Diabetic Bone Disease

Basic and Translational Research and Clinical Applications

Beata Lecka-Czernik
John L. Fowlkes
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Preface

The World Health Organization (WHO) estimates that worldwide, diabetes occurs in more than 180 million people. Because the incidence of Type 1 diabetes mellitus (T1D) and Type 2 diabetes mellitus (T2D) is increasing globally, it is estimated that the number of people with diabetes will more than double by 2030. In parallel, it is anticipated that comorbid states associated with diabetes will also rise; thus, understanding and treating complications of diabetes will be a very high priority going forward in order to decrease morbidity and mortality, as well as to better control health care expenditures. Historically, most attention has been focused on four major complications known to afflict many individuals with T1DM and T2DM: retinopathy, neuropathy, nephropathy, and cardiovascular disease. However, epidemiological data now show that other tissues and organs may be significantly impacted by the diabetic state—and the skeletal system is now emerging as a primary target of diabetes-mediated damage (i.e., diabetic bone disease).

Studies have demonstrated that osteopenia and osteoporosis may be frequent complications of T1D, both in children and adults, and that T1D is associated with decreased bone density and increased fracture risk. In contrast to T1D, T2D has typically not been associated with osteopenia or osteoporosis and, in fact, has been more often associated with increased BMD. However, newer data show that bone quality and bone microarchitecture may be compromised in both conditions, suggesting that underlying mechanisms related to increased risk to fracture may be contributory to both forms of diabetes.

In this volume, we provide the reader with up-to-date information about what is currently known about diabetic bone disease and what are the challenges still facing the research and clinical care communities. In the first two chapters, the clinical and epidemiological data about diabetic bone disease is evaluated and reviewed for T1D and T2D, respectively. Chapter 3 discusses how the propensity to fracture in diabetic bone disease can impact fracture risk assessments and how it can be adjusted for using current clinically relevant fracture risk models. Chapter 4 provides a comprehensive overview of orthopedic complications observed in diabetes, and Chapter 5 focuses on the consequences of diabetes on periodontal disease. The utility

of skeletal biomarkers in assessing diabetic bone disease is reviewed in Chapter 6. Chapter 7 shows how drugs used to treat diabetes may also have skeletal consequences. Diabetes may fundamentally impact early progenitor cells of various bone lineages, and through this mechanism globally impact bone; Chapter 8 reviews the literature related to this possibility. How diabetes ultimately may impact the architecture, integrity, and quality of bone is discussed in Chapters 9–11.

As editors, we are truly indebted to the authors who have allowed us to catalogue their unique insights and expertise in diabetic bone disease into one comprehensive text. We hope the reader will find this volume, the first ever to be devoted specifically to diabetic bone disease, to be a useful and thought-provoking resource.

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Part I
Clinical and Pre-clinical Applications and Research

Chapter 1
Skeletal Deficits in Type 1 Diabetes Mellitus

Kathryn M. Thrailkill

While type 1 diabetes mellitus (T1D) accounts for <10 % of all diabetes, studies suggest that the global incidence of T1D appears to be increasing by 2–3 % per year [1, 2], attributed to a variety of possible immune-modulatory factors which include societal changes in hygiene, infectious exposures, vitamin D deficiency, and/or infant diets [1]. Diagnosis of the disease most commonly occurs in the pediatric population [3], with peaks in presentation occurring at school entry (5–7 years) and puberty (10–14 years) [2]. In the USA, prevalence of T1D has recently been estimated at 1 in every 433 youth <20 years of age [4].

T1D is, foremost, a state of insulin deficiency due to the progressive, predominantly autoimmune-mediated, destruction of pancreatic beta-cells. As such, unlike type 2 diabetes (T2D), insulinopenia, rather than insulin secretory dysregulation, becomes the overriding phenotype of this disease and insulin replacement therapy is a necessity. In the last two decades, however, therapeutic options (insulin analogues, insulin pumps, continuous glucose sensors) [5–7] have advanced tremendously. And, in recent years, the use of insulin-pump therapy, in particular, continues to increase in an effort to more-tightly regulate glycemic control [8, 9]. Hence, understanding the protracted pathophysiology of *adult* diabetic bone disease in T1D requires an appreciation of the individual context in which this comorbidity has developed over an individual lifetime. The following pages will review skeletal deficits in T1D as they are currently understood, recognizing that the disease phenotype may change in the future, as treatment options continue to improve.

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Human Bone Phenotype in T1D

Bone Mineral Density

In patients with T1D, an increased incidence of osteopenia and osteoporosis has been recognized for over three decades [10–14], occurring not only in adults, but in children as well [15–17]. Many more recent studies have since validated these early findings, demonstrating a reduced bone mineral density (BMD) in T1D [18–22]. Clinical factors associated with lower bone density include: male gender [18, 19, 23, 24]; longer duration of disease [14, 25]; younger age at diagnosis [26]; lower endogenous insulin or C-peptide levels [27]; low body mass index (BMI) [18, 28]; and possibly the presence of chronic diabetes comorbidities or associated autoimmunity [29]. Some studies also suggest that greater longitudinal decrements in BMD occur over time in males [24].

In most studies, poor glycemic control does not seem to be strongly associated with a reduced BMD [18–20, 22, 23, 30, 31], other than in the context of fracture risk (see below) or as a prerequisite for diabetes complications [28]. However, a single point-in-time HbA1c determination used by many of these studies does not reflect long-term hyperglycemia exposure. It is interesting, therefore, that in a few studies with repeated measures of HbA1c over time, chronically poor glycemic control does correlate with low BMD [32], even in children [33]. In addition, in studies of bone quality using phalangeal quantitative ultrasound (QUS) techniques, poor metabolic control is associated with lower QUS scores in premenopausal women [34]. Chronic hypercalciuria, as a consequence of chronic osmotic diuresis in patients with poorly controlled disease, also contributes to negative calcium balance and loss of bone mineral content [30]. Moreover, microvascular complications [35], including the presence of diabetic retinopathy [36], neuropathy [37, 38], and/or nephropathy [39], are associated with a greater occurrence of osteopenia or osteoporosis, in most, but not all studies [30, 40]. Together, these findings would seem to support the intuitive concept that chronically poor metabolic control, particularly of childhood onset [41], either directly or indirectly has some detrimental impact on the skeleton.

While both the axial and appendicular skeleton can be impacted, skeletal sites at greater risk for osteopenia or osteoporosis in males include the spine and femoral neck [18, 19, 23], whereas in females low BMD of the hip or femoral neck is prevalent [19, 30, 40]. Reduced BMD at distal limb sites has also been reported [32, 38]. Even so, a few studies with exclusively premenopausal female enrollment have demonstrated normal BMD in T1D, relative to healthy controls [42, 43].

Fracture Risk

T1D is also associated with an increased risk for fracture, higher than the risk in type 2 diabetes (T2D) [22], though the specific nature and circumstances of this risk are still undergoing scrutiny. Many initial studies examining the risk of fracture in

patients with diabetes either did not, or were not able to distinguish between T1D and T2D patients in their reported analyses [44–46]. Typically, these studies were conducted in older adults (≥50 years of age), considered generally at greater risk for fracture, and diabetes was subcategorized only as to the presence or absence of insulin treatment. Hence, some studies demonstrated an increased risk for fracture [44, 46, 47], while others did not [45, 48]. However, among risk factors for hip fracture in >33,000 *middle-aged* adults in Sweden (~25–60 years), the strongest risk factor for both women (RR = 3.89; 95 % CI 1.69–8.93, $p = 0.001$) and men (RR = 6.13, 95 % CI 3.19–11.8, $p = 0.001$) was diabetes [49], suggesting that the presence of diabetes was a major risk determinant for this age group. Similar findings had been reported years before in middle-aged Norwegian women and men [50]. To better delineate the relative risk for fracture in T1D, Table 1.1 summarizes a majority of studies conducted during the last 15 years specifically examining patients with T1D, compared with nondiabetics, from selected populations. Together, these studies demonstrate an unequivocally increased fracture risk at the hip, with most demonstrating a six to ninefold increase in relative risk. Moreover, the increase in hip fracture risk is greater than would be expected on the basis of BMD decrements alone [22, 51]. Two more recent studies examining mixed T1D and T2D populations using health-care databases in Canada [52] and Taiwan [53] have also confirmed an increased risk for hip fracture in persons with diabetes, though these studies were, again, predominantly composed of T2D patients.

Though only a very few studies have examined fracture risk at other skeletal sites [51, 54], an increased risk for vertebral fracture is also a consistent finding in studies that have quantified this. This includes the elevated prevalence of asymptomatic vertebral fractures in T1D, independent of BMD [51]. And, in one study, an approximate threefold increase in risk for all non-vertebral fractures was reported in men with T1D [55]. Even in case–control studies comparing lifetime fracture history at any site, an increased frequency of fracture is noted in persons with T1D (odds ratios of ~2) [30, 32]. And, as in other conditions, a more common site for osteoporotic fracture in adults with T1D remains limb fractures, related to accident or falls [44, 47, 56].

In diabetes, an increased risk for fracture has been associated with longer duration of disease [44]; then again, few studies of fracture risk in *pediatric* patients with T1D have been conducted. However, from one study with relatively small sample size, when comparing the history of any fracture in pediatric T1D patients ≤13 years of age to age-matched control subjects, no significant increase in fracture occurrence was yet seen in these early years [57].

Whether fracture risk is influenced by long-term glycemic control remains unclear, as studies exist to both support and refute this idea (Table 1.1). Nevertheless, in T1D, the presence of diabetic microvascular complications [58], and specifically including retinopathy [44], and nephropathy [59], are associated with a higher risk for fracture, though this increased fracture risk might relate, in part, to the health consequences of these comorbidities (decreased visual acuity or kidney function). Certainly, in persons with long-standing T1D, fracture risk is amplified by an increased fall risk, as might result from hypoglycemic events, visual deficits, peripheral neuropathy, or physical disability [60].

Table 1.1 Studies of fracture risk in type 1 diabetes

Authors	Years	Locations	Study methods	*N*	(F, M)	Age at enroll (years)	Fracture sites	RR [OR][a]	Associations, impact of comorbidities	CGC
Zhukouskaya [51]	2013	Belarus	Case–control	82/82	F+M	20–55	Vertebral	4	NR[b]	No
Neumann [30]	2011	Germany	Case–control	128/77	F+M	20–70	Any	[2.6] (F) [1.9] (M)	*Not* associated with microvascular complications	Yes
Danielson [32]	2009	USA (WI)	Case–control	75/75	F	18–50	Any	[2.3]	Poor control associated with low BMD	NR
Vestergaard [22]	2007	Mixed	Meta-analysis	5 studies	F+M	–	Hip	6.9	–	NR
Janghorbani [175]	2007	Mixed	Meta-analysis	6 studies	F+M	–	Hip	6.3	–	NR
Ahmed [55]	2006	Norway	Prospective cohort—6 years	27,159 (T1D=81)	F+M	25–98	All non-vert. hip	3.1 (M) 8.9 (F) 17.8 (M)	–	NR
Strotmeyer [40]	2006	USA (PA)	Case–control	67/237	F	35–55	Any	[1.9]	–	NR
Janghorbani [176]	2006	USA	Nurses' health study cohort	101,343 (T1D=292)	F	30–55	Hip	6.4	–	NR
Vestergaard [54]	2005	Denmark	Case–control	1745/2618	F+M	43±27	Hip Spine	[1.7] [2.5]	Hypoglycemia	NR
Miao [58]	2005	Sweden	Prospective cohort—10 years	24,605 (all T1D)	F+M	20.7±10.9	Hip	9.8 (F) 7.6 (M)	Microvascular, Cardiovascular complications	NR
Nicodemus [177]	2001	USA (Iowa)	Prospective cohort—11 years	32,089 (T1D=47)	F	55–69	Hip	12.25	–	NR
Forsen [178]	1999	Norway	Prospective cohort—9 years	35,444 (T1D=54)	F+M	≥50	Hip	6.9 (F)	Impaired vision, stroke, motor deficits	No

[a]RR is reported for most studies; [OR] indicates that odds ratio was reported
[b]*NR* not reported, *CGC* fracture risk correlated with glycemic control

Recognizing that diabetic bone has a greater propensity for fracture than is predicted by BMD, attributes of diabetic bone quality have been examined. A role for the skeletal accumulation of advanced glycation end products (AGEs; see section "Advanced Glycosylation End Products," below) [61, 62], chronic hyperglycemia [30], oxidative stress [63], and microarchitectural bone defects [64] have all been proposed, and it is expected that the pathological mechanisms leading to bone fragility in T1D are multifactorial [65]. To date, however, most information concerning diabetic bone quality is provided by data from animal models. This topic is reviewed in greater detail in other chapters (see Chaps. 9 and 10).

Beyond fragility fractures, other skeletal complications also occur disproportionately in persons with T1D, including fracture-healing complications (nonunion, malunion) [66], Charcot osteoarthropathy [67], osteomyelitis, and diabetic foot syndrome. These orthopedic complications are discussed in Chap. 4.

Onset of Skeletal Pathology: Studies in Children and Adolescents

When focused on *pediatric* patients with T1D, measurements of bone mineral density by dual X-ray absorptiometry (DXA) alone have provided conflicting results [20], with specific reports demonstrating global reductions in BMD [68], site-specific reductions in BMD [69, 70], or minimal to no effect on bone density [71, 72]. By providing only a two-dimensional estimate of BMD, however, DXA neglects parameters of bone size, bone geometry and bone compartment, which are critical components of the integrity of the growing skeleton. Other investigations using QUS or peripheral quantitative computed tomography (pQCT) provide additional information on bone mineral content, bone size, and bone structure. Collectively, these studies do suggest that cumulative changes in bone architecture are beginning early in childhood, particularly in those diagnosed with T1D at very young ages [73]. Compared with nondiabetic children, reductions in BMD [68, 74–78] and bone size, specifically total cross-sectional area (CSA) [73, 79] and cortical area [15, 80], are relatively consistent findings. Consequently, total bone mineral content of these generally smaller bones is also reduced in pediatric patients with T1D [80].

A mild delay in skeletal maturation, acquired after diagnosis, has been reported in some studies [81, 82]. In addition, the age of peak bone mass acquisition may be delayed in individuals with T1D [68, 72] and chronically poor glycemic control may negatively impact growth velocity [83]. However, unlike historical reports of short stature (i.e., Mauriac syndrome) in poorly controlled T1D [84], in the modern era of tighter glycemic management the impact of T1D on final height is minimal [73, 82, 85].

As with adults, the impact of T1D on the pediatric skeleton may again be gender-discrepant. Several studies report a more significant impact in young males [80]. Additionally, in a study of females, 13–19 years of age, no differences in BMD,

Table 1.2 Pediatric risk factors for diabetic bone disease

Possible pediatric risk factors for DBD in adulthood	References
Younger age at diagnosis	[73]
Longer duration of disease	[41, 77, 179]
Longer duration of poor glycemic control	[33, 41]
Lower BMI	[18, 22, 28]
Delayed skeletal maturation	[81]
Male gender	[80]
Accompanying celiac disease	[151, 154]
Vitamin D deficiency	[110, 111]

measured by DXA, were seen when comparing T1D with control subjects [72]. Other comorbidities, including the diagnosis of associated autoimmune disorders during childhood [86], may also exacerbate the impact of DBD on the growing skeleton (see section "Effects of Ancillary Diagnoses: Celiac Disease, Autoimmune Thyroid Disease, Addison's Disease"). Possible pediatric risk factors for DBD are listed in Table 1.2. Evidence to date would suggest that in patients with one or more of these risk factors, earlier screening for DBD may be warranted.

Contributing Pathological Mechanisms

Decreased Bone Formation

Analysis of bone turnover markers in persons with T1D suggest that bone homeostasis is altered so as to create, predominantly, a state of lowered bone formation, osteoblast dysfunction, and low bone turnover. Numerous studies have demonstrated a decrease in serum osteocalcin (OC) concentration, a marker of bone formation, in children and adolescents [87–90], young adults [31], and middle-aged adults [32, 90–92] with T1D. Even at the time of diagnosis, OC levels in children with T1D appear to be lower than in healthy children [93]. Additionally, lower OC concentrations, typically, are associated with indices of poorer glycemic control [32, 88, 91, 94–96] implying a disease-specific effect. A decrease in serum concentrations of insulin-like growth factor-I (IGF-I), an anabolic regulator of osteoblast function, is also very common in patients of all ages with T1D [31, 97–99], and IGF-I levels correlate with residual beta-cell function, again implying a potential impact of disease severity on bone health in T1D [100]. Similarly, a decrease in serum levels of bone-specific alkaline phosphatase (bALP), a marker of active bone formation and osteoblast activity, has been reported in adults with T1D [84]. However, somewhat contrary to expectations for a state of low bone turnover, an *increase* in bALP has been reported by others [91, 98] although this has been postulated to reflect impaired osteoblast differentiation. Finally, serum levels of sclerostin, an osteocyte product that antagonizes the Wnt signaling pathway and hence inhibits

bone formation, are increased in T1D [101] perhaps more-so in women [102]. As a whole, these studies suggest that systemic markers of bone formation in T1D are generally indicative of a condition in which bone formation is reduced.

In T1D, an uncoupling of bone formation and bone resorption is also apparent. In young [31] and middle-aged [32] women and in men [103] markers of bone resorption, including urine N-terminal telopeptides (NTx), are comparable in concentration between T1D cases and controls. Similarly, urine deoxypyridinoline (DPD) [91, 104] and C-terminal telopeptide (CTX) [21, 104] values are comparable between patients and healthy controls. Osteoprotegerin (OPG), an inhibitor of osteoclast formation, and subsequently of bone resorption, has also been assessed in T1D. Plasma OPG concentrations and OPG mRNA expression [88] are typically, but not unequivocally [105], higher in persons with T1D. This has been demonstrated both in pediatric [57, 88] and in adult populations [21]. Whether this finding suggests a state of constrained bone resorption, however, is unclear since OPG levels are also increased in periodontal disease [106] and can be indicative of vascular pathology or endothelial dysfunction. Finally, average serum PTH concentrations in T1D are often elevated relative to a matched control population, but frequently not above normal ranges in patients without renal dysfunction or vitamin D deficiency [25, 107]. Taken together, it would appear that T1D is characterized best as a state of inappropriately lowered bone turnover which exists in conjunction with relative osteoblast dysfunction [90] and, hence, low bone formation [103].

Vitamin D Deficiency

Hypovitaminosis D is an important risk factor for decreased bone mineralization [108, 109]. Hence, studies have examined the prevalence of vitamin D deficiency or insufficiency among individuals with T1D, both in childhood and adulthood, and across a variety of geographic locations; many suggest that vitamin D insufficiency/deficiency is more common in T1D compared with the general population [110–112]. Additionally, circulating levels of 25OHD are very often found to be significantly lower in case–control comparisons of T1D with the general population [107, 112–114], even when absolute levels do not meet diagnostic cut-points for deficiency. Even so, a 13-year Danish study of 25OHD levels in 907 children with newly diagnosed T1D, compared with 896 nondiabetic siblings, a study intended to eliminate genetic and environmental confounding, showed no difference in vitamin D levels [95]. Hence, the nature of this relationship between vitamin D deficiency and T1D, as being a preexisting condition or an acquired condition, remains controversial.

Several mechanisms could contribute to vitamin D deficiency in T1D. By cross-sectional analysis, data from National Health and Nutrition Examination Surveys (NHANES III and NHANES 2001–2006) identified an association between vitamin D deficiency and/or insufficiency and albuminuria [115, 116]. In addition, excess urinary loss of vitamin D binding protein (DBP) in T1D, particularly in persons with albuminuria, might contribute mechanistically to vitamin D deficiency.

Consistent with this hypothesis, we have demonstrated a significant increase in the urinary excretion of DBP in persons with T1D compared with controls; additionally, urine DBP concentrations correlated with urinary albumin excretion, and vitamin D deficiency or insufficiency was again more prevalent in diabetic subjects with albuminuria [107]. Decreased serum DBP levels in T1D have also been reported [117]. Finally, population-specific genetic variation in the vitamin D axis has been proposed. Vitamin D receptor (VDR) gene polymorphisms (FokI), for instance, have been associated with vitamin D deficiency [118]. However, while polymorphisms in the VDR gene (e.g., FokI, BsmI, ApaI, TaqI) have inconsistently been linked to possible susceptibility for T1D in certain populations, VDR genotypes have *not* been associated with differences in bone turnover markers seen in T1D [119, 120] or with measurements or bone mineral density [120, 121].

While numerous studies have examined the prevalence of vitamin D insufficiency/deficiency in various T1D populations, clinical data examining a direct role for vitamin D in the pathogenesis of diabetic bone disease are scarce, and largely historical. In 1981, a study of 45 Caucasian children with T1D (age 7–18 years), reported no difference in circulating 25OHD levels between non-osteopenic vs. osteopenic diabetic patients (cortical thickness >2SD below the mean normal value) [122], though much less stringent glucometabolic control would have been standard-of-care at the time. However, a more recent study of 58 children with T1D, ages 9–19 years, found that children with vitamin D deficiency and T1D did have lower lumbar spine BMD Z-score [89]. Additionally, serum 25OHD levels have been shown to negatively correlate with serum collagen type 1 C-terminal propeptide [123], yet positively correlate with GLA-carboxylated osteocalcin [124], perhaps inferring a positive effect of 25OHD on bone quality in T1D. Finally, some studies suggest that vitamin D insufficiency may also increase the risk for insulin resistance in T1D [111, 125], hence perhaps impeding the anabolic benefits of insulin signaling in bone [20] in these patients.

Advanced Glycosylation End Products

In the presence of hyperglycemia, the non-enzymatic addition of reduced sugar moieties to amine groups on both tissue-specific and circulating proteins occurs. The generation of one subtype, the advanced glycosylation end products (AGEs), in the milieu of chronic hyperglycemia and the interaction of AGEs with their receptors (RAGEs) are thought to play a significant role in the pathogenesis of diabetic complications [126] including diabetic cardiovascular disease [127], diabetic nephropathy [128], and diabetic retinopathy [129], via activation of pro-inflammatory pathways. While enzymatic cross-link formation between bone collagen molecules is an important element of the mechanical strength and material properties of healthy bone, the dysregulated accumulation of AGEs in bone is thought to negatively impact the integrity of skeletal tissues [130] possibly by causing

micro-damage, hindering osteoblast function and differentiation [131], reducing bone turnover, and/or by competitively inhibiting normal enzymatic cross-link formation [132]. For example, evidence exists to suggest that AGE accumulation is operative in age-related bone fragility [93].

Because diabetic bone has a greater propensity for fracture than is predicted by BMD deficits alone, reduced bone quality secondary to AGE accumulation [133] has also been hypothesized. However, in T1D this mechanism has not yet been established. Measurements of urine and serum pentosidine have been used as biomarkers for AGE accumulation in bone, particularly trabecular bone [134]. Adults with diabetes have increased levels of tissue AGEs compared with chronologically age-matched nondiabetics [96]; and, in older adults with type 2 diabetes, higher urine [135], and serum pentosidine [136] levels correlate with increased vertebral fracture prevalence. Similar data linking serum pentosidine levels and fracture risk in T1D have only recently been reported [137]. Nevertheless, serum AGE concentrations are clearly elevated in T1D during childhood [138], even during preschool and prepubertal years [139]. In addition, skin AGEs, estimated from measurements of skin intrinsic florescence (SIF), are increased in children with both T1D and T2D, to the extent that "approximately 4–6 years of diabetes exposure in some children may be sufficient to increase skin AGEs to levels that would naturally accumulate only after ~25 years of chronological aging" [140]. A similar elevation in SIF is observed in adults with T1D, and is clearly related to long-term glycemic control [141–143]. Gingival AGEs are also increased in T1D-associated periodontitis [144]. Even so, while studies to date suggest an association of AGEs with micro- and macrovascular complications of diabetes, future research will be required to establish a link between bone AGE biomarkers and skeletal phenotype in T1D-associated diabetic bone disease.

Effects of Ancillary Diagnoses: Celiac Disease, Autoimmune Thyroid Disease, Addison's Disease

The relationships between autoimmune disease and bone loss are multifactorial, with outcomes dictated by: (1) direct immune cell regulation of bone homeostasis; (2) disease-specific systemic or local inflammation (e.g., rheumatoid arthritis); (3) disease-specific systemic hormone alterations (e.g., hypothyroidism/hyperthyroidism); (4) ancillary organ damage; or (5) negative skeletal consequences of therapy (i.e., corticosteroids) (For Review, [145]). Certain autoimmune disorders in particular, including autoimmune thyroid disease (ATD), celiac disease (CD), and Addison's disease (AD) are more prevalent in persons with T1D, and all can independently confer detrimental effects on skeletal homeostasis, compounding the impact of diabetic bone disease. The prevalence of celiac autoimmunity or CD in persons with T1D has been estimated at between 4 and 12 % [86, 146–149] and osteoporosis, along with an increase in the risk of bone fractures, is present in the

majority of celiac patients [150]. It is interesting, therefore, that in children with T1D, high-titer seropositivity to celiac antigens in otherwise asymptomatic children is associated with lower bone mineralization [151]. Similarly, in studies of BMD in adults with T1D, a greater reduction in BMD, as well as a higher incidence of fractures [149], is seen among the subset of T1D patients with concurrent celiac autoimmunity [149, 152, 153]. In patients with T1D and active CD, a significant increase in the prevalence of osteopenia (compared to T1D alone) is seen in those with seropositivity and non-adherence to dietary gluten restrictions [154]. Finally, autoantibodies against osteoprotegerin have been reported in a few patients with celiac disease, possibly further contributing to osteoporosis [155]. Hence, in persons with a long-standing coexistence of T1D and CD, the risk for poor bone health is almost undoubtedly higher.

Autoimmune thyroid disease (ATD), either Hashimoto's disease or Grave's disease can occur in 12–30 % of persons with T1D [86, 147], likely related to a common genetic susceptibility for the two disorders [156]. Whether related to the primary hormonal disruption, or to the antithyroid autoimmunity [157], these thyroid disorders can independently contribute to a decrement in BMD. Prevalence rates for adrenal autoantibodies and/or Addison's disease (AD) among persons with T1D vary from 0.5 to 1.4 % [86, 147, 158]. While Addison's disease is a rare condition, AD is also associated with a higher prevalence of osteopenia or osteoporosis [159] and an increased risk of fracture [160], which is unrelated to glucocorticoid replacement therapy. At present, it is not clear whether the diagnosis of ATD or of AD in a person with T1D further increases the risk of osteoporosis or fracture; however, the combination of multiple autoimmune diseases in some persons with T1D may synergistically increase the risk of low bone mineral density [29].

Treatment of Diabetic Bone Disease in T1D

Because diabetic bone disease in type 1 diabetes represents a deficit in osteoblast function and bone formation, antiresorptive therapies for osteoporosis (e.g., bisphosphonates, denosumab) may be ineffective in this form of secondary osteoporosis, other than as combination therapy in conditions of increased bone resorption (i.e., postmenopausal females with T1D) [161]. Calcium and vitamin D supplementation, where indicated, is considered standard-of-care for osteoporosis treatment [162]. Nonetheless, 1 year of calcitriol supplementation in young adults with recent-onset T1D did not significantly change circulating markers of bone turnover, specifically levels of OC or beta-Crosslaps [160]. Moreover, very little information from comparative effectiveness studies is available on the treatment of osteoporosis in T1D. In animal models, intermittent PTH treatment has been shown to improve osteoblast survival and reverse diabetes-associated bone loss [163]. In clinical trials examining the effectiveness of injectable PTH (teriparatide) for treatment of osteoporosis, however, diabetes has typically been an exclusion criteria preventing

enrollment, though positive results with off-label use of teriparatide for nonunion fracture repair [164] or Charcot osteoarthropathy provide some early support for this concept. Clearly, additional clinical investigation in this area is needed.

Prevention of Diabetic Bone Disease in T1D

Greater than 90 % of peak adult bone mass is typically achieved by the end of the second decade of life, and ~40 % of this bone mass is acquired during the adolescent growth spurt; hence, adolescence is a period of particular importance for prevention strategies. Optimal T1D disease management in children and adolescents, according to best practice guidelines [3], would seem a prudent approach to minimizing the impact of T1D on skeletal tissues. This would include effectively managing blood glucose to achieve age-appropriate treatment goals for plasma glucose and HbA1c, as published [3]; additionally, adequate insulin replacement may, in and of itself, have beneficial anabolic effects on bone [20]. Routine screening for associated autoimmune disorders, such as celiac disease and hypothyroidism, as is recommended, is also a necessary component to prevention [3].

Optimal bone mineralization during puberty should also become an important objective for the pediatric clinician, utilizing the following general guidelines:

- Weight-bearing exercise during childhood and adolescence is an important determinant of bone mineral density [165, 166]. Regular physical exercise and sustained physical activity should be encouraged for all adolescents, in accordance with physical activity recommendations of the Centers for Disease Control [167], and as further delineated for persons with T1D by the American Diabetes Association [3]; specifically, it is recommended that children and adolescents perform 60 min or more of physical activity daily. Demonstrating the importance of exercise, a 9-month weight bearing physical activity program comparing 27 children with T1D and 32 healthy children demonstrated improved bone mineral accretion in T1D children, comparable in magnitude to the improvements seen in healthy children [160].
- Adequate daily intake of calcium is important. During puberty, normal skeletal mineralization is dependent upon calcium accrual; calcium acquisition/retention rates vary by gender and race, but are estimated at ~160–500 mg/day [168–171]. To achieve this, the most recent Institute of Medicine dietary guidelines for the Recommended Dietary Allowance for calcium for adolescents is 1300 mg per day [172]. Recognizing that urinary calcium excretion is increased as a consequence of osmotic diuresis in T1D, recommendations for daily calcium intake in the adolescent with T1D may, in fact, be greater.
- Recommended Dietary Allowance (RDA) for vitamin D in children and adolescents 1–18 years of age is 600 IU daily [172]. Because vitamin D insufficiency is more common in persons with T1D, the periodic assessment of vitamin D status along with the diagnosis and treatment of vitamin D deficiency in this population are also important goals.

Consensus Recommendations

The FRAX® tool, or World Health Organization Fracture Risk Assessment tool, was developed to integrate BMD measurements and patient-specific clinical risk factors in determining an individual's 10-year probability of fracture. Current American Diabetes Association (ADA) Standards of Medical Care for fracture assessment are largely derived from studies of fracture risk in type 2 diabetes, which suggest that for a given FRAX® score, the risk of fracture is higher in persons with diabetes [3, 173]. ADA guidelines state that "it is appropriate to assess fracture history and risk factors in older patients with diabetes and recommend BMD testing if appropriate for the patient's age and sex" [3]. Additionally, the fracture risk prediction algorithm used in the United Kingdom (QFracture algorithm) has been recently updated to incorporate type 1 diabetes as a specific variable in their hazard assessment [174]. Guidelines for BMD testing in children and adolescents with T1D, however, do not currently exist.

Summary

Persons with type 1 diabetes, particularly those with long-standing disease, are at significantly increased risk for osteopenia or osteoporosis, fractures, and poor bone healing, collectively now termed diabetic bone disease. Many factors contribute to these deficits in skeletal integrity, including: (1) a disease-specific reduction in new bone formation; (2) an increased occurrence of vitamin D insufficiency; (3) a variety of bone-adverse comorbid conditions, including diabetic microvascular complications and associated autoimmune diseases; and, very possibly (4) glucose-related alterations in the material properties and mechanical strength of diabetic bone. Because T1D is most commonly diagnosed in childhood and adolescence, the early assessment of bone health and skeletal risk factors in youth with T1D will be important, in an effort to maximize the acquisition of peak bone mass in these patients, and to minimize the impact of T1D on the skeleton going forward.

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Chapter 2
Type 2 Diabetes Mellitus and Skeletal Health

Ann V. Schwartz

Fractures, Type 2 Diabetes and an Aging Population

Fractures and type 2 diabetes (T2D) are both more prevalent with older age. At age 50 years, the lifetime risk of a hip fracture is estimated to be 17.5 % for women and 6 % for men in the United States [1]. The lifetime risk of a vertebral fracture is even higher [2]. Fractures exact a substantial public health toll among older adults [3]. Hip fractures in particular are associated with increased mortality and functional decline, but vertebral and other fractures also have substantial consequences [4–8]. The global trend towards an aging population is likely to produce profound changes in the number and geographic distribution of fractures. In the next decades, the number of older adults (60+) in the world population is expected to more than double, from 841 million in 2013 to 2 billion in 2050 [9]. The population of adults who are aged 80+ is projected to increase even more rapidly from 120 million in 2013 to 392 million in 2050. The proportion of older adults (60+) is expected to increase from 23 % in 2012 to 32 % in 2050 in the developed countries and from 9 to 19 % in developing countries. With these trends, the number of fractures is predicted to increase. The number of hip fractures is projected to reach 2.6 million by 2025 and 4.5 million in 2050 [10].

Type 2 diabetes affects over 25 % of older adults in the United States, including diagnosed and undiagnosed cases [11]. In the world population, a recent estimate indicates that over 15 % of adults age 55 years and older have T2D [12]. Given this high prevalence of T2D in older adults, efforts to prevent fractures must necessarily consider the epidemiology and underlying etiology of fracture in those with diabetes to guide effective prevention.

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Hip Fracture Incidence and Type 2 Diabetes

Hip Fracture Risk Higher with T2D

Type 2 diabetes is characterized by greater weight and by higher bone density [13]. Both of these factors are protective for most fractures, including hip fracture. Based on this information alone, one would expect reduced fracture risk in those with T2D. However, most studies have instead identified a higher risk of fracture associated with diabetes. The most extensive studies of the effects of T2D on fracture incidence have focused on hip fracture. Because nearly all cases of hip fracture are admitted to a hospital for care, this fracture outcome is easier to study in the large datasets necessary to compare fracture incidence in those with and without diabetes. In 2007 Vestergaard published a meta-analysis of hip fracture results that included eight studies and reported an age-adjusted summary relative risk for hip fracture of 1.38 (1.25–1.53), comparing those with and without T2D [14]. This increase in fracture risk with T2D occurred in spite of higher bone density in those with T2D. The estimated BMD *Z*-score, also assessed in this meta-analysis, was +0.41 for lumbar spine and +0.27 for total hip.

Most [15–21], but not all [22, 23], subsequent studies have reported increased rates of hip fracture with T2D in age-adjusted models. Among older women in WHI, the relative rate of hip fracture, comparing women with and without T2D, was 1.41 (1.17–1.70) [17]. Among Rochester MN residents, hip fracture was increased with T2D after 10 years of follow-up (standardized incidence ratio = 1.5; 1.1–1.9) [16]. A study in Manitoba Canada found an increased hip fracture rate for diagnosed diabetes but not for newly identified diabetes [15]. In a cohort in Ontario Canada, women (HR = 1.20; 1.16–1.24) and men (HR = 1.22; 1.16–1.28) with diabetes had higher hip fracture rates than those without diabetes [19]. In contrast, a study in nursing home residents found no difference in hip fracture rates in age and weight-adjusted models (HR = 0.90; 0.60–1.34) although, as discussed below, hip fracture rates were higher in those with diabetes after adjustment for BMD [22].

In a recent large cohort study from Scotland that reported hip fracture rates for the period 2005 through 2007, the age- and calendar-year adjusted results showed a small increased hip fracture rate with T2D in women (1.05; 95 % CI 1.01–1.10) and no evidence of increased hip fracture with T2D in men (0.97; 95 % CI 0.92–1.02) [18]. The authors discuss several possible reasons, other than chance, for the lower relative rates in their study compared with the higher associations reported in the Vestergaard meta-analysis and several subsequent studies. One possible influence is the increase in the proportion of diabetic adults who are overweight or obese, a phenomenon reported in Scotland. In the United States as well, the proportion of those who are obese among adults with T2D increased from 35 % in 1994 to 57 % in 2010, a change that would be expected to reduce hip fracture risk among those with T2D [24]. Better screening and earlier detection of diabetes in recent years would increase the proportion of diabetic patients with shorter duration of the disease, a factor that is associated with fracture risk. Indeed, in the Scotland cohort, those with diabetes

duration of 7 years or more had a higher relative rate of hip fracture (women: rate ratio 1.55; 1.38–1.75. men: rate ratio 1.25; 1.08–1.45) [18]. Another possible influence on the relative rate of hip fracture might be improvements in medical care with improved glycemic control and reductions in diabetic complications. These and other factors are likely to result in variation in age-adjusted associations between diabetes and fracture across countries and time periods.

Nearly all of the studies of diabetes and hip fracture included in the Vestergaard meta-analysis were conducted in Western populations where most of those with T2D are also overweight or obese. In contrast, in East Asian countries, a substantial proportion of those with T2D are normal weight. In this setting, T2D may be associated with a greater relative risk of fracture since fewer diabetic adults have the protective effects of higher BMI. Limited studies indicate that the age-adjusted relative risk is as strong or stronger compared with studies in Western countries. In Taiwan, diabetes was associated with hip fracture risk in women (HR 1.72; 1.66–1.78) and men (HR 1.28; 1.21–1.34) in models adjusted for age, geographic area and urbanization status, but not for BMI or BMD [20]. A study of diabetes and hip fracture in Singapore Chinese reported a rate ratio of 2.00 (1.73–2.31), adjusted for age, sex, dialect group and SES, but not for BMI or BMD [21].

The association between T2D and hip fracture may vary by fracture location although only limited data are currently available. In a study of hip fracture cases in the Netherlands, diabetes was more prevalent among those with a subtrochanteric or femoral shaft fracture, compared with a femoral neck or peritrochanteric fracture (OR = 3.62; 1.45–9.07). In older white women in the United States, the relative rate of intertrochanteric (multivariable adjusted HR = 1.76; 1.37–2.27) and subtrochanteric (3.25; 1.55–6.82), comparing T2D and nondiabetic women, was higher than the relative rate for femoral neck (1.20; 0.90–1.58) fractures [25].

Hip Fracture Risk Higher for a Given BMD in T2D

Some studies of T2D and hip fracture have been able to adjust for the higher BMI and/or BMD that characterizes T2D. These studies have generally found that T2D is associated with higher hip fracture risk *for a given BMD*. A meta-analysis (12 studies) by Janghorbani et al. assessed the relationship between T2D and hip fracture from this perspective, using reported results adjusted for BMI and, where data were available, BMD in those with T2D [26]. The summary relative risk for hip fracture reported in this meta-analysis was 1.7 (1.3–2.2) (Fig. 2.1). Results for subsequent studies with adjustment for BMD have been consistent with this meta-analysis. Among older women in WHI, hip BMD was available on a subset, and the BMD-adjusted model was consistent with increased hip fracture risk although not statistically significant (HR = 1.82; 0.90–3.64) [17]. In a study among nursing home residents, BMD-adjusted relative rate for hip fracture, comparing those with and without DM, was 1.46 (1.25–1.81) [22].

