnostic performance of lumbar spine DXA in patients with T2D: from an image processing perspective, TBS is based on two-dimensional texture-analysis of DXA images. Applying methodologies originally used in pattern recognition (for example, in geostatistics characterizing landscapes from aerial views), textural variations are quantified between neighboring gray-scale pixels.[49] In clinical practise, operators and clinicians are only presented with the end-result of these calculations: A single (TBS) index which is higher in images with fewer inter-pixel variations (that is, indirect measure of better spinal bone microarchitecture) and lower in images with higher inter-pixel variations (that is, indirect measure of worse spinal bone microarchitecture).[50] While BMD is typically normal to higher in subjects with diabetes, TBS appears to be lower in diabetics than in non-diabetic subjects.[51] In the same publication, Leslie *et al*.[51] also showed that lumbar spine TBS can predict osteoporotic fractures irrespective of the presence or absence of diabetes. In addition, TBS has been shown to be positively associated with good glycemic control.[52–53] While these results are of major clinical interest and relevance, it remains to be stressed that—from an imaging standpoint—TBS only provides an indirect measure of bone microarchitecture at low resolution and reduced image quality.

While DXA is a projectional technique, central QCT provides volumetric data on BMD and bone geometry of the spine and the proximal femur. For QCT, a clinical multidetector-CT scanner, a calibration phantom mat, and dedicated software are needed.[54] Alterations in volumetric hip BMD—as determined by QCT in diabetic versus non-diabetic subjects—have been confirmed by two independent studies.[29,53–55] One of these studies also concluded that patients with T2D appear to have little benefit from elevated BMD due to unchanged load-to-strength ratios.[55]

HIGH-RESOLUTION PERIPHERAL QCT

Within the last two decades, dedicated peripheral QCT scanners have been developed to capture volumetric BMD of peripheral sites (that is, the extremities) at varying resolution. In addition to BMD and geometric measures, high-resolution peripheral QCT (HR-pQCT) provides quantitative access to bone microarchitecture, an important surrogate of bone quality and bone strength.[56–57] Many micro-architectural parameters quantifiable by HR-pQCT refer to those initially coined by static histomorphometry (for example, trabecular number, trabecular thickness).[58] In the first generation HR-pQCT scanner, which has a spatial resolution of ~80 μm, only two microstructural parameters—trabecular number and trabecular heterogeneity—are directly measured by three-dimensional distance transformations.[59] Trabecular separation and trabecular thickness are derived from trabecular BMD and trabecular number, which are determined by threshold-based segmentation and a ridge-counting technique.[60] Recently, a second generation scanner with shorter scan time and higher spatial resolution has been introduced. Using this second generation scanner, all micro-architectural indices can be calculated directly.[61] It is noteworthy that so far, all published HR-pQCT data on bone microarchitecture in T2D have been acquired with scanners of the first generation.[62–65]

First evidence of associations between impaired cortical strength and T2D originally came from a pQCT study in a cohort of elderly men.[66] The first HR-pQCT study investigating bone microarchitecture in T2D then raised the topic of high cortical porosity as a potential predominant structural phenotype:[62] while trabecular BMD and trabecular thickness were greater in postmenopausal women with T2D than in those without, high cortical porosity was observed (Figure 2). Based on the unstratified enrollment of diabetic women with and without fractures, this study lead to the formation of a key hypothesis for subsequent research: could cortical porosity serve as a discriminative feature between diabetic women with and without fragility fractures? In line with this hypothesis, Shu *et al*,[63] also reported no differences in peripheral bone microarchitecture between postmenopausal women with and without T2D (without history of fragility fractures). Specifically enrolling postmenopausal diabetic women with and without fragility fractures and comparing them with non-diabetic controls with and without fractures, the importance of high cortical porosity with relatively maintained trabecular properties could be confirmed.[64] Interestingly, diabetic women with and without fractures were comparable with respect to clinical characteristics: age, BMI, glycemic control, 25(OH)-vitamin D, kidney function, and PTH levels were similar. The only significant clinical difference was the duration of diabetes: diabetic women with