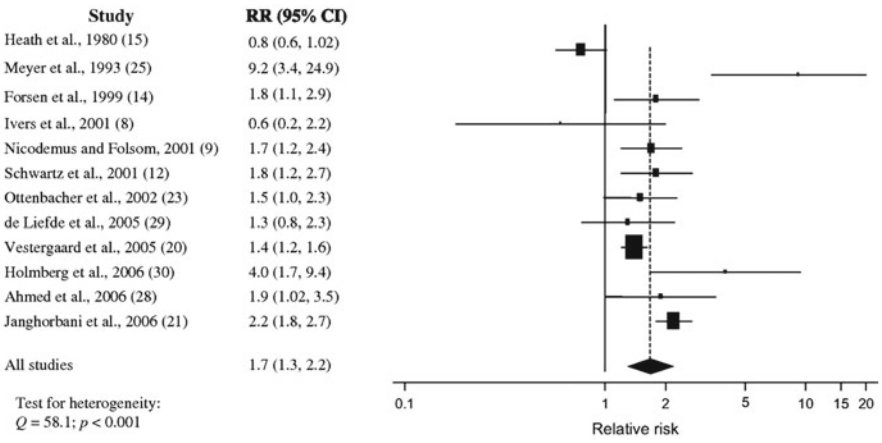

Fig. 2.1 Association between type 2 diabetes mellitus and risk of hip fracture in case–control and cohort studies. Each square shows the study-specific relative risk (RR) estimate (the size of the square reflects the study-specific statistical weight, that is, the inverse of the variance), and the horizontal line shows the related 95 confidence interval (CI). The *diamond* shows the summary RR estimate, and its width represents the corresponding 95 % CI. All statistical tests were two-sided. Statistical heterogeneity between studies was assessed with Cochran's Q test. Reprinted with permission from Janghorbani et al. [26]

Interaction with Age But Not Gender

There is limited evidence that the relationship between diabetes and hip fracture may be stronger at younger ages. A study in Manitoba, Canada, reported an increased rate of hip fracture with diabetes in those <65 years old (6.27; 95 % CI 3.62–10.87) that was greater (p for interaction = 0.002) than the relative rate in those ≥65 years old (2.22; 95 % CI 1.71–2.90) [27]. A study of diabetes and hip fracture in Taiwan reported a similar interaction between diabetes and age [20]. For gender, the association between diabetes and hip fracture appears to be similar for women and men. In the meta-analysis by Janghorbani et al., the association between T2D and hip fracture did not differ by gender (p for interaction = 0.51). The summary relative risk was 2.1 (1.6–2.7) among women (eight studies), and 2.8 (1.2–6.6) among men (five studies) [26]. Similarly, in the Canadian cohort, there was no evidence of interaction by gender for diabetes and hip fracture [27].

Incidence of Any Fracture and Type 2 Diabetes

The two meta-analyses discussed earlier provided evidence of a modest increase in the risk of any fracture with T2D. As with hip fracture, studies of all fractures that have adjusted for the higher BMD associated with type 2 diabetes have generally

found that those with T2D have an elevated risk *for a given BMD*. Vestergaard reported an age-adjusted relative risk of 0.96 (0.57–1.61), combining five studies, with strong evidence of heterogeneity ($p<0.01$) [14]. When two studies reporting reduced fracture risk with T2D were excluded, heterogeneity was reduced ($p=0.88$), and the estimated relative risk indicated a modest increase in the risk of any fracture with T2D (1.19; 95 % CI 1.11–1.27). Janghorbani et al. reported an increase in non-vertebral fractures with T2D in results from eight studies, adjusted for BMI and/or BMD (adjusted RR 1.2; 95 % CI 1.01–1.5) [26]. Since these meta-analyses, the WHI study reported an increased risk of any fracture in women (age-adjusted RR 1.29; 1.20–1.38) [17], and a similar increased risk was reported for Rochester MN residents (Standardized incidence ratio = 1.3; 1.2–1.4) [16]. In a cohort of older US men, risk of non-vertebral fracture was not increased in age-adjusted models (HR = 1.12; 0.94–1.34) but was modestly elevated after adjustment for BMD (HR = 1.30; 1.09–1.54) [28].

A few studies have considered whether diabetes interacts with age, gender, or race for the outcome of any fracture. A recent study in the United States using NHANES data reported an interaction ($p<0.05$) between diabetes and race for the outcome of non-skull fracture, comparing age- and sex-adjusted results for the relative rate of fracture associated with T2D in Mexican-American (HR = 2.29; 1.41–3.73), non-Hispanic black (1.86; 1.05–3.30), and non-Hispanic white (HR = 1.17; 0.89–1.52) participants [29]. The study found no evidence of interaction between diabetes and age or gender for the outcome of non-skull fracture. In WHI there was a suggestion of an increased relative rate of any fracture in black women (HR = 1.33; 1.00–1.75) compared with non-Hispanic white women (HR 1.18; 1.08–1.29) [17]. In a smaller cohort of older black and white adults, there was no evidence of interaction between diabetes and gender or race for the outcome of any fracture [30].

The data available for specific fracture sites other than hip are more limited. Janghorbani et al. summarized results for studies published before 2006. With the exception of distal forearm fracture (summary RR = 0.98; 95 % CI 0.8–1.2), the point estimates for the summary relative risks were modestly elevated (ankle 1.3, proximal humerus 1.3, vertebra 1.2, foot 1.3), but only the RR for foot fracture was statistically significant. Studies published since this meta-analysis with results for specific non-hip sites include the WHI cohort [17] and Rochester, Minnesota population [16]. Results from WHI were quite similar to the Janghorbani et al. meta-analysis with increased rates for all fracture sites considered with the exception of the lower arm/wrist [17]. Many vertebral fractures do not come to clinical attention, but can be identified on spine X-rays as morphometric vertebral fractures. Some studies of T2D and morphometric vertebral fractures have reported increased prevalence of vertebral fractures [31, 32], but others have not found evidence of an association [33, 34]. A study among postmenopausal women in Beijing found no difference in prevalence of vertebral fracture between women with and without diabetes (OR = 1.04; 0.58–1.88) [35]. However, when women were stratified by BMI, diabetes was associated with higher vertebral fracture prevalence in the non-obese (BMI <25 kg/m^2) women (OR = 2.79; 1.16–6.68).

Risk Factors for Fracture in T2D

Traditional risk factors for fracture include lower bone density, lower BMI, and increased frequency of falls. These factors are also associated with fracture in those with T2D [28, 30, 36].

Bone Density

As noted above, T2D presents a paradox of increased fracture risk in spite of higher bone density. A meta-analysis by Vestergaard reported higher BMD, measured by dual X-ray absorptiometry (DXA) and expressed as Z-score, at the lumbar spine and total hip in those with T2D [14]. Higher BMD was also found by Ma et al. in a more recent meta-analysis of age-adjusted results at the lumbar spine, total hip, and femoral neck [13]. BMD at the radius did not differ by diabetes status. BMI is positively associated with BMD in T2D [13] and broader populations [37] and may account for at least some of the higher BMD with T2D. However, when Ma et al. calculated pooled estimates using results with further adjustment for BMI and other factors, BMD remained higher at the hip (Fig. 2.2) and spine (Fig. 2.3) in T2D. All

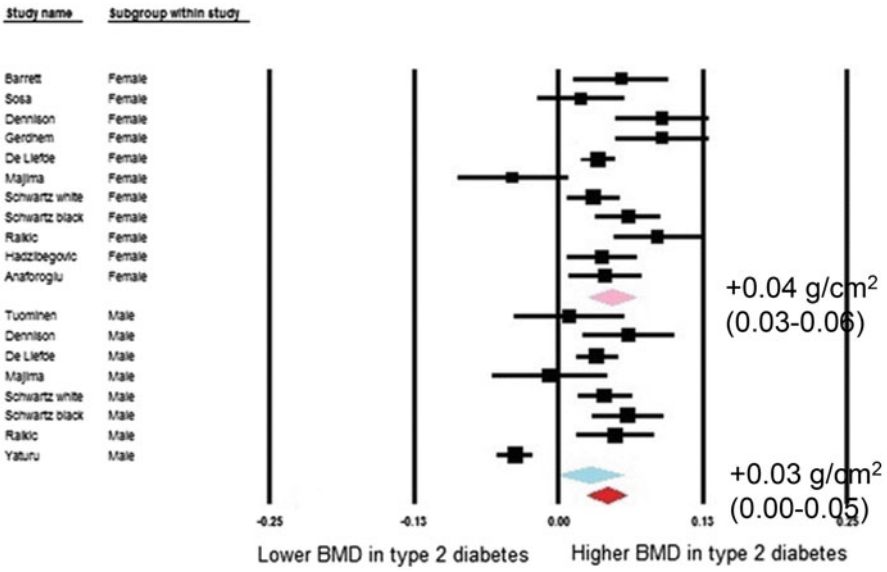

Fig. 2.2 Forest plot for mean femoral neck bone mineral density. Difference in means (g/cm^2) and 95 % confidence interval for femoral neck bone mineral density between comparison groups with and without type 2 diabetes mellitus, stratified per study and gender. *Diamonds* represent joint estimate for subgroups of available studies for women (*upper*) and men (*middle*), respectively. Pooled estimate for all studies displayed with the *diamond* at the bottom [13]

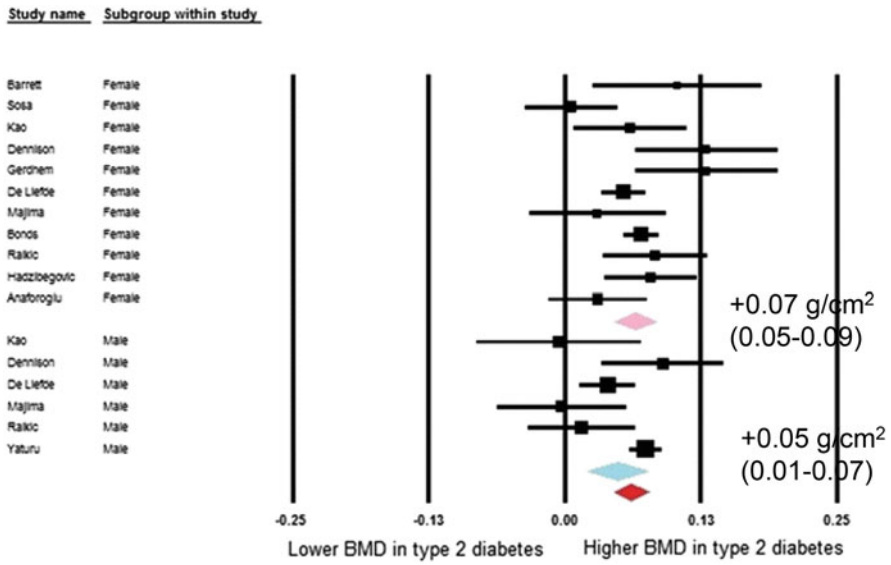

Fig. 2.3 Forest plot for mean spine mineral density. Difference in means (g/cm^2) and 95 % confidence interval for femoral neck bone mineral density between comparison groups with and without type 2 diabetes mellitus, stratified per study and gender. *Diamonds* represent joint estimate for subgroups of available studies for women (*upper*) and men (*middle*), respectively. Pooled estimate for all studies displayed with the *diamond* at the bottom [13]

but one of the studies were conducted in Western countries, characterized by a high prevalence of obesity with T2D. Notably, the one study conducted in East Asia that was included in this meta-analysis found no statistically significant difference in BMD by diabetes status at the femoral neck or lumbar spine while BMD at the radius was lower with T2D [38]. Other studies in East Asian countries have found lower [39–41], similar [42–44] and higher [42–46] BMD at the hip and/or spine in those with diabetes. Two studies in China have assessed the relationship between DM and BMD, stratified by BMI. Among postmenopausal women in Shenyang, Zhou et al. reported lower BMD at the femoral neck and total hip with DM among non-obese (BMI <25 kg/m^2) women [47]. Among obese women, BMD was higher but not statistically different between those with and without DM. However, the Peking Vertebral Fracture Study among women in Beijing found higher spine BMD with DM among the non-obese and no difference by diabetes status in the obese women [35]. A recent study in the United States, using high resolution pQCT rather than DXA, found evidence of reduced cortical bone density and thickness with greater cortical porosity, in non-obese T2D women compared with controls or with obese T2D women [48].

A few studies have considered change in BMD in those with diabetes. The results appear to differ by skeletal site. Somewhat surprisingly, several have reported greater bone loss with T2D at the hip in spite of higher baseline BMD. In contrast,

bone loss at the radius does not appear to differ with diabetes status. Results for spine BMD have been inconsistent. In the Study of Osteoporotic Fractures (SOF) among older white women, bone loss was more rapid in those with T2D at the total hip, femoral neck, spine, and calcaneus but was not different at the distal radius [49]. Greater bone loss at the femoral neck with diabetes was also reported among white women in a cohort study of older adults; bone loss did not differ by diabetes status in white or black men or black women [50]. In the placebo group of the Fracture Intervention Trial, women with diabetes had faster bone loss at the total hip [51]. In a longitudinal study of perimenopausal women, those with diabetes lost bone more rapidly at the total hip, but preserved bone relative to nondiabetic women at the spine [52]. Krakauer et al. reported no differences in bone loss at the radius by T2D status over 12.5 years of follow-up [53].

The relationship between bone density and fracture in T2D has been an important focus of research. Longitudinal studies have demonstrated that lower BMD is a risk factor for fracture in T2D as in broader populations [27, 54]. However, at any given BMD those with T2D have a higher risk of fracture than those without diabetes. This discrepancy has implications for fracture risk assessment, discussed in Chap. 3. It also implies that there are other factors contributing to fracture risk in T2D, beyond BMD. In broad terms, this additional fracture risk at a given BMD might be the result of increased frequency of falls or reduced bone quality that is not captured by BMD measurements, or both. Evidence that more frequent falls do not fully account for increased fracture risk with T2D (discussed in more detail below), combined with evidence from rodent models [55], has led to the conclusion that diabetic bone is more fragile for a given BMD. Understanding the aspects of bone that are affected by diabetes and that result in fragile bone has been an important focus of research on diabetes and skeletal health.

Body Size

Higher BMI is associated with lower risk of fracture in those with T2D [16, 36, 56]. A study of adults (40+ years old) with a DXA scan record in the Manitoba health registry made an explicit comparison of BMI as a risk factor for fracture in those with ($n=6455$) and without ($n=55{,}958$) diabetes [36]. BMI was higher in those with diabetes, but the relationship with fracture did not differ. In models adjusted for femoral neck BMD and other risk factors in the FRAX calculator, a 5 kg/m^2 increase in BMI was associated with a lower rate of hip fracture in those with (HR = 0.81; 0.69–0.95) and without (HR = 0.82; 0.76–0.89) diabetes (p for interaction = 0.891). For the outcome of "major osteoporotic fracture" the relationship was also not statistically different (p for interaction = 0.080). If anything, the relationship was stronger in those with (HR = 0.90; 0.83–0.98) compared to those without (HR = 0.98; 0.95–1.02) diabetes.

Falls

About 90 % of fractures are due to a fall although less than 5 % of falls result in a fracture [57]. Falls are associated with fracture risk in those with T2D just as in broader populations [28, 58]. Those with T2D have a moderately increased risk of falling. A meta-analysis of eight studies estimated an increased risk of 1.19 (95 % CI 1.08–1.31), comparing those with and without diabetes [59]. However, insulin-treated patients appear to have a 2–3 times higher risk of falls compared with non-diabetic patients [60, 61]. In addition, several [62–64] although not all [65] studies of serious fall injuries resulting in a hospital or emergency room visit have reported higher incidence for T2D patients. These studies did not include information on hypoglycemia, discussed below.

This increased frequency of falls has been proposed as an explanation for the increased fracture risk observed among T2D for a given BMD. Importantly, as noted earlier, several studies of diabetes and fracture have included data on falls and have been able to address this question. In these observational studies, more frequent falls did not fully account for the increased risk of fracture observed with T2D [17, 33, 58, 66]. Higher fracture risk associated with T2D persisted even with adjustment for increased frequency of falls in these cohorts.

Diabetes-Related Risk Factors for Fracture

Investigations into diabetes-related risk factors for fracture have assessed the possible contributions of diabetes duration, presence of diabetes-related complications, glycemic control, and diabetes medications. Evidence that diabetes medications influence skeletal health is discussed in Chap. 7.

Diabetes Duration

Those with longer duration of diabetes have a higher risk of fracture [15, 16, 18, 19, 21, 33, 66–69]. For example, in a longitudinal cohort of Chinese in Singapore, the relative rate of hip fracture was 1.40 (1.08–1.82) in diabetic participants with duration of less than 5 years, compared with nondiabetic participants, and 2.66 (2.04–3.47) in diabetic participants with duration of 15+ years [21]. Longer duration of diabetes also appears to be associated with greater frequency of falls [70]. Reasons for this higher risk of fractures and falls with greater duration of diabetes may include increased frequency of insulin therapy and diabetes-related complications.

Glycemic Control

The effect of glycemic control on fracture risk, BMD, and falls remains poorly understood and controversial. On the one hand, reducing A1C levels is a standard goal of diabetes care that has been shown to reduce microvascular complications [71]. It is reasonable to assume that improved control might also have positive effects on bone health. On the other hand, lower A1C levels increase the frequency of hypoglycemic episodes which may increase the risk of falls and fractures. These hypotheses have been tested in a randomized trial, the Action to Control Cardiovascular Risk in Diabetes (ACCORD), a comparison of intensive and standard glycemic control in a cohort of patients with long-term T2D at increased risk of cardiovascular disease age 40–79 years [72]. The median achieved A1C in ACCORD was 6.4 % in the intensive and 7.5 % in the standard glycemic control groups [73]. In an ancillary study, ACCORD BONE, assessment of incident fractures and falls was added to the trial. This trial found no difference in fracture rates between the two treatment groups (HR = 1.04; 0.86–1.27) [74]. These results may have been influenced by the greater degree of TZD use in the intensive glycemic control group. As discussed in Chap. 7, fracture rates are doubled in women, but not men, using a TZD [75]. However, considering the fracture results only among men, whose fracture rates are less likely to be affected by TZD use, there was no evidence of a difference in fracture rates between the intensive and standard glycemic control groups (HR = 0.93; 0.70–1.25).

The trial also considered the effect of intensive versus standard glycemic control on falls. There were more hypoglycemic episodes in the intensive treatment group [73], but there was no overall effect of intensive control on the rate of falls (rate ratio = 1.10; 0.84–1.43). In analyses stratified by age, there was no evidence of increased falls with intensive control among those 65–79 years old (HR = 0.75; 0.55–1.01). In sum, the ACCORD trial provides evidence that reducing average A1C to 6.4 % does not reduce or increase fracture or fall risk compared with an average A1C of 7.5 % in older adults (40–79 years old). While the intensive treatment did not reduce fractures, it was also safe with regard to fractures and falls.

The ACCORD trial does not address the effects of *poor* glycemic control compared with standard (or intensive) control on fractures or falls. This question has been considered recently in several observational studies [76–79]. Three longitudinal studies have reported increased fracture risk with poor control. The largest study, using health registry data in Taiwan, included 1514 hip fracture cases in T2D patients [76]. Those with baseline A1C levels of 9–10 % (HR = 1.24; 1.02–1.49) and 10 % + (HR = 1.32; 1.09–1.58) had increased rates of hip fracture compared with patients whose A1C level was 6–7 %. There was a suggestion of a higher rate in those with A1C <6 % (HR = 1.19; 0.97–1.45) compared with 6–7 %, but the difference was not statistically significant. Those with a baseline A1C of 7–8 % had a slight increase in hip fracture rate (HR = 1.07; 0.92–1.25) compared with 6–7 %. This result is generally consistent with the report from the ACCORD BONE trial comparing fracture rates in men in the standard (median A1C 7.5 %) and intensive (median A1C 6.4 %) control groups (HR = 1.08; 0.80–1.43) [74].

In the Rotterdam cohort of older adults, baseline measurements of fructosamine were converted to A1C equivalent units; the median A1C was 7.5 % among those with type 2 diabetes [77]. Diabetic participants with A1C ≥7.5 % had a higher rate of fracture, in spite of higher BMD, compared with diabetic participants with A1C <7.5 % (HR = 1.54; 95 % CI 1.04–2.29, adjusted for age, sex, height, and weight). The fracture incidence was 31.1 per 1000 person-year in those with A1C ≥7.5 % and 23.0 per 1000 person-year for those with A1C <7.5 %, an absolute difference in fracture rates of 8 per 1000 person-years. A second longitudinal study, based on the Atherosclerosis Risk in Communities (ARIC) Study, compared rates of fractures identified through hospitalizations [78]. Among those with diagnosed diabetes, the average A1C was 8.3 (SD 2.3). Those with A1C ≥8 % had a higher rate of fracture than those with lower A1C (HR = 1.63; 95 % CI 1.09–2.44, adjusted for age, sex, race, BMI, and other factors). Ten-year cumulative incidence of hospitalized fracture was 4.9 % in those with A1C ≥8 % and 4.4 % in those with A1C <8 %.

In contrast to these three studies that considered long-term effects of poor glycemic control on fracture risk, Puar et al. considered a somewhat different question, analyzing hip fracture risk within 3 months after assessment of A1C, among diabetic patients admitted to Changi General Hospital in Singapore over a 5-year period [79]. In this analysis of short-term effects of glycemic control, *lower* A1C in the previous 3 months was associated with reduced hip fracture risk. Compared with the reference group (A1C >8 %), diabetic patients with A1C <6 % (OR = 3.0; 2.0–4.5), A1C between 6.1 and 7 % (OR = 2.4; 95 % 1.7–3.2), or A1C between 7.1 and 8.0 % (OR = 1.2; 95 % CI 0.8–1.6) had higher odds of a hip fracture. In contrast, the ACCORD trial did not find increased fracture risk with sustained intensive glycemic control (median A1C 6.4 %) compared with standard control (median A1C 7.5 %) [74].

Studies of the effect of A1C on falls have yielded inconsistent results. In a study of incident falls among older white and African-American adults in the United States, there was no association between glycemic control and falls for those using an oral antidiabetes medication [80]. However, among those using insulin, low baseline A1C (≤6 %) was associated with increased risk of falling. A study of US adults 75 years of age and older also found increased risk of falls with lower A1C (≤7 %). In contrast, a study in London reported increased falls in older adults (65+ years) in those with poor glycemic control (A1C >8 %) [81]. A study in older African-American adults in the United States found no association between glycemic control, assessed with fructosamine, and falls [82]. In a study that was limited to falls resulting in an injury requiring hospitalization, poor glycemic control (A1C of 8 % or higher) was associated with increased risk among older adults with T2D [64].

The most appropriate A1C goal for treatment of diabetes in older adults remains controversial [71, 83, 84]. There are unanswered questions regarding the net benefit of a lower target in this age group, and the effects on falls and fractures are an important part of this equation. The ACCORD trial suggests that an A1C target of 6.4 % is safe with regards to falls and fractures but notably the trial did not include any participants over age 79 years. ACCORD also indicates that prevention of

fractures and falls is not a motivation for maintaining A1C levels below 7.5 %. Observational studies to date are limited but suggest that an A1C target of less than 8 % could reduce fractures.

Hypoglycemia

Severe hypoglycemia is relatively common in T2D [85, 86]. A recent survey in a California HMO found that about 10 % of T2D patients experience an episode of severe hypoglycemia during a year [86]. Severe episodes were more prevalent among those with near normal glycemia and those with poor control (A1C ≥9 %). It is believed that hypoglycemic episodes lead to falls and increase the risk of fractures. However, there has been surprisingly little study of these associations. A study using Danish health registry data found higher fracture risk associated with a prior episode of hypoglycemia (HR = 1.13; 1.00–1.26) [87]. In a study designed to assess the relationship between hypoglycemic events and fracture, using a healthcare claims database in the United States, T2D patients with a hypoglycemic event requiring medical care during a 1-year period of observation had an increased risk of a fall-related fracture during the same year (adjusted OR 1.70; 95 % CI 1.58–1.83) [88].

Diabetes-Related Complications

Diabetes duration may increase fracture risk in part through an increase in the prevalence of microvascular and macrovascular diabetic complications. Certainly in broader populations there is evidence that the manifestations of microvascular complications, particularly reduced vision [89] and kidney disease [90], contribute to fracture risk. Similarly, the manifestations of macrovascular complications increase fracture risk in broader populations, including stroke [91], myocardial infarction [92] and peripheral arterial disease [93].

Diabetic patients with multiple complications appear to be at higher risk of fracture, but results are mixed for the association between specific complications and fracture. A study using Danish registry data found increased risk of any fracture in T2 diabetic patients with eye disease (OR = 2.1; 95 % CI 1.8–2.4), kidney disease (RO = 2.0; 1.6–2.5), diabetic neuropathy (1.9; 1.6–2.2), or macrovascular complications (OR = 1.9; 95 % CI 1.6–2.3) while T2 diabetes without complications was associated with a more modest increase in fracture risk (OR = 1.4; 95 % CI 1.4–1.5), all compared with nondiabetic patients in unadjusted models [94]. However, after multiple adjustment, including for the presence of other complications, these estimates were attenuated. Those with multiple complications had an increased risk of fracture (adjusted OR = 1.3; 95 % CI 1.2–1.5) as did those with uncomplicated T2 diabetes (adjusted OR = 1.13; 95 % CI 1.06–1.22), but specific complications were not associated with fracture risk in adjusted models. A population-based study using

medical records in Rochester, Minnesota, assessed diabetic complications as risk factors for fracture in models limited to those with T2D [16]. Increased fracture risk was reported for neuropathy (age-adjusted HR 1.4; 95 % CI 1.2–1.7) but not for clinically diagnosed nephropathy (age-adjusted HR 1.1; 0.8–1.3) or retinopathy (age-adjusted HR 1.0; 0.8–1.2). However, renal failure was associated with higher fracture risk (age-adjusted HR = 1.6; 1.2–2.2).

A study using fundus photography to identify retinopathy, conducted as part of the Blue Mountains Eye Study in Australia, found increased fracture risk with diabetic retinopathy [68]. In a US study with direct measurements of monofilament detection and serum creatinine, Strotmeyer et al. reported higher fracture risk among diabetic participants with inability to detect 10 g monofilament, but no increased risk associated with high creatinine [58]. A study of risk factors for prevalent vertebral fractures among T2D women in Brazil found increased prevalence of fractures in those with diabetic retinopathy, identified by funduscopy, and those with lower creatinine clearance, but no difference in fracture prevalence based on clinical nephropathy or peripheral neuropathy [95].

For macrovascular complications, history of stroke is associated with higher fracture risk in older adults with T2D [58]. A mediation analysis, based on data from the Cardiovascular Health Study, found that ankle-arm index, a measure of peripheral arterial disease, accounted for a substantial portion of the higher hip fracture risk associated with diabetes [56].

Prediabetes and Fracture Risk

Those with glucose levels below the threshold defining diabetes but above normal levels have a higher risk of developing diabetes. Primarily on this basis, a category of "prediabetes" has been defined using fasting glucose, the oral glucose tolerance test, or A1C levels [71]. Prediabetes predicts the development of cardiovascular disease as well as the development of diabetes [96]. The effect of prediabetes on fracture risk has been assessed in a limited number of studies with inconsistent results. None of the studies have reported a statistically significant increased risk of fracture with prediabetes although power in each study was limited. Some have reported lower risk of fracture among those with elevated 2 h oral glucose tolerance test (OGTT) results, but elevated fasting glucose (FG) has not been associated with reduced risk of fracture. In the Rotterdam study, participants with impaired glucose tolerance based on OGTT (7.8–11.1 mmol/L) had a lower risk of non-vertebral fracture compared with nondiabetic participants (RR = 0.80; 0.63–1.00) in multivariable models including BMD [66]. The Malmo Preventive Project also found evidence of lower osteoporotic fracture risk among those without diabetes in the highest quartile of OGTT but not among those with elevated fasting glucose (FG) [97]. The AusDiab study reported a similar pattern in women, but not men. Nondiabetic women in the highest quartile of OGTT had a reduced risk of a low-trauma fracture (age and BMI-adjusted OR = 0.59; 0.40–0.88) but fracture risk did

not differ across levels of FG [98]. For men, FG and OGTT (age and BMI-adjusted OR = 1.39; 0.60–3.26, comparing upper and lower quartiles of OGTT) were not associated with fracture risk. The Osteoporotic Fractures in Men (MrOS) study in the United States also found no association between prediabetes, defined by FG, and risk of non-vertebral fracture (age and BMD-adjusted HR = 1.04; 0.89–1.21) [28]. In contrast, in older US adults, fracture risk in those with impaired fasting glucose (110–125 mg/dL) was modestly elevated but not statistically different compared with participants who had normal fasting glucose in multivariable models including BMD (HR = 1.34; 0.67–2.67) [58]. A larger US study based on NHANES data similarly reported modestly elevated, but not statistically different, risk of any fracture in those with prediabetes, defined by A1C (5.7–6.4 %), compared with no diabetes (A1C <5.7 %) in multivariable models including BMI [29]. The adjusted HR for any fracture was 1.20 (95 % CI 0.96–1.51) in non-Hispanic whites and 1.42 (95 % CI 0.72–2.81) in Mexican-Americans.

Conclusion

Those with T2D are at increased risk of fracture, especially when their higher bone density is taken into account. The reasons for this higher fracture risk are not clearly understood. Observational studies exploring the role of falls as an intermediary have concluded that falls do not fully account for the increased fracture risk with T2D. Instead, the epidemiology of T2D and fracture points to aspects of bone strength—that cannot be measured with standard DXA—as the underlying factor responsible for increased fracture risk.

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Chapter 3
Fracture Risk Assessment in Diabetes

William D. Leslie and Stephen Hough

Introduction

As highlighted in earlier chapters, diabetes mellitus is associated with an increased risk for low-trauma fractures [1, 2]. In the case of Type 1 diabetes (T1DM) this is at least partially mediated through lower bone mineral density (BMD) as reflected by routine clinical measurements such as dual energy X-ray absorptiometry (DXA) [2]. The situation with Type 2 diabetes (T2DM) is clearly more complicated since BMD measurements are typically increased. In view of the differences in underlying pathophysiology and how this may mediate its effects on subsequent fractures, it is unlikely that a single approach for fracture risk assessment will be equally applicable to T1DM and T2DM. Given the preponderance of T2DM among older individuals, the segment of the population at highest risk for osteoporotic fractures, and the BMD-fracture paradox alluded to earlier, this chapter will emphasize considerations in T2DM.

In the absence of a fragility fracture, osteoporosis is diagnosed from bone mineral density (BMD) measured with dual X-ray absorptiometry (DXA). The World Health Organization operational definition of osteoporosis is a BMD that lies 2.5 standard deviations (SD) or more below the average mean value for young healthy women (T-score ≤ -2.5) based upon a standardized reference site (the femoral neck) and reference population (National Health and Nutrition Examination Survey [NHANES] III data for White women aged 20–29 years) [3–5]. This definition

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serves as a reference standard for older adults independent of sex, ethnicity, and underlying conditions including obesity and diabetes. Despite the deceptive simplicity of a BMD-based approach to osteoporosis management, many studies show that most fractures occur in individuals who have a BMD *T*-score above the defining cut-off for osteoporosis [6–9]. This suboptimal performance of BMD alone for fracture prediction has led to the development of new risk prediction algorithms that estimate fracture probability by integrating the effects of multiple risk factors for fracture.

Fracture Risk Assessment in the General Population

At the present time, no fracture prediction tool has been developed for the diabetes population. This is not unique to diabetes, however, and is a limitation for risk assessment in other complex conditions such as organ transplantation, chronic kidney disease, and glucocorticoid-induced osteoporosis. Therefore, we start with a review of screening and fracture risk assessment systems developed for the general population before considering their applicability to patients with diabetes. Broadly speaking, there are tools to identify individuals with osteoporotic BMD, and tools that integrate BMD with other clinical risk factors to identify individuals at high risk for fracture in order to address the suboptimal performance of BMD alone [6–9]. These tools were systematically reviewed by Rubin et al. [10] and therefore we will focus on those that had the highest levels of evidence for their use and at least one independent assessment. Of a total of 48 tools, only 6 had been tested more than once in a population-based setting with acceptable methodology (defined a Quality Assessment Tool for Diagnostic Accuracy Studies [QUADAS] score above 60 % [11]). Model-based fracture prediction algorithms include: the World Health Organization FRAX tool, the Garvan Fracture Risk Calculator, and the QResearch Database's QFracture. The basic components of these tools are summarized in Table 3.1. Discrimination (the model's ability to distinguish between individuals who do or do not experience the event of interest) and calibration (agreement between observed and predicted event rates for groups of individuals) are key performance aspects in risk prediction. Procedures for development and validation of fracture prediction models are reviewed elsewhere [12–16]. There was no consistent evidence that more complex tools had better performance characteristics than simpler tools, however, the paucity of head-to-head comparisons limits any definitive conclusions. Larger, high-quality studies with different case mixes should address this important question.

Simple Screening Tools

Tools developed to identify individuals with low BMD (e.g., SCORE, OST, ORAI [17–19]) do not provide a direct estimate of fracture probability, though some of these have also been shown to stratify fracture risk [20–23]. SCORE, ORAI, and

Table 3.1 Screening and fracture risk prediction tools for the general population

Screening tool, URL	*Risk factors*	*Outputs*
SCORE (Simple Calculated Risk Estimation Score) [19]	• Age, weight, previous fracture, estrogen use, rheumatoid arthritis, race	• Risk score for femoral neck *T*-scores ≤ −2.0
OST (Osteoporosis Self-Assessment Tool) [18]	• Age, weight	• Risk score for femoral neck *T*-scores ≤ −2.5
ORAI (Osteoporosis Risk Assessment Instrument) [17]	• Age, weight, current estrogen use	• Risk score for femoral neck *T*-scores ≤ −2.0
Prediction tool, URL	*Risk factors*	*Outputs*
FRAX (Fracture Risk Assessment Tool) [27], www.shef.ac.uk/FRAX	• Age, sex, BMI	• 10 year major osteoporotic fracture (clinical vertebrae, hip, forearm, proximal humerus)
	• Prior fragility fracture, glucocorticoid use ≥3 months, secondary osteoporosis, rheumatoid arthritis, parental hip fracture, current cigarette smoking, alcohol intake of ≥3 units/day (yes/no)	• 10 year hip fracture
	• Femoral neck BMD or *T*-score (optional)	
Garvan Fracture Risk Calculator (Dubbo nomogram) [40, 41], www.garvan.org.au/bone-fracture-risk	• Age, sex	• 5 or 10 year any osteoporotic fracture (hip, clinical vertebrae, wrist, metacarpal, humerus, scapula, clavicle, distal femur, proximal tibia, patella, pelvis, and sternum)
	• Number of fractures after age 50 (none, 0, 1, 2, ≥3)	• 5- or 10-year hip fracture
	• Number of falls in the previous 12 months (none, 0, 1, 2, ≥3)	
	• Femoral neck BMD (or *T*-score) or weight (if BMD not entered)	

(continued)

Table 3.1 (continued)

Screening tool, URL	*Risk factors*	*Outputs*
QFracture-2013 [43, 44], www.qfracture.org	• Age, sex, 10 ethnic origins	• 1–10 year osteoporotic fracture (clinical spine, hip, distal forearm, humerus fracture)
	• Height, weight	• 1–10-year hip fracture
	• Smoking (4 levels), alcohol intake (5 levels), diabetes (type 1, type 2), previous fracture, parental osteoporosis or hip fracture, living in a nursing or care home, history of falls, dementia, cancer, asthma/COPD, cardiovascular disease, chronic liver disease, chronic kidney disease, Parkinson's disease, rheumatoid arthritis/SLE, malabsorption, endocrine problems, epilepsy or anticonvulsant use, antidepressant use, steroid use, HRT use	

BMI body mass index, *BMD* bone mineral density, *HRT* hormone replacement therapy, *COPD* chronic obstructive pulmonary disease

OST have been validated as screening tools for BMD testing, and outperform a simple body weight criterion [24, 25]. In the review of Rubin et al. [10], none of the tools performed consistently better than others when tested in external validation studies, and simple tools with fewer risk factors (i.e., OST, ORAI) did as well as more complex tools with more risk factors (i.e., SCORE).

FRAX (www.shef.ac.uk/FRAX)

FRAX was developed by the WHO Collaborating Centre for Metabolic Bone Diseases to estimate an individual's 10-year probability of major osteoporotic fracture (MOF, composite of clinical spine, hip, forearm, proximal humerus) and hip fracture [26]. The input variables were selected following a series of meta-analyses using data from nine prospective international population-based cohorts [27]. In addition to age, sex, and body mass index (BMI), additional clinical risk factors (CRFs) for fractures include prior fragility fracture, a parental history of hip fracture, prolonged use of glucocorticoids, rheumatoid arthritis, current cigarette

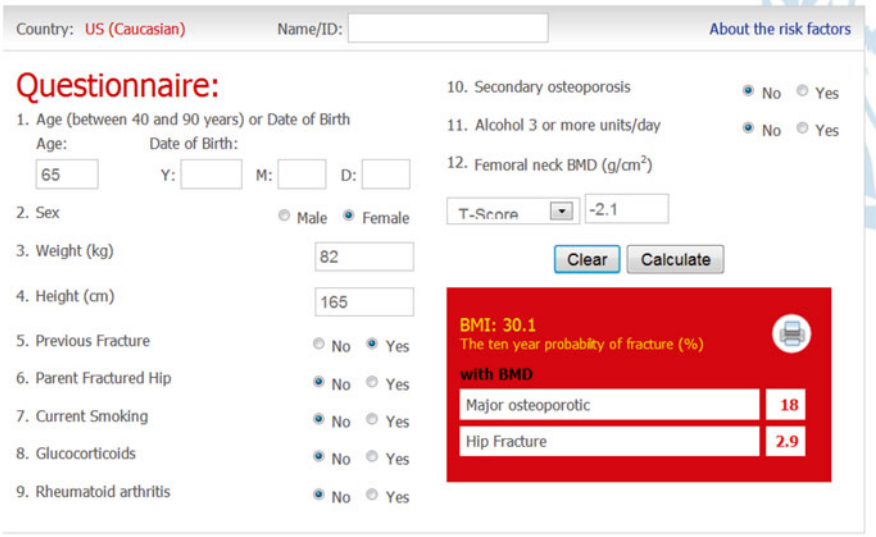

Fig. 3.1 Sample screenshot for FRAX® (US Caucasian tool). Ten-year probability for major osteoporotic fracture is 18 % and for hip fracture is 2.9 % in a woman age 65 years, weight 82 kg, height 165 cm, previous fracture, and femoral neck *T*-score −2.1. (Note that more than one fracture, type 2 diabetes or fall in the prior year does not affect the calculation)

smoking, alcohol intake of three or more units/day, and secondary osteoporosis (Fig. 3.1). Femoral neck BMD is an optional input that can refine the risk estimate, though even in its absence FRAX performs very well [28, 29]. Interactions among CRFs are also incorporated into the FRAX algorithm. More recently, specific adjustments were developed that can be applied to FRAX-derived risk scores to accommodate discordantly lower or higher lumbar spine BMD (more than 1 SD difference from femoral neck BMD) or glucocorticoid doses that are above or below average (average use defined as daily 2.5–7.5 mg prednisone-equivalent) [30, 31].

In survival analysis, the time at which a subject experiences an event of interest may be altered by another event, known as competing risk events [32]. For fracture, competing death is particularly important to consider in order to produce unbiased estimates of fracture risk since, following death, fracture is no longer possible. FRAX adjusts for competing mortality, and this is unique among the risk prediction models. Individuals may have equivalent hazards for fracture but if they differ in terms of hazard for death then this will affect the 10-year fracture probability. For example, smoking is a risk factor for fracture but also increases the risk for death. Thus, the increased mortality associated with smoking reduces the importance of smoking as a risk factor for fracture. Ten-year major fracture probability tends to increase with age to peak around 80–85 years and then declines as the death hazard rises faster than the fracture hazard (Table 3.2). Failure to account for competing mortality has been shown to overestimate major fracture probability by 15–56 % and hip fracture probability by 17–36 % in those with high mortality [33].

Table 3.2 Relative change in fracture probability versus no other risk factors from type 1 diabetes, type 2 diabetes, and inflammatory arthropathy (rheumatoid arthritis or SLE) with QFracture®-2013 or from rheumatoid arthritis with FRAX

Age	40	50	60	70	80	90	Mean
	Women—major osteoporotic fractures						
QFracture—type 1 diabetes	1.88	1.88	1.91	1.86	2.25	2.74	2.08
QFracture—type 2 diabetes	1.25	1.25	1.28	1.25	1.25	1.24	1.25
QFracture—rheumatoid arthritis or SLE	1.25	1.31	1.34	1.32	1.31	1.30	1.30
FRAX—rheumatoid arthritis (no BMD)	1.35	1.35	1.37	1.36	1.41	1.45	1.38
FRAX—rheumatoid arthritis (*T*-score −2.5)	1.33	1.32	1.35	1.29	1.29	1.29	1.31
	Men—major osteoporotic fractures						
QFracture—type 1 diabetes	2.20	2.33	2.30	2.61	3.38	3.34	2.69
QFracture—type 2 diabetes	1.20	1.33	1.20	1.28	1.23	1.24	1.25
QFracture—rheumatoid arthritis or SLE	1.40	1.50	1.50	1.56	1.54	1.52	1.50
FRAX—rheumatoid arthritis (no BMD)	1.32	1.33	1.39	1.41	1.53	1.58	1.43
FRAX—rheumatoid arthritis (*T*-score −2.5)	1.32	1.34	1.29	1.30	1.29	1.33	1.31
	Women—hip fracture						
QFracture—type 1 diabetes			4.83	4.36	4.13	3.73	4.27
QFracture—type 2 diabetes			1.67	1.55	1.53	1.51	1.56
QFracture—rheumatoid arthritis or SLE			1.83	1.64	1.65	1.61	1.68
FRAX—rheumatoid arthritis (no BMD)			1.71	1.74	1.74	1.67	1.71
FRAX—rheumatoid arthritis (*T*-score −2.5)			1.42	1.39	1.41	1.39	1.40
	Men—hip fracture						
QFracture—type 1 diabetes			5.33	4.70	4.55	4.24	4.71
QFracture—type 2 diabetes			1.33	1.30	1.31	1.33	1.32
QFracture—rheumatoid arthritis or SLE			2.00	1.90	1.86	1.86	1.90
FRAX—rheumatoid arthritis (no BMD)			2.00	1.69	1.76	1.77	1.81
FRAX—rheumatoid arthritis (*T*-score −2.5)			1.41	1.42	1.40	1.41	1.41

In recognition of the large international variability in fracture and mortality rates [34], population-specific FRAX tools are customized to the fracture and mortality epidemiology in a specific region, with the most recent version containing over 50 countries [26]. Minimum data requirements for constructing a new FRAX tool are sex and age-specific mortality and hip fracture rates (5 year subgroups). In many countries, such data are relatively easy to obtain. In contrast, non-hip fracture data considered by FRAX (clinical spine, distal forearm, proximal humerus) are difficult to accurately collect at the population level. Where high-quality data are not available, country-specific FRAX tools can be calibrated under the assumption that the ratio of these non-hip to hip fracture rates is similar to that observed in the Swedish population [35, 36]. Whether these ratios are applicable in all populations has been questioned, but currently they provide a valuable reference standard [37].

Fracture discrimination with FRAX was initially assessed in 9 primary derivation cohorts (46,340 subjects with 189,852 person years follow-up) and then in 11 additional validation cohorts (230,486 persons with 1,208,528 person years of follow-up) [38]. Risk stratification with FRAX including BMD was superior to FRAX without BMD or to BMD alone. In the primary derivation cohorts, the gradient of risk for hip fracture increased from 1.84 to 2.91 (area under the curve [AUC] from 0.67 to 0.78) with the inclusion of BMD, and for MOF increased from 1.55 to 1.61 (AUC from 0.62 to 0.63) with the inclusion of BMD. In the validation cohorts, the averaged hip fracture gradient of risk (1.83 without BMD and 2.52 with BMD) and AUC (0.66 without BMD and 0.74 with BMD) was similar to that of the derivation cohorts but the gradient of risk for other osteoporotic fractures (1.53 without BMD, 1.57 with BMD) and AUC (0.60 without BMD and 0.62 with BMD) was slightly lower.

A large number of studies have performed independent assessments of FRAX to predict subsequent fracture [16], but studies differ widely in their sample size, methodology (particularly incorporation of competing mortality risk) and techniques used to assess the performance of the fracture prediction tool (discrimination versus calibration), which can affect the validity of these validation studies [39]. As a general rule, these studies confirm the validity of FRAX for fracture risk assessment but highlight the importance of using high-quality fracture data to ensure accurate calibration of the FRAX tool.

Garvan Fracture Risk Calculator (www.garvan.org.au/bone-fracture-risk)

The Dubbo Osteoporosis Epidemiology Study (DOES) was initiated in 1989 and involves follow-up of over 3500 participants. Using information on 426 clinical fractures in women (96 hip) and 149 clinical fractures in men (31 hip) excluding digits, 5- and 10-year fracture probability nomograms were constructed [40, 41]. Inputs include age, sex, femoral neck BMD (optional), history of prior fractures after age 50 years (none, 0, 1, 2, 3, or more) and history of falls in the previous 12 months (none, 0, 1, 2, 3, or more) (Fig. 3.2). If femoral neck BMD is not available, then weight is used as a proxy. Risk factors that are relatively uncommon in the general population (e.g., glucocorticoid use and specific medical conditions) are not included. The model has only been calibrated for the Australian population and does not include an explicit competing mortality risk adjustment. However, the algorithm has been independently validated in one North American population of women and men [42].

QFracture (www.qfracture.org)

The largest prospective database for osteoporotic fracture prediction is from England and Wales using patients from 357 general practices for derivation and patients from 178 practices for validation in the initial analysis (QResearch Database) [43]. This

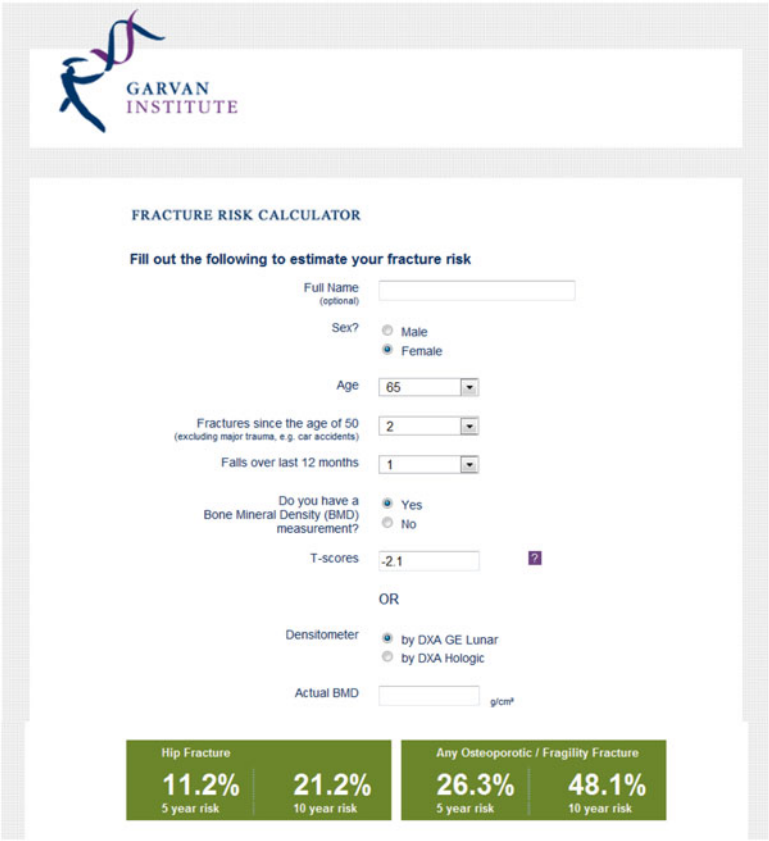

Fig. 3.2 Sample screenshot for Garvan fracture risk calculator. Ten-year probability for major osteoporotic fracture is 53.7 % and for hip fracture is 28.4 % in a woman age 65 years, two previous fractures, one previous fall, and femoral neck *T*-score −2.1. (Note that weight 82 kg, height 165 cm, and type 2 diabetes does not affect the calculation)

provided more than one million women and more than one million men age 30–85 years in the derivation cohort with 24,350 incident osteoporotic fractures in women (9302 hip fractures) and 7934 osteoporotic fractures in men (5424 hip fractures). The risk calculator includes numerous CRFs, but not BMD (Fig. 3.3). It provides outputs of any osteoporotic fracture (hip, wrist, or spine) and hip fracture over a user selected follow-up period from 1 year to 10 years. The QFracture algorithm was updated in 2012, with inclusion of a number of new CRFs, removal of several others, and the addition of humerus fractures as one of the osteoporotic fractures [44]. In addition to age, sex, and ethnicity (10 different ethnic origins) the algorithm includes smoking status (4 levels), alcohol consumption (5 levels), diabetes (2 levels, T1DM or T2DM), previous fracture, parental osteoporosis or hip fracture, living in a nursing or care home, history of falls, dementia, cancer, asthma/COPD,

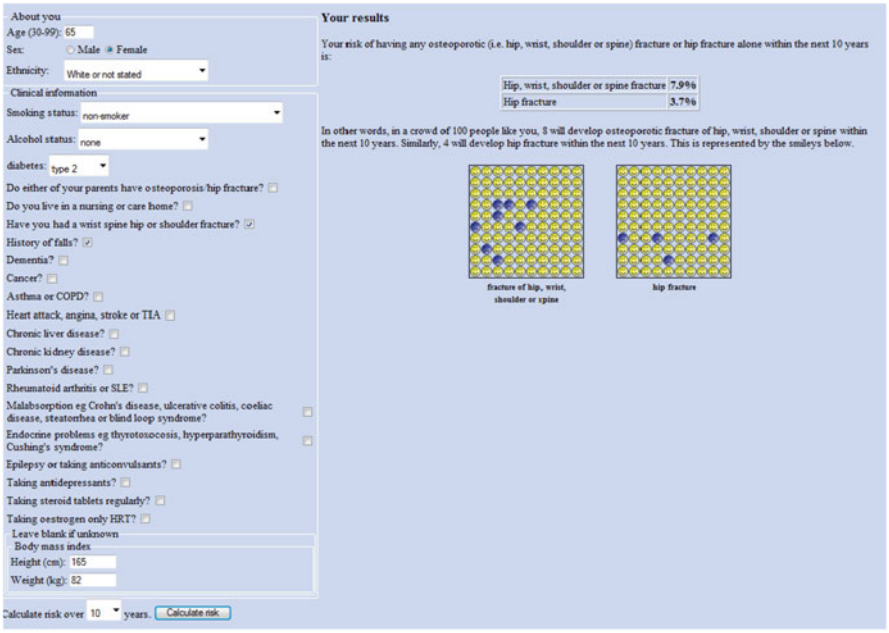

Fig. 3.3 Sample screenshot for QFracture®-2013 risk calculator. Ten-year probability for major osteoporotic fracture is 7.9 % and for hip fracture is 3.7 % in a White woman age 65 years, weight 82 kg, height 165 cm, type 2 diabetes, previous fracture, history of falls. (Note that more than one fracture, and femoral neck *T*-score does not affect the calculation)

cardiovascular disease, chronic liver disease, chronic kidney disease, Parkinson's disease, rheumatoid arthritis/SLE, malabsorption, endocrine problems, epilepsy or anticonvulsant use, antidepressant use, steroid use, HRT use, height, and weight.

The 2012 version reported very good performance for osteoporotic fracture prediction (AUC 0.79 in women and 0.71 in men) and excellent performance for hip fracture prediction (AUC 0.89 in women and 0.88 in men). An independent validation study was performed using patients from 364 general practices in the UK Health Improvement Network (THIN) database (2.2 million adults aged 30–85 years with 25,208 osteoporotic and 12,188 hip fractures) [45]. The validation cohort gave AUC discrimination for osteoporotic fracture of 0.82 in women and 0.74 in men, and for hip fracture of 0.89 in women and 0.86 in men. Calibration plots adhered closely to the line of identity. QFracture explained 63 % of the variation in hip fracture risk in women and 60 % of the variation in men (49 and 38 % for osteoporotic fracture risk).

A small retrospective comparison of FRAX and QFracture was conducted in 246 postmenopausal women aged 50–85 years with recent low trauma fracture and 338 nonfracture control women from six centers in Ireland and the UK [46]. AUC for fracture discrimination were similar in QFracture and FRAX (0.668 versus 0.665) and also for hip fractures (0.637 versus 0.710). The striking difference with AUC

measures from the THIN database validation study is unexplained. The broad age range used in the initial validation work may have inflated the performance measures since osteoporotic fractures are unlikely before age 50, and additional assessments of QFracture in older women and men are needed.

Risk Factors for Fracture in Diabetes

General Risk Factors

Individual risk factors for fracture in individuals with diabetes can be divided into those that are applicable to the general population and those that are specific to diabetes. Most of the risk factors for osteoporotic fractures that apply to the general population, particularly those that are included in the most common risk assessment tools (Table 3.1), have been shown to predict fracture in individuals with diabetes [47]. For example, older age, lower BMI and previous osteoporotic fracture help to identify patients with diabetes who are at greater fracture risk [47]. BMD measurement from DXA provides a robust estimate of fracture risk, increasing 1.4–2.6 fold for every SD decrease in BMD in the general population [48, 49] and in those with diabetes [47]. Two reports from the population-based clinical BMD repository for Manitoba, Canada, have examined whether individual risk factors behave the same in those with and without diabetes, or whether there is effect moderation (interaction) [47, 50]. For MOF, no significant effect moderation was observed for FRAX clinical risk factors. Prior fracture was examined in relation to fracture site, and also behaved similarly in those with and without diabetes. Prior vertebral fracture was associated with the highest risk for subsequent MOF, while prior ankle fracture (which is common in individuals with diabetes) was not associated with an increased risk for subsequent fracture. Age was a significant effect modifier for hip fracture risk, however. Specifically, younger individuals with diabetes were at much higher risk for hip fracture than were those of similar age without diabetes and this difference narrowed with age (adjusted hazard ratio age <60 years: 4.67 [95 % CI 2.76–7.89], age 60–69 years: 2.68 [1.77–4.04], age 70–79 years: 1.57 [1.20–2.04], age ≥80 years: 1.42 [1.10–1.99]; P-interaction <0.001). Unfortunately, this study was unable to distinguish T1DM and T2DM which differ substantially in their pathophysiology as reviewed in previous chapters. Other studies have noted much higher risk for T1DM than T2DM which would be expected to be relatively more prevalent in younger versus older diabetic [1, 2].

Obesity

It is impossible to overlook the parallel between the BMD-fracture paradox in T2DM and in obesity, and implications for fracture risk assessment. Historically, obesity has been considered a protective factor for osteoporosis but recent

observations have called this into question based upon reports that obesity can be protective, neutral, or a risk factor for osteoporosis and related fractures [51–54]. Mechanisms whereby obesity might be associated with fractures are poorly defined but potentially include: biomechanical effects (increased skeletal loading), metabolic changes (fat-derived adipocytokines, inflammatory mediators), and statistical (failure to adequately consider variable collinearity, differential effects of lean versus fat mass) [54–57]. For some sites (particularly the upper arm), obesity appears to be a risk factor for some fractures even after adjustment for higher BMD [52, 58]. A recent meta-analysis examined the association between BMI and fracture risk in prospective cohorts from 25 countries (398,610 women average age 63 years, 2.2 million person-years follow-up) with a reported 22 % prevalence of obesity [58]. Risk for MOF and hip fractures decreased monotonically with increasing BMI when the modifying effect of BMD was excluded. When BMD was considered, the effect of BMI was greatly reduced and, at least for MOF, showed a biphasic response such that fracture risk decreased as BMI increased to the normal range and then slightly increased for overweight and obese individuals. Obese women were at lower risk for MOF when BMD was not considered (hazard ratio 0.87 for BMI 35 kg/m^2 versus 25 kg/m^2) but at higher risk when BMD was considered (HR 1.16). Obesity was still protective against hip fractures, even when adjusted for higher BMD, possibly reflecting energy absorption from soft tissue padding [59].

However, weight associations become more complicated when separated into their lean mass and fat mass components. BMI does not distinguish the very different skeletal effects from lean versus fat tissue where significant nonlinearities have been observed. Most studies find that lean mass is positively associated with higher BMD, whereas fat mass shows neutral or negative effects though there are exceptions to this generalization and a dual (biphasic) effect has even been reported [57, 60]. A meta-analysis found that among premenopausal women lean mass exerted a greater effect on femoral neck BMD than fat mass ($r=0.45$ versus $r=0.30$), whereas in postmenopausal women the effects of lean mass and fat mass were similar ($r=0.33$ versus $r=0.31$) [57]. A recent population-based study found that the effect of BMI on fracture risk was largely mediated by its effect on BMD, with little if any direct effect of BMI on fracture risk [61]. This awaits confirmation in additional larger studies where the mediating effects of other body composition variables can be studied.

Biomechanical models have not yet been incorporated into fracture prediction tools. Based upon engineering principles, such as beam theory, these have the potential to incorporate the opposing effects of greater BMD (protective) versus increased skeletal loading from falls (risk factor) that occur during specific fall configurations (boundary conditions). Melton et al. [62] compared estimated bone load to bone strength ratios in 49 T2DM patients to age and sex-matched controls. Hip BMD from DXA was greater in diabetic subjects due to greater trabecular volumetric BMD (vBMD) while cortical vBMD was similar. Derived bone strength measures were generally better in diabetic subjects, but bone loads were higher from

their greater weight, such that load to strength ratios (i.e., factor-of-risk) were similar. A subsequent analysis of older men with ($n=190$) and without ($n=981$) T2DM found that although patients with T2DM had lower bone bending strength at the mostly cortical bone midshaft sites of the radius and tibia after adjusting for body weight (−2 to −5 %, $p<0.05$) despite the lack of difference in cortical vBMD at these sites. Indices of femoral neck strength in diabetic versus nondiabetic women (adjusted for age, race/ethnicity, menopausal stage, body mass index, smoking, physical activity, calcium and vitamin D supplementation, and study site) showed lower composite strength indices for compression, bending, or impact despite higher femoral neck BMD [63]. Furthermore, there was an inverse relationship between insulin resistance (homeostasis model) and all three strength indices. These studies suggest that patients with T2DM do not benefit from elevated BMD in terms of improved bone load to strength ratios. However, these predictions may not always align with clinical observations. In a large registry-based cohort (40,050 women and 3600 men), the femoral strength index (FSI) showed a progressive decline with increasing fat mass index but did not improve fracture prediction over conventional FRAX probability measurements [56]. The FSI does not consider soft tissue padding effects, which may significantly attenuate the fracture risk relationship [59]. Additional work is needed to evaluate these indices derived from biomechanical principles in individuals with obesity and diabetes.

Importantly, some measure of body size (weight or body mass index [BMI]) is included in each of the systems summarized in Table 3.1. Given that obesity strongly predisposes to T2DM, one would anticipate that these tools would be insensitive to the effect of T2DM and would underestimate risk. A demonstration of the differing approaches to including BMI (or weight equivalent) on fracture risk assessment is illustrated in Fig. 3.4. When BMD is not included, FRAX predicts a linear decrease in fracture probability with increasing BMI. A similar relationship is seen with the Garvan FRC (which uses weight when BMD is not available) and QFracture risk (which does not have a BMD input). When BMD is included in the FRAX calculation, BMI has a very different relationship such that fracture probability increases with greater BMI up to 25 kg/m^2, and subsequently declines. The lower fracture probability for individuals with BMI much less than 25 kg/m^2 likely relates to the effect of competing mortality since underweight is associated with a higher likelihood of death from comorbid conditions. The protective effect of BMI in the overweight and obese range that is independent of BMD may reflect soft tissue padding [59].

Together, the foregoing observations challenge conventional concepts of weight as a risk factor for osteoporosis and fractures, and beg the question of why something that is associated with preserved BMD would be a risk factor for fracture. Potential explanations include greater falls risk, adverse changes on skeletal biomechanics, alterations in energy homeostasis, inflammation, insulin resistance, overt T2DM or their associated treatments and comorbidities are potential contributors [52, 64]. Despite these questions, lean and fat tissue mass indices did not have an independent effect on MOF or hip fracture risk when adjusted for FRAX scores [56].

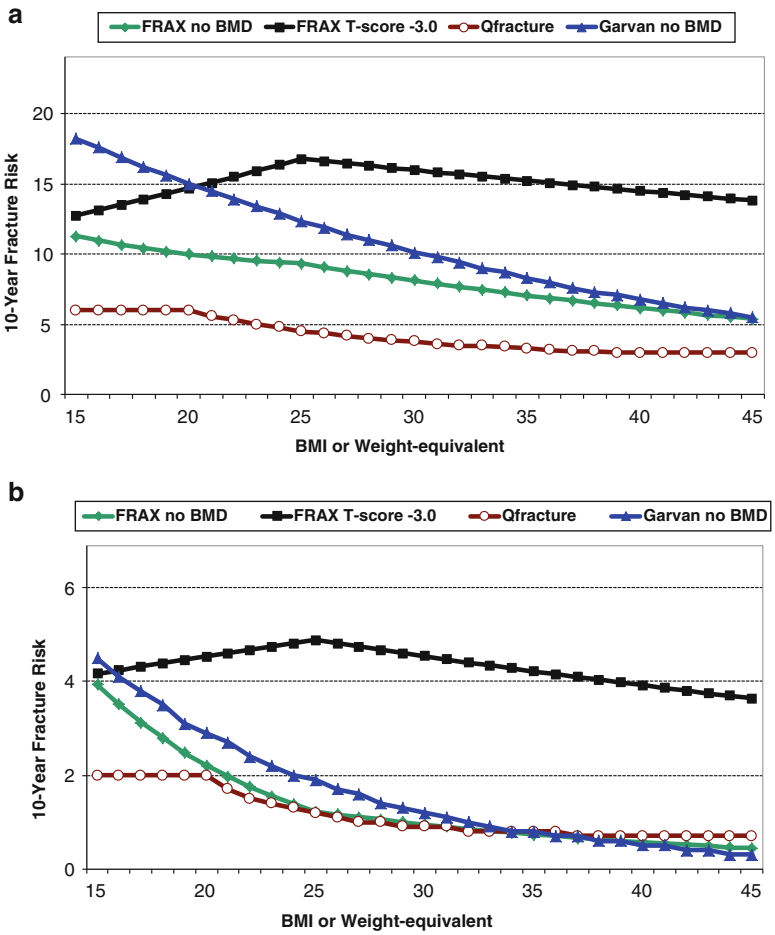

Fig. 3.4 Effect of increasing body mass index (BMI) or weight-equivalent (height 165 cm) on fracture predictions from US White FRAX (with and without BMD), Garvan FRC (without BMD), and QFracture®-2013 (without BMD) for a 65-year-old woman with *T*-score −3.0 and no other risk factors. (**a**) Non-hip fractures (MOF for FRAX and QFracture®-2013, any fracture for Garvan FRC). (**b**) Hip fractures

Of potential relevance to T2DM, Premaor et al. [65] examined the question of whether FRAX was applicable to obese older women using 6049 white women from the US Study of Osteoporotic Fractures (SOF) cohort. Fracture discrimination from AUC was similar in obese and nonobese women. Calibration was good in both groups for prediction of MOF using FRAX with BMD, but hip fracture risk was found to be underestimated, most markedly among obese women in the lowest category for FRAX probability with BMD (though based on only four predicted versus nine observed hip fractures).

Diabetes-Specific Risk Factors

It is logical to consider whether factors specific to diabetes should be included in the risk assessment since these may mediate some of the excess fracture risk. This could include increased risk for falls and fractures related to diabetic complications (including retinopathy, neuropathy), hypoglycemic episodes, or the adverse effect of some medications (most specifically the thiazolidinediones) [66, 67]. Indeed, there are population-based data that duration of diabetes, which correlates with long-term complications, is associated with increased fracture risk [68]. Use of insulin (HR 1.3) and neuropathy (HR 1.3) are independent risk factors for fracture in T2DM [69]. The effect of insulin may not be causally related to the higher fracture risk as patients on insulin tend to have more severe dysglycemia and are at higher risk for complications and falls, which have not been incorporated into risk assessment tools for individuals with diabetes. In part, this relates to the requirements for large cohorts for the derivation of the appropriate weight to be accorded the risk factor and verification that it improves fracture discrimination and calibration. The challenge of developing a diabetes-specific risk assessment tool becomes moot if it does not enter widespread clinical practice. Primary care physicians are already overwhelmed with the number of clinical practice guidelines and risk assessment tools, and the availability of more than one fracture risk assessment tool may generate confusion and inactivity rather than an improvement in care. Therefore, methods that can be incorporated into existing risk assessment tools with the minimum of effort on the part of practicing physicians are likely to be more successful in terms of enhanced clinical care.

Risk Prediction in Diabetes

Simple Screening Tools

To date, none of the BMD screening tools referred to above (SCORE, OST, ORAI [17–19]) have been specifically evaluated in diabetes. Moreover, they each include weight in the calculation given its strong correlation with BMD. Therefore, it is very unlikely that these tools would be satisfactory for use in individuals with T2DM where increased fracture risk is not mediated through lower BMD or lower weight. It is possible that these tools could be modified to incorporate T1DM and/or T2DM with a numeric "penalty" included in the score, but these would need to be worked out.

Multifactorial Models for Absolute Fracture Prediction

As noted earlier, FRAX does not include a diabetes input variable. T1DM is considered as one of the secondary osteoporosis causes and only affects fracture probability when BMD is not included in the calculation [27]. When BMD is available for

the FRAX calculation, the secondary osteoporosis variable does not affect the fracture probability calculation since it is assumed that these conditions mediate their effect through lower BMD. All secondary osteoporosis conditions are assigned the same weight in the FRAX calculation (equal to rheumatoid arthritis) which may not be applicable to T1DM, particularly among younger individuals.

The predictive performance of FRAX in patients with diabetes has been evaluated in two large studies. Schwartz et al. [70] combined data in older community-dwelling adults (9449 women and 7436 men) from three prospective observational studies with adjudicated fracture outcomes (Study of Osteoporotic Fractures; Osteoporotic Fractures in Men Study; and Health, Aging, and Body Composition study). Of 770 women with T2DM, 84 experienced a hip fracture and 262 a non-spine fracture; among 1199 men with T2DM, 32 experienced a hip fracture and 133 a nonspine fracture. Although both femoral neck BMD and FRAX score were associated with fracture risk in participants with DM, for a given *T*-score and age or for a given FRAX score, participants with T2DM had a higher fracture risk than those without diabetes. Similar results were seen in a large BMD registry analysis from Manitoba, Canada [71]. Among 3518 men and women with diabetes and 36,085 nondiabetics aged ≥50 years at the time of BMD, diabetes was a significant risk factor for subsequent MOF (hazard ratio [HR] = 1.61, 95 % confidence interval [CI] 1.42–1.83) after controlling for age, sex, medication use, and FRAX risk factors including BMD. Similar results were seen after adjusting for FRAX probability directly (HR = 1.59, 95 % CI 1.40–1.79). Diabetes was also associated with significantly higher risk for hip fractures ($p < 0.001$). FRAX underestimated observed major osteoporotic and hip fracture risk in diabetics (adjusted for competing mortality) but demonstrated good concordance with observed fractures for nondiabetics. An even larger discrepancy between observed and predict fracture risk was seen if competing mortality was not considered. Smaller cross-sectional studies have shown similar findings [72, 73]. A multicenter cross-sectional study found that 974 patients with T2DM had mean FRAX scores lower than 777 control subjects despite a greater number of previous fractures [72]. Of interest, HbA1c and hypoglycemia were significantly associated with higher FRAX scores independent of other covariates. Together, these studies indicate that FRAX systematically underestimates fracture risk in patients with T2DM despite the competing effect of increased mortality associated with diabetes.

The Garvan FRC has not been evaluated in relation to diabetes. Based upon its construction, it is likely to underestimate fracture risk in T2DM for the same reasons that affect FRAX. This discrepancy would be amplified by not considering competing mortality, but potentially attenuated by considering falls which is one of the mediating factors between diabetes and fracture. Therefore, it remains important to directly assess how the Garvan FRC performs in patients with T2DM.

Only the QFracture tool has direct inputs for diabetes, stratified as T1DM and T2DM. The magnitude of the adjustment is illustrated in Fig. 3.4. For T1DM, the average effect (as a ratio versus an individual with no other risk factors) across all ages and sexes is 2.37 for MOF and 4.46 for hip fracture in T1DM, 1.25 for MOF, and 1.45 for hip fracture in T2DM. The effect of T2DM (compared with an individual

with no other risk factors) is relatively constant across the age spectrum of women and men. The relative effect of T1DM on MOF increases with age (from 1.88 to 2.74 in women, from 2.20 to 3.34 in men) while the effect on hip fracture risk shows little variation with age or sex. Although QFracture appears to be well calibrated for the general population, it has not been specifically evaluated with regard to calibration in individuals with diabetes nor does it explicitly consider competing mortality.

Comparative Performance

Figures 3.1, 3.2, and 3.3 illustrate some of the differences between the fracture prediction systems discussed above for a 65-year-old obese woman (BMI 30.1 kg/m^2) with femoral neck *T*-score −2.1 and having the following CRFs: two previous fragility fractures (distal radius and humerus), T2DM and one fall in the prior year. None of the systems uses all of the available information and this contributes to the range in fracture predictions. For example, 10-year hip fracture probability varies from 2.9 % for FRAX, to 3.7 % for QFracture-2013, to 21.2 % for the Garvan calculator (18 % MOF, 7.9 % MOF, and 48.1 % any fracture, respectively). It is clear that where an important CRF is not captured by a tool, clinical judgment must be used to make some estimate of the importance of the missing information.

Identification of High Risk in Diabetes

Clinical Practice Guidelines

Validated prognostic models for fracture risk assessment can guide clinicians and individuals in understanding the risk of suffering an osteoporosis-related fracture and inform their decision making to mitigate this risk. Integration of risk prediction tools into clinical practice guidelines can in turn support better clinical decision making and improved patient outcomes.

The lack of significant interaction (effect modification) means that conventional risk factors, including BMD and BMI, remain valid in the population with diabetes [47, 50]. For example, all other factors being equal, a 70-year-old woman with T2DM and BMD *T*-score −2.5 or BMI 22 kg/m^2 is at higher risk than a woman with the same risk profile who has BMD *T*-score −1.0 or BMI 28 kg/m^2. Based upon the expectation and empirical observations that fracture prediction tools underestimate fracture risk in diabetes (particularly T2DM), it is reasonable to assume that an individual with diabetes who meets the high risk (intervention) threshold for the general population should also be considered at high risk. Similarly, since prior fracture and low BMD predict fractures in individuals with diabetes as well as in individuals without diabetes (no effect modification), these can also be used as

indicators of high risk (intervention). For example, under the National Osteoporosis Foundation (NOF) guidelines, prior low-trauma fracture (vertebral or hip), osteoporotic BMD (*T*-score −2.5 or lower), or high FRAX probability for low bone mass (osteopenic) individuals (MOF 20 % or greater, hip 3 % or greater) would be considered intervention thresholds for the general population [74]. An individual with diabetes (T1DM or T2DM) could likewise be considered for treatment using the same thresholds. Since QFracture was calibrated for use in the UK, its applicability to other populations remains uncertain. However, within the UK (or countries with similar fracture and mortality rates), QFracture may be a good tool for use in individuals with T1DM or T2DM although this tool does not incorporate BMD in the calculation.

Potential Risk Adjustments for Diabetes

Although appealing, whether it is possible to develop simple adjustments to FRAX or the Garvan FRC that would accommodate the risk attributable to diabetes is uncertain. Several options on tempering clinical judgment with the existing output of the FRAX models have been proposed [75]. In the context of a specific FRAX-based intervention thresholds (e.g., NOF-based 20 % probability of MOF or 3 % probability of hip fracture [74]), a patient with diabetes below but close to the intervention threshold such as the one in Fig. 3.2 (MOF probability 18 %, hip fracture probability 2.9 %) could be recommended for treatment. (The upward revision of a patient with a fracture probability already exceeding the treatment threshold has little clinical utility in making a decision to treat.) A second measure might be to model the potential impact of diabetes on FRAX using a percentage adjustment to the probability score in much the same way that has been done when considering the effects of glucocorticoid dose or lumbar spine BMD on fracture probability [30, 31]. A third option is to use the existing input variable rheumatoid arthritis as a substitute for diabetes. As shown in Table 3.2 and Fig. 3.5, the weight attributed to rheumatoid arthritis in FRAX is similar to the weight of rheumatoid arthritis or SLE in QFracture and T2DM in QFracture, but considerably less than the effect of T1DM in QFracture. Therefore, this simple adjustment would not adequately address underestimation in fracture risk for T1DM. The prevalence of individuals with both rheumatoid arthritis and diabetes in the population is quite low, therefore the rheumatoid arthritis input to FRAX is usually available for individuals with diabetes.

Whether a similar approach would work for the Garvan FRC has not yet been evaluated. It is conceivable that either the fracture input or the falls input (which can accommodate 0, 1, 2, or 3+ events) could be used by incrementing the input that would be used in the absence of diabetes. For example, in an individual with no prior fractures and one fall in the last year, incrementing the falls input to 2 for T2DM and to 3+ for T1DM could conceptually be considered but requires careful study to determine the need and accuracy of such an adjustment.

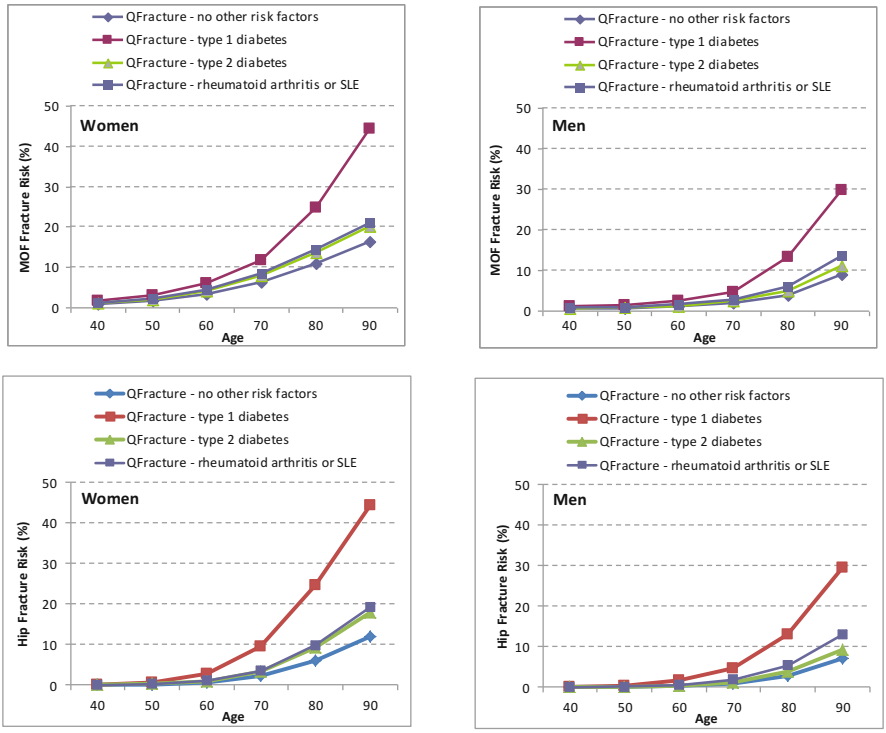

Fig. 3.5 Comparative effects of type 1 diabetes, type 2 diabetes, and inflammatory arthropathy (rheumatoid arthritis or SLE) versus no other risk factors on 10-year probability for major osteoporotic fracture (*upper panel*) and hip fracture (*lower panel*) with QFracture®-2013

Lumbar Spine Trabecular Bone Score

The trabecular bone score (TBS) is a novel texture parameter that evaluates pixel gray-level variations in the spine DXA image and is related to bone microarchitecture and fracture risk, providing information independent of BMD [76–79]. TBS is not a direct physical measurement of bone microarchitecture, but rather an overall score computed by the projection of the 3D structure onto a 2D plane [77]. TBS is calculated as the slope of the log-log transform of the 2D variogram from the projected DXA image (calculated as the sum of the squared gray-level differences between pixels at a specific distance). While the TBS result is given for each vertebra, the usually reported TBS value represents the average of L1–L4. TBS has the potential to discern differences between DXA scans that show similar BMD measurements. A recent review by Silva et al. [80] concluded that: (1) TBS gives lower values in postmenopausal women and in men with previous fragility fractures than their nonfractured counterparts; (2) TBS is complementary to data available by

lumbar spine DXA measurements; (3) TBS results are lower in women who have sustained a fragility fracture but in whom DXA does not indicate osteoporosis or even osteopenia; (4) TBS predicts fracture risk as well as lumbar spine BMD measurements in postmenopausal women; (5) efficacious therapies for osteoporosis differ in the extent to which they influence the TBS; (6) TBS is associated with fracture risk in individuals with conditions related to reduced bone mass or bone quality.

Although there is accumulating evidence that TBS is able to discriminate between fracture and nonfracture subjects and predict future fracture risk in the general population [80], it is less certain whether TBS is useful in specific conditions that modify fracture risk. Several studies have evaluated TBS in individuals with conditions or diseases related to increased fracture risk, and TBS was associated with fragility fracture in subjects with diabetes, rheumatoid arthritis, primary hyperparathyroidism, adrenal incidentaloma, individuals on long-term glucocorticoid therapy and chronic kidney disease [80].

To date, four published studies have evaluated the ability of lumbar spine TBS to account for the increased fracture risk in diabetes. A retrospective cohort study using BMD results (Prodigy, GE HealthCare) from a large clinical registry for the province of Manitoba, Canada [81]. This included 29,407 women ≥50 years with baseline DXA examinations, among whom 2356 had diagnosed diabetes. Diabetes was associated with higher BMD at all sites but lower lumbar spine TBS in unadjusted and adjusted models (all $p<0.001$). The adjusted odds ratio (aOR) for a measurement in the lowest versus highest tertile was less than 1 for BMD (all $p<0.001$) but was increased for lumbar spine TBS (aOR 2.61, 95 % CI 2.30–2.97). During mean 4.7 years of observation, MOF were identified in 175 women (7.4 %) with and 1493 (5.5 %) without diabetes ($p<0.001$). Lumbar spine TBS was a BMD-independent predictor of fracture, and predicted fractures in those with diabetes (adjusted HR 1.27, 95 % CI 1.10–1.46) and without diabetes (HR 1.31, 95 % CI 1.24–1.38). The effect of diabetes on fracture was reduced when lumbar spine TBS was added to a regression model, but was paradoxically increased from adding BMD measurements.

Similar effects of T2DM were seen in a subsequent retrospective analysis of 56 postmenopausal women with T2DM and 61 women without DM or impaired glucose tolerance [67] using equipment from the second major DXA manufacturer (Hologic). T2DM was associated with higher BMD at all sites but lower lumbar spine TBS (all $p<0.05$). The adjusted odds ratio (aOR) for a measurement in the lowest versus the highest tertile was less than 1 for BMD ($p<0.05$) but was increased for lumbar spine TBS (aOR 2.39, 95 % CI 2.22–2.81). In a small cross-sectional study, Dhaliwal et al. [82] examined lumbar spine BMD (iDXA, GE HealthCare) and TBS in 57 women with T2DM and 43 women without diabetes, ages 30–90 years. Mean TBS was lower in T2DM ($p=0.013$), irrespective of age, while mean BMD was higher ($p=0.001$). Within the T2DM group, TBS was higher in subjects with good glycemic control (A1c ≤ 7.5 %) compared to those with poor glycemic control (A1c > 7.5 %). Rubin et al. [83] reported the association of TBS with trabecular heterogeneity by high resolution pQCT (HRpQCT) and biochemical markers of bone turnover in 14 postmenopausal T2DM women and 21 age-matched

controls. Despite similar lumbar spine BMD, TBS was significantly lower in T2DM versus controls ($p=0.01$). Consistent with this, trabecular heterogeneity (SD of 1/Tb Number) at the tibia was greater in T2DM ($p=0.05$). The authors concluded that while BMD does not differ from controls in T2DM, TBS is worse and is consistent with the greater trabecular heterogeneity seen on HRpQCT.