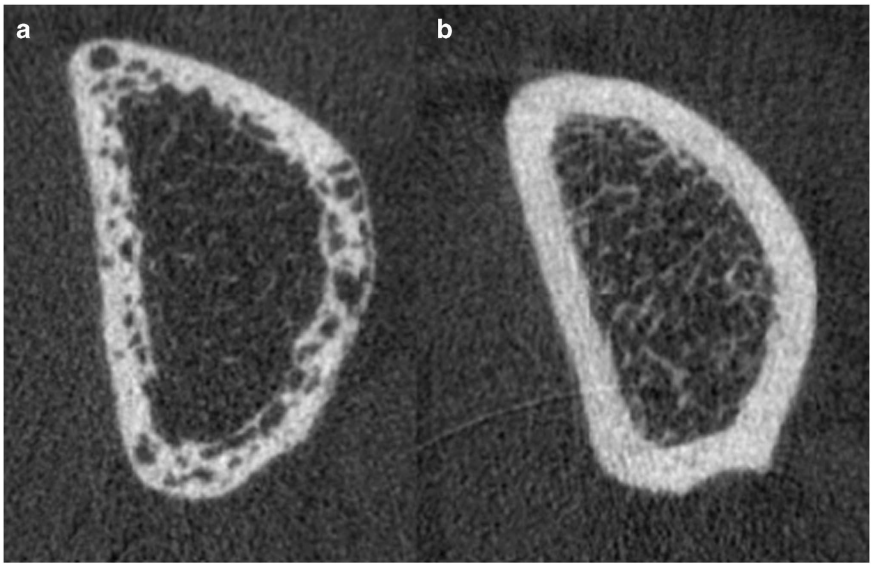

Figure 2. Cortical porosity in diabetic bone disease with fractures. High-resolution peripheral quantitative computed tomography (HR-pQCT) of the distal radius in type 2 diabetic women with (**a**) and without (**b**) fragility fractures.[64] Image courtesy: Thomas M. Link, Department of Radiology and Biomedical Imaging, The University of California, San Francisco.

fractures had been suffering from diabetes significantly longer than diabetic women without fractures (13 years versus 8 years). Using finite element analyses, deficits in stiffness, failure load, and cortical load fraction could be detected and attributed to cortical porosity.[64] Considering the high prevalence of insulin resistance and T2D in the African-American population, a forth HR-pQCT study specifically focused on the assessment of bone microarchitecture in postmenopausal African-American women: In line with above mentionned studies of mixed racial recruitment, Yu *et al.*[65] found T2D and unfavorable cortical bone microarchitecture to be similarly associated in African-American women.

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) provides an alternative method of depicting and quantifying bone geometry and bone microarchitecture.[47] High-resolution MRI has been used to study bone microarchitecture in postmenopausal and age-related osteoporosis,[67–69] secondary osteoporosis,[70] chronic kidney disease,[71] and with respect to treatment effects of anti-osteoporotic drugs.[72–73] Regarding diabetic bone disease, there is only a limited number of publications using MRI. Specifically, a Canadian researcher reported poorer bone microarchitecture expressed as greater trabecular heterogeneity in subjects with diabetes mellitus than in healthy controls.[74] Although compared with HR-pQCT, MRI has certain disadvantages in studying bone microarchitecture (for example, lower resolution, higher technical complexity in acquisition and postprocessing of image data, no option of measuring BMD, and fewer reference data), the method does hold significant potential for bone research. Specifically, MRI offers the option of imaging the skeleton beyond its geometry and structure. Using techniques such as magnetic resonance-perfusion and MR-spectrocopy, novel imaging surrogates of bone strength (for example, skeletal blood flow and regional biochemical composition of bone marrow) have been introduced.[75–76] Recent MR-imaging data have demonstrated that altered bone marrow fat composition is linked with T2D and fragility fractures in postmenopausal women.[77] Even after adjustment for age, race, and local (that is, spinal) BMD, lower unsaturated bone marrow fat and higher saturated bone marrow fat as determined by MR-spectroscopy were significantly associated with T2D. Of interest, postmenopausal diabetic women with a history of fragility fractures displayed the lowest unsaturation and the highest saturation levels. The relevance of these findings is supported by epidemiologic data linking low dietary intake of n-3 poly-unsaturated fatty acids (PUFA) with low BMD[78–80] and the risk of hip fractures.[81] Preclinical data support a potential osteoprotective role of PUFA.[82–85] Vice versa, saturated dietary fatty acids appear to be linked with osteoporotic fractures.[86] With poor glycemic control also leading to accelerated aging of adipocytes and thus adipocyte dysfunction,[87–90] changes in bone marrow fat composition of subjects with T2D are likely to result from a multitude of interacting etiologic factors. It is conceivable that bone