Notwithstanding these promising reports, some notes of caution are warranted. Spine TBS is (negatively) affected by obesity and tissue thickness [84], though recent changes to the algorithm have greatly attenuated this effect [85]. Increased soft tissue thickness may have the same effect on TBS as noise, i.e., lower TBS. The TBS algorithm includes an adjustment for BMI that is optimized for BMI ranges from 15 to 35 kg/m^2, so that the assessment of TBS may not be as reliable in subjects with a BMI beyond these limits. The use of BMI as a proxy for soft tissue thickness is limited since it can overestimate adiposity in subjects with a high lean body mass, and underestimate adiposity in subjects with low lean body mass. Furthermore, higher BMI does not distinguish abdominal weight accumulation (which would directly affect spine TBS) from weight accumulation at other sites. Truncal obesity in T2DM could contribute to the lower apparent TBS in diabetes, though this does not negative the fact that TBS is still able to predict fractures in those with diabetes. While results from clinical studies have confirmed the fracture-discriminating ability of TBS in a substantial number of postmenopausal women, data in men are still preliminary with only one cross-sectional [86] and one longitudinal study of fractures in men [87]. A final limitation for the use of TBS in the clinical practice is the lack of a well-established TBS cutoff point that defines normal and abnormal TBS values, and how this would be used clinically in conjunction with other data. Methods to incorporate this information into risk assessment are still evolving, but the feasibility of a TBS-based adjustment to FRAX probability has been reported [88]. Additional work is needed to extend this preliminary observation to hip fracture prediction with validation in independent cohorts.

In summary, lumbar spine TBS predicts osteoporotic fractures in those with diabetes, and captures a larger portion of the diabetes-associated fracture risk than BMD [81]. In T2DM, TBS is lower and associated with poor glycemic control [82]. Abnormal trabecular microarchitecture may help explain the paradox of increased fractures at a higher BMD in T2DM [83]. Further studies are needed to better understand the relationship of T2DM with glycemic control and trabecular bone quality.

Emerging and Future Methods

There are many additional fracture risk predictors that could be considered in individuals with diabetes that are not currently incorporated in any of the fracture prediction systems discussed earlier. Some of these are based upon advanced imaging such as HRpQCT, such as cortical porosity, or minimally invasive measurements of tissue properties, such as microindentation [67, 89]. Such methods are likely to remain as research methods for the foreseeable future. The imaging method most

likely to be helpful in routine clinical practice is lumbar spine TBS where several studies now indicate that this captures some of the skeletal effect of T2DM that is missed by conventional BMD [67, 81–83]. The use of biochemical parameters as discussed elsewhere (such as osteoblast- and osteoclast-derived bone turnover markers, advanced glycation end products such as pentosidine, measures of diabetes control, serum sclerostin, insulin-like growth factor-1), or clinical markers of diabetes-related complications (neuropathy, retinopathy, nephropathy, microvascular) represent an alternative direction based upon clinical parameters [66, 67, 90, 91]. Incorporation of falls risk into FRAX (already considered by Garvan FRC and QFracture) is another option. Meanwhile, the need for improved methods for identification of fracture risk in older adults with diabetes is an important priority for osteoporosis research.

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Chapter 4
Diabetes-Related Conditions and Complications in Orthopaedic Surgery

Daniel J. Gehling and Nabil A. Ebraheim

Introduction

Diabetes mellitus (DM) is a significant clinical problem, with the treatment costs in 2012 estimated at $245 billion in the USA alone [1]. This amount is a 41 % increase over the estimated $174 billion in 2007 [2]. Medical expenditures account for the largest proportion of general costs. However, there are also many indirect costs of diabetes as a consequence of increased absenteeism, reduced productivity in the workplace, unemployment from disease-related disability, and productive capacity loss due to early mortality [1, 2]. In orthopaedics, patients with diabetes have a number of associated disorders, and these present a challenge as many have an increased hospital stay, higher risk of infection, and higher risk of complications after orthopaedic treatment.

The orthopaedic-related problems in diabetes are varied, and the true causal links between diabetes and the disorders are largely unknown. Hip fractures have a higher incidence in patients with diabetes, while ankle fractures are important in diabetic patients because there is a unique concern of treatment of patients with diabetes and the clearly associated worse outcomes. Infection with or without surgery is also a concern in diabetes. Plantar ulcers are discussed because there is an association of ulcers in diabetes and they are predictive of poor outcomes. Finally, there are a number of upper extremity disorders including trigger finger, carpal tunnel syndrome, Dupuytren's disease, and adhesive capsulitis that have clear associations with diabetes.

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Hip Fracture

Fractures around the hip in the general population are associated with decreased function and an increased mortality [3–12]. These fractures can be either intracapsular, usually femoral neck fractures, or extra-capsular, usually referred to as intertrochanteric or subtrochanteric fractures (Fig. 4.1) [13]. The cause of these fractures has largely been attributed to decreased bone mass, as most of these fractures are low energy falls in patients older than 65 years old [3]. In diabetic patients, the concern with hip fracture is the increased incidence, with a clear increase in type I DM, and a probable increase in those patients with type II DM [14].

The overall incidence of femoral neck fractures in the USA is between 0.4 and 1.0 % [15, 16], and the estimated cost of treatment of femoral neck fracture alone has been estimated at $19,000–$23,000 for 1 year of treatment [17–19]. With the demographical aging of the population, the number of fractures is expected to increase, and it is reasonable to expect that cost of treatment will increase as well. Over 12 years in a British hospital, there has been a 10 % increase in admissions from hip fracture from 2000 to 2012, and it has been considered to be a nonlinear increase over time [20].

Type I DM has a definite effect on the incidence of hip fractures with reported relative risks of between 1.7 and 12 times higher than controls, with a suggested average of sixfold increase [21–24]. In addition, type I DM patients have hip fractures at a younger age on average, with a mean of 43 for women and 41 for men in one study. Almost 7 % of people with type I DM can be expected to have sustained a hip fracture by age 65 [7]. The mechanisms that cause these fractures in type I DM are unclear, but three main reasons have been speculated.

First, bone mineral density (BMD) in patients with DM type I is decreased as compared with controls [25]. One large meta-analysis estimates that the hip Z-scores are –0.37 from expected, and this decrease in BMD suggests a 1.42 increase in risk of hip fracture. However the relative risk is somewhere about sixfold over nondiabetic

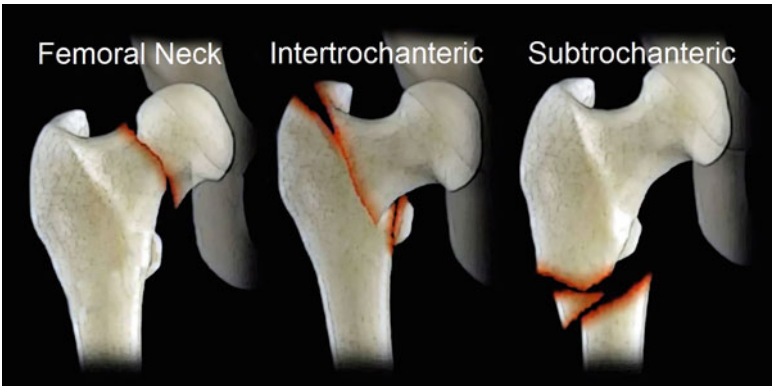

Fig. 4.1 General classification of hip fractures

people. This discrepancy has suggested that other pathological mechanisms should be explored to help explain the increased risk of hip fracture in type I DM [26].

Second, type I DM is commonly associated with decreased proprioception, neuropathic changes, and retinopathy with increasing duration and severity of disease process. Each of these comorbidities can potentially contribute to falls, increasing the chances of hip fracture over time [7, 27, 28]. In addition, these disease processes could serve as a marker of severity, and may also have some contribution to pathological bone homeostasis. Supporting these hypotheses, the presence of ophthalmic, neurological, and cardiovascular complications increase the risks of hip fracture by 20, 41, and 29 times expected general population values. Furthermore, as compared to others with type I DM without complications, the risks are 1.3, 2.0, and 1.7, respectively [7]. Finally, hypoglycemia has been suggested as a possible cause, as type I DM is treated with various types of insulin, but all can lead to hypoglycemia [27, 29].

Compared to patients with type I DM, the increased incidence of hip fracture in patients with type II DM is less certain, but is likely. Conflicting evidence has suggested an increased risk, no change in risk, and even a decreased risk of fracture [10, 21, 28, 30–35]. However, the reports on the largest enrollments generally put the relative risk between 1.2 and 2.2, and most recent studies suggest an increased relative risk [14]. It is somewhat confusing that patients with type II DM also have increased BMD on average; however, some propose that the BMD difference is related to the confounding variable of obesity rather than solely a direct effect [25, 26, 30, 33, 34]. The duration of diabetes has also been suggested as a risk factor [31], but age is a significant confounding variable to be considered.

The medical treatment regimens for patients with diabetes have also been suggested as a possible risk factor, given a two times higher risk of hip fracture in patients with a HbA1c of <6.0 vs. those with HbA1c levels greater than 8 %. The tighter control of diabetes could result in more episodes of hypoglycemia, which in turn can potentially lead to more falls [27]. To support this hypothesis, the use of metformin and acarbose has been associated with a decreased risk of hip fractures in type II DM as compared to patients treated with insulin or sulfonylureas. Metformin and acarbose have a much smaller risk of hypoglycemia, and hypothetically may lead to less falls. Thiazolidinediones have also been implicated in higher rates of hip fracture, but not universally [21, 27, 36]. However, hypoglycemia as a potential reason for falls is controversial, as some research suggests that intensive glucose control does not increase the propensity for falls [37].

Treatment for hip fractures is almost exclusively operative, as nonsurgical treatments result in high rates of nonunion, pain, decubitus ulcers, pneumonia, deep venous thrombosis, and pulmonary embolus [38, 39]. For femoral neck fractures, treatment options are open reduction and internal fixation (ORIF), percutaneous screw fixation, hemiarthroplasty, and total hip arthroplasty (THA). Although somewhat controversial, there has been a trend towards total hip replacement in patients with femoral neck fracture despite many having intact cartilage. This trend is because the long-term outcomes after THA are superior to other methods of treatment, in spite of having some increase in early complications [40–43]. With ORIF, there are late complications of fracture nonunion and avascular necrosis of

the femoral head. These complications are thought to be related to damage of the femoral head blood supply and the resulting hemarthrosis after displaced femoral neck fractures [44, 45]. In hip arthroplasty, the proximal segment of the femur is replaced and the problems of nonunion and avascular necrosis of the femoral head are no longer issues. For intertrochanteric and subtrochanteric hip fractures, the most accepted treatment is with ORIF, as the blood supply generally allows the fracture to heal, without significant risk of avascular necrosis [46].

Mortality rates after hip fracture at 1 year range from 18 to 33 % [3, 5, 8, 9, 47], and the rates remain higher than the general public for at least 10 years postoperatively [11, 12]. An analysis in the Danish registry noted that the 1 year mortality is nearly double that of those without femoral neck fracture, and that the excess mortality is approximately 1.8 % increased each year after. At 20 years follow-up, the survival is 57 % of what would be expected from the control group [11]. Patients with DM and hip fracture are at a higher risk of mortality than patients without DM, with 1-year rates as high as 32 % vs. 13 % of nondiabetic patients [3, 4]. In addition, DM is associated with an increased risk of cardiovascular, pressure ulcer, and infectious complications after hip fracture [4, 9, 48]. These complications of hip fracture treatment are thought to be the cause for increase in the risk of mortality, not just the presence of pre-existing diabetes [11].

Ankle Fracture

Fractures around the ankle are some of the most commonly seen fractures in the hospital setting and the incidence appears to be increasing, as a Finnish study has suggested a threefold increase in incidence by 2030 [49]. In diabetic patients, the concern is not necessarily an increased risk of fracture, but of treatment complications. Diabetic patients have significant increased risks of infection, malunion, neuropathic ankle, and amputation after ankle fractures, even those that are low energy injuries [50–58].

Ankle fractures consist of one or more fractures of the medial malleolus of the tibia, the lateral malleolus of the fibula (Fig. 4.2), and sometimes the posterior malleolus of the tibia [59]. A more significant, but related injury is a tibial plafond fracture. The plafond fracture is usually a higher energy injury with axial load, rather than a routine ankle fracture, which is a rotational injury [60]. The specific direction of forces that have created an ankle fracture classically determines the different fracture patterns [59]. Patient with complicated diabetes and no trauma may have Charcot or neuropathic arthropathy, and this type of presentation must be distinguished from those patients with a traumatic ankle fracture [55, 61, 62]. The ankle is an area of relative paucity of muscle tissue, and therefore the bony structures are covered with skin and subcutaneous tissue alone. Therefore, the associated soft tissue injury after ankle fracture is a more significant problem than with fractures in other locations in the body [63].

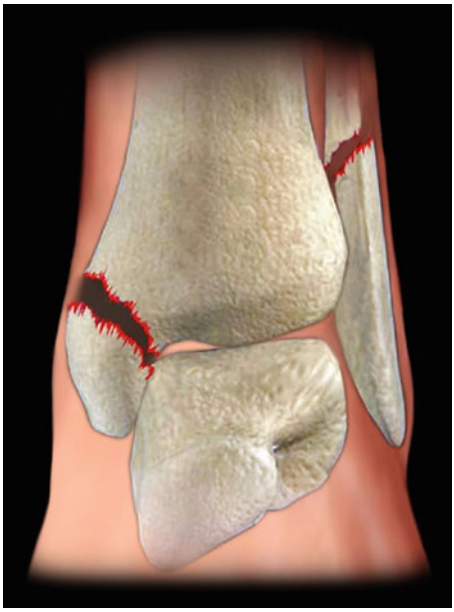

Fig. 4.2 Unstable ankle fracture

Treatment of ankle fractures can be either nonoperative or operative, depending on the stability of the ankle joint. The stability of ankle fractures is based on the integrity of the medial malleolus and the deltoid ligament. In an unstable ankle fracture, treatment is generally operative with anatomic reduction and fixation. In stable ankle fractures, the treatment is general nonoperative [64, 65].

Given the microvascular complications in diabetic patients, and with the relative paucity of soft tissue around the ankle, there is a great deal of concern with diabetic patients with unstable ankle fractures [66]. With nonoperative treatment, the complication rate in diabetic patients is high, with some studies stating an up to 83 % risk of complication in diabetics [57, 67]. Most of these complications relate to malunion and pressure sores from cast treatment, and the risks are understandably higher in those patients with neuropathy [50]. Although counter-intuitive, these studies are evidence that operative management of ankle fractures is indicated in those patients with more severe and complicated diabetes.

In diabetic patients treated with reduction and internal fixation, the complication rate is improved from nonoperative treatment, but still higher than controls at between 13 and 50 % [52, 56, 63, 68, 69]. This rate is compared to the reported risk of infections in controls of 1.4–7 % [55, 63, 68, 69]. The amputation rates are even more striking, with between 3.8 and 5 % of diabetics eventually ending with amputation after ankle fracture treatment, versus well under 1 % of nondiabetics [52, 56, 57, 67, 70, 71]. In open ankle fractures, the amputation rate in diabetic patients has been reported to be as high as 42 % [51], and is a strong predictor of increased complication rates overall [51, 70].

Peripheral neuropathy and microvascular complications are commonly cited as the underlying cause of these complications. First, the microvascular supply and the healing response are compromised in diabetic patients [66]. Second, peripheral neuropathy has loss of protective sensation in the foot and ankle, leading to pressure sores, noncompliance postoperatively, failure of fixation, and malunion of fractures. Indeed, patients with neuropathy have a reported 75 % complication rate after ORIF, and a 100 % complication rate treated with closed methods [50, 52, 55, 56, 67, 71]. Overall, those patients with peripheral neuropathy have a 15.5 odds ratio of a postoperative wound complication versus a control group [63].

Higher complication rates are seen in patients with HbA1c levels greater than 6.5 %, and optimization of blood glucose control is likely to be of benefit for overall outcomes [53, 72]. Emphasizing the importance of blood glucose control long-term, recent results suggest that the complication rate for those patients with uncomplicated diabetes is similar to nondiabetics [53]. More than 90 % of nondiabetic patients with ankle fractures will regain nearly all function after treatment, but only about 70 % of diabetic patients functionally recover, have longer hospital stays, and have higher overall hospital costs [54, 73]. There are two consensus recommendations for treatment of ankle fractures in diabetic patients. First, the ORIF fixation strength should be increased over what would be normally used for nondiabetic patients. Second, the postoperative nonweightbearing period should be lengthened over what would be used routinely, especially in those patients with complicated DM [58, 66].

Infection

Although there is a risk of infection associated with any orthopaedic surgery, patients with diabetes are at a higher risk of developing surgical site infections (SSIs) [51, 52, 57, 68, 74–77]. SSIs are associated with increased hospital stay, increased hospital cost, and reduced quality of life. A review of one hospital in the 1990s showed double mortality, double length of hospital stay, and double the cost expenditure in patients with SSIs compared to controls [78]. In orthopaedic surgery, SSIs are also associated with increased amputation rates, and worse functional outcomes [57, 74, 76, 77, 79–81].

SSIs are defined as superficial incisional, deep incisional, or organ/space infections. A superficial incisional SSI occurs within 30 days, and involves only the skin and subcutaneous tissues. These type of SSI are usually treated with antibiotics alone, or possibly a minor debridement if there is dehiscence of the incision. A deep incisional or organ/space SSI is one that occurs within 30 days or alternatively within 1 year if implants are in place [82]. A deep SSI usually requires aggressive management and almost always some form of surgical debridement.

Orthopaedic spine surgery has an infection rate of about 2 % in elective cases, but has been reported up to 5 % at 1 year following surgery for traumatic spine injury [74, 77, 83]. Diabetes is strongly associated with SSI in these patients, with

the risk being three times that of nondiabetic patients [74, 77, 83]. Furthermore, a preoperative HbA1c greater than 7.0 % is highly associated with SSI in spine surgery, with up to 35 % incidence in patients with uncontrolled DM [84]. Interestingly, increased serum glucose in patients without DM may also be an independent risk of SSI in spine surgery [74]. Correspondingly, in orthopaedic trauma, the stress-response increase in serum glucose also is associated with an increased risk of infection, even without history of diabetes [85–87]. Considering these studies, it appears that perioperative glycemic control is important to prevent SSI.

Periprosthetic infection (PPI) in total joint arthroplasty is also a serious epidemiologic issue. In 2003, there were about 200,000 total hip and 400,000 total knee arthroplasties performed in the USA. The projection to 2030 is a concerning 572,000 total hip and 3.5 million total knee arthroplasties annually [88]. A recent update seems to largely confirm those previous estimates [89]. In the general population, the arthroplasty infection rate is about 0.7 % [90]. Even this low rate of infection will generate a significant health burden of PPI, if the numbers of arthroplasty patients follows the predicted future trends.

Historically, rates of PPI in patients with diabetes have been as high as 7 % [76, 91]. An additional report has suggested a nearly fourfold increased risk of infection in those with diabetes [92]. However, a more recent report suggests a 1.2 times increase in the relative risk of PPI in diabetic patients [93]. Patients with uncontrolled DM preoperatively have a particularly high risk of PPI, urinary tract infection, transfusion, stroke, infection, and death [94]. In addition, even nondiabetic patients with a fasting preoperative glucose greater than 124 mg/dL have a significantly higher rate of PPI [95]. These studies strongly suggest preoperative DM optimization and glycemic control prior to orthopaedic procedures in order to prevent PPI and other complications.

Plantar Ulcerations

Plantar ulcers are a serious concern in patient with diabetes. Ulcers have predictive value for infection, sepsis, and amputation; and prophylactic care decreases patient morbidity and lowers expense of care [96]. The lifetime risk of plantar ulcer in diabetic patients has been estimated as high as 25 %, and the recurrence is 50 % at 3 years. In the past, up to 10 % of patients initially diagnosed with diabetes will have presented with an ulceration [97].

Peripheral neuropathy is the primary determinant of ulcer development, and 20–50 % of patients with diabetes have some degree of neuropathy [98–100]. With neuropathy, the loss of protective sensation in the foot allows an ulcer to start unchecked, as there is no painful feedback to the patient [101]. In addition, perhaps only half of patients with clinically significant diabetic neuropathy self-report neuropathic symptoms [98]. Therefore screening for sensation loss is the primary clinical method to determine those patients at risk for ulceration and to assign appropriate care measures accordingly [102, 103]. In addition to neuropathy, those

patients with minor foot trauma and deformity are at very high risk of ulceration. The triad of neuropathy, trauma, and deformity is present in up to two thirds of patients with ulcers [103].

Screening has been suggested in the primary care setting, and is most commonly recommended with a Semmes-Weinstein 5.07 monofilament, which is a simple and cost-effective diagnostic strategy [96, 97, 100, 104, 105]. Additional screening methods are evaluation of vibratory sensation, temperature sensation, the presence of an Achilles tendon reflex, and transcutaneous oxygenation measurements [96, 102, 104–107]. Prevention is targeted at patient education, appropriate foot wear, optimization of blood glucose control, smoking cessation, identification of deformity and orthopaedic consultation, identification of large vessel disease and vascular consultation, and consistent follow-up care [96, 105].

Treatment of ulcers, once formed, is targeted to offload the ulcer with appropriate foot wear, orthotics, limited weightbearing, or total contact casting [108–111]. Additionally, optimization of blood glucose control, cessation of smoking, orthopaedic debridement or deformity correction, and vascular intervention complete the multidisciplinary approach to treatment of ulceration [112–116]. This treatment comes at a great cost, as the treatment of single foot ulcer over 2 years is estimated at around $28,000 [117, 118].

The time to successful healing of a plantar ulcer is usually on the scale of months, not days. The average healing time is variable, but many will heal by 2–3 months, with the more severe cases taking 4 months or longer [119, 120]. Most of the cross-sectional area of healing takes place at the beginning of treatment, but complete healing takes a much longer time [121]. The average time to healing is lower in those patients with smaller initial wounds, and those with more acute wounds [119, 120]. Transcutaneous oxygen tension may also predict ultimate healing potential [122]. Those ulcerations that ultimately are not able to be healed have very high incidences of osteomyelitis and eventual amputation. Of those diabetic patients who eventually end with amputation, up to 85 % had previous ulcerations [123–125]. In those ulcers with infection and ischemia, the outcomes are very poor. Patients with a deep wound, infection, and ischemia have an amputation rate of at least 50 %, and even a superficial wound can be 17–50 % [126].

Trigger Finger

Also known as stenosing tenosynovitis, trigger finger is an overuse disorder of the flexor tendon at the region of the A1 pulley. This pulley is the first in a set of pulleys in the finger that maintain the tendon–bone relationship while allowing the tendon to slide freely during movement [127]. The trigger finger is caused when there is a mismatch of tendon thickness to the internal diameter of the A1 pulley, and the tendon becomes stuck in the pulley after movement of the finger into flexion (Fig. 4.3) [127–129]. Patients commonly report using force to “pop” the finger back into extension.

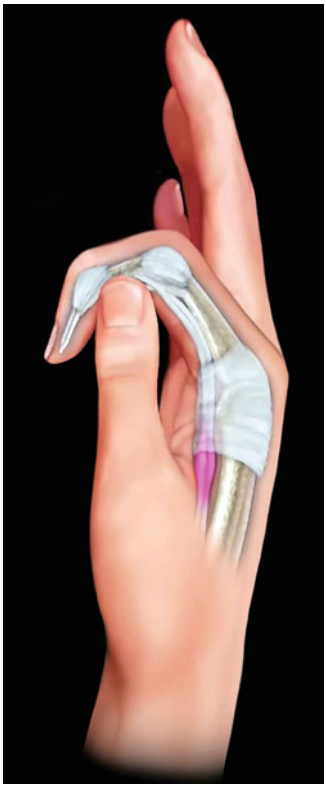

Fig. 4.3 Trigger finger

The incidence of trigger finger is 7–20 % of patients with diabetes comparing to only about 1–2 % in nondiabetic patients [130–132]. Trigger finger is also more common in women than men, except for a possible equal association in type I DM [133, 134]. When associated with diabetes, it commonly presents with multiple digit involvement, with a mean of 2.2 digits vs. 1.3 digits in nondiabetic patients [135, 136]. The duration of diabetes and insulin dependence is also associated with an increased incidence of trigger finger [130, 131, 134, 137]. However, the overall effectiveness of glycemic control does not seem to be correlated with the prevalence of trigger digit [131, 137].

Treatment of trigger finger is similar among patients with and without diabetes. Non-operative treatment includes stretching exercises to the flexor tendon, especially before and after activity, or injections of corticosteroid into the region around or inside the A1 pulley [138, 139]. Diabetic patients may have less successful non-operative treatment overall, especially if insulin dependent [136]. Finally, surgical treatment is generally reserved for patients who fail non-operative management, and is almost always a release of the A1 pulley. The pulley is not absolutely necessary for proper function of the fingers, and has a reported 97 % success rate [140].

Dupuytren's Disease

Dupuytren's disease (DD) is a hand disorder that is primarily symptomatic in older individuals, but can have relatively asymptomatic signs in younger patients as well. It was described by and named after Baron Dupuytren in the 1830s, but he was not the first to describe it [141]. The disease clearly has a genetic component, but with incomplete penetrance, and is most common in men and in patients of Northern European ancestry. It is also associated with a number of other factors including a history of trauma, epilepsy, alcoholism, smoking, adhesive capsulitis, and diabetes [142, 143].

Symptoms of DD begin with palmar nodules, and later with pretendinous cords. Later, the cords begin to contract and cause flexion of the digits, limiting extension and causing flexion contractures (Fig. 4.4). These contractures are frequently the presenting symptoms, as they have a direct limitation of patient activity and overall hand function. The cords contain myofibroblasts and organize along predictable, but abnormal routes in the hand and fingers [144, 145]. Most often, the ring and small fingers are involved in nondiabetic patients, but any digit can be affected. Curiously, diabetic patients with DD are more likely to have long and ring finger involvement instead of ring and small finger involvement [133]. Also, the symptoms of the disease tend to be milder, and progression is slower in those diabetic patients than the general population [141]. Although men are more prone to develop the disease, diabetic women have a high chance of also being affected [133, 146].

The prevalence of DD in the general population is estimated to be 1–8 %, but depends on the diagnostic criteria used. Those patients who present with cords may be closer to 1 %, but those with palmar nodules are probably closer to 7–11 % of the population [130, 133]. The risk of developing Dupuytren's disease in diabetic

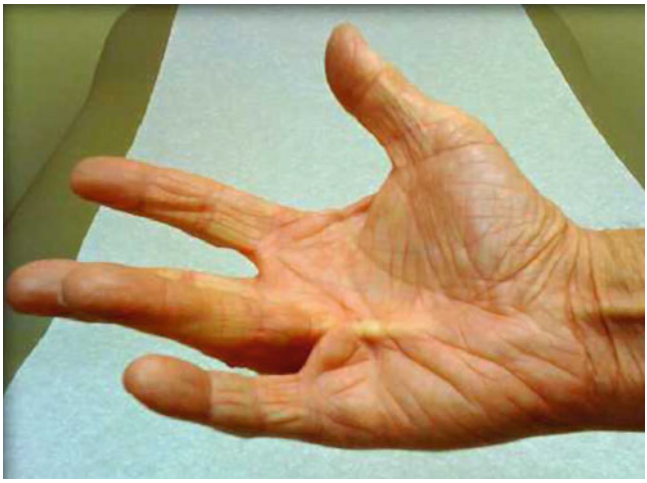

Fig. 4.4 Dupuytren's disease

patients is significantly higher than that of general population at between 19 and 42 % [130, 133, 141]. Dupuytren's disease has been reported to have incidence rates in type I diabetes patients comparable to type II diabetes patients, and correlates to the duration of diabetes [133, 134, 147, 148]. Impaired glycemic control as measured by HbA1c, mean glucose, and presence of diabetic comorbidities seem to correlate with the prevalence of DD [130, 141].

Noninvasive treatment is largely unsuccessful in improving the features of the disease. One minimally invasive treatment option is injection of collagenase followed by manipulation of the digits, and this method has been shown to be an effective treatment [149]. Percutaneous release of the cords is another treatment option that does not have the risk of wound complications postoperatively compared to open fasciectomy, but may not have as significant a correction overall [150, 151]. Open surgical treatment has historically been a complete or partial fasciectomy, which is aggressive removal of the cords of abnormal tissue. Complications of partial fasciectomy are incomplete correction, recurrence, infection, and wound problems. Even with surgical treatment, however, recurrence is up to 80 %, but only about 12 % have additional treatment from loss of function or recurrence [146]. Surgical treatment in diabetic patients does not seem to have an increased risk of complication [152].

Carpal Tunnel Syndrome

Carpal tunnel syndrome (CTS) is the compression of the median nerve within the carpal tunnel in the wrist, and it classically manifests as pain and paresthesias of the thumb, index, long, and the radial half of the ring finger. Symptoms are usually worse at nighttime, and patients frequently report being woken up from numbness in the hand. CTS is typically an overuse injury related to occupational and daily activities, and is more common in women [131, 153–155]. However, it can also present in cases with extrinsic compression of the carpal tunnel from masses, or acutely from trauma.

The prevalence of CTS in patients with diabetes has been estimated at 11–30 % [130, 133, 153, 156], and is dependent on the duration of diabetes. In contrast, the incidence is estimated at 2–3.8 % of the general population [155, 157]. Bilateral involvement of CTS is common in diabetic patients, but both sides are not necessarily symptomatic simultaneously. Type I DM patients have a high prevalence of CTS with increasing duration of disease, up to 85 % after 54 years of DM. However the prevalence does not seem to be associated with glycemic control [130, 158].

Overall, the basic science mechanism in which DM is associated with CTS is not well understood, but microangiopathy has been suggested as a likely contributing factor [159, 160]. However, nephropathy, retinopathy, and polyneuropathy have not been consistently identified as risk factors for CTS, possibly from the confounding effect of duration of diabetes [130, 157, 158].

Initial treatment of CTS is generally non-operative and initiated with splinting, especially at night. Injections into the carpal tunnel are sometimes advised in milder

disease, and can sometimes be effective long-term [161]. Surgical treatment consists of a release of the transverse carpal ligament, which decompresses the median nerve and is a highly effective treatment of patients' symptoms [160, 162]. However, there may be a slightly less efficacious outcome in patients with DM [159, 160].

Adhesive Capsulitis

Adhesive capsulitis, also known as frozen shoulder, is a disorder of the shoulder characterized by pain and stiffness [163], and is commonly arbitrarily divided into phases [164–166]. The first phase is painful, without significant loss of shoulder range of motion. The second phase remains painful, but the shoulder becomes significantly stiffened. In the third phase, the shoulder remains stiff, but is less painful. Finally, the fourth phase is spontaneous resolution, which is sometimes mistakenly believed to happen in all patients [164, 165, 167].

Adhesive capsulitis is a term coined by Nevassier for a disorder of the shoulder in which the glenohumeral joint capsule thickens and has an adhesive appearance when opened to be released [163]. Although used interchangeably, frozen shoulder describes the clinical presentation of a stiff shoulder, either from a primary condition of capsular thickening or a secondary cause from another shoulder diagnosis such as rotator cuff pathology. Adhesive capsulitis is used as a description of the pathological condition of capsular thickening and hypertrophy resulting in shoulder stiffness [163, 164].

Adhesive capsulitis is associated with a number of other medical conditions including diabetes, both type 1 and 2 [166–171]. Other associated conditions reported are trauma, cervical spine disease, Dupuytren's disease, coronary heart disease, tuberculosis, carcinoma, hyperthyroidism, and epilepsy. However, the relationship to these medical comorbidities is unclear, including diabetes [171]. The incidence of adhesive capsulitis is up to 11–20 % in diabetic patients, in comparison to 2–5 % of control patients [171, 172]. Serum glycosylated hemoglobin levels have been studied, but the levels do not seem to correlate with a risk for adhesive capsulitis [166, 168]. Supporting this finding, histologically measured glycosylation of tissues does not seem to directly correlate with the risk of other secondary complications of diabetes including joint stiffness [173]. In type I diabetic patients, the prevalence of adhesive capsulitis is increased after 20 years duration of the disease process [168, 174]. Type II patients do not have a measurable association with duration of disease [168]. However, patients in the past were not commonly diagnosed with DM until years after the process actually commenced. DM patients with a history of myocardial infarction [168, 171] and autonomic neuropathy have been independently associated with an increased risk of adhesive capsulitis [168].

Diagnosis of adhesive capsulitis is based predominately on a loss of passive range of motion in a shoulder, and the exclusion of other diagnoses that may also cause a secondary loss of range of motion from pain [167, 171, 175]. A diagnostic injection into the subacromial space may aid in diagnosis to eliminate pain from other coexisting diagnoses such as rotator cuff pathology, as even in those cases the

shoulder range of motion is limited. Range of motion in adhesive capsulitis is limited in multiple directions, but especially in external rotation and abducted external rotation [167]. This ROM can be compared against the contralateral side to determine a patient's normal ROM, as it can differ among patients [176].

The natural history of adhesive capsulitis has commonly been cited as a self-limiting process. This citation would suggest that the condition resolves on its own, but careful review of past literature would suggest otherwise. Up to 60 % of patients followed for an average 7 years have some persistent symptoms and disability [177]. In addition, about 50 % of patients have incomplete resolution of symptoms at 1 year [164, 165, 167]. These persistent symptoms support early treatment in most patients.

Treatment of adhesive capsulitis is overall similar in diabetic and nondiabetic patients; however, the results are worse in diabetic patients. Diabetic patients have less range of motion, more pain, and more disability after treatment [169, 170]. Treatment options include physical therapy, intra-articular corticosteroid injection, manipulation under anesthesia, and surgical release [178].

One study of aggressive vs. gentle ROM would suggest that gentle ROM may be better than aggressive manipulation [179]. However, it has been unclear which intervention or combination of interventions are the most successful at treatment [180–182]. Corticosteroid injection may improve with pain, but it is unclear if the long-term outcomes are changed [165, 181, 182]. Oral corticosteroid does not appear to affect outcomes positively [165, 183, 184].

Manipulation under anesthesia is indicated in some cases that have failed a course of physical therapy [175]. This manipulation can be under regional or general anesthesia, and may be followed or preceded with an intra-articular injection of steroid solution. Follow-up with physical therapy immediately afterwards is considered standard. A complication to be avoided is fracture of the humerus, which if occurs, may require additional surgical fixation to continue physical therapy, as immobilization would likely result in treatment failure.

Finally, arthroscopic capsular release is indicated in those patients with 3–6 months of failure of other non-operative options, especially those with significantly stiff shoulders. The result of capsular release is marginally improved over manipulation, but is considered in refractory cases [185]. Risks of the procedure include fracture and axillary nerve injury, where the nerve is located in the axillary recess, immediately underneath the capsular tissue.

Conclusion

Diabetes mellitus is a significant medical issue that increases the risk of certain orthopaedic conditions and complications, and leads to less successful outcomes than in nondiabetic patients. Glycemic control is certainly recommended before any elective procedure, and has become more recognized to be a primary goal after traumatic injuries as well.

Hip fractures are a varied group of fractures around the hip, of which DM significantly increases the risks fracture, the risks of complications, and the risk of

mortality after treatment. Ankle fractures are a major source of complications in patients with diabetes after treatment, both non-operatively and operatively. Overall, operative treatment of unstable ankle fractures is superior to that of non-operative treatment. Some patients will ultimately end with amputation despite best efforts.

Infection risk is increased postoperatively after orthopaedic procedures, and there is an increasing emphasis on glycemic control perioperatively to reduce the risk of SSI. Plantar ulcerations are largely a complication of diabetic neuropathy, deformity, and minor trauma. Prevention is more cost-effective than treatment, as the presence of an ulcer is a predictor of ultimately poor outcomes.

Trigger finger has a high incidence in patients with DM, and more fingers are generally involved at presentation in patients with DM. Treatment options are no different than the general population, but successful treatment may be slightly less efficacious in patients with DM than in nondiabetic patients. Dupuytren's disease has a high prevalence in patients with DM, and there is a predilection for long finger and ring finger involvement rather than the small and ring finger involvement seen in the general population. DD is associated with the duration of diabetes, but the presentation in DM patients is less severe on the whole. The treatment of DD is similar in patients with or without DM. Carpal tunnel syndrome is a common orthopaedic hand issue in patients with diabetes, at least four times the general population incidence, and is associated with the duration of diabetes. The incidence of CTS may be even higher in type I DM than in type II DM. Treatment of CTS in DM and nondiabetic patients is not significantly different than the general population. Surgical treatment is usually successful, but possibly less improvement in patients with DM. Adhesive capsulitis has a high incidence in DM patients as well. The overall presentation is similar, but the treatments may be less successful overall. After treatment, patients with DM have more pain and stiffness on average vs. patients without DM.

Diabetic-associated orthopaedic conditions and complications are commonly seen in the general orthopaedic practice, and are challenging to treat. Although some of the conditions associated with DM do not depend on glycemic control, most orthopaedic complications of treatment do have increased risks in those patients with either acutely or chronically poor blood glucose control. In the future, the medical maintenance of DM and attention to perioperative glycemic control may be the most effective interventions to limit these set of orthopaedic complications in patients with DM.

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Chapter 5
Impact of Diabetes on Periodontal Disease

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Diabetes mellitus is characterized by high blood glucose levels [1]. Type 1 diabetes mellitus (T1DM) caused by a deficiency of insulin is most commonly due to autoimmune destruction of pancreatic β-cells. Type 2 diabetes mellitus (T2DM) is caused by insulin resistance and a failure of β-cells to compensate for the reduced effect of insulin stimulation [2, 3].

Metabolic Changes in Diabetes That Contribute to Periodontal Bone Loss

The primary morbidity and mortality associated with DM is from diabetic complications. There are several underlying mechanisms that link diabetic complications including those that affect bone. In particular, advanced glycation end products,

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excessive production of reactive oxygen species (ROS), and enhanced cytokine expression such as tumor necrosis factor have been experimentally linked to diabetes-enhanced periodontal bone loss [4]. These underlined causes are also associated with several other diabetic complications.

Both systemic and local inflammation have been shown to be increased by diabetes. Macrophages in adipose tissue produce inflammatory mediators that contribute but are not solely responsible for insulin resistance and may promote diabetic complications [5]. Pro-inflammatory factors such as TNF-α, IL-1β, IL-6, and IL-18 are reported to be increased in diabetes mellitus [6, 7] and contribute to destruction of beta cells in the pancreas and diabetic complications [8, 9].

Oxidative stress plays an important role in diabetic complications. Hyperglycemia leads to generation of ROS by overproduction of superoxides in mitochondria [10]. Increased intracellular ROS enhances inflammation [11]. Furthermore, increased ROS combined with depleted antioxidant defense mechanisms render tissues more susceptible to oxidative damage [12]. ROS also function as intracellular second messengers that regulate signaling cascades and modulate gene expression [13].

AGEs are formed by the nonenzymatic creation of glucose and other glycating compounds derived from both glucose and increased fatty acid oxidation. Proteins may be structurally modified by glycosylation thereby affecting their function. Alternatively, AGEs bind to AGE receptors inducing production of inflammatory mediators such as tumor necrosis alpha (TNF) and activation of NF-κB [14]. There are a number of receptors for AGEs that are linked to increased inflammation including receptor for AGE (RAGE).

Impact of Diabetes on Bone

Diabetes negatively impacts bone metabolism and affects bone quantity and quality [15]. T1DM and T2DM have increased fracture risk. Bone mineral density (BMD) is reduced in T1DM [16]. T2DM has equal or increased BMD [17] but still has greater fracture risk [18]. Diabetes increases the severity and risk of periodontitis, the most common lytic disease of bone and a frequent complication of diabetes [9, 19]. Both increased bone resorption and reduced bone formation have been linked to the negative effect of diabetes on periodontal bone loss [20].

Periodontal Disease

Periodontitis involves the loss of supporting structure for the tooth consisting of connective tissue attachment and bone. Periodontitis is one of the most widespread oral diseases [21]. Severe periodontitis, which may result in tooth loss, is found in 5–20 % of most adult population worldwide. The 2010 National Health and Nutrition Examination Survey estimates that over 47 % of American adults have

periodontitis [22]. Almost 25 % of adults in Australia aged 35–54 years have moderate to severe periodontitis [23]. Children and adolescents also have forms of periodontitis such as aggressive periodontitis [24].

Under physiological conditions, the tooth is supported by alveolar bone and periodontal ligament in the tooth socket. The surface of the alveolar bone and periodontal ligament are covered by gingival connective tissue and epithelium. The periodontium consists of gingival tissue, periodontal ligament, and alveolar bone. Gingival tissues act as a physical barrier to reduce invasion by bacteria that form a biofilm on the tooth surface. Bacteria or their products penetrate the epithelial barrier of the gingiva to penetrate the connective tissue and thereby stimulate inflammation that induces an inflammatory response. The primary distinction between gingivitis and periodontitis is the irreversible loss of bone and connective tissue which attaches the gingiva to the tooth surface [20] (Fig. 5.1). Thus, gingivitis may cause reversible damage that is repaired whereas periodontitis involves irreversible damage to the periodontal tissues [20]. It is thought that an essential component of periodontitis is suppression of repair processes by inflammation. Periodontal disease includes both periodontitis and gingivitis. In some cases, periodontitis is referred to as moderate to severe periodontal disease. For the purpose of this review, we will use the term periodontitis to mean inflammation that causes irreversible bone loss.

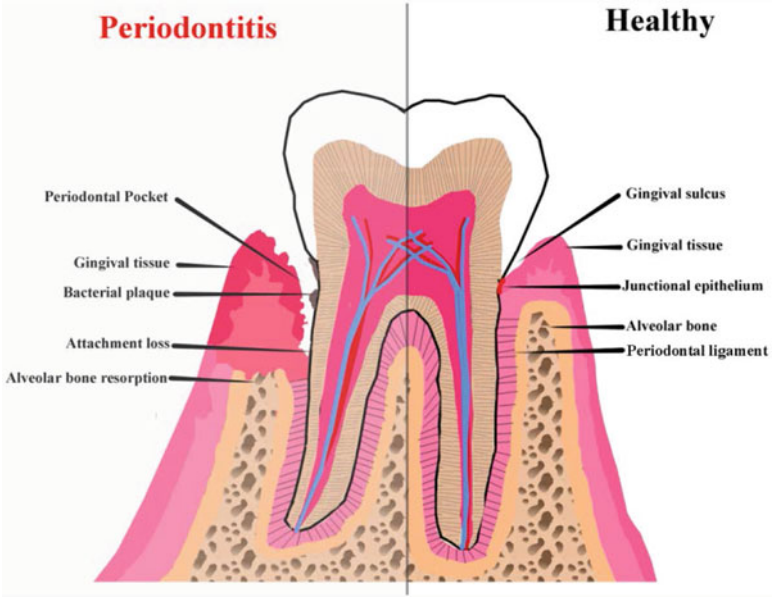

Fig. 5.1 (*Right side*) Healthy periodontal tissue. (*Left half side*) Effects of periodontitis with subgingival bacterial plaque formation, gingival tissue inflammation, periodontal attachment loss, periodontal pocket formation, and alveolar bone loss

Pathogenic Mechanisms

The chronic inflammatory condition of periodontitis is induced by pathogenic biofilm or dental plaque, which accumulates on the tooth surface. Over 500 bacterial species have been detected in dental plaque; however, the composition of the causative bacterial species is still debated [25–28]. The classic periodontal pathogens are Gram-negative bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* [29]. Recent studies have identified other bacteria that may be pathogenic [30]. Although bacteria are necessary for periodontal disease to take place, a susceptible host is also needed [27]. The predisposing factors that make an individual susceptible have not yet been defined though periodontal disease increases considerably in aged humans and animals including dogs and mice and by conditions that increase inflammation such as diabetes [31]. The inflammatory process occurring in periodontitis is characterized by the infiltration of leukocytes, which limit the level of bacterial invasion and at the same time may be harmful to the periodontal tissue [31]. The destruction of periodontal ligament and bone is thought to be the result of a disruption of the homeostatic balance between the host response and bacteria that result in inflammation in close proximity to bone [31–33]. The host immune response to bacteria or their products stimulates production of osteoclastogenic factors by immune cells and cells of osteoblastic lineage, which then induce bone loss. Periodontal disease in humans and in experimental animal models is linked to both the innate and adaptive immune response. Several studies have reported that individuals with periodontitis exhibit increased levels of interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in gingiva and crevicular fluid in the gingival sulcus [34]. Both genetic deletion and specific inhibition of these cytokines have been found to reduce periodontal disease progression [34–37]. Similarly, mediators of the adaptive immune response are elevated in individuals with periodontal disease and inhibition or genetic deletion of mediators such as receptor activator of nuclear factor kappa-B ligand (RANKL) and interferon-γ (IFN-γ) result in reduced periodontal disease progression [36, 38, 39]. Our laboratory has recently shown that the production of factors by osteoblasts and osteocytes also contributes to osteoclast formation and activity in periodontal disease (unpublished data). Evidence that the host response plays a critical role was also shown when treatment with a prostaglandin inhibitor reduced periodontitis-related bone loss [40] or inhibition of inflammatory cytokines such as IL-1 and TNFα [35, 37]. Thus, periodontitis is a complex disease, where multiple causal risk factors play simultaneous and interactive roles. Periodontal bone loss is affected by the bacterial biofilm which forms, ability of bacteria or their products to pass through the epithelial barrier into connective tissue, the status of the host response, and the presence of environmental stressors and/or systemic disease such as diabetes [41–43].

Impact of Diabetes on Periodontal Disease

Diabetes and periodontitis are two chronic diseases that are biologically linked [44, 45]. Periodontitis is one of the first clinical manifestations of diabetes [19]. Diabetes is an important risk factor for periodontitis [4, 46]. The risk of periodontitis is increased approximately 2–4 times in diabetic versus nondiabetic subjects [4, 47]. In one study, periodontitis was found in 60 % of T1DM patients compared to 15 % without diabetes [48]. Patients with diabetes are at higher risk of severe periodontitis compared with nondiabetic subjects [49]. A study in African Americans found 70 % T2DM patients had moderate periodontitis and 29 % had severe disease, which is significantly higher than the prevalence of 11 % without diabetes [50]. The severity of periodontitis is directly proportional to the level of glucose control [48, 51].

Impact of Diabetes on Gingiva/Gingivitis

Periodontitis is preceded by various stages of gingival inflammation referred to as gingivitis. Gingivitis in T1DM children and adolescents is twice that of matched control subjects [52]. Similarly, higher rates of gingival inflammation occur in T2DM adults compared to adults without diabetes. Nearly, 64 % of patients with T2DM have gingivitis compared with 50 % of subjects without diabetes [52]. The degree of metabolic control of diabetes is an important factor in the development and progression of gingivitis; good metabolic control significantly reduces gingivitis [53, 54].

Diabetes may affect periodontal disease through a number of different avenues including an impaired antibacterial defense. However, studies investigating whether diabetes causes a change in oral flora have had inconsistent results and the issue has not yet been settled. An alternative explanation is that diabetes alters the inflammatory response to periodontal pathogens. This is supported by studies examining the response to a well-defined inoculum of periodopathic bacteria [55]. Under normal circumstances, inflammation normally resolves through an active process regulated by cellular signals [56, 57]. However, resolution of the inflammation is impaired in the diabetic animals [58]. Thus, one explanation for enhanced risk of periodontal disease in diabetic individuals is the presence of a greater inflammatory response to bacteria on the tooth surface that stimulates the formation of osteoclasts and suppresses the repair process [55, 59].

In animal models, diabetes leads to increased production of TNF in the epithelium and connective tissue [60]. Periodontal infection causes an increase in apoptosis of gingival epithelial cells, fibroblasts, and bone lining cells that is significantly enhanced by diabetes in a caspase-3-dependent mechanism [60]. This may be important because diabetes-enhanced inflammation and apoptosis negatively impact the gingiva by causing a loss of epithelial barrier function and inhibiting repair processes [61, 62]. High levels of TNF-α can stimulate the expression of pro-apoptotic genes in diabetics [58, 63].

Impact of Diabetes on Periodontal Ligament/Loss of Attachment

The gingiva is attached to the root surface of teeth by collagen bundles that integrate with the tooth surface. Loss of this attachment is one of the hallmarks of periodontitis and occurs in conjunction with bone loss. Diabetes increases attachment loss, which is worsened by poor glycemic control [48, 64]. More than 25 % of T1DM patients with poor metabolic control have sites with moderate to severe signs of periodontitis compared to only 10 % of subjects with good metabolic control [52]. Moreover, the severity is proportional to the duration of diabetes [51, 65].

Impact of Diabetes on Alveolar Bone Loss

Periodontitis is the most common osteolytic disease in humans and is aggravated by diabetes [66]. Diabetes potentiates the severity of periodontitis and accelerates bone resorption. The number of sites with bone loss in poorly controlled T1DM individuals is twice that of nondiabetic subjects [67]. Animal studies demonstrate that periodontitis is increased threefold in T1DM rats compared to normal rats [68] and is significantly higher in T2DM rats [61]. The risk and degree of alveolar bone loss is positively correlated with the lack of metabolic control [69].

Effect of Diabetes on Osteoclasts in Periodontitis

Bone remodeling begins with the activity of osteoclasts, followed by new bone formation through the activity of osteoblasts. Under physiological conditions, the two activities are coupled, but the two processes are uncoupled in pathologic processes. Human studies generally indicate that diabetes mellitus increases osteoclastogenesis. Individuals with DM generally have increased systemic levels of bone resorption markers as indicated by higher circulating levels of tartrate-resistant acid phosphatase [70]. Animal studies show similar results [71]. T2DM rats have increased osteoclastic bone resorption in periodontal bone compared to normoglycemic controls [61]. Increased inflammation, ROS, and AGEs are thought to increase osteoclast activity.

Increased Inflammation Activate Osteoclasts in Diabetes

Diabetes has been shown to enhance osteoclast formation in inflammatory areas. Type 2 diabetic rats have a ~2–4-fold increase of the osteoclast number after bacterial infection by oral inoculation of a periodontal pathogen or ligature-induced periodontitis compared with control rats [59–61]. T1DM with periodontitis also have a 2–4-fold

increase in the number of osteoclasts compared to nondiabetic rats with periodontitis [72]. A higher degree of inflammation and a more persistent inflammatory response following periodontitis [55, 73] may lead to greater stimuli for osteoclastogenesis.

In diabetic mice, TNF-α, macrophage colony-stimulating factor, RANKL, and vascular endothelial growth factor-A (VEGF-A) are up-regulated which can directly promote osteoclast differentiation and activation [74, 75]. Diabetes increases TNF levels that has been shown to prevent downregulation of genes associated with host defense, apoptosis, cell signaling and activity, and coagulation/hemostasis/complement [58]. Similarly, patients with periodontitis and diabetes have significantly higher levels of IL-1β, TNF-α, and prostaglandin E_2, which result in more prolonged osteoclast formation and activity [76, 77]. Enhancement of IL-17, IL-23 in periodontitis in type 1 diabetic subjects, and overexpression of IL-1β, IL-6 in type 2 diabetic patients have been reported, which result in enhanced osteoclastogenesis and prolonged duration of inflammatory responses [78, 79]. T2DM patients with periodontal disease have increased levels of TNF-α and IL-6, which was also associated with increased dyslipidemia and lipid peroxidation [80]. Increased fatty acid levels in diabetes mellitus may also enhance osteoclastogenesis [81]. In addition to increasing inflammation, diabetes also impairs the resolution of periodontal inflammation. The importance of resolving inflammation has been demonstrated by treatment of animals with periodontitis with resolvins [82] or by treatment of diabetic animals with TNF inhibitors [58, 59].

Increased ROS Activate Osteoclasts in Diabetes

High levels of ROS contribute to diabetes-related periodontitis. Invading bacteria trigger the release of cytokines and chemokines that induce neutrophil recruitment and activity and which subsequently release ROS in periodontal tissues [83, 84]. Neutrophils from diabetic patients produce more superoxide than neutrophils from normal subjects [14]. The imbalance between production of ROS and antioxidant defense results in increased oxidative stress [85]. The formation of AGEs also increases oxidative stress in the periodontal tissues. It has been shown that ROS such as superoxide and hydrogen peroxide activate osteoclasts and promote osteoclast formation [86]. A related process, lipid peroxidation, is also linked to increased periodontal disease T2DM and a greater inflammatory response in the periodontal tissues in humans [80, 87]. Patients with T2DM show elevated mitochondrial ROS which promotes RANKL-mediated osteoclast differentiation and function [88].

Increased AGEs Activate Osteoclasts in Diabetes

In vitro studies suggest that hyperglycemia predisposes to increased osteoclast formation [89]. AGEs also increase osteoclasts activity [90]. Osteoclast-like cells express RAGE, which serves as a positive factor to regulate the osteoclast formation [91].

Mice that lack the RAGE have increased bone mass and decreased osteoclast numbers compared to wild-type mice [91], supporting the concept that AGEs contribute to osteoclast formation in diabetes. Diabetes enhances the formation of AGEs in the periodontium and increases expression of RAGE [92]. The level of AGEs in the gingiva is increased in both type 1 and type 2 diabetes-associated periodontitis [93]. It has been shown that AGE–RAGE interaction on monocytes activates the transcription factor NF-κB, which alters the phenotype of the monocyte/macrophage and results in the increased production of proinflammatory cytokines [94]. RAGE stimulation may contribute to osteoclastogenesis via increased expression of receptor activator of RANKL and downregulation of osteoprotegerin (OPG) [95].

RANKL interacts with its receptor on the surface of osteoclast precursors to induce osteoclast formation and activity. OPG inhibits osteoclast formation by binding to RANKL [96]. A number of studies focusing on osteoclastogenesis-related factors have reported elevated expression of RANKL and TNF in diabetes-associated periodontal tissues [59, 96]. Studies with animals suggest that RANKL/OPG ratios and the level of other inflammatory cytokines such as TNF are critical mediators for the enhanced osteoclastogenesis in diabetes in periodontal disease [59, 97]. TNF levels and high RANKL/OPG ratios in the periodontium in humans are negatively influenced by poor glycemic control in subjects with diabetes [80, 98]

Effect of Diabetes on Osteoblasts in Periodontitis

Bone resorption is followed by a period of bone formation, a coupling process that limits the amount of net bone loss, which occurs during the resolution of inflammation in the periodontium [20]. We found that T2DM rats do not generate a burst of bone formation that normally occurs following induction of periodontal disease [61]. Inflammation plays an important role in this effect by limiting repair of resorbed bone. This occurs by reducing osteoblast numbers through decreased proliferation of precursors and greater apoptosis of mature osteoblasts [59, 63]. These studies suggest a molecular basis for the negative impact of T2DM on bone by the effect of diabetes-enhanced inflammation on suppressing the expression of factors such as fibroblast growth factor or bone morphogenetic proteins that are needed for new bone formation.

Diabetes Inhibits Osteoblasts Differentiation and Function

Diabetes also interferes with the bone formation by reducing the expression of transcription factors that regulate osteoblast differentiation [99]. In T1DM and T2DM rats, osteoblasts exhibit lower alkaline phosphatase activity and mineralized matrix formation [100, 101]. Inflammation has a significant effect on bone [102, 103]. Inflammation impairs the function of bone-forming osteoblasts by suppressing

mature osteoblast function such as the production of bone matrix [104]. One of the striking features of diabetes is elevated levels of inflammatory mediators, particularly TNF [76]. Diabetic animals have higher levels of TNF in bone, which is associated with reduced bone formation and repair. TNF blocks the differentiation of osteoblasts, where inflammation is thought to be present [105]. This is consistent with reports that TNF inhibits differentiation of osteoblasts in vitro [106, 107] and also interferes with bone morphogenetic protein signaling [108].

Periodontal infection-induced alveolar bone loss in diabetic subjects is accompanied by enhanced expression of RAGE and production of AGEs in the gingival tissue [109]. AGEs have been shown to interfere with osteoblast differentiation and induce apoptosis of osteoblasts in diabetes via the mitogen-activated protein kinase and cytosolic apoptotic pathway [110]. When AGEs are applied to osseous wounds in normal animals, the rate of healing is reduced in half, indicating that AGEs, which are elevated in diabetes, contribute to impaired bone formation [111]. In addition, RAGE is expressed at higher levels in osteoblasts in diabetic conditions rendering diabetic animals even more sensitive to the effects of AGEs [111].

Mesenchymal stem cells (MSCs) represent a precursor pool of osteoblasts that are bone-forming cells. Inflammation, which is elevated in diabetic bone healing [112], has a significant effect on reducing MSC differentiation [113]. A mechanism through which inflammation affects MSC is through induction of NF-κB activation. Increased NF-κB activity interferes with wnt-stimulated MSC differentiation by increasing beta-catenin degradation [102]. Moreover, TNF suppresses activation of the Osx promoter [114]. This interferes with MSC differentiation to osteoblasts since osterix is needed in early steps of differentiation. AGEs also inhibit MSC differentiation. One mechanism involves AGE-induced up-regulation of ROS in MSCs that leads to reduced MSC differentiation [115, 116]. In human MSCs and mouse stromal ST2 cells, AGEs suppress osteogenic differentiation [117]. T2DM mice have fewer MSCs and these MSCs appear to have poor homing capability to injury sites [118]. T1DM rats have more numerous apoptotic cells in the bone marrow, and the size of osteoprogenitor pool is significantly reduced [101]. Thus, diabetes reduces the number of progenitors and inhibits differentiation of MSC to osteoblasts.

Diabetes Promote Osteoblast Apoptosis

Apoptosis of osteoblasts is significantly increased by diabetes. Diabetes leads to the up-regulation of pro-apoptotic mediators including TNF-α, AGEs, and the formation of ROS [119]. TNF can induce apoptosis by binding to the TNF receptor-1, which triggers the initial events in apoptosis [120]. Some of the detrimental effects of diabetes-enhanced TNF-α levels may be due to the induction of cell death by triggering caspase activity. Caspases are a family of cysteine proteases that can act as either initiators (caspase-2, 8, and 9) or executioners (caspase-3, 6, and 7) of apoptosis [121]. Caspase-3 appears to play a central role in bacteria and lipopolysaccharide (LPS)-mediated apoptosis [122, 123]. Additionally, TNF stimulates the

expression of several pro-apoptotic genes, many of which are regulated by the pro-apoptotic transcription factor, forkhead box-O1 (FOXO1) [124]. There is evidence that both diabetes and bacterial infection in periodontitis enhances apoptosis of osteoblastic cells to reduce osseous coupling [63, 125], which may involve stimulatory signals from both the innate and adaptive immune response [112, 126]. CML-collagen, one of the AGEs found in bone, stimulates apoptosis of bone-lining cells in vivo and in various osteoblastic cell cultures mediated through RAGE [127] via the MAP kinase pathway [110].

The production of ROS is another mechanism of diabetes increasing apoptosis. Persistent inflammation and hyperglycemia leads to the cellular accumulation of ROS, which is linked to diabetic complications [66, 128]. Increased oxidative stress in periodontal tissue has been shown to lead to greater osteoblast apoptosis [129] and involve activation of caspase-3 [130].

Diabetes also increases loss of cells in the periodontal ligament from periodontal infection [61, 131]. This is significant since the periodontal ligament is a rich source of cells capable of differentiating into osteoblasts. Studies in diabetic animals indicate that diabetes causes a more than twofold induction of genes that regulate the apoptosis of osteoblasts and fibroblasts following bacterial infection and a fivefold increase in osteoblast apoptosis [122, 132].

Blocking apoptosis by treatment of diabetic rats with a caspase-3 inhibitor significantly increases the number of osteoblasts, which in turn leads to significantly greater amounts of new bone formation. Furthermore, the number of osteoclasts and their activity is increased by treatment with a caspase-3 inhibitor with the net effect increasing bone formation due to greater bone coupling. This is consistent with previous findings that a pancaspase inhibitor reduces apoptosis and increases new bone formation following bacterial infection [122]. Taken together the results indicate that bacterial infection in diabetic animals has a significant impact on periodontal disease through enhanced apoptosis of osteoblasts or their precursors.

Conclusion

In summary, individuals with diabetes mellitus have increased risk and severity of periodontal disease [9, 19, 133]. Periodontitis is one of the first clinical manifestations of diabetes [19]. Diabetes aggravates periodontitis by an increase in the inflammatory response to bacterial infection and reducing the capacity to down-regulate inflammation [9, 55]. There is a direct link between persistent hyperglycemia, an exaggerated inflammatory response to periodontal pathogens and periodontal bone loss [31, 66]. The impact of diabetes on the periodontium involves inflammation associated with both the innate and adaptive immune response [8, 31]. Diabetes-enhanced inflammation increases osteoclastogenesis and decreases reparative bone formation. A number of factors are increased by diabetes including RANKL, AGEs, ROS, and TNF that stimulate osteoclasts. Moreover, diabetes prolongs inflammation leading to longer periods on osteoclast activity as well as interfering with

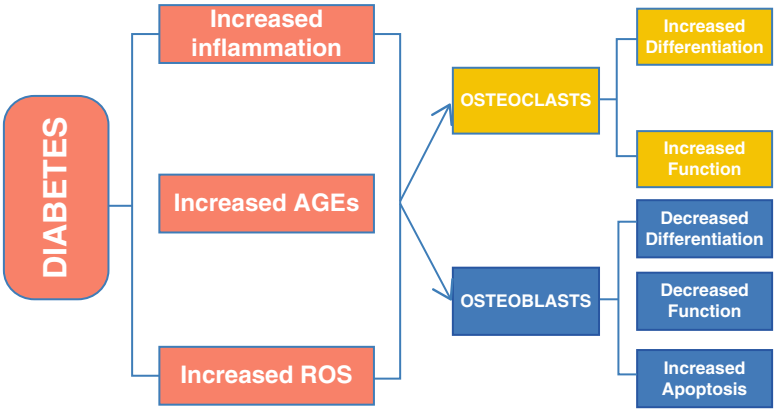

Fig. 5.2 Impact of diabetic mellitus on periodontitis. Diabetes increases inflammation (increased cytokine production such as TNFα), and the production of AGEs and ROS that can affect both osteoclasts and osteoblasts. These factors may increase osteoclast formation and activity and inhibit osteoblast differentiation, activity, and survival

subsequent bone coupling. The negative effect of inflammation on bone coupling is likely to be an important factor in the disease process [59, 112] as inflammatory cytokines interferes with bone morphogenetic protein and Wnt signaling and also stimulates osteoblast apoptosis [104, 108, 134, 135]. The mechanisms by which diabetes affects periodontitis are summarized in Fig. 5.2.

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Chapter 6
Biomarkers of Diabetic Bone Disease

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Introduction

Diabetes mellitus is the most common endocrine disorder with a prevalence of approximately 327 million worldwide [1]. Substantial evidence exists that in addition to the well-known complications of diabetes, such as neuropathy, nephropathy, and retinopathy, increased fracture risk is an important morbidity [2, 3]. Individuals with type 1 diabetes (T1D) have a very high risk of hip fracture, approximately six times greater than those without diabetes [2, 3]. Although the fracture risk is not as high in type 2 diabetes (T2D), it is nevertheless increased as well [4, 5]. A meta-analysis of 12 studies reported a relative risk of 1.7 (95 % CI: 1.3–2.2) for hip fracture in both men and women with T2D [2]. The risk of all clinical fractures was also increased, with a summary RR of 1.2 (95 % CI: 1.0–1.5) [2]. Subsequent studies have reported similar results [6, 7], with a direct association between the duration of diabetes and increased fracture risk [8]. Fractures in all diabetic individuals are particularly problematic because they are associated with poor fracture healing, greater morbidity [9, 10], and greater healthcare costs [11] as compared to nondiabetics.

Fracture risk can be explained by a decrease in measurement of bone mineral density (BMD) by dual energy X-ray absorptiometry in T1D but not in T2D [3]. In T1D, hip and spine BMD are reduced compared with normative reference populations [3] or with healthy controls [12]. As in broader populations, reduced BMD is associated with higher fracture prevalence among those with T1D [13]. In contrast, BMD is generally higher in those with T2D when compared to nondiabetics [3].

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In a meta-analysis, Vestergaard reported an increased *Z*-score of +0.41 at the spine and +0.27 at the hip associated with T2D [3]. Similarly, the WHO fracture risk assessment tool, FRAX, a key clinical instrument, has been shown to underestimate fracture risk in diabetes in several US study cohorts and in a large clinical cohort in Manitoba, Canada [4, 5]. In the Canadian study, diabetes was a significant predictor of subsequent major osteoporotic fracture (adjusted hazard ratio [aHR] = 1.61, 95 % confidence interval [CI] 1.42–1.83) and hip fractures (aHR 6.27, 95 % CI 3.62–10.87 in those aged <65 years, aHR 2.22, 95 % CI 1.71–2.90 in those ≥65 years) even when adjusted for competing mortality. These data have led to discussion of how to accommodate the risk associated with T2D in the FRAX algorithm [14].

The paradox of higher BMD in association with increased fractures in T2D could potentially be explained by more frequent trauma, as diabetes is associated with an increased frequency of falls. However, in studies of diabetes and fracture that controlled for fall frequency, diabetes still remained independently associated with increased fracture risk [6, 15]. Thiazolidinediones (TZD) 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 [16]. However, TZD use does not 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.

It is thus apparent that regardless of falls and TZD use, fracture risk is increased in both T1D and T2D. Yet BMD and FRAX, the clinical mainstays for predicting fractures, do not fully capture fracture risk in these populations. It is therefore imperative to identify biochemical markers in diabetes that can predict fracture risk independent of bone density assessment.

Glycemia

Chronic hyperglycemia, as reflected by HbA1c, is a marker of increased fracture risk in observational studies of patients with T2D. In the ARIC study, an increased risk of fractures was observed among diabetic individuals who had an average HbA1c ≥8 % as compared to diabetics with HbA1c <8 % (HR 1.63; 95 % CI 1.09–2.44) [17]. Similarly, in the Rotterdam study, participants with HbA1c levels ≥7.5 % had a 62 % higher fracture risk than diabetics with HbA1c levels <7.5 % (HR 1.62; 95 % CI 1.09–2.40), whereas those with HbA1c levels <7.5 % had a risk similar to those without diabetes [18]. However, chronic glycemia might not be consistently predictive of fracture risk. In one study, lower HbA1c levels were associated with increased hip fracture risk [19]. In addition, an ancillary analysis of fractures from the ACCORD study, which compared intensive vs. standard glycemic control [20], found no difference with lower HbA1c levels in the rate of first non-spine fracture (hazard ratio 1.04 [95 % CI 0.86–1.27]) over 3.8 years [21], suggesting that changes in HbA1c levels may not predict fracture risk. However, these data are not

conclusive because there was a high prevalence in ACCORD of TZD use, a known skeletal toxin [16], particularly in the intensive group, which may have obscured a relationship between fracture risk and HgbA1c .

Insulin

Whether insulin is a biomarker for diabetic bone disease is unclear. Insulin itself is typically considered to be anabolic for bone [22, 23], but higher endogenous insulin levels might also be associated with adverse skeletal effects such as decreases in cortical BMD and periosteal circumference [24]. Studies in T1D regarding the effect of exogenous insulin dosing have yielded mixed results [25, 26]. In T2D patients, insulin use does not appear to explain the higher rates of bone loss or the increased incidence of fractures [27]. In both T1D and T2D, higher insulin dosages may be markers of more severe disease, including increased inflammation and comorbidities that could lead to more bone damage [23].

Biochemical Markers of Bone Remodeling

Numerous lines of evidence suggest that diabetic bone disease is associated with alterations in biochemical markers of bone remodeling. High glucose levels, even in the absence of diabetes, can adversely affect bone formation. In healthy individuals, ingestion of 75 g of glucose leads to a decrease in markers of both bone formation and resorption [28] and in vitro data show that exposure to high glucose levels impairs osteoblast function [29–31]. With regard to T1D, in vitro data [30] and in vivo studies involving rodent models [32] indicate that bone formation is characteristically impaired, as shown by the expression of osteoblastic transcription factors such as RUNX2, biochemical markers, and histomorphometric indices [33]. The skeletal effect is generally rescued by insulin treatment and normalization of glycemia [34]. An association between T1D and low bone formation in clinical studies has also been shown [35–37]. In the largest histomorphometry study to date, iliac crest biopsies in 18 otherwise healthy subjects with T1D were compared with those from healthy age- and sex-matched nondiabetic control subjects [38]. Diabetic subjects, when compared to controls, had no significant differences in mineral apposition rate (MAR), mineralizing surface (MS/BS), osteoid maturation time (Omt), mineralizing osteoid (MS/OS), mineralization lag time (Mlt), bone formation rate (BFR/BS or BFR/BV), formation period (FP), remodeling period (Rm.P), or activation frequency (Ac.F) [38]. However, in a subset of diabetic patients who had fractured, dynamic variables such as BFR/BS, BFR/BV, and Ac.F tended to be lower in the fracturing subjects, perhaps indicating lower remodeling in those T1D subjects [38].

Decreased bone remodeling in T2D has also been demonstrated in a number of reports. Clinically, circulating biochemical markers of bone formation, including

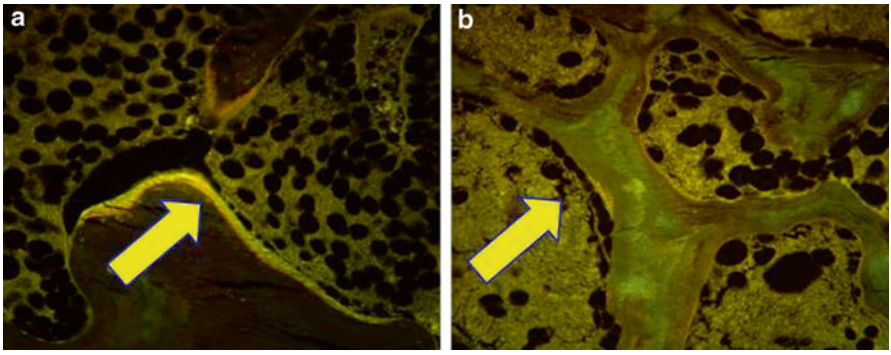

Fig. 6.1 Histomorphometric changes in bone formation. (**a**, **b**) Tetracycline double-labeled bone biopsies in a 58-year-old T2D Caucasian woman (**a**) and a 57-year-old Caucasian female control (**b**). Bone formation is decreased in T2D with reduced mineralizing surface. The *arrows* highlight tetracycline uptake in the control subject and the absence of uptake in the diabetic subject. Adapted with permission from [46]

P1NP, osteocalcin [39, 40], and bone-specific alkaline phosphatase [41] have been found to be decreased in T2D. Osteocalcin levels were found to increase in diabetic men concomitant with improved glycemic control [42]. Impaired formation measures in T2D are associated with reductions in bone resorption markers including serum CTx [39–41, 43] and DPD [44]. 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 osteocalcin levels had nearly a fivefold increased risk of vertebral fractures independent of lumbar spine BMD [39].

Alterations in dynamic histomorphometry in T2D were 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 [45]. In a more recent pilot study, low bone formation was observed in 6 T2D postmenopausal women and 6 postmenopausal age-matched nondiabetic controls, where tetracycline double-labeled iliac crest bone biopsies showed virtually no uptake of label in diabetic subjects (Fig. 6.1), with reduced mineralizing surface, osteoid surface, and osteoblast surface (Fig. 6.2) [46]. These preliminary histological data corroborate the decrease in biochemical markers of bone turnover.

PTH

Levels of PTH, a key regulator of bone remodeling, are altered with glycemia and diabetes. In healthy subjects, a glucose load leads to a slight decrease in ionized calcium and an increase in PTH, after an initial temporary decrease [28]. In T1D, blunted PTH responses have been observed [47]. In T2D, levels of PTH tend to be 20–50 % lower than in controls, even in the setting of reduced eGFR, suggesting a

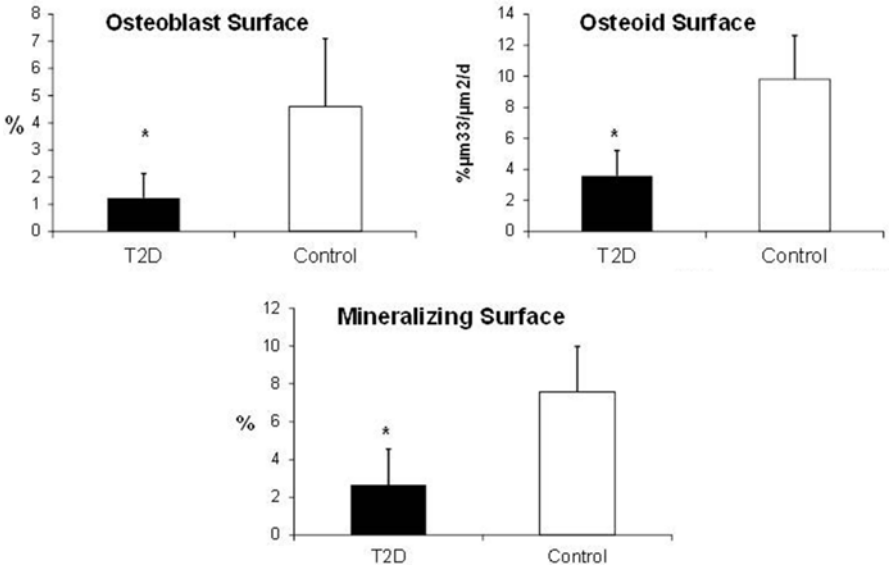

Fig. 6.2 Quantitative measures of bone formation were lower in T2D postmenopausal women than in controls. Adapted with permission from [46]

state of reduced PTH secretion [39, 40, 43]. The importance of PTH in diabetic bone disease has been corroborated by the beneficial skeletal effects of PTH treatment in T2D rats [48]. Specifically, PTH treatment partially reversed the adverse skeletal effects of T2D on bone mass, bone strength, and bone defect repair [48].

Nonclassical Bone Biomarkers

IGF-1, an anabolic factor which stimulates osteoblast proliferation, has been inversely associated with the risk and number of vertebral fractures in T2D postmenopausal women independent of BMD [40, 49]. Circulating osteogenic precursor (COP) cells [50] might also be a biomarker for diabetic bone disease. COP cells can be detected in the peripheral blood by flow cytometry using antibodies specific for the osteoblast matrix protein osteocalcin (OCN) [50, 51] or alkaline phosphatase [52] and are capable of mineralization in vitro [52]. Certain subpopulations of OCN+ cells (OCN+/CD133+/CD34−/KDR+, known as calcifying cells) were increased in subjects with an HbA1c in the prediabetic range [53], although in another report, peripheral blood mononuclear cells that were positive for osteocalcin were lower in postmenopausal women with T2D as compared to nondiabetic controls [46]. An additional potential biomarker of diabetic bone disease is RANKL. Kiechl et al. showed that increased levels of soluble RANKL were associated with the development of diabetes in 844 subjects (OR = 3.37; 95 % CI: 1.63–6.97) [54]. Another

novel bone marker in T2D may be sphingosine 1-phosphate (S1P), a lipid mediator which increases osteoclastogenesis by increasing RANKL [55]. S1P was found to be increased in T2D women ($n=482$) as compared to controls and was associated with increasing numbers of vertebral fractures and bone resorption markers [55]. Interestingly, this marker suggests an elevation in bone resorption in T2D, in contrast to the reports of reduced biochemical markers of bone resorption [39–41, 43].

Sclerostin

Sclerostin, an osteocyte product, is a negative regulator of bone formation which competes with the anabolic Wnt b-catenin pathway by binding to LRP5 or 6 [56]. In healthy adults, sclerostin levels are increased by factors including age, BMI, inactivity, bone mineral content, and possibly fractures [56]. It was first reported in 2012 that sclerostin levels were higher in 74 T2D women and men vs. 50 nondiabetic controls, and that higher levels correlated with age, male gender, and BMD [57]. In a different study which included T1D ($n=43$), T2D ($n=40$) and matched controls, sclerostin levels did not differ between T1D subjects and controls, but were twofold higher in T2D than in controls or T1D, even after adjusting for age and BMI [58]. These data suggest that the Wnt signaling pathway may be impaired in T2D, although no relationship between markers of bone formation and sclerostin was found [58]. Interestingly, T2D subjects also had lower PTH levels [58], raising the possibility that since PTH inhibits sclerostin [59], perhaps the higher sclerostin levels were due to a decrease in the usual inhibitory effect of PTH. A correlation between Wnt disruption and decreased osteoblast activity was further observed in 40 T2D postmenopausal women who, as compared to controls, had decreased β-catenin levels which correlated with lower BAP [41]. 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 [60]. It could be posited from these data that because sclerostin is secreted from deeply embedded osteocytes that are in mature bone tissue, the higher sclerostin levels reflect an increase in the amount of aged bone mass that is more likely to accumulate microfractures independent of BMD. Further work will be needed to clarify this point. In a rat model of T2D, treatment with an anti-sclerostin antibody enhanced bone mass and reversed femoral defects [61], although this model did not fully replicate adult-onset diabetes because diabetes developed before skeletal maturity.

Advanced Glycation End Products

Increased levels of advanced glycation end products (AGEs) have been shown to be biomarkers for diabetic bone disease. In the setting of chronic hyperglycemia, AGEs accumulate in the organic bone matrix by a process known as nonenzymatic

glycation (the Maillard reaction) [62–69]. Accumulation of AGEs in the organic bone matrix leads to more biomechanically brittle bone that has lost its toughness and is less able to deform before fracturing [63]. Circulating levels of pentosidine, one of the best studied AGEs, have been shown to correlate with bone AGE levels [70]. In 104 nondiabetic patients undergoing orthopedic surgery, plasma pentosidine levels correlated with cortical bone pentosidine [70]. In a nondiabetic population of 765 postmenopausal women, an increase in urinary pentosidine levels predicted a 20 % increase in vertebral and long bone fractures over 5 years [71]. However, pentosidine levels do not consistently distinguish diabetics and nondiabetics when measured in urine [72] or serum [73]. Nevertheless, pentosidine levels appear to provide information about diabetic bone fragility that is separate from BMD parameters. In 1000 patients followed for 7.5 years, urinary pentosidine levels in those with diabetes were associated with a 42 % increase in clinical fracture incidence [relative hazard, 1.42; 95 % confidence interval (CI), 1.10, 1.83] and a nearly sixfold increase in vertebral fracture prevalence, independently of BMD [72]. Similarly, in 153 Japanese men and women with T2D, serum pentosidine levels were significantly higher in the women who had vertebral fractures, independent of BMD (OR 2.50, CI: 1.09–5.73) [73]. With regard to direct measurement of pentosidine in diabetic bone, preliminary data in T1D patients showed that iliac crest bone biopsies in T1D who had fractured had higher pentosidine levels than controls, in association with a greater degree of mineralization [74]. In addition to pentosidine, circulating levels of another AGE, carboxy-methyl-lysine (CML), might indicate bone fragility. When 3373 nondiabetic patients were followed for 9 years, an increase in CML predicted a 27 % increase in hip fracture risk, independent of hip BMD [75].