marrow adiposity might reflect a shift in stem cell lineage away from osteoblastogenesis toward adipogenesis.[91] In the AGEs–Reykjavik cohort (115 men and 134 women; mean age 79; 7% with diabetes), sclerostin levels were positively associated with marrow fat by MRI in men independent of BMD.[91] This positive relationship could be explained by a shift in precursor stem cell lineage away from osteoblastogenesis, as reflected by higher sclerostin levels, toward adipogenesis, as reflected by higher bone marrow fat.[91]

OBESITY AS A POTENTIAL CONFOUNDER

Confounding greater understanding of the mechanisms responsible for increased skeletal fragility in T2D is the frequent concurrence of obesity, which has also recently been shown to be a risk factor for fractures.[92–94] Mechanisms proposed to be responsible for increased skeletal fragility in both obesity and T2D include structural, metabolic, material, dynamic, and imaging abnormalities. However, the relative contribution of each condition separately and their combined effects on skeletal fragility remain unclear. The concurrence of obesity and T2D in most individuals has undermined attempts to gain pathogenetic clarity. For example, obese women have been reported to have trabecular and cortical microarchitectural abnormalities, but their diabetic status was not considered.[95] Moreover, obese diabetics have lower biochemical and quantitative histomorphometric indices of bone formation,[17–96] elevated circulating levels of the osteocyte product sclerostin,[27] increased adipocyte markers,[97] and abnormal bone marrow fat composition,[77] however, these abnormalities are also found in obese individuals without T2D.[98–101] Whether T2D has negative effects on specific skeletal parameters that are distinct from the adverse skeletal effects of obesity remains to be clarified.

A CONTINUING QUEST FOR NOVEL NON-INVASIVE BIOMARKERS REFLECTING HIGH FRACTURE RISK IN PATIENTS WITH T2D

Minimal invasive testing of bone strength ("micro-indentation") is nowadays possible in the setting of clinical research. After local anesthesia at the mid-shaft point of the anterior tibia, a handheld device is inserted through soft tissue and periosteum, to touch the bone surface. Using triggered, pre-defined impacts small bony indentations (that is, local superficial microfractures) are created ($< 350\,\mu m$). The deeper the mean indentation distance, the weaker bone material properties are at the testing site of an individual subject.[102–103] Using the most recent generation *in vivo* testing device, Farr *et al*.[104] have shown that postmenopausal diabetic women had significantly lower bone material strength than non-diabetic controls. Poor bone material strength correlated with poor long-term glycemic control over the past 10 years.[104] *In vivo* micro-indentation has been used to quantify reduced bone strength in patients with osteoporotic hip fractures and atypical femoral fractures.[105–106] To the best of our knowledge, *in vivo* micro-indentation data are still lacking for patients with T1D, men, and patients with fragility fractures.

Given significant advances in knowledge within the last decade, dedicated research in diabetic bone disease with and without fractures and development of novel patient-oriented biomarkers continue to be warranted.

SUMMARY AND CONCLUSION

There is growing evidence that the skeleton is an important target organ for complications of T2D. Many large prospective studies have established that T2D is associated with an increased risk of fractures. Yet it remains unknown how to identify which patients with T2D are at increased risk because it is uncertain why bone fragility is increased when aBMD is normal. Available evidence suggests that compromised bone quality, not deficits in aBMD, is the underlying basis for fragility fractures in patients with T2D. Clues supporting this hypothesis include alterations in bone remodeling, increased cortical porosity, and decreased bone material properties. More information is needed about the pathogenesis of these abnormalities and their relationship to the increased fracture risk observed in T2D. Whether a circulating marker, such as an AGE or sclerostin, would be predictive of alterations in bone material properties or cortical porosity remains to be determined. Further investigation of skeletal parameters would shed light on the issue of greater bone fragility in T2D and potentially offset serious challenges in this population as they age.

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