Architectural Properties in T2D

In addition to alterations in remodeling and matrix properties, another factor that may contribute to the paradox of increased fractures despite normal areal BMD in T2D is microarchitectural abnormality. Increased cortical porosity, a key determinant of bone fragility [76], has been reported at the radius and tibia in female diabetics who have fractured, as measured by intra-cortical pore volume fraction via high-resolution peripheral quantitative computed tomography (HR-pQCT) [77]. In a recent community-based study of women and men, T2D and increased HbA1c levels were associated with deficits in cortical microstructure and density at the distal tibia [78]. Microarchitectural deficits in bone geometry might also explain reduced bone strength in T2D. Strength-to-load ratios (QCT) at the spine and femoral neck were not improved in older adults with T2D although areal BMD (DXA) was higher [79]. In a study of older men, volumetric BMD (pQCT) was higher but bone area was smaller at the distal radius and tibia [80]. Smaller cross-sectional area suggests that stimulation of periosteal apposition, normally observed with greater loading, may be reduced in diabetes. These data seem to suggest that the higher areal BMD in diabetics does not result in the expected biomechanical advantage and supports the likelihood of abnormalities in dynamic and material properties.

Conclusions

At a time when classical complications of diabetes mellitus are becoming less common due to improved glucose control, the skeleton has emerged as a target organ for disease complications. It is now well established that fracture risk is increased in both T1D and T2D. Yet BMD and FRAX, the clinical mainstays for predicting fractures, do not fully capture fracture risk in these populations. Identification of biomarkers in diabetic individuals that predict fracture risk, independent of bone density assessment, will help to diagnose and treat bone fragility in diabetes. Use of biomarkers in diabetes will hopefully offset serious skeletal challenges in this population as they age.

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Chapter 7
Safety of Antidiabetic Therapies on Bone

Beata Lecka-Czernik and Ann V. Schwartz

Bone Remodeling

Maintenance of bone homeostasis throughout life relies on the bone remodeling process, which continually replaces old and damaged bone with new bone in order to maintain strength and elasticity [1]. In a healthy state, bone resorption is balanced with bone formation. Changes in the milieu of local and systemic factors may alter this balance leading to changes in the bone mass and/or bone biomechanical properties. Aging, estrogen deficiency, and metabolic diseases negatively affect bone mass and/or bone quality leading to the development of osteoporosis and increased fracture rate (Fig. 7.1).

Three types of cells are involved in bone remodeling: osteoclasts which resorb an old or damaged bone, osteoblasts which form new bone at the site of the resorbed cavity, and osteocytes which orchestrate the whole process. Osteoclasts and osteoblasts/osteocytes develop from two distinct populations of stem cells residing in the bone marrow, hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC), respectively. Osteoclast differentiation is determined by both, factors produced by cells of osteoclast lineage and factors produced by other bone marrow cells including

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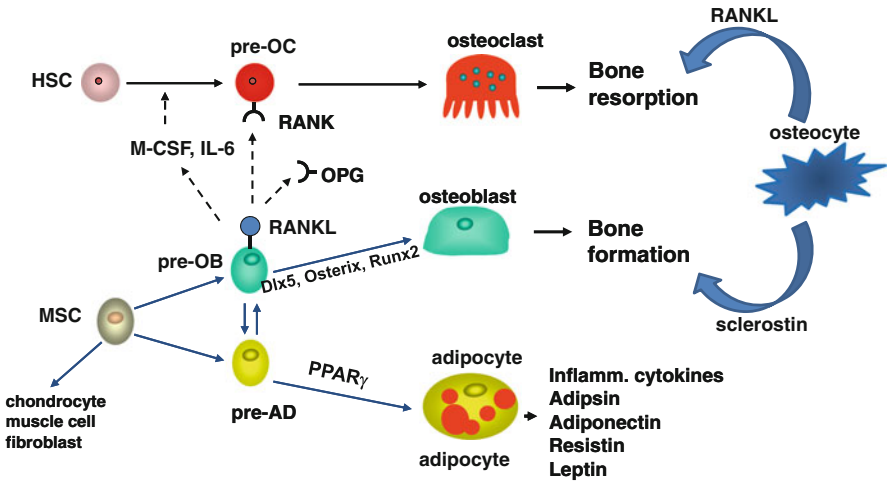

Fig. 7.1 Schematic representation of coupling between osteoblast, osteocyte, and osteoclast development and function. *MSC* mesenchymal stem cells, *HSC* hematopoietic stem cells

cells of osteoblast lineage [2]. Osteoclast recruitment from the HSC pool and their maturation is controlled by osteoblast-derived cytokines: M-CSF, IL-6, and RANKL. Osteoblasts originate in a marrow MSC compartment which also produces adipocytes [3, 4]. The commitment of MSC toward either the osteoblast or adipocyte lineage occurs by a stochastic mechanism [5]; lineage-specific transcription factors, such as Runx2, Dlx5, and Osterix for osteoblasts and PPARγ2 and C/EBPs for adipocytes are activated [6–11]. Activation of osteoblast-specific transcription factors is determined by a milieu of extracellular factors, which regulate the cellular activity of Wnt, TGFβ/BMP, and IGF-1 signaling pathways [12]. Process of bone remodeling is controlled by osteocytes, which represent specialized cells of osteoblast lineage [13]. They are located inside of bone mineralized matrix and communicate with other osteocytes and bone marrow environment through the system of dendrite-like processes. Osteocytes control dynamics of bone remodeling process by secreting RANKL to control bone resorption and sclerostin to control bone formation [13].

Bone as an Integral Part of Energy Metabolism System

Bone is closely integrated with the system regulating energy balance. Organs involved in this regulation including brain, fat, gastrointestinal system, and pancreas are secreting hormones which in endocrine manner regulate both energy metabolism and bone mass. Their effect on bone is possible because bone marrow cells, both mesenchymal and hematopoietic lineage, are equipped with necessary receptors to respond to these signaling (Fig. 7.2).

Integration of bone metabolism with energy metabolism has been presented recently as a model which links anabolic effect of insulin signaling in osteoblasts

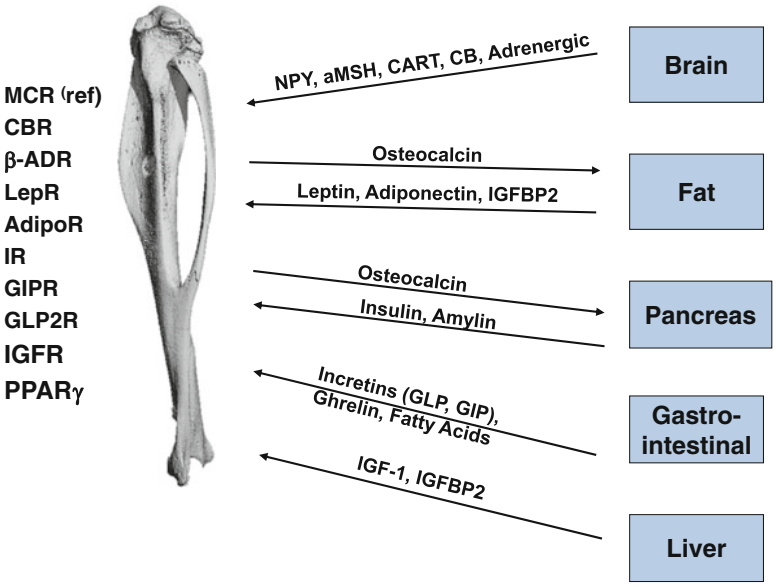

Fig. 7.2 Bone is a part of energy metabolism network. Factors and their receptors which mediate a cross-talk between bone and other organs involved in regulation of energy metabolism. *MCR* melanocortin receptor, *CBR* cannabinoid receptor, *LepR* leptin receptor, *AdipoR* adiponectin receptor, *GIPR* glucose inhibitory protein receptor, *GLP2R* glucagon inhibitory peptide receptor, *β-ADR* beta adrenergic receptor, *IR* insulin receptor, *IGFR* insulin growth factor receptor, *PPARγ* peroxisome proliferator activator receptor γ

with bone turnover and regulation of insulin sensitivity in peripheral organs [14, 15]. Thus, in osteoblasts insulin signaling regulates an expression of Runx2 and osteocalcin production. In addition, insulin increases support for osteoclastogenesis by decreasing an expression of OPG, a decoy receptor for RANKL. As a result, insulin increases bone turnover and production of undercarboxylated osteocalcin, which in endocrine fashion regulates insulin release from β-cells in pancreas and production of adiponectin in fat tissue [14–17]. Although it is not clear whether this regulatory circuit is affected in diabetes, several studies suggest that patients with T2DM have decreased bone turnover [18–20]. If so, it would result in the decrease in osteocalcin production, especially its undercarboxylated form, which would lead to the attenuation of signaling responsible for increasing of insulin release from the pancreas and increasing fat sensitivity to insulin.

Anti-hyperglycemic Therapies and Their Effects on Bone

The most common form of diabetes is insulin-independent T2DM, which is characterized by insulin and glucose intolerance, and is associated with development of hyperglycemia and hyperinsulinemia. Therapies, either approved by FDA or in

Table 7.1 Antidiabetic drugs and their effects on skeleton

Target	Mode of action	Class of drugs	Drugs	Skeletal effect
Insulin	Sensitizers	Biguanides	Metformin[a]	Neutral
		TZDs (PPARγ agonists)	Pioglitazone[b], Rosiglitazone[b]	Bone loss; increased fractures
		Dual PPARα/ PPARγ agonists	Aleglitazar[c]	Unknown
	Secretagogues	K+ ATP	Sulfonylureas (e.g. Glyburide[a])	Neutral
			Meglitinides (e.g. Nateglinide)	Unknown
		GLP-1 analogs	Exenatide, Liraglutide, Taspoglutide[c], Albiglutide[c], Lixisenatide[c]	Unknown
		DPP-4 inhibitors	Alogliptin[c], Saxagliptin, Sitagliptin, Vildagliptin, Linagliptin	Decreased fractures
		Analogs/other insulins[a]	Insulin lispro, Insulin aspart, Insulin glargine	Increased fractures
Other	SGLT2 inhibitors		Canagliflozin, Dapagliflozin	Increased fractures
	Amylin analog		Pramlintide	Unknown
	Alpha-glucosidase inhibitors		Acarbose, Miglitol, Voglibose	Unknown

[a]World Health Organization Essential Medicine (WHO-EM)
[b]Restricted use in USA and Europe
[c]Phase III clinical trial was halted due to unfavorable renal effects

Phase III clinical trial, include insulin sensitizers, insulin secretagogues, and drugs which increase glucose excretion in the urine (SGLT2 inhibitors), regulate glucose absorption in intestine (amylin analog), and prevent digestion of carbohydrates (Alpha-glucosidase inhibitors) (Table 7.1).

In general, there is a lack of rigorous clinical evidence regarding the skeletal effects of these medications. Fracture is the primary outcome of interest but is a relatively rare outcome, requiring large studies. Randomized controlled trials (RCT) of diabetes medications have not included fracture as a primary endpoint, but increasingly studies are reporting the evidence from fractures identified as adverse events in RCTs. This provides the best evidence available to us regarding the clinical effects of these medications on the skeleton. Large observational studies have also considered the effects of diabetes medications on fracture risk. However, medications may be systematically prescribed to patients with different risk profiles for fracture, making it difficult to distinguish effects of the medications themselves on fracture, a problem known as "allocation bias" or "confounding by indication." Changes in bone mineral density are an important marker for skeletal health, but are not always a consistent predictor of the effects of a medication on fracture risk [21]. In addition, diabetic bone is compromised by other deficits, distinct from BMD, that have not been clearly delineated but may include increased cortical porosity and

greater accumulation of advanced glycation endproducts in bone collagen (see Chap. 9). As research clarifies which secondary markers are important predictors of fracture risk in diabetic patients, it will be essential to clarify how they are affected by diabetic medications.

Biguanides (Metformin)

Metformin is the most commonly used to increase insulin sensitivity in diabetic patients. Biguanides class of drugs decreases hepatic glucose production and increases glucose uptake in muscle. Metformin is considered by the World Health Organization an essential medicine satisfying the criteria of the public health relevance, evidence on efficacy and safety, and comparative cost effectiveness (www.who.int/medicines). Metformin mechanism of insulin sensitization includes activation of hepatic and muscle AMP-activated protein kinase (AMPK), which results in suppression of fatty acid synthesis, stimulation of fatty acid oxidation in liver and increase in muscle glucose uptake [22]. AMPK also decreases expression of sterol-regulatory element-binding-protein 1 (SREBP-1), a transcription factor involved in adipocyte differentiation and pathogenesis of insulin resistance, dislipidemia and diabetes. Animal studies indicate that metformin has a positive effect on osteoblast differentiation due to increased activity of osteoblast-specific Runx2 transcription factor via AMPK/USF-1/SHP regulatory cascade [23] and it has a negative effect on osteoclast differentiation and bone loss after ovariectomy by decreasing RANKL and increasing osteoprotegerin levels [24]. Interestingly, in rodent models metformin can prevent the adverse effects of TZDs on bone by either inducing reossification of bone after rosiglitazone treatment or preventing rosiglitazone effects when applied in combination with rosiglitazone [25].

Human studies of the effects of metformin on the skeleton are limited in design and number. The only RCT with a fracture outcome that included metformin is the ADOPT trial, discussed in more detail in the section on "Thiazolidinediones (Rosiglitazone, Pioglitazone)" [26]. Briefly, this trial randomized participants to receive metformin, glyburide (a sulfonylurea), or rosiglitazone; the primary outcome was time to monotherapy failure. Fractures were identified as adverse events. The fracture rates were similar in those randomized to metformin or glyburide. During the first 12 months of ADOPT, changes in the levels of the bone resorption marker CTX were similar in women (difference in 12-months change: +2.0 %) and modestly greater in men (−8.4 %) in those assigned to metformin compared with glyburide [27]. The metformin group had greater decreases in levels of the bone formation marker P1NP (difference in 12-months change: −9.4 % women; −19.5 % men), compared with glyburide.

Several observational studies have reported a lower risk of fracture with metformin use. In a study of the Danish population, metformin use was associated with lower fracture risk, compared with nondiabetic residents [28]. In the Rochester cohort, metformin use was associated with a lower rate of fracture in T2DM patients

(adjusted hazard ratio 0.7; 95% CI 0.6–0.96) [29]. However, other studies have found no difference in fracture risk with metformin use [30–35]. Interestingly, results from a study of hip fracture in Scotland suggest that metformin tends to be prescribed to patients with a lower overall risk of fracture while sulfonylureas are prescribed to those at higher risk [33]. This finding suggests that some of the reduction in fracture risk reported with metformin use in observational studies may be due to the underlying prescribing pattern.

Insulin

There are no randomized trials of insulin therapy with fracture or BMD outcomes. Most observational studies have identified increased fracture risk in those using insulin [29–31, 36–38] although others have not found an increased risk [28, 33]. Insulin treatment is also associated with a higher risk of falls [39, 40], and this is likely a contributing factor to the increased fracture risk. Insulin does not appear to have a negative effect on bone; indeed, preclinical studies suggest an anabolic effect. Increased falls and fractures may be the result of more frequent episodes of hypoglycemia and greater frailty due to diabetic complications.

Thiazolidinediones (Rosiglitazone, Pioglitazone)

TZDs increase insulin sensitivity via activation of peroxisome proliferator-activated receptor (PPARγ). Two TZDs, rosiglitazone and pioglitazone, have been used clinically since 1999. A number of studies showed superior efficacy of TZDs over other available antidiabetic therapies in the control of diabetic hyperglycemia [41]. However, their prolonged use is associated with several adverse effects. Strong clinical evidence points to the connection between rosiglitazone use and a significant increase in risk of myocardial infarction and death from cardiovascular causes [42]. This association resulted in a recent review of rosiglitazone safety by the FDA and recommendation for its restricted use in the United States. Interestingly, pioglitazone use is associated with a significantly lower risk of death and lower number of myocardial infarction and stroke incidence [43], indicating that cardiovascular effects of TZDs are not a drug class effect, but rather specifically associated with the TZD type. However, increased risk of bladder cancer in long time pioglitazone users resulted in recent restriction of its use by FDA. Both TZDs exhibit drug class properties of fluid retention and weight gain [44]. Although the use of both rosiglitazone and pioglitazone is currently restricted, the new TZDs with better safety profile are in development. Therefore, understanding TZDs mechanism of action on bone is needed in respect to improvement of safety for bone of new line of TZDs.

Although they possess beneficial anti-hyperglycemic profiles, rosiglitazone and pioglitazone use is associated with adverse effects on the skeleton [45, 46]. The crucial clinical evidence of a causal connection between TZD therapy and

increased fracture risk was determined from secondary analyses of results from randomized clinical trials of rosiglitazone and pioglitazone. The first demonstration of increased fracture risk was reported from ADOPT (A Diabetes Outcome Progression Trial), designed to compare time to monotherapy failure of rosiglitazone, metformin and glyburide in recently diagnosed T2DM patients [41]. Because of growing concern, based on rodent models and clinical trials, that TZDs might have a negative effect on bone, the investigators undertook a post hoc analysis of fracture rates in the three groups, using adverse event reports to identify fractures. In 1840 women and 2511 men with a median follow-up of 4.0 years and an average age of 56 (SD 10) years, fracture rates in men did not differ across treatment groups [26, 41]. However, in women, the cumulative incidence of fractures at 5 years was 15.1 % (11.2–19.1) with rosiglitazone, 7.3 % (95 % CI 4.4–10.1) with metformin, and 7.7 % (95 % CI 3.7–11.7) with glyburide, representing hazard ratios of 1.81 (95 % CI 1.17–2.80) and 2.13 (95 % CI 1.30–3.51) for rosiglitazone compared with metformin and glyburide, respectively. Increased fracture rates were seen in the lower and upper limbs. The incidence of hip and clinical vertebral fractures did not differ across treatment assignments, but only four hip and three clinical vertebral fractures were reported in women, as expected in the age range of this trial. Rosiglitazone was associated with higher fracture rates in both pre-and postmenopausal women, suggesting that estrogen status does not modify the effect on bone.

Soon after the ADOPT findings were published, Takeda performed a meta-analysis of pioglitazone trials and reported a similar pattern of increased fracture risk in women, but not men [47]. These observations were subsequently corroborated by other randomized trials. An early meta-analysis of data from ten different randomized controlled trials confirmed that TZD use doubles the risk of fractures exclusively in women [48]. More recently, a meta-analysis of 22 randomized controlled trials, including 896 fracture events, reported increased fracture incidence in women (OR = 1.94; 95 % CI 1.60–2.35) but not in men (OR = 1.02; 95 % CI 0.83–1.27) [49]. Effects in women were similar for rosiglitazone (OR = 2.10; 95 % CI 1.61–2.51) and pioglitazone (OR = 1.73; 95 % CI 1.18–2.55).

Because the TZD trials have included few hip or vertebral fractures, it is necessary to rely on observational studies to assess whether TZD use increases fractures at these particular skeletal sites. A study using registry data in Scotland focused exclusively on hip fracture risk and reported increased risk with greater cumulative TZD use among those with any use (OR per year of exposure 1.18; 95 % CI 1.09–1.28) [33]. Results were similar for pioglitazone and rosiglitazone considered separately. In contrast to reports from randomized trials, increased hip fracture risk was found in men (OR per year of exposure 1.20; 95 % CI 1.03, 1.41) as well as women (OR per year of exposure 1.18; 95 % CI 1.07, 1.29). Those with >4 years of TZD use had OR for hip fracture of 1.94 (95 % CI 1.28, 2.94), compared with those who used a TZD for up to 2 years. Finally, results were similar when evaluated in a subset with adjustment for use of other antidiabetic medications (insulin, metformin, or sulfonylurea). A large observational study using the UK General Practice Research Database also concluded that TZD use increased hip fracture incidence (Rate Ratio 2.09; 95 % CI 1.29–3.40) as well as spine fracture incidence (RR 2.72; 95 % CI

1.29–5.73) [50]. This study reported similar increases in risk of any fracture in men (RR 1.44; 95 % CI 1.18–1.77) and women (RR 1.42; 95 % CI 1.20–1.69).

The principal mechanism underlying increased fracture risk with TZD use appears to be bone loss. A recent meta-analysis of ten randomized clinical trials that assessed change in BMD reported greater bone loss at the lumbar spine, total hip and femoral neck in women randomized to TZD treatment compared with placebo or other antidiabetic medication [49]. Only one trial included men.

Clinical studies of changes in bone turnover markers with TZD treatment have not provided consistent results. In the largest study to date, 12-month changes in serum markers were assessed in 1605 participants in ADOPT [27]. This analysis showed modest but statistically significant increases in levels of resorption marker C-terminal telopeptide (CTX) in women on rosiglitazone therapy compared with glyburide (10.7 % difference, $p=0.002$) or metformin (7.3 % difference, $p=0.029$). In men, CTX was elevated in the rosiglitazone group compared with metformin (12.2%, $p<0.001$) but not compared with glyburide. Both genders had modest reductions in levels of the marker of bone formation P1NP (women −4.4 %, men −14.4 %), but those in the metformin arm experienced greater reductions. For women, changes in P1NP did not differ between the rosiglitazone and glyburide groups while in men losses were greater in the rosiglitazone group. Although rodent models suggest an important role for reduced bone formation as a mechanism of bone loss with TZD treatment, the ADOPT results instead indicate that increases in bone resorption may explain at least in part the increased fracture rate in women on TZD therapy [27].

Smaller trials of rosiglitazone treatment have also reported relative increases in markers of bone resorption compared to placebo [51, 52] and to metformin [53]. However, others have reported a relative reduction in bone formation markers with rosiglitazone treatment, compared with placebo [54] or with diet only treatment [55], and others have reported no difference [56]. For pioglitazone, the largest trial included 156 postmenopausal women with prediabetes and found no differences in bone turnover markers after 12 months compared with placebo [57]. In contrast, a trial in 71 diabetic men reported relative increases in markers of bone resorption (CTX) and formation (P1NP) in the pioglitazone group compared with metformin [58] while a trial in 86 diabetic men and women found a relative increase in a formation but not a resorption marker with pioglitazone treatment compared with placebo [59].

Taken together, results of available clinical studies indicate the following regarding TZD use (1) women are at increased risk of fractures; however, some studies point to elevated risk in men as well; (2) the increased fracture risk appears to be a class effect of currently available TZDs; (3) bone loss is an underlying mechanism; (4) fracture risk is increased in the extremities and most likely at the hip and spine as well.

Mechanism of TZD-Induced Bone Loss

PPARγ, an essential regulator of lipid, glucose, and insulin metabolism [10], is a target for TZDs. The PPARγ protein is expressed in mice and humans in two isoforms, PPARγ1 and PPARγ2. PPARγ1 is expressed in a variety of cell types,

including cells of hematopoietic lineage macrophages and osteoclasts [60], whereas PPARγ2 expression is restricted to cells of mesenchymal lineage adipocytes [61]. In bone, PPARγ2 plays an important role in regulation of MSC differentiation toward osteoblasts and adipocytes, and the maintenance of bone mass. Activation of the PPARγ2 isoform with rosiglitazone converts cells of osteoblast lineage to terminally differentiated adipocytes and irreversibly suppresses both the osteoblast phenotype and osteoblast-specific gene expression. Thus, in MSCs PPARγ2 acts as a positive regulator of adipocyte differentiation and a dominant-negative regulator of osteoblast differentiation [11, 62]. In contrast, PPARγ1 expressed in HSC promotes osteoclast differentiation and bone resorption [60]. It controls an expression of c-fos protein, an important determinant of osteoclast lineage commitment and development.

An essential role of PPARγ in maintenance of bone homeostasis was demonstrated in several animal models of either bone accrual or bone loss depending on the status of PPARγ activity [63–68]. In models of bone accrual, a decrease in PPARγ activity in either heterozygous PPARγ-deficient mice or mice carrying a hypomorphic mutation in the PPARγ gene locus led to increased bone mass due to increased quantity of osteoblasts [66, 68]. Interestingly, mice deficient in PPARγ expression in cells of hematopoietic lineage develop osteopetrosis and are less sensitive to the TZD-induced bone loss than control mice [60]. In contrast, in rodent models of bone loss due to PPARγ activation, administration of rosiglitazone resulted in significant decreases in BMD, bone volume, and changes in bone microarchitecture [63, 67, 69]. Observed bone loss was associated with expected changes in the structure and function of bone marrow, which included decreased number of osteoblasts, increased number of adipocytes, and increased support for osteoclastogenesis. The degree of bone loss in response to rosiglitazone correlated with the animal age and the level of PPARγ expression. In younger animals with less PPARγ, bone loss was less extensive than in older animals [69]. Moreover, age determined the mechanism by which bone loss occurred. In younger animals it occurred due to decreased bone formation, whereas in older animals due to increased bone resorption [69]. In addition, studies of rosiglitazone effects in estrogen deficient rats showed that bone loss occurred mainly due to increased bone resorption [64]. In conclusion, animal studies suggest that aging and estrogen deficiency confound TZD-induced bone loss and determine its mechanism.

The negative effect of TZDs on osteoblastogenesis includes decreased activity of Runx2, Dlx5, and Osterix, which are osteoblast-specific transcription factors, and decreased activity of osteoblast-specific signaling pathways controlling bone homeostasis, among them Wnt, TGF-β/BMP, and IGF-1 [70, 71]. The effect of TZDs on the expression of genes essential for osteoblast development is strikingly similar to changes observed during aging. Due to the type of bone loss and similarities to aging, some speculate that TZDs may accelerate the aging of bone [69, 72]. The complexity of TZDs effects on bone remodeling resulting from changes on osteoblast and osteoclast differentiation and alterations in bone marrow milieu supporting remodeling are summarized in Fig. 7.3.

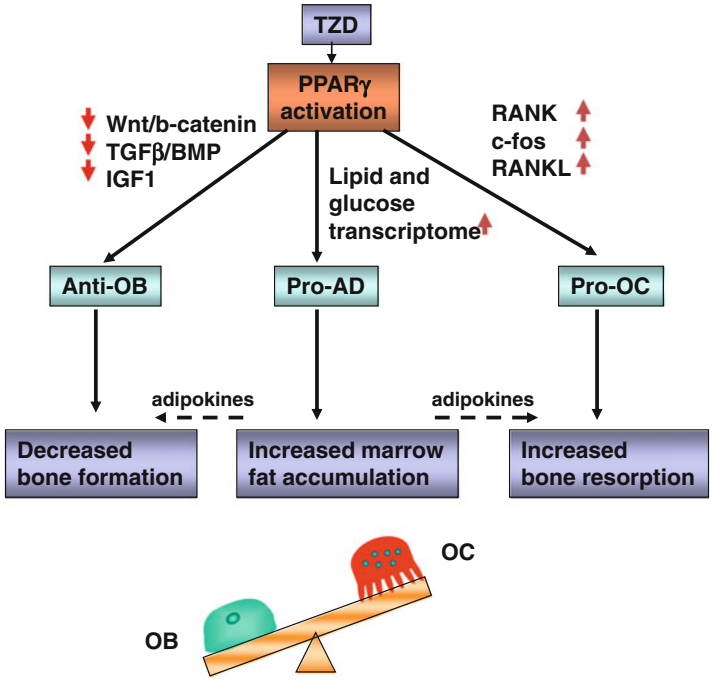

Fig. 7.3 PPARγ activation with TZDs leads to multiple direct and indirect effects in the bone marrow which result in changes in bone cell differentiation, unbalanced bone remodeling and ultimately bone loss. *Anti-OB* PPARγ activity inhibiting osteoblast differentiation, *pro-AD* PPARγ activity stimulating adipocyte differentiation, and *pro-OC* PPARγ activity stimulating osteoclast differentiation

Novel Selective PPARγ Modulators with Beneficial Effect of Insulin Sensitizers and No Effect on Adipocyte Differentiation

The PPARγ ligand-binding domain contains a large binding pocket capable of encompassing a variety of ligands. This provides a wide array of potential contact points that can result in various PPARγ post-translational modifications (PTMs), including phosphorylation, acetylation and sumoylation, and differential recruitment of coactivators, which determine specific activities of this nuclear receptor [73]. The molecular studies provide evidence for distinct mechanisms regulating the proadipocytic, antiosteoblastic, and insulin sensitizing activities of PPARγ and include the levels of Serine 273 and Serine 112 phosphorylation and functional interaction with other proteins such as β-catenin and molecular chaperons FKBP51 and PP5 [74–77].

The concern of TZDs adverse effects has prompted pharmaceutical efforts to develop selective PPARγ modulators which will retain high potency to treat diabetic disease with minimal adverse effects [78]. The PPARγ selective activators, with a decreased proadipocytic activity but intact insulin sensitizing activity such as

netoglitazone, INT131, MSDC-0602 and telmisartan do not affect bone mass in mice treated with the therapeutic doses [79–82]. A new class of insulin sensitizers with structural similarities to telmisartan, which block Serine 273 phosphorylation but do not stimulate PPARγ transcriptional proadipocytic activity, has been recently developed [74, 83], however their safety for bone is not as yet determined.

Sulfonylureas

Sulfonylureas function as insulin secretagogues. This class of drugs activates sulfonylurea receptors on the surface of pancreatic β cells and stimulates exocytosis of insulin from vesicles. In addition, sulfonylureas are associated with greater frequency of hypoglycemia which may increase the risk of falls and fractures [84].

In the ADOPT trial, described earlier, fracture incidence was similar in those randomized to a sulfonylurea (glyburide) versus metformin [41]. The results of observational studies have been inconsistent with reports of increased [34], decreased [28, 31] and no difference [29, 30, 32, 35, 50, 85, 86] in fracture risk among those using a sulfonylurea. As noted in a recent review of current literature [87], although a large number of studies have reported no association with fracture, the majority of these studies were not specifically intended to assess the impact of sulfonylureas in particular on fracture. Allocation bias is an important consideration in observational studies, and results from a study in Scotland suggest that patients using sulfonylureas tend to have a higher background fracture risk [33]. Although sulfonylureas increase hypoglycemic episodes, an observational study conducted among Kaiser Permanente members did not find an association between sulfonylurea use and incident falls, identified through inpatient and outpatient medical records [88].

Incretin Analogs and DPP4 Protease Inhibitors

This newest class of antidiabetic drugs enhances the mechanism by which enteric hormones stimulate insulin release from β-cells and inhibit glucagon production in the liver [89]. Glucose-dependent insulinotropic peptide (GIP), and glucagon-like peptides (GLP-1 and GLP-2), are released by gut endocrine cells in response to nutrient intake. Bioactivity of incretin hormones is limited by their rapid degradation and inactivation by dipeptidyl peptidase-4 (DPP-4), a serine protease that is present in a soluble form in plasma and is expressed in most tissues [90]. Recently, incretin mimetics (GLP1 receptor agonists) and DPP-4 inhibitors have emerged as a new class of pharmacological agents to enhance incretins action and improve glycemic control in patients with T2DM. Incretin mimetics and DPP-4 inhibitors have a major advantage over other diabetic medications in that glucose control remains stable with little or no rise in HbA1c levels after long periods of use. The side effects common for incretin-based therapies, including incretin receptors

agonists and DPP-4 inhibitors, consist of gastrointestinal, immune system and pancreatic reactions. Since DPP-4 enzyme is known to be involved in the suppression of certain malignancies, particularly in limiting the tissue invasion of tumors, there is a concern that DPP-4 inhibitors may allow some cancers to progress, however clinical data are not as yet available [91–93].

Nutritional hormones are known to be important in bone turnover; as soon as a meal is ingested, bone breakdown is suppressed [94, 95]. Osteoblasts and osteoclasts express receptors for both GIP and GLP incretins. A number of studies indicate that GLP-2 acts mainly as an antiresorptive hormone [96], while GIP can act both as an antiresorptive and anabolic hormone [97, 98]. Mice deficient in GLP-1 receptor develop cortical osteopenia and have more fragile bone as well as increased quantity of osteoclasts and increased bone resorption [99]. GLP-1 receptor signaling may play an essential role in the control of bone resorption indirectly, through a calcitonin-dependent pathway. Calcitonin treatment effectively suppressed bone resorption markers in Glp-1r(−/−) mice, and the GLP-1 receptor agonist exendin-4 increased calcitonin gene expression in the thyroid of wild-type mice [99]. Interestingly, although animal studies showed that DPP-4 inhibitor sitagliptin did not affect bone density, however the absence of DPP-4 in Dpp-4(−/−) mice lead to the greater bone loss after ovariectomy as compared to animals with unaltered DPP-4 expression [100]. In summary, a number of animal studies indicate that incretins have beneficial effects on bone mass and protective effects on bone quality. Therefore, antidiabetic therapies which increase GIP and GLP hormone levels and their bioactivity might exert beneficial effects on human bone.

Since incretin-based therapy is relatively new, the clinical data of its safety for bone is just emerging. The 44-week treatment of T2DM patients with incretin mimetic exenatide did not decrease total body BMD, although it decreased body weight by 6 % [101]. Currently, two meta-analyses of incretin mimetics and fracture outcomes, reported as serious adverse events, have been published. The first meta-analysis included seven trials with placebo or other antidiabetic medications as the comparison group and a total of 19 fractures [102]. There was no difference in fracture incidence between incretin mimetic treatment and the comparator groups (MH OR 0.75; 95 % CI 0.28–2.02), but confidence intervals were wide. A second meta-analysis included 14 trials with 38 fractures and also found no difference in fracture incidence (MH OR 1.05; 95 % CI 0.59–1.87) [103]. However, when examined separately, the investigators found decreased fracture incidence for liraglutide treatment (MH OR 0.38; 95 % CI 0.17–0.87) and increased fracture incidence for exenatide treatment (MH OR 2.09; 95 % CI 1.03–4.21), both compared with placebo or other antidiabetic medications. The reason for this difference is not immediately apparent and may be a chance finding. Liraglutide and exenatide have similar effects on blood glucose and body weight without an increased frequency of hypoglycemia. An important limitation of these trials is their relatively short length for the purposes of assessing fracture risk. Only five of the trials were 52 weeks or longer, and effects on fracture risk that are operating through changes in bone would be expected to develop over periods of a year or more.

A meta-analysis of 28 clinical trials of DPP-4 inhibitors with duration of at least 24 weeks included 63 fractures reported as serious adverse events. Treatment with DPP-4 inhibitors was associated with a reduced risk of fractures (Mantel Haenszel Odds Ratio 0.60; 95 % CI 0.37–0.99) compared to placebo and other treatments [104]. Excluding TZDs or sulfonylurea as comparators yielded similar results (MH OR 0.56; 95 % CI 0.33–0.93). As noted for the trials of incretin mimetics, an important weakness of these results is the short duration of the trials. Only 7 of the 28 trials were 52 weeks or longer. More clinical studies on the effects of incretin mimetics and DPP-4 inhibitors on BMD and fracture risk with stratification according to gender, postmenopausal status, and age are needed.

SGLT2 Inhibitors

In 2013 and 2014 the first of two SGLT2 inhibitors class of diabetes drugs, canagliflozin and dapagliflozin, were approved by FDA for improving glycemic control in T2DM patients in conjunction with diet and exercise. Both drugs are selective and reversible inhibitors of sodium glucose co-transporter 2 (SGLT2), which is responsible for the majority of glucose reabsorption in kidney. Bone safety of dapagliflozin has been evaluated in a randomized trial, adding study drug or placebo to metformin in T2DM patients with inadequate control on metformin. Results have been reported after 50 weeks [105] and 102 weeks [106] of treatment. At 102 weeks, the trial was completed by 140 patients, men and postmenopausal women. The dapagliflozin group lost more weight than the placebo group (difference of −2.42 kg; 95 % CI −3.64, −1.21). In spite of the greater weight loss, no significant differences were identified in changes from baseline in markers of bone formation (P1NP) and bone resorption (CTX). Bone loss was greater at the femoral neck in those treated with dapagliflozin but the difference was not statistically significant (difference −0.94 %; 95 % CI −2.21, 0.35). Differences in BMD changes at the lumbar spine and total hip were smaller and also not statistically significant. There were no significant treatment-by-gender interactions. In a 104-week trial of dapagliflozin to assess efficacy among T2DM patients with moderate renal impairment, 252 patients were randomized to dapagliflozin (5 or 10 mg) or placebo. The treated groups lost weight compared with placebo but glycemic control was not different. More fractures were reported in the treated groups ($N=13$) than the placebo group ($N=0$). Dapagliflozin is not recommended for use in patients with moderate renal impairment [107].

There are no available studies of the effects of canagliflozin on bone turnover or BMD. Increased incidence of upper extremity fractures with canagliflozin treatment was reported in the Prescribing Information by the manufacturer [108]. In a meta-analysis of eight clinical trials with longer mean duration of treatment (68 weeks), the incidence rate of fracture was 14.2, 18.7, and 17.6 per 1000 patient-years of exposure to comparator, canagliflozin 100 mg and canagliflozin 300 mg. FDA approval for canagliflozin included a requirement for postmarketing studies to monitor for bone safety [109].

Amylin Analogs

Amylin, also known as Islet Amyloid Polypeptide (IAPP), is a 37-residue peptide hormone produced in pancreatic β-cells. Amylin is co-secreted with insulin and plays a role in glycemic regulation by slowing gastric emptying, promoting satiety and decreasing glucose levels in circulation. Amylin, like insulin, is absent in individuals with T1DM. Amylin belongs to the family of regulatory hormones that are structurally and functionally related to calcitonin, calcitonin gene-related peptide and adrenomedulin, and signals through the calcitonin receptor modified for amylin-specific activity by binding to receptor activity modifying proteins (RAMP) [110]. Cellular studies have shown that amylin may stimulate osteoblast proliferation and may inhibit osteoclast development and activity through increasing cyclic AMP [111]. These activities have been confirmed in several animal studies which showed a positive effect of amylin on trabecular and cortical bone volume [111] and some of them are indicative that the bone response to amylin may differ depending on diabetes status [112].

Human data suggest that there is a functional link between amylin and skeletal health. Amylin levels decrease with aging and correlate inversely with osteoporosis [113]. In addition, reduced amylin levels are associated with low BMD in women with anorexia nervosa and are significant predictors of BMD and of *Z*-scores at the femoral neck and at the total hip in this group of patients [114].

An analog of amylin, pramlintide, had been approved for therapy in 2005 by FDA to treat diabetes. It is used as an adjunctive therapy with insulin in both T1DM and T2DM. Pramlintide allows patients to use less insulin, because it improves hemoglobin 1Ac levels, lowers average blood sugar levels, and substantially reduces blood sugar that occurs in diabetic individuals right after eating.

Although animal studies suggest positive effect of amylin on bone, the clinical studies do not provide supporting results. Pramlintide safety on bone was assessed in a study conducted on patients with T1DM who injected the drug for 12 months. BMD measurements of the lumbar spine by dual-energy X-ray absorptiometry (DXA), and biochemical markers of bone metabolism (serum-calcium, PTH, osteocalcin, urinary pyridinium cross-links) before and one year after starting pramlintide therapy showed no significant changes. It is concluded that a 1-year pramlintide therapy does not affect bone density or bone metabolism in patients with type 1 diabetes mellitus without osteopenia (based on the markers used) [115].

Alpha-Glucosidase Inhibitors

Alpha-glucosidase inhibitors are saccharides that act as competitive inhibitors of enzymes needed to digest carbohydrates, specifically alpha-glucosidase enzymes in the brush border of the small intestines, which subsequently leads to the reduction of blood sugar levels. Therapy with alpha-glucosidase inhibitors is associated with

several side effects which are in close relation to their mechanism of action and include increased levels of carbohydrates in the intestine causing flatulence and diarrhea. There are no available information on bone safety of alpha-glucosidase inhibitors.

Conclusions

In conclusion, the available evidence indicates that antidiabetic therapies may either increase fracture risk (TZDs and insulin), may not affect this risk (sulfonylureas and metformin) or may possibly decrease the risk (DPP-4 inhibitors). From a bone perspective, metformin and sulphonylureas are safer than TZDs; randomised trials have shown that TZDs decrease BMD and increase fracture risk. The mechanism of TZD-induced bone loss includes unbalanced bone remodeling processes resulting from decreased bone formation and increased bone resorption. Animal studies suggest that aging and estrogen deficiency may modify the effects of TZDs on bone and determine the mechanism of bone loss. The emerging potential of incretin-based therapies as sparing or perhaps even beneficial for bones requires systematic clinical assessment in the future.

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Chapter 8
Bone Marrow Stem Cells and Bone Turnover in Diabetic Disease

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Introduction

The Centers for Disease Control and Prevention in 2012 indicated that over 29 million Americans have diabetes. Hyperglycemia is a diagnostic indicator of diabetes. Diabetes has two main forms: type 1 (T1D) and type 2 (T2D). In T1D, hyperglycemia occurs as a result of little or no insulin production by pancreatic beta cells, thus glucose is unable to be taken up by cells that have insulin-dependent glucose channels. In T2D, hyperglycemia results from insulin resistance that leads to reduced insulin signaling and glucose uptake in insulin-dependent cells. Long-term diabetes affects multiple organ systems resulting in complications such as neuropathy, nephropathy, and retinopathy as well as less well-known complications such as increased fracture risk. Both T1D and T2D patients are at risk for fractures but the underlying pathophysiology is somewhat different [1–6]. T1D is associated with reduced bone density while T2D is often linked with increased or unaltered bone density. Thus, there are likely separate and overlapping mechanisms. In this chapter we will focus on understanding the effects of T1D and T2D on the bone microenvironment, stem cell maturation and bone remodeling with particular emphasis on osteoblast, osteoclast, and immune cell activity and composition. Understanding the underlying mechanisms of diabetic bone changes is critical for identifying and developing effective therapeutics.

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The Bone Marrow Microenvironment

The bone marrow contains many different cell types including stem cells, hematopoetic cells, and immune cells that are in direct contact with the cells that ultimately determine bone density: osteoblasts and osteoclasts. Stem cells are defined as undifferentiated cells capable of self-renewing that have the potential to differentiate into specialized cell types such as red blood cells, bone cells, muscle, adipocytes and immune cells. They are also able of differentiating into cells of non-hematopoietic tissue such as the heart and pancreas [7, 8].

Bone marrow stem cells give rise to both hematopoietic and non-hematopoietic stem cells. Hematopoietic stems cells (HSCs) are important for generating the immune system and renewing blood cells. HSCs can differentiate into lymphoid progenitor cells (i.e., B-lymphocytes, T-lymphocytes, Natural killer (NK) cells) and myeloid progenitor cells (i.e., monocytes, macrophages, Langerhans cells, dendritic cells, megakaryocytes, and granulocytes). HSCs respond to different environmental signals, such as cytokines, to maintain cellular homeostasis and respond to the body's need for certain cell types. Non-hematopoietic mesenchymal stem cells (MSCs) differentiate into osteoblasts, osteocytes, chondrocytes, and adipocytes as well as into myocytes, neurons, and astrocytes in vitro and in vivo. MSCs can also differentiate into endothelial stem cells (ESCs), which are important in blood vessels and lymphatic vessels, and are thought to contribute to the healing of other organs including intestine [9, 10].

The cross talk among cells located within the bone (both non-hematopoietic and hematopoietic) is critical in the regulation of bone remodeling which involves both osteoblasts and osteoclasts. Osteoblasts, derived from MSCs, interact with the hematopoietic precursors through local and systemic factors (i.e., receptor activator of nuclear factor kappa-B ligand (RANKL)) and cells (particularly macrophages and T-cells) to enhance the differentiation and activity of osteoclasts. Osteoclasts, derived from HSCs of the monocyte/macrophage lineage, are induced to differentiate and become active (resorb bone) in the presence of macrophage colony stimulating factor (M-CSF) and RANKL [11] which is further enhanced by the presence of factors such as tumor necrosis factor alpha (TNF-α) [11]. Osteoblasts and osteoclasts work together during bone remodeling to maintain skeletal integrity. This balance is vital to bone health and therefore the two cell types communicate with each other extensively. This cross-talk begins even before the cells are fully mature. Diabetes is one of many diseases that can result in altered bone marrow stem cell maturation leading to changes in bone remodeling.

The Regulation of Osteoblasts and Bone Formation by Diabetes

Regulation of Osteoblast Activity: Normal Physiology

Osteoblast activity (bone formation) is regulated at three main levels: lineage selection, maturation, and apoptosis [12] (Fig. 8.1). Lineage selection, the balance of MSCs maturation into osteoblasts (bone) versus adipocytes (marrow adiposity) [13], is a well-understood mechanism that regulates bone density [14]. MSCs progression to the osteoblast lineage is reduced under conditions of age-related or disease-related osteoporotic conditions as well as with certain pharmacologic therapies such as Rosiglizaone [14–20]. While expression of Runx2 (an osteogenic transcription factor) induces osteogenesis [21], expression and activation of peroxisome proliferator-activated receptor-γ (PPAR-γ, an adipogenic transcription factor) promotes adipogenesis. When PPARγ2 is activated (by binding fatty acid ligands) or when PPARγ2 is overexpressed, mice display increased marrow adiposity, suppressed osteoblast maturation and bone loss [22–27]. In contrast, PPARγ2-deficient mice have reduced marrow adiposity and increased bone density [28, 29]. In vitro studies also show that stimulation of MSC towards adipogenesis reduces osteogenesis [30, 31]. Factors that regulate selection of osteogenesis over adipogenesis include transforming growth factor beta (TGF-β), bone morphogenetic proteins (BMPs), cytokines, adipokines, thyroid hormones, metabolic stress, and Wnt signaling [11, 32–41].

Osteoblast activity is also regulated through modifying the rate/extent of cell maturation and death. During early stages of maturation, osteoblasts express collagen I, the major extracellular matrix component in bone [42]. Collagen I provides the foundation for subsequent matrix maturation and mineralization. During late stages of maturation, osteoblasts express osteocalcin, which is carboxylated at three residues to increase affinity to the bone matrix [43]. Not all osteocalcin is carboxylated. The undercarboxylated form can enter into the circulation to stimulate insulin secretion, insulin signaling sensitivity, and glucose homeostasis [43–46]; thus bone may affect and regulate diabetes. BMP and Wnt signaling promote osteoblast differentiation while many pro-inflammatory cytokines such as TNF-α reduce maturation. Once bone mineral is made, osteoblasts become osteocytes and embed within the mineralized bone, become bone lining cells that lie atop the newly formed bone, or undergo apoptosis [13, 21, 47, 48]. Increased osteoblast death is associated with decreased bone density due to the lack of bone forming cells. Metabolic stress (hypoxia, hyperglycemia) and pro-inflammatory cytokines can promote osteoblast apoptosis and bone loss [48–52].

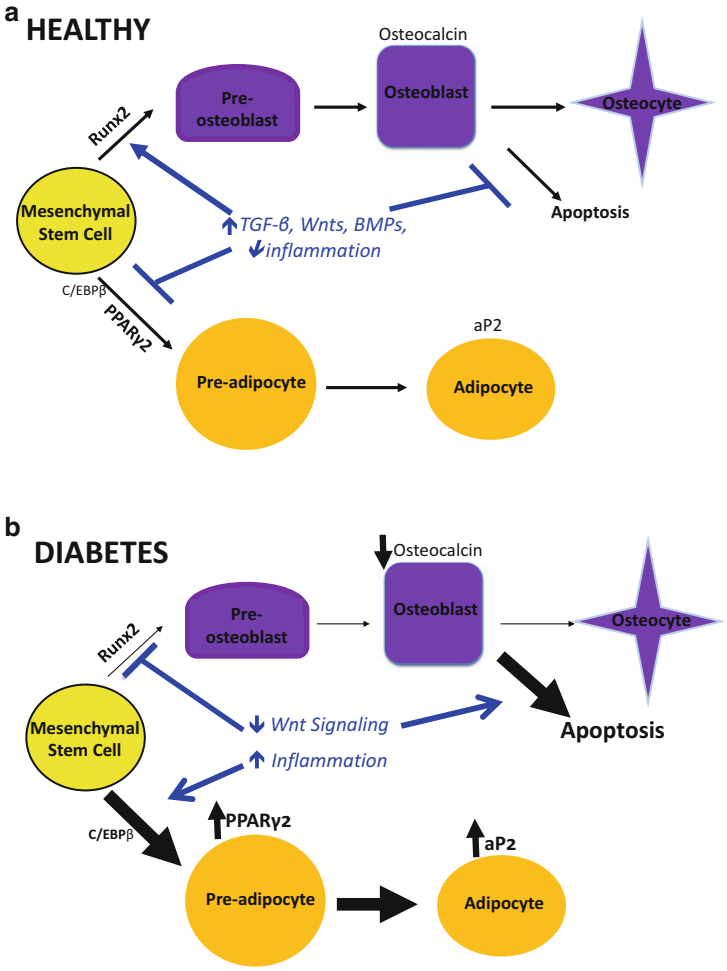

Fig. 8.1 Diabetes significantly affects osteoblast activity at multiple levels. Mesenchymal stem cells can differentiate into pre-osteoblasts or pre-adipocytes as well as other cell types. Runx2 is expressed when mesenchymal stem cells commit to osteogenesis and is required for osteoblast (bone formation cells) lineage selection. During the progression of osteoblast maturation stage, specific genes are expressed including osteocalcin, which is a late stage marker of osteoblast maturation. Following maturation, osteoblasts can undergo apoptosis or become embedded in the bone (osteocytes) during mineralization. Mesenchymal stems cells can also commit to adipogenesis. C/EBPβ promotes the expression of PPARγ2 which induces expression of other adipogenic genes such as aP2 (a protein that binds fatty acids). Osteoblast activity can be decreased by (1) promoting adipogenesis rather than osteogenesis, (2) inhibiting osteoblast maturation, and (3) increasing osteoblast apoptosis. Factors including TGF-β, Wnts, and BMPs increase osteogenesis, osteoblast maturation, and osteoblast viability. Diabetes can reduce osteoblast activity at all three levels of regulation. Factors such as diabetes-induced inflammation and reduced Wnt signaling can lead to suppressed osteoblast activity through modulation of the three levels of osteoblast regulation. These changes are thought to contribute to the decrease in bone formation seen in diabetes

Effect of T1 Diabetes on Osteoblasts

Decreased osteoblast activity is the key contributor to decreased bone mineral density observed in T1D children and adults [53–58]. Consistent with a decrease in bone mineral density, serum osteocalcin levels are typically lower in diabetic patients compared to nondiabetic subjects [46, 48, 53, 59–73] and are inversely proportional to glycosylated hemoglobin levels, a measure of metabolic control [74, 75]. Levels of serum undercaboxylated osteocalcin, which stimulates insulin secretion [59, 62, 76, 77], are negatively correlated with glycosylated hemoglobin levels in T1D male patients [78]. T1D rodents display bone loss similar to diabetic patients [68, 69, 71–73, 77, 79–82] and are useful models to study mechanisms of T1D bone loss. Specifically, diabetic mice and rats exhibit decreased trabecular bone volume and reduced cortical thickness (in some reports) within 4 weeks of T1D induction. T1D mouse and rat models also display decreased serum osteocalcin levels [64, 71, 73, 77, 80, 83] as well as decreased bone osteocalcin mRNA levels [46, 60, 62, 73, 84]. Histologic analyses indicate that osteoblast surface, mineral apposition and dynamic bone formation rates at trabecular, endosteal, and periosteal sites are reduced in T1D bone [52, 62, 76, 77, 85]. Bone implant integration and fracture healing are also suppressed in diabetic rodent models [72, 85, 86] consistent with an overall reduction in bone formation. Thus, a critical area of investigation is determining at what level (lineage selection, maturation, death) osteoblast activity is modified by T1D.

Lineage Selection

Several lines of evidence support that T1D alters MSC lineage selection. Most notably, mouse models of T1D display an increase in bone marrow adipocyte number and a decrease in the number of surface osteoblasts [14, 16, 18, 19, 45, 60, 87]. In vitro studies further indicate that factors present in T1D patients and mice (i.e., high glucose, pro-inflammatory cytokines) are capable of promoting MSC adipogenesis at the cost of osteogenesis [45, 49, 50, 60, 83, 88–90]. In addition, a recent study in rats found that the osteoprogenitor pool in T1D rat bone marrow was significantly depleted [88], further suggesting decreased lineage selection towards osteogenesis and/or a reduction in overall MSC number. Interestingly, the T1D adiposity is bone marrow specific, as T1D mice have depleted subcutaneous and visceral fat stores and exhibit weight loss [48, 83, 87]. This is consistent with the weight loss seen in T1D patients [87, 91]. Both T1D male and female mice display increased bone marrow adiposity in tibia, femur, and calvaria, and correspondingly display increased levels of adipocyte markers such as PPARγ2 and adipocyte protein 2 [aP2, also known as fatty acid binding protein 4 (FABP4)] in bone [45, 62]. T1D-induced marrow adiposity could be either an active or passive shunt of MSCs towards adipogenesis and away from osteogenesis [17–19, 92, 93] (see section on "Potential Mechanisms Contributing to Altered Bone Remodeling").

Several in vivo studies have examined the role of T1D-induced marrow adiposity in mediating bone loss in T1D mouse models. Pharmacologically, an antagonist of PPARγ (bisphenol-A-diglycidyl ether) was given to T1D mice to inhibit adipogenesis. This approach was successful in preventing T1D-induced marrow adiposity, but T1D bone loss still occurred [23–25, 79]. Genetic manipulations, such as the knockout of C/EBP-β (a transcription factor required early in adipogenesis) have not been effective in preventing T1D bone loss [94, 95]. The lack of an association between bone marrow adiposity and bone loss was also observed in T1D patients [96], though marrow adiposity did correlate with serum lipid levels [96] suggesting a link to hyperlipidemia. Larger clinical studies are needed to determine significant relationships. Consistent with the lack of a link between marrow fat and T1D bone loss, mouse vertebral bone which has reduced density in T1D does not display an increase in marrow adiposity [45]. Taken together, these studies indicate that T1D is associated with changes in MSC lineage selection but T1D bone loss is not the direct result of increased marrow adiposity. Additional pathways regulating osteoblast activity must be involved.

Maturation

Once osteoblast lineage is selected, pre-osteoblasts progressively mature through stages of matrix production and mineralization. Identifying a specific change in maturation is somewhat difficult since altered lineage selection and increased death can also reduce markers of maturation in T1D bone. It is known that bone defect healing is decreased in T1D rats [97]. Similarly, T1D decreases bone formation on titanium and hydroxyapatite implants [80, 98, 99]. Though a reduction in pre-osteoblasts would cause similar outcomes, cell culture studies provide evidence to suggest that T1D reduces osteoblast maturation. Pre-osteoblast cell lines (i.e., MC3T3-E1 cells) cultured under conditions associated with T1D (high glucose or pro-inflammatory cytokines such as TNFα) display reduced levels of maturation markers [100–104]. In many of these conditions there is also evidence of enhanced expression of adipocyte markers (PPARγ and aP2) [45, 60, 62] suggesting a lineage or functional shift to an adipocyte.

Apoptosis

Increased osteoblast apoptosis can also contribute to reduced T1D osteoblast activity. T1D associated factors, such as reactive oxygen species (ROS), advanced glycation end-products (AGEs) and pro-inflammatory cytokines, are linked to increased osteoblast death [33, 48, 105–107]. In T1D mouse models, the number of osteoblasts stained by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), a method of detecting cellular apoptosis, was increased twofold compared to levels in control mouse femurs and tibias [52, 108]. The increase in apoptotic osteoblasts was confirmed in spontaneously diabetic *Ins2*$^{+/-}$ mice where the

increase was over threefold when compared to control [52]. Similarly, mRNA levels of Bax (a pro-apoptotic protein) were increased in streptozotocin (STZ)-induced diabetic mice and *Ins2*$^{+/-}$ mice [52]. When osteoblasts (primary cells and cell lines) were cocultured with control versus T1D mouse bone marrow, the T1D marrow caused a twofold increase in osteoblast caspase-3 activity, a marker of cell apoptosis [52]. These findings support a role for the diabetic bone marrow microenvironment in inducing osteoblast death [52].

Effect of T2 Diabetes on Osteoblasts

In clinical studies, T2D patients display an increase, no change or decreased bone density [1–6]. Some studies found T2D to be associated with a decrease in serum osteocalcin levels in men and postmenopausal women [78, 109]. Variations in bone responses are likely a consequence of the complexity of the pathogenesis of T2D. Some T2D patients with above average weight and normal to high bone mineral density (factors typically associated with decreased risk fracture) also have increased risk fracture [110]. T2D can occur in non-obese and obese patients and thus bone changes can be associated with increased load, hyperlipidemia, hyperglycemia, inflammation and/or other complications that can contribute to altered bone density.

Accordingly, there are several T2D mouse models including obese and non-obese. In obese models such as T2D obese Wistar fatty rats, serum osteocalcin and bone osteocalcin levels are decreased as well as bone parameters such as length, strength, and weight [111]. Bone formation and turnover are both decreased in these T2D rodent models [112, 113]. In Zucker diabetic fatty (ZDF) rats (harbor a mutation in the leptin receptor, become obese rats and develop T2D at 9 week of age), a 55 % decrease in mineralized matrix formation was shown when bone marrow cells were stimulated by osteogenic media [114]. The same cells showed a 40–80 % reduction in Runx2, osteopontin, BMP-2, and osteocalcin mRNA expression. This data suggests that T2D may decrease lineage selection away from osteogenesis and decrease osteoblast maturation. The *ob*/*ob* mouse, a severe obesity model for T2D that harbors a mutation in the leptin gene, displays decreased amounts of trabecular and cortical bone and reduced femoral bone length, but increased trabecular bone volume in the lumbar vertebrae [115–119]. These mice also have decreased bone formation in both long bones and vertebrae [115, 119]. In the *db*/*db* T2D model (which harbor a mutation in the leptin receptor and become diabetic at 6–8 weeks of age) *P. gingivalis*-induced inflammation is exacerbated [112]. Bone formation was impaired and osteocalcin mRNA expression in the calvaria was decreased [112]. Caspase-3 and caspase-8 mRNA levels in the calvaria (apoptotic proteins) were increased over twofold when compared to control [83]. While osteoblast activity is decreased in these models, interpretations are complicated by not being able to distinguish bone response from T2D versus decreased leptin signaling.

Non-obese Goto-Kakizaki mice display reduced cortical bone thickness but this model showed abnormal pancreatic signs in the embryonic stage [33, 111]. Another non-obese T2D model, Torii rats, display suppressed osteoid surface, bone strength, and osteoblast numbers, which are all reversed by treatment with insulin [120]. A spontaneous T2D model (muscle IGF-1-1R-lysine-arginine, MKR) has a mutation in the skeletal muscle IGF-1 that leads to hyperglycemia by 7–8 weeks of age [110, 121–124]. MKR mice have decreased bone volume/total volume at the distal femoral metaphyses, stiffness, and failure load when compared to controls [122].

The Regulation of Osteoclasts and Bone Resorption by Diabetes

Regulation of Osteoclast Activity

Osteoclasts are multinucleated cells responsible for the resorption phase of the bone remodeling process. These cells are derived from HSCs, which reside in the bone marrow. Differentiation of HSCs into active osteoclasts is a multistep process (Fig. 8.2) and is regulated by three main cytokines: macrophage colony stimulating factor (M-CSF), RANKL and osteoprotegerin (OPG). These factors are derived

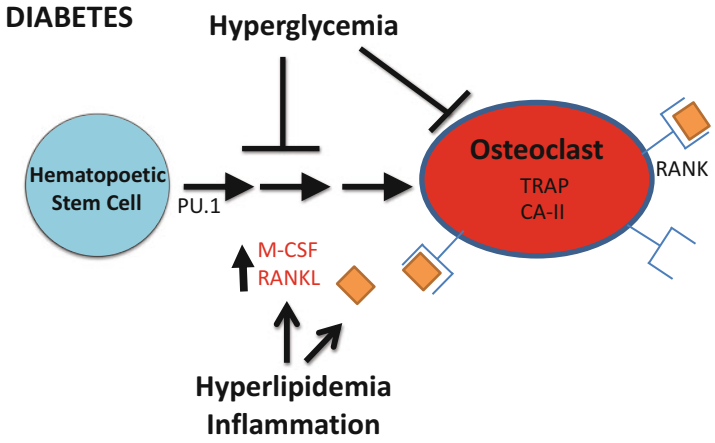

Fig. 8.2 Model for diabetes induced changes in osteoclast activity. Osteoclastogenesis involves early expression of PU.1 which regulates expression of the receptor for M-CSF. Early osteoclast maturation is induced by M-CSF and RANKL (expressed by osteoblasts). RANKL binds the RANK receptor on osteoclasts to activate NFkB activity, initiating the transcription of genes necessary for bone resorption. Diabetes-associated conditions such as inflammation (TNFα) and hyperlipidemia are known to activate osteoclast activity through increasing osteoclastogenesis and maturation. However, diabetes is typically associated with decreased bone resorption. This likely occurs due to other diabetes-associated factors such as hyperglycemia as well as expression of anti-inflammatory cytokines

from osteoblasts as well as other cells of the immune system including T-cells and B-cells [131]. While RANKL binds to RANK on osteoclasts to activate resorption, OPG (a secreted as well as membrane-bound protein produced by osteoblasts) acts as a decoy for RANKL, preventing it from binding to the transmembrane receptor RANK [137–139]. Both M-CSF and RANKL can increase nuclear factor-kappa (NF-kB) activity to stimulate osteoclast maturation [132–136]. RANKL does this by first inducing TNF receptor-associated factor (TRAF) proteins to start a signaling pathway that ends in the activation of NF-kB. NF-kB is typically bound in the cytoplasm by the inhibitor of kB (IkB). Binding of RANKL induces the phosphorylation of IkB to allow NF-kB to enter the nucleus to stimulate genes required for osteoclast function. Mutations in this signaling pathway lead to defective osteoclast formation and function [140–143]. Similarly, mutations in the gene encoding M-CSF causes osteopetrosis due to the inability of osteoclasts to fully mature.

Other factors involved in osteoclast differentiation include PU.1, which is a transcription factor that is required for the normal formation of osteoclasts. PU.1 regulates the receptor for macrophage colony stimulating actor (M-CSF) and integrin proteins needed for osteoclast maturation and binding of the osteoclast to the bone surface [125–127]. Mice lacking PU.1 die immediately after being born and have symptoms of osteopetrosis, an increase in bone density [128]. PU.1 interacts with other transcription factors (MITF, micro-ophthalmia-associated transcription factor) to regulate genes important for osteoclast function such as tartrate-resistant acid phosphatase (TRAP) and carbonic anhydrase 2 (CA-II) [129, 130].

Effect of T1 Diabetes on Osteoclasts

The majority of rodent studies examining T1D show no change or decreases in markers of osteoclastic resorption [33, 48, 62, 85, 144–147]. However, there is some controversy since a few studies have found increased osteoclast parameters in animals [147]. Clinical studies support no change in T1D osteoclast activity as determined by levels of serum markers of resorption such as deoxypyridinoline and c-terminal telopeptide of type 1 collagen (CTX) [53, 69, 86, 148]. In mice, the typical STZ dosing regimen used to induce T1D does not cause a change in osteoclast parameters (consistent with spontaneous mouse models [60]), however, increasing the dose causes an increase in osteoclast activity possibly due to more rapid onset of T1D and/or inflammation (as seen in the liver with high STZ dose) [67]. As noted previously, T1D increases bone marrow adiposity [60, 62] and thus MSC lineage selection. Not only does this change the overall physical morphology of the bone marrow compartment, but it also reduces the number of MSC maturing to the osteoblasts that are needed for HSC maintenance and maturation to osteoclasts (via M-CSF and RANKL) [149]; this can contribute to reduced osteoclastogenesis in the presence of inflammation. Other contributors to reduced resorption in T1D include hyperglycemia which can directly decrease RANKL-induced osteoclastogenesis [150], a reduced number of osteoblasts which can reduce RANKL levels in bone, and elevated levels of anti-inflammatory cytokines.

Effect of T2 Diabetes on Osteoclasts

T2D effects on patient bone densities are variable with reports indicating lower, the same, or higher bone density than nondiabetic individuals. In T2D animal models, such as the leptin receptor knock out *db*/*db* mouse, mice display suppression of bone remodeling, with both bone formation and resorption decreased [151]. Yet, osteoclast activity in T2D male patients has been reported to increase [152, 153]. Serum concentrations of TRAP and urine markers for bone resorption, such as CTX, deoxypyridinoline, and N-telopeptide of type I collagen (NTX), are significantly elevated in T2D patients [154–156]. However, T2D patients can also exhibit increased levels of OPG which can reduce the increased resorption caused by T2D [157].

Role of Marrow Immune Cells in the Regulation of Bone Remodeling in Diabetes

Bone Marrow: Bone Remodeling Signaling

Immune cells are capable of regulating osteoblast and osteoclast activity and vice versa. The role of immune cell populations and their effect on bone marrow hematopoiesis has been demonstrated in a variety of pathology conditions associated with bone loss. For example, activated T-cells are involved in regulating bone homeostasis in estrogen deficiency, an effect that was mediated by IFNγ [158]. In ovariectomized (ovx) mice, T-cells express high levels of TNF-α (enough to induce osteoclastogenesis) [159]. T-cell deficiency prevents ovx induced bone loss, a benefit lost when mice are given T-cells from WT mice but not T-cells from TNFα-deficient mice [160]. Similarly, blockade of TNF-α and IL-1 by Anakinra or Etanercept decreases bone resorption in postmenopausal women [161]. This suggests that an increase in these cytokines can be one of the causes of bone loss. However, TNF-α, IFNγ, and IL-1 are not the only cytokines associated with bone loss. Another cytokine IL-7 promotes osteoclastogenesis by upregulating T- and B-cell-derived RANKL [162]. IL-23 also stimulates the differentiation of human osteoclasts from peripheral blood mononuclear cells (PBMC) in the absence of osteoblasts or exogenous RANKL and in vivo blockade of IL-23 activity prevents inflammation and bone destruction in collagen-induced arthritis in rats [163]. These findings provide a new insight into the interaction between immune system, bone marrow and bone loss.

Another intracellular system that is associated with changes in the bone marrow, immune cells, and bone density is Wnt signaling. Wnt signaling regulates the reciprocal relationship between adipocytes and osteoblast. Part of the mechanism whereby Wnt10b inhibits adipogenesis and stimulates osteoblastogenesis is by suppression of PPAR-γ. In addition, it has been found that expression of Wnt10b in

marrow increases trabecular bone mass and strength [37]. Wnt ligands are also required for hematopoietic progenitor cells (HSPC) expansion and survival. Experiments in ovx mice has shown that T-cell-expressed CD40 ligand (CD40L) enhance T-cell production of Wnt10b which results in activation of stromal cell (SCs) and HSPCs, along with improvement of cytokine production by SCs [164]. In summary, these results recognized the impact of cellular interaction between immune cells, HSPCs and the effect in bone homeostasis.

Effect of T1D on Marrow Cells

T1D is an autoimmune disease characterized by destruction of pancreatic β-cells producing insulin. White blood cells, like T-cells and macrophages, and the interaction and secretion of cytokines, nitric oxide, and free radicals are involved in this autoimmune pathology. Cytokines, such as IFNγ, IL-1β, and TNFα, can act together to induce beta cell death and can also affect the expression of genes that are protective or harmful for beta cell survival such as signal transducers and activators of transcription 1 (STAT-1) and interferon regulatory factor 1 (IRF-1) [169]. One study examining T-helper cell cytokine profiles indicated higher intracellular TNFα in $CD8^+$T cells at the time of T1D diagnosis and higher intracellular TNFα in $CD4^+$T lymphocytes in patients at 3 months post-diagnosis, compared with controls [170]. This suggests that there are distinct roles for cytokine release during different stages of T1D. Diabetic mouse models also display elevated TNFα levels in serum and increased TNFα mRNA level/expression in bone [52, 72]. In the STZ-induced diabetic mouse model IL-1α, IL-6, IFNγ, and TNFα expression are increased in bone with the onset of T1D and during the onset of altered expression of bone phenotype markers [72] (Fig. 8.3). Furthermore, it has been shown that T1D reduces the number of MSCs found at sites of fracture repair and that suppression of TNFα with Pegsunercept treatment restores the level of MSCs to control levels. It was further determined that TNFα is capable of reducing MSC proliferation and increasing MSC apoptosis [171].

Emerging evidence indicates that T1D directly compromises the function of the bone marrow [57]. Increased local cytokine levels in the bone marrow of mice under hyperglycemic conditions can disrupt hematopoiesis by shifting monocytes towards a pro-inflammatory phenotype [167, 172]. This change generated more monocytes and less endothelial progenitor cells (EPCs) in T1D, which contribute to the development of microvascular complications in this condition [166]. This finding was also evident in individuals with non-proliferative diabetic retinopathy [173]. In another experiment using Yorkshire pigs, after STZ-induction of diabetes a significant impairment of bone marrow and circulating EPCs as well as endothelial cells function were observed [174]. Chronic diabetes is also associated with dysfunction in the number and repopulation activity of HSPC, which is likely mediated by alterations in cytokines involved in stem cell renewal and maintenance [165]. Longstanding STZ-induced and spontaneous T1D mice further display impairment

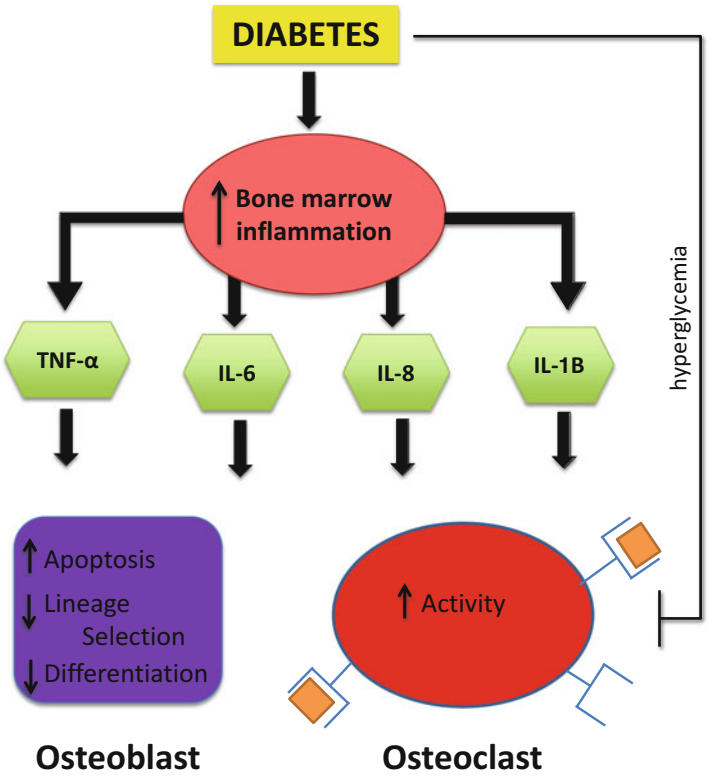

Fig. 8.3 Model for the role of inflammation in diabetes induced bone pathology. Both T1D and T2D conditions can increase bone marrow inflammation. The release of inflammatory cytokines into the bone marrow microenvironment by immune and bone cells can directly impact osteoblast and osteoclast activity which can potentially result in reduced bone turnover (T2D) or bone loss (T1D). TNF-α has extensively been shown to reduce osteoblast viability, lineage selection, and maturation. Similarly, IL-6, IL-8, and IL-1B can negatively affect osteoblast activity. Interestingly, while these cytokines are all capable of promoting osteoclast activity, conditions of diabetes such as hyperglycemia as well as expression of other inhibitory cytokines likely lead to the overall outcome of suppressed osteoclast activity typically seen in diabetes

of colony-forming capability of the bone marrow and an increase in the number of $Ly6C^{hi}$ (pro-inflammatory) monocytes in the peripheral blood [166].

The bone marrow is also the source of cells that contribute to diabetes-associated inflammation. Bone marrow transplantation experiments done in both mice and rats, demonstrate that most of the extrapancreatic proinsulin producing cells (found in liver, adipose tissue, spleen, and thymus) originated from the bone marrow [168]. These cells were also demonstrated to produce the pro-inflammatory cytokine TNF-α which could contribute to damage of the target organs [168]. This suggests that the damage seen in organs such as bone during diabetes may be due to the irregularity in bone marrow stem cells.

Effect of T2D on Marrow Cells

T2D is not an autoimmune disease and is not always associated with bone loss [175]. However, there are changes in hematopoetic profiles. For example, T2D patients have 33 % less circulating progenitor cells (CPCs) and 40 % less circulating EPCs compared with healthy subjects [176]. Peripheral vascular complications of T2D mellitus are associated with an extensively low number of EPCs [176] which could result from alterations in bone marrow function. Altered Wnt signaling is also associated with T2D. A single nucleotide polymorphism locus in the Wnt5b gene conferred susceptibility to T2D by modifying adipocyte function [177]. Wnt signaling is also altered in immune cells. A link between cellular glucose sensing and the Wnt/b-catenin pathway was recently reported in two macrophage cell lines (J774.2 and RAW264.7 cells) [178]. T2D is also accompanied by an increased inflammatory state that can disrupt osteoclast function and survival [67]. However, a small study in T2D patients examining changes in bone turnover markers found that treatment with nonsteroidal anti-inflammatory drugs causes an increase in osteocalcin levels in men (not in women) and increases serum C-terminal telopeptide (s-CTx) [179]. This small study suggests that T2D inflammation decreases bone turnover.

Potential Mechanisms Contributing to Altered Bone Remodeling

Several mechanisms are thought to contribute to diabetes complications and the bone remodeling changes that occur with diabetes. Diabetes is associated with hyperglycemia, hyperlipidemia decreased insulin signaling, decreased IGF-1, oxidative stress, and inflammation; all of which can contribute to suppressed osteoblast activity among other additional regulators including amylin and incretins. We will discuss several of these mechanisms briefly below.

Hyperglycemia

Hyperglycemia has many negative effects on bone and bone formation. High glucose can result in the production of ROS [180, 181], induction of cellular osmotic responses [182, 183], and increased nonenzymatic glycosylation of proteins and DNA [144, 184–188] as well as suppress calciotropic hormones and growth factors [62, 144, 180–185, 187–189], all of which can lead to decreased osteoblast activity. In vitro studies exposing MC3T3-E1 cells (osteoblastic cell line) to high glucose conditions (30 mM) showed a significant decrease in markers of osteoblast maturation, such as alkaline phosphatase and osteocalcin [90, 190]. Even when MC3T3-E1 cells are exposed to hyperglycemic conditions for short time periods, 48 h,

osteocalcin mRNA levels are significantly decreased compared to cells cultured under normal (5 mM) glucose levels [90]. MC3T3-E1 cells cultured in high glucose also show decreased mineralization [190, 191]. These in vitro results suggest hyperglycemia decreases osteoblast maturation.

Hyperglycemia also promotes MSC adipogenesis over osteogenesis [45, 60, 88–90]. Specifically, culturing primary rat osteoblasts under high glucose conditions (25.5 and 35.5 mM) increases levels of adipogenic markers (PPARy and aP2) and decreases levels of osteogenic markers (osteocalcin) [192]. A similar lineage switch was seen in human osteoblastic MG-63 cells cultured under high glucose conditions. The decrease in osteogenesis appears to be dependent on cAMP/protein kinase A (PKA)/extracellular signal-regulated kinase (ERK) signaling [193] and the PI3K/Akt signaling [192]. Hyperglycemia can also reduce osteoblast growth by 40 % [192] and induce osteoblast apoptosis. Both UMR and MC3T3-E1 osteoblasts undergo increased apoptosis when exposed for 24 h to AGE-BSA compared to unmodified BSA [194]. Thus, hyperglycemia can affect osteoblast activity through alterations in MSC lineage selection, osteoblast maturation, and osteoblast death.

Hyperglycemia decreases osteoclastogenesis in vitro. Using RAW264.7 cells and bone marrow macrophages, it was found that high levels of D(+)glucose inhibited osteoclast formation, ROS production (needed for bone resorption), caspase-3 activity (necessary for RANKL-induced differentiation) and migration to bone resorption pits [150]. In addition to this, osteoclasts derived from bone marrow cells extracted from mice which were exposed to high glucose had decreased mRNA expression of RANK and cathepsin K, decreased TRAP activity and decreased resorption activity [195]. Together, these studies indicate that a high glucose environment decreases osteoclast differentiation and activity.

Hyperglycemia also promotes a nonenzymatic reaction between glucose and proteins that results in AGEs. AGEs build up over time in diabetic tissues [196, 197] because they are irreversible modifications [198]. For example, T2D elderly patients display increased urine pentosidine (an AGE) which was associated with increased clinical fracture incidence and increased vertebral fracture prevalence in those with diabetes but not in those without diabetes [199]. Pentosidine, pyrraline and *N*(carboxymethyl)lysine (CML) are some of the more commonly characterized AGE products whose levels increase in tissues with collagen matrices, particularly pentosidine, under conditions of diabetes [200–202] and aging [199, 203, 204]. AGE modifications can change the structural and functional properties of proteins and correspondingly their levels correlate with diabetes complication severity, including glycosylated hemoglobin levels and bone loss [188, 201, 202, 205–209]. Type 1 collagen, the most abundant constituent of the bone matrix, is a target for AGE [210, 211], which can accumulate and alter the mechanical properties of bone, decreasing its toughness and could therefore contribute to skeletal fragility [212–214]. AGE increases collagen crosslinking, which can decrease bone strength and ductility, and increase brittleness [209, 214–218]. An increase in AGEs have been linked to a reduction in serum osteocalcin levels and are positively associated with levels of pentosidine which impairs the biomechanical properties of bone [216, 219–221]. AGEs can modify bone cell behavior by interacting with specific cellular

receptors, such as RAGE (receptor for AGEs). When AGEs bind to RAGE on osteoclasts, NF-kB activity and ROS increase [222, 223]. RAGE is also expressed in osteoblasts and AGE treatment of osteoblasts causes decreased osteoblast maturation [184, 222]. In vitro and in vivo studies showed that AGEs increase calvarial periosteal cell apoptosis and induced apoptosis in primary cultures of human or neonatal rat osteoblastic cells. This effect was mediated through RAGE and increased p38 and c-Jun N-terminal kinase (JNK) [224]. The presence of AGE can also induce osteoblast apoptosis through activation of caspase-3 and caspase-8 [105, 108, 197]. Consistent with these findings, deficiency of RAGE results in an increased BMD compared with control mice [225]. Interestingly, in vitro studies using AGE-modified bone slices and osteoclastogenesis assays have shown that resorption was markedly inhibited. This was confirmed by a marked decrease in the release of type I collagen fragments generated by the collagenolytic enzymes secreted by osteoclasts [226]. This decrease in bone resorption is thought to be due not only to the alteration of the structural integrity of the bone matrix by AGEs, but also their effect on osteoclastic differentiation [226]. AGEs also increase inflammation by monocytes and macrophages due to upregulation of TNFα and IL-1β [227].

Hyperglycemia can also increase oxidative stress. Oxidative stress is triggered through two ways: the mitochondria becoming overloaded with glucose or through AGEs and Polyol signaling [88]. Oxidative DNA damage markers such as 8-hydroxydeoxyguanosine are increased in hyperglycemic diabetic mice but decreased when the mice are treated with insulin and become euglycemic [120, 228]. This oxidative stress marker is also elevated in T2D rats and could contribute to decreased osteoblast maturation based on the in vitro findings of oxidative stress negatively affecting osteoblast maturation [120, 122, 229, 230]. In addition, hyperglycemia-induced osteoblast apoptosis has been linked with an increase in oxidative stress [51, 231]. ER-stress activates the unfolded protein response mechanism leading to increased C/EBP homologous protein (CHOP) signaling and cellular apoptosis [51]. T1D rats showed an increase in CHOP positive osteoblasts in the femurs which also showed decreased bone mineral density [51].

Wnt Signaling

An important regulator of bone formation is Wnt10b [37, 232]. Wnt10b is critical for promoting MSCs toward the osteoblast lineage [232], enhancing osteoblast maturation and suppressing osteoblast apoptosis. Wnt10b activity increases expression of osteogenic transcription factors such as Runx2, Dlx5, and osterix [37]. In addition, targeted overexpression of Wnt10b in mouse bone leads to increased trabecular bone density [234], while Wnt10b knockout mice display significant bone loss [37]. Wnt10b binds its receptors LRP5, LRP6 and frizzled to increase intracellular beta-catenin levels and stimulate gene expression through TCF/LEF transcription factor binding to gene promoter regions. Alterations in the level or in the activity of LRP5 markedly affect bone density. Specifically, activating mutations lead to increased bone density [235] while inactivating mutations or LRP5 deficiency

results in osteoporosis [35, 37, 236]. Similarly LRP5 mutations in humans can increase or decrease bone density [237].

Given that diabetes is marked by reduced bone remodeling and reduced osteoblast activity, a role for altered Wnt signaling has been suggested. It was recently found that TNFα transgenic mice display decreased Wnt activity implying a potential interaction between TNFα and the Wnt signaling pathway [238]. As noted previously TNF α is increased with diabetes [227, 228] and its expression is elevated in T1D mouse bone [72]. Correspondingly, Wnt10b expression is decreased in T1D bone (unpublished data, McCabe) suggesting that Wnt10b downregulation could contribute to the T1D bone phenotype [239, 240]. This is supported by decreased β-catenin staining of osteocytes and osteoblasts in diabetic mouse bones [241]. Interestingly, sclerostin, a circulating inhibitor of Wnt signaling made by osteocytes, is demonstrated to be elevated in T2D patient serum [242]. This could function to suppress Wnt10b and overall bone remodeling in T2D. Future studies will further determine the role of Wnts and other regulators of bone remodeling such as BMPs in the pathophysiology of diabetic bone.

Insulin and IGF-1 Signaling

Because this is already discussed in Chap. 1, we will only briefly discuss the role of insulin and insulin growth factor-1 (IGF-1) changes in diabetes that lead to altered osteoblast activity. Osteoblasts express insulin receptors and can readily respond to insulin treatment [33]. IGF-1 binds to insulin growth factor-1 receptors (IGF1R) and can activate osteoblast maturation, proliferation, and increased matrix generation [244]. Bone resorption is also regulated by IGF-1 acting through osteoblasts [244].

A hormone co-secreted with insulin, amylin is also suppressed in TD1 [48, 145]. IGF-1 has been reported to be decreased in T1D patients and animal models [245, 246]. Decreased IGF-1 in serum has been linked to the reduction in bone mineral density, increased adiposity in bone marrow, and decreased osteoblast differentiation. IGF-1 treatment in diabetic rats was able to increase the mineral apposition rate (rate at which new bone is formed) when compared to diabetic controls [65].

To determine if the lack of insulin is responsible for bone loss, insulin receptor knockout mice were analyzed for osteogenic and adipogenic markers. There was no change in osteogenic markers when compared to the control mice but adipogenic markers were decreased [62, 72, 79]. To analyze the effects of insulin-resistance (T2D) on osteoblasts, a mouse strain was generated to reproduce osteoblast insulin-resistance [247]. These mice were then fed a high-fat diet to induce T2D. Circulating osteocalcin levels were decreased and glucose intolerance worsened. The results implicate osteoblast insulin signaling in whole-body glucose homeostasis. Mice that lack insulin receptor signaling in osteoblasts exhibit either no change in osteoclast number [44] or a marked decrease in bone resorption markers [43]. The latter suggests a role for osteoblast insulin signals in enhancing resorption, consistent with studies demonstrating reduced or unaltered resorption in humans and mouse models of T1D. In both studies, OPG was increased and may be involved in suppressing osteoclast activity in T1 diabetics similar to other studies in T1-diabetic mice [67].

Role of Hyperlipidemia

Diabetes is associated with increased serum lipids. Hyperlipidemia has a number of adverse effects, including osteoporosis [250–254]. Hyperlipidemia results in increased amounts of LDL particles which can accumulate in the subendothelial space and are oxidatively modified to increase the generation of lipid oxidation products [255]. These lipid oxidation products can accumulate in bone [256]. Studies examining the effect of hyperlipidemia on bone health revealed increased levels of parathyroid hormone, TNF-α, calcium and phosphorus, and carboxyl-terminal collagen cross-links (a marker of bone resorption) [257]. Diabetic patients exhibit changes in serum lipid profiles along with hyperlipidemia linked with a decrease in metabolic control [258–260]. In female T1D patients, there was a significant correlation between increased serum lipid levels and decreased bone density [96]. Serum lipids can serve as ligands to activate PPARγ2 and promote altered MSC lineage selection and thus could play a role in suppressing osteoblast activity. However, in T1D mice, inhibition of PPARγ2 activity prevented hyperlipidemia but did not prevent bone loss [79]. This suggests that hyperlipidemia may not play a major role in T1D bone loss but could contribute to the altered lineage selection of MSC.

Studies examining the role of hyperlipidemia during osteoclastogenesis utilized pre-osteoclasts from hyperlipidemic mice (*LDLR*−/− mice) and control mice. After induction with M-CSF and RANKL, there was an increased number of resorption pits indicating an increase in osteoclastic activity [256]. In addition to this, there was also an increase in TRAP activity, indicating increased osteoclast differentiation [256]. Another study using *LDLR*−/− mice showed that serum levels of CTX, a marker for bone resorption was significantly increased in hyperlipidemic mice [257]. Together, these studies indicate a role for hyperlipidemia in osteoclast differentiation and activation. Although hyperlipidemia is associated with diabetes and is linked with osteoclast activity, hyperglycemia, the driving force of diabetes, decreases osteoclast activity and may work to override the effects of hyperlipidemia seen in patients.

Role of Calcitropic Hormones and Leptin

Studies are controversial regarding the effect of T1D on the serum level of vitamin D, which is key for enhancing intestinal calcium absorption and enhancing some stages of osteoblast maturation. While some studies report no changes in vitamin D levels, others report decreased levels in over 75 % of diabetic children and adults in the US northeast [261–263]. However, it is difficult to distinguish T1D associated vitamin D deficiency since more than 50 % of control subjects in winter months experience vitamin D deficiency [261]. In addition to the controversy about vitamin D deficiency, PTH levels in T1D patients are unchanged or decreased and in T2D patients are decreased [71, 72, 77, 264–266].

Another regulator of bone density is leptin [46]. Leptin is produced and secreted by adipocytes and has been shown to regulate lineage selection by stimulating MSC to differentiate into osteoblasts over adipocytes [189, 267, 268]. In T1D patients,

serum leptin levels vary between patients [45, 269] although some studies report a decrease [270, 271]. Chronic treatment of hypoleptinemic T1D mice with leptin did not prevent bone loss but did reduce marrow adiposity [46]. T2D patients are even more complex with various factors affecting leptin levels including insulin secretion and resistance, gender and fat mass [33]. In vitro experiments show that leptin stimulates osteogenesis over adipogenesis [118, 189, 268].

Inflammation

Both obesity (which can result in T2D) and diabetic conditions can increase bone marrow inflammation. Inflammatory cytokines can directly impact osteoblast activity through changes in lineage selection, maturation, and death. T1D patients and mice have increased inflammation [108, 272–274], and elevated expression of inflammatory cytokines have been identified in T1D mouse bone [72]. Similarly, serum pro-inflammatory cytokines are increased in T2D patients [275–278]. Pro-apoptotic inflammatory cytokines like TNF-α induce osteoblast apoptosis and have been shown to be elevated in both T1D and T2D patients [46, 83, 147]. Elevated TNF-α mRNA levels are increased in T1D mouse whole crushed bone [83, 147], bone marrow [52], and gingival tissue [279]. When TNF-α binding to its receptor is inhibited by Pegsunercept, diabetes-associated osteoblast apoptosis is reduced [49, 280]. TNF-α and other pro-inflammatory cytokines such as IL-6 and IL-1 can also negatively affect osteoblast differentiation/activity and therefore suppress bone formation [281]. For example, in vitro studies show when cultured osteoblasts are treated with inflammatory cytokines (IFN-γ), there is a decrease in alkaline phosphatase activity [282].

Inflammation in the bone marrow favors expansion of osteoclasts and an increase in their activity. This increase in number can not only lead to bone loss, but also lead to impaired HSC maintenance and homeostasis [283]. Osteoclastic bone resorption can be activated by infection and inflammation. Because osteoclasts come from the same lineage as many immune cells, they express many innate immune cell receptors, such as toll-like receptors which can respond to antigen stimulation [284–286]. In addition to antigen stimulation, pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6 can induce osteoclast-mediated resorption [287, 288]. Studies in T1D mice have shown that induction of an immune response by the bacterial antigen LPS induces altered osteoclast differentiation with mature osteoclasts having increased bone resorption activity and increased release of osteoclastogenic factors (cathepsin K and MMP-9) compared to diabetic mice without LPS stimulation [289]. Cytokines secreted from adipose tissue, IL-6, IL-8, and TNFα, can both activate osteoclast bone resorption and decrease bone formation through the suppression of osteoblast differentiation [281]. While inflammatory conditions can directly activate osteoclasts, the effect of inflammation on osteoblasts can indirectly decreases osteoclast maturation and activity due to the extensive cross talk between the two cell types. A study examining the effects of bacterial-induced inflammation in T2D animals (*ob*/*ob*) showed that bone loss was not due to increased osteoclastogenesis but was dependent on the decreased levels of bone formation [112].

Summary

Diabetes affects bone remodeling through multiple mechanisms and alterations in multiple cell types. T1D and T2D have similar and different mechanisms at play that result ultimately in increased fracture risk. While we have mentioned several mechanisms here, there is still much to be learned and more contributors to the bone phenotype are likely.

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Part II
Biomechanics and Bone Quality

Chapter 9
Material Properties of Diabetic Bone

Jeffry S. Nyman and Amy Creecy

Role of Bone Structure in the Diabetes-Related Decrease in Fracture Resistance

As described in a number of review articles [1–3], the hierarchical organization of bone (Fig. 9.1) confers multiple mechanisms of fracture resistance. As such, diabetes-related changes to any level of organization (collagen fibrils at the nanoscale to cortical thickness at the macro-scale) can affect the ability of bone to resist failure [4]. Material properties depend on all aspects of bone organization except the macrostructure. In order to delineate the role of material properties in fracture resistance, bone structure is first defined with respect to diabetes.

Structural properties are derived from the cross-sectional geometry of cortical bone (the analog for trabecular bone is architectural properties) and include the moment of inertia (or second moment of area), endocortical circumference, periosteal circumference, bone cross-sectional area (cortex only), and total cross-sectional area (cortex and marrow space). They are related to one another; but from the perspective of engineering mechanics, moment of inertia and bone cross-sectional area characterize the ability of whole-bone to resist bending/torsion and compression/tension, respectively. Also, an increase in the periosteal circumference—for example, during growth—imparts a greater increase in the principal moment of

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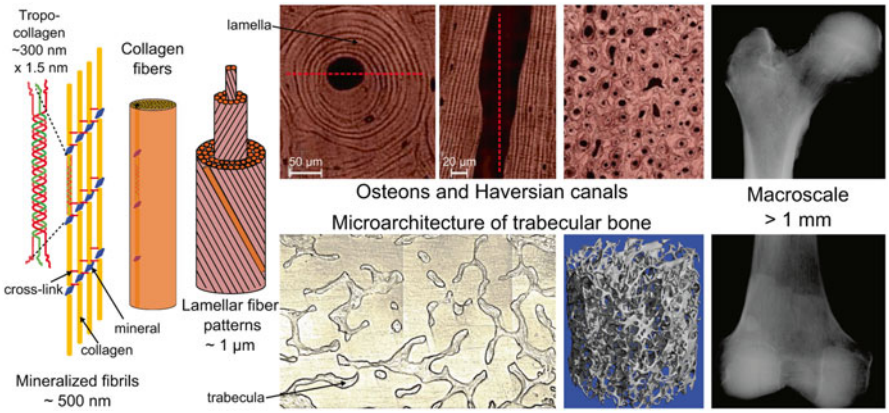

Fig. 9.1 The ability of bone to resist fracture depends on each level of organizational hierarchy from the triple helix of collagen being cross-linked (*left*) and filled with mineral crystals through arrangement of osteons or trabeculae to the shape (*right*)

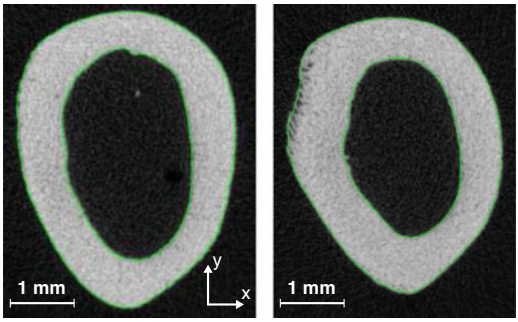

Fig. 9.2 The cross-sectional area (Ct.Ar) of a femur mid-shaft can be similar between two different rats, as an example, while the moment of inertia (I_{min}) is considerably different. If the material properties are equivalent between the animals, then the strength in compression will be similar but weaker in bending for the diabetic than for the nondiabetic rat

inertia (I_{min} or I_{max}) than an increase in bone cross-sectional area (Ct.Ar). Given equivalent material properties, normal bone and diabetic bone would break at the same force when loaded in compression if there is no difference in Ct.Ar, but the same diabetic bone would break at a lower force when loaded in bending if the I_{min} is less for the diabetic bone than for the normal bone (Fig. 9.2). That is, two different bones can have the same Ct.Ar but different values of I_{min} or polar moment of inertia ($J=(I_{min}+I_{max})/2$) in the case of torsion. Also, the moment of inertia varies with the orientation of the bone relative to the bending axis (Fig. 9.3). Therefore, the invariant calculations are often reported in the literature (I_{min} corresponds to bending about the minor axis of the bone cross section or the minimum distance between centroid and the periosteal surface).

From human imaging studies, there is evidence that type 1 diabetes (T1D) affects bone structure in deleterious way [5], especially if glucose is poorly controlled or if T1D starts at a young age [6]. However, since most T1D patients are treated with

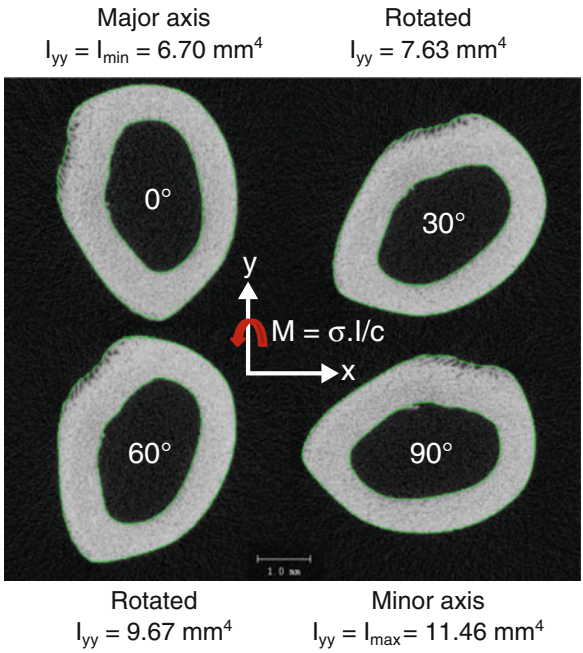

Fig. 9.3 As a bone rotates with respect to the axis of bending, the moment of inertia changes. For a full rotation, there is maximum value and minimum value. In bending tests of rodent femurs to determine material properties, the minimum principal moment of inertia corresponds to bending about the medial-lateral axis

insulin, bone structure can normalize with age [7] and structural indices of the hip do not necessarily differ between men (43.6 ± 5.1 years of age) with long-lasting T1D and those without diabetes [8]. For the most part, cortical bone structure of individuals with type 2 diabetes (T2D) does not significantly vary from that of age- and gender-matched nondiabetics as determined by various X-ray computed tomography (CT) scans [9–11], though there are reports of hip strength indices being adversely affected by T2D [12–14]. Additional information on bone structure can be found in Chap. 11.

The consequence of diabetes on size-dependent, structural strength is fairly well established for rodents (see reviews by Nyman [4] and Fajardo et al. [15]), but the mechanism of action is not particularly clear. Whether there is a loss of insulin production or an increase in insulin resistance in rodents, bones become structurally weaker and less rigid [16–19]. Of note though, diabetes develops before skeletal maturation in most rodent models of T2D, which can explain the discrepancy between rodent and human studies of bone structure involving T2D. Differences in bone structure between control mice and mice injected with streptozotocin (STZ), a toxin that decreases insulin production by the pancreas, explain the lower whole bone strength that occurs with the onset of T1D (Fig. 9.4) [19]. The primary cause for the reduction in bone size with diabetes is difficult to delineate in rodents as insulin, leptin, insulin-like growth factor-1 (IGF-1), and glucose levels cannot be independently controlled. Certainly, insulin and IGF-1 signaling are important to bone mass accrual [20, 21]. When the insulin receptor (IR) was conditionally deleted in cells of the osteoblast lineage (Osterix-cre), the femur mid-shafts of the mutant mice were strikingly narrow or slender and thus weaker than those from control littermates [22]. This effect was independent of body weight. Loss of

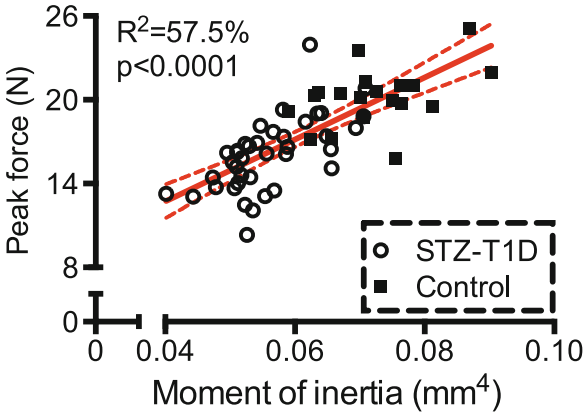

Fig. 9.4 In the streptozotocin (STZ) model of type 1 diabetes, a lower moment of inertia (structure) explains a significant portion of the lower bone strength in the diabetic mice compared to the age- and strain-matched controls

insulin signaling however is likely not the only cause for deficits in diabetic bone structure. Rats with early onset T2D are hyperinsulinemic before 16 weeks of age, but yet, their bones have a much smaller moment of inertia than do the nondiabetic control rats before and after skeletal maturity [16] suggesting insulin resistance in osteoblasts or the action of other factors contribute to the deficit in cortical bone structure. Also, when human IRs were activated in the pancreas, liver, and brain of mice lacking IR in all cells including osteoblasts, there were no difference in bone properties between genotypes (euglycemic condition) [23]. These mutant mice are known to be hyperinsulinemic [24] and express more IGF-1 receptors in bone (insulin can bind IGF-1 receptors and vice versa) [23]. When a structural deficit in bone does occur with diabetes, it is likely the result of both a loss of anabolic action of insulin/IGF-1 on osteoblasts (especially in early-onset diabetes) and inhibition of bone formation by another mechanism (e.g., oxidative stress due to hyperglycemia).

While poor bone structure (e.g., thinning of cortex) can lower areal bone mineral density (aBMD) as determined by dual-energy X-ray absorptiometry, the increase in fracture risk with T1D is disproportionate to the reduced aBMD [25]. Even though the elevated fracture risk is not as high for individuals with T2D, this increase also occurs independently of aBMD and bone structure [26–28]. Thus, diabetes must cause other changes to bone that lower fracture resistance. What follows is a tutorial on bone mechanics and review of studies reporting whether diabetes affects the material properties of bone.

Engineering Mechanics: Assessing Material Properties of Bone

Engineering materials (e.g., metal alloys, polymers, ceramics) break in many ways, and bone is no different. Intuitively, a bone fracture occurs when a region of the skeleton (e.g., femoral neck or distal radius) experiences a load that exceeds the

maximum force that the bone region can sustain. Perhaps less obvious is that a healthy bone can yield (i.e., experience permanent damage) but not break. As long as that bone does not experience another high load too soon, remodeling removes the microdamage by replacing it with new tissue and returning the bone tissue to its normal material strength. In the case of unhealthy bone that cannot sustain damage after yielding, there is a "brittle" fracture. Through locomotion, bones—especially the femur, tibia, and spine—experience repetitive loads that are well below the yield force. Because there are numerous "flaws" in bone (e.g., lacunae, canaliculi, Haversian canals, Howship's lacuna, and vacancies in the mineral) acting as stress risers, this cyclic loading at a low force causes microdamage formation over time. In the case of trabecular bone, microdamage accumulation from a bout of overloading [29] or fatigue loading [30] lowers the apparent-level strength. For either cortical or trabecular bone, the accumulation of microdamage eventually causes fatigue failure if allowed to proceed unchecked. Clinically, this leads to a stress fracture [31]. Such a bone stress injury has been postulated as a pathological mechanism of stage 0 diabetic Charcot foot [32].

Because materials can fail in a number of ways, such as an overload, insufficient energy dissipation, and fatigue, there are multiple engineering techniques to assess material properties. Comprehensive descriptions of these techniques can be found in several books on mechanical testing of bone or biomaterials [33–35]. Briefly, in all the techniques, force vs. displacement (f vs. d) data is recorded as a bone specimen is loaded to failure (i.e., stretched, compressed, twisted, or flexed). This is not the case for tests characterizing elastic or viscoelastic properties but is necessary to characterize post-yield behavior. After testing the specimen with an instrument that has a load cell (force) and an actuator (displacement), the f vs. d is processed in such way to remove specimen size (or structure) from the mechanical behavior. As a simple example, f vs. d from a monotonic, load-to-failure, tensile test of cortical bone is converted to engineering stress (σ) vs. engineering strain (ε) in which σ is f divided by the cross-sectional area of the specimen and ε is d divided by the original gage length of the extensometer, a device that clips to the specimen and records displacement between two knife edges (Fig. 9.5). The material properties are then determined from the resulting σ vs. ε curve (Fig. 9.5). Similar analysis occurs for bending tests (three point or four point) except that the equations to determine σ vs. ε differ as they are derived from beam theory and use the moment of inertia, instead of cross-sectional area of the specimen [36]. Also, in bending tests, displacement can be recorded from a deflectometer or the instrument's linear variable displacement transducer that is part of the actuator.

With respect to fatigue testing, the primary outcome is fatigue life in which failure can be defined several ways: (1) actual numbers of cycles of loading experienced by the specimen before breaking (N_f), (2) the number of cycles to reach a specified level of creep (permanent deformation or subsidence in the case of compressive testing) or a specified level of modulus loss, and (3) the steady-state creep rate (Fig. 9.6). Unlike most monotonic tests, fatigue tests are run in force control imparting a constant stress-amplitude (σ_a = maximum σ minus the minimum σ during cyclic loading divided by 2). Although difficult in bone studies because the amount of material to generate replicates is limited, characterizing fatigue properties

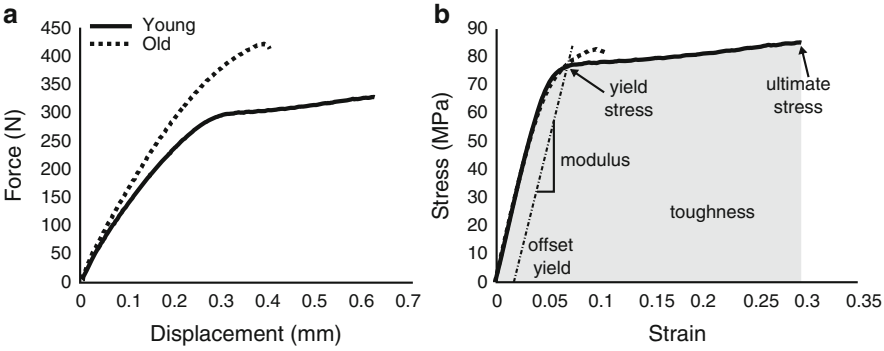

Fig. 9.5 Using the cross-sectional area of the specimen and gage length of the extensometer, the resulting force vs. displacement curve (**a**) from a tensile test is converted to an engineering stress vs. engineering strain curve (**b**) from which the material properties are determined. Determination of material properties are marked on curve for the bone specimen from a relatively young donor

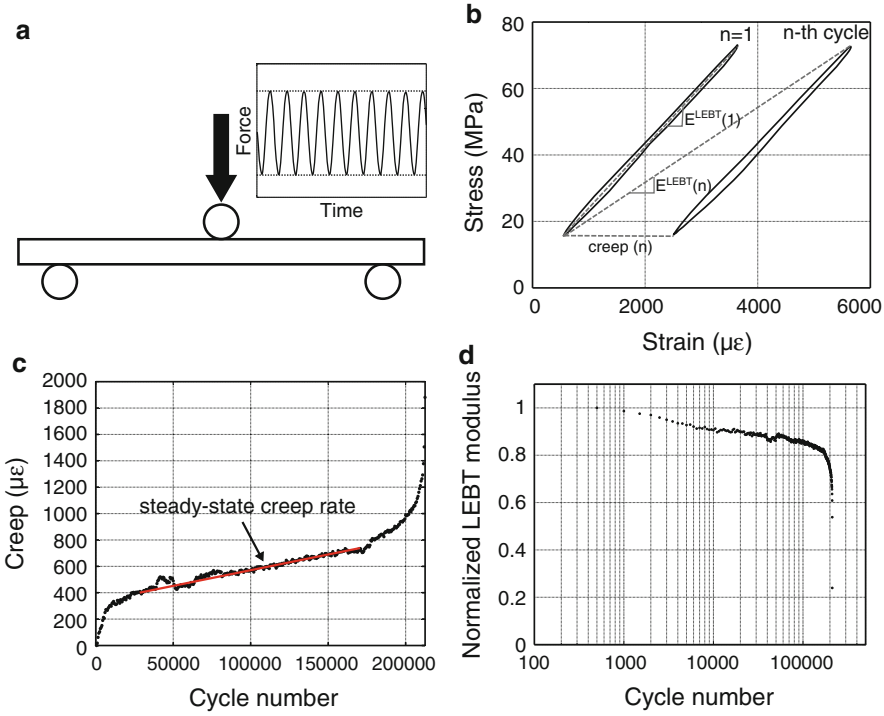

Fig. 9.6 In a fatigue test, the bone is dynamically loaded (sinusoidal wave profile) below the yield force (**a**). The force vs. displacement data is converted to stress vs. strain data to calculate changes in modulus, as determined by linear elastic beam theory (LEBT), and creep or permanent deformation (**b**). Creep occurs at a constant rate during most of the life of the specimen (**c**). As the number of cycles increases, the modulus decreases during fatigue (**d**)

typically involves loading multiple sets of specimens at different stress amplitudes to generate a σ_a vs. N_f plot or *S–N* curve. Alternatively, σ_a can vary across specimens to ensure that the initial maximum strain experienced by each specimen is constant (e.g., 4000 microstrain). Comparing results across fatigue studies is difficult because a number of extrinsic factors affect fatigue life (e.g., frequency of loading, waveform, initial strain, specimen geometry, loading mode, and the ratio of σ_{max} to σ_{min}).

Because fatigue testing does not delineate the ability of bone to resist microcrack initiation from its ability to resist microcrack propagation and accumulation, there is another type of material characterization technique known as fracture toughness testing. Involving a sharp notch that acts as the "worst flaw" in the material, a fracture toughness specimen can be loaded cyclically (fatigue crack growth resistance), monotonically (crack initiation toughness or total nonlinear strain energy release rate), or progressively (R-curve method to measure crack initiation and crack propagation toughness simultaneously).

Fracture toughness tests essentially characterize the ability of a material to resist fracture when a flaw is present. Engineers use this material property to determine what is the tolerable flaw size that a particular structure can have for a given service load without failing. There are acoustic devices to determine the size of flaws or cracks in structures such as airplane wings, but no such method is available for detecting microcracks in bone in vivo. Acoustic techniques such as ultrasound or acoustic emission are however being developed to monitor microdamage progression in in vitro tests [37, 38]. Traditionally, the assessment of microdamage morphology ex vivo involves bulk staining with a chelating agent and fluorescence microscopy [39] and more recently contrast-enhanced micro-CT (μCT) using an agent (e.g., barium sulfate) with higher X-ray attenuation than bone [40]. Such methods have been used to demonstrate associations between loading mode (compression, tension, torsion) and damage morphology (linear microcracks, diffuse damage) [41, 42]. To the best of our knowledge, differences in microdamage morphology between diabetic and otherwise normal bone has not been investigated.

Combining Material Properties with Bone Structure Using Finite Element Analysis

There is a way to incorporate the contributions of both structure and material to fracture resistance: apply the finite element method to the boundary value problem for a continuum body like bone. Two centuries after the first application of mechanics to bone by Galileo (1638), anatomists and engineers—Ward, Engel, von Meyer, and Culmann—connected the structure and architecture of bone to the stresses acting within bone [43]. While elegant graphical techniques matched bone structure/architecture to principal stress directions, the basis of Wolff's law (1892), bone geometry is too complex for deriving analytical solutions using the theory of elasticity to determine the displacement response to prescribed boundary conditions. This was overcome in the 1960s for all complex structures when a numerical method

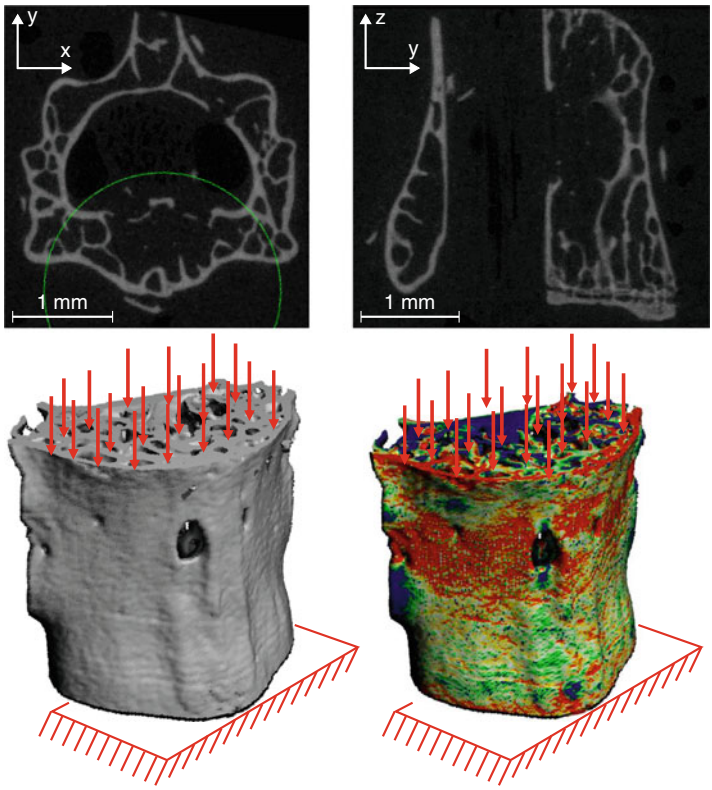

Fig. 9.7 A finite element model can be directly converted from the voxels of a micro-computed tomography scan of a bone. The finite element analysis calculates the strain distribution throughout the bone. In this example, the boundary conditions of the mouse lumbar vertebra simulate compression loading

was developed to solve the equilibrium, strain–displacement, and stress–strain equations comprising the boundary value problem in solid mechanics [44]. Since then, finite element analysis (FEA) has been the cornerstone of engineering design. Beginning in the 1990s, researchers in the field of orthopaedic biomechanics began developing three-dimensional finite element models of bone [45] and bone implants [46]. This became possible with the advent of quantitative CT (QCT) because voxels in the CT scan can be directly converted to elements that discretize the complex bone geometry into a mesh (Fig. 9.7) allowing for the creation of the linear algebra problem that is then solved in an iterative fashion by a finite element solver. Moreover, CT attenuation can be converted to material property definitions when incorporating a hydroxyapatite (HA) phantom into the scan.

With ample evidence that predictions of stiffness and strength by QCT-FEA strongly correlate with experimental measurements as determined by whole bone testing of cadaveric tissue, namely the proximal femur [45, 47, 48], distal radius [49, 50],

and vertebra [51], the methodology has been applied to QCT scans of patients in clinical trials testing the efficacy of drugs to prevent fractures in mainly postmenopausal women (but also men in the MrOS cohort). For example, differences in predicted strength between placebo and therapy have been reported for alendronate (a bisphosphonate) [52, 53], combined teriparatide (recombinant parathyroid hormone 1–34) and alendronate [54], alendronate and then teriparatide [55], teriparatide to treat glucocorticoid-induced osteoporosis [56], odanacatib (cathepsin K inhibitor) [57, 58], denosumab (anti-RANKL antibody) [59], combined odanacatib and teriparatide [60], and combined teriparatide and denosumab [61]. An open question is how well these strength predictions explain the drug-related reduction in fracture incidence after adjusting for aBMD, which of course increases with treatment. QCT-FEAs are capable of fracture discrimination [52, 62–66], though some overlap in predicted strength exists between non-fracture and fracture cases [63, 67]. In a 5-year case–control study (>65 years of age) using baseline QCT scans of the spine and hip to develop the finite element model, the age-adjusted odds ratio for vertebral and hip strength predictions was significant for women and men [68]. That is, women and men with low vertebral strength or low hip strength, as predicted by QCT-FEA, were ~2 or ~4 times more likely, respectively, to suffer a fracture, and these odd ratios were still significant even if adjusted for most aBMD measurements.

To date, there is a paucity of studies investigating whether CT-derived FEA is useful for identifying diabetics who are at risk of a fracture (see Chap. 3). QCT-FEA or even high-resolution (HR)-peripheral CT (pCT)-FEA might not be particularly effective in the case of diabetes, unlike osteoporosis. The power of FEA is its ability to account for the structure in the response of a continuum body to loading or specifically boundary conditions, and as previously mentioned, diabetes does not necessarily affect bone structure. Moreover, the material properties must be defined in the FEA, and they are typically based on assumed empirical relationships between elastic modulus and bone density or strength and bone density acquired from mechanical testing of cadaveric tissue. There are no such relationships derived from diabetic bone. FEA calculates the stress–strain distribution throughout the bone as well as displacement for a given prescribed boundary condition (e.g., side-ways fall in which the trochanter is fixed against a rigid surface and the femoral head experiences a high joint reaction force). It does not actually calculate the strength of the bone. FEA predicted strength requires failure criteria (strain at which elasticity no longer holds), and yield strain or the damage tolerance of diabetic bone relative to normal bone is not yet known.

Age- and Diabetes-Related Changes in Material Properties of Bone

There is little known about the effect of T1D or T2D on the material properties of human bone or bone from nonhuman primates. This is likely to change in the coming years, as there is a growing recognition for the need to understand how diabetes

is increasing fracture risk. The challenge to the assessment of material properties is the procurement of bone samples that can be machined into the proper test geometry. Traditionally, this is done using cadaveric bone, but information about whether the donor had diabetes and for how long is rarely provided by willed body programs and tissue allograft banks. Alternatively, bone biopsies from surgeries or from the iliac crest can be analyzed by other techniques such as μCT, Raman spectroscopy, Fourier transform infrared spectroscopy, high performance liquid chromatography, and quantitative back-scatter electron microscopy [69] to identify organizational and compositional differences between diabetic and nondiabetic bone. Such information would inform as to whether diabetes affects bone quality. In on-going work, trabecular bone biopsies are being collected from patients undergoing total hip arthroplasty (osteoarthritis, not hip fracture), and those with glycated hemoglobin levels (HbA1c) greater than six tend to have bone with lower apparent strength and higher AGEs [70].

To the best of our knowledge, there are only three studies that reported material properties of human bone from donors with diabetes. In the earliest study (1998), metatarsals were acquired from amputations (30 diabetics and 19 nondiabetics) performed for a variety of reasons (elective, trauma, infection, tumor) [71]. The type of diabetes was not specified nor the duration. An additional set of metatarsals was acquired from 29 cadavers. Tested in the three-point bending, the estimated modulus and strength of the diaphysis (cortical bone) did not differ between the two groups. However, there was an age discrepancy in which the diabetics were 51.3 ± 8 years of age and the normal controls were 72.4 ± 10 years of age, suggesting the effect of diabetes on bone material properties is similar to the effect of aging. In a follow-up study by the same group (2006), beam specimens (nominal dimensions of $30 \times 4 \times 2$ mm) were extracted from the anterior aspect of tibia, which were acquired from amputations (seven diabetics and seven nondiabetics) [72]. Again, there were no differences in the material properties (modulus, strength, and fracture toughness) between the younger diabetic bone and the elderly nondiabetic bone. While an extended period of non-ambulation (unspecified) was part of the exclusion criteria, confounding factors related to the amputation limit the interpretation of these findings. Nonetheless, the idea that diabetes is a form of accelerated aging with respect to material properties of bone is intriguing. In the third study, the fracture toughness of cadaveric bone from one donor with diabetes (duration not specified) was 40 % lower than cadaveric bone from several donors without diabetes [73].

Given the dearth of information about how diabetes affects the material properties of human bone, we turn to the literature reporting how material properties of cadaveric bone change with age. Evidence that the material properties of human cortical bone changed with age began to appear in the literature around the time that the finite element method was being developed [74–76]. However, there were several studies that did not find age-related changes in tensile strength [77, 78] owing to discrepancies in sample size (sometimes less than ten) and storage conditions (frozen vs. fixed). Then, testing 175 tensile specimens from 33 cadaveric femurs acquired from donors spanning 20–89 years of age, Burstein et al. showed that yield and ultimate stress (i.e., material strength) significantly decreased 2.2 % per decade

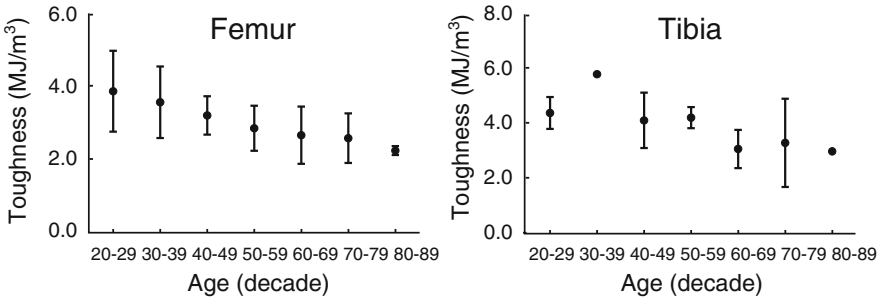

Fig. 9.8 The toughness of human cortical bone decreases over life-span (adapted from Burstein et al. [79])

and that energy dissipation (i.e., toughness) significantly decreased by 6.8 % per decade (Fig. 9.8) [79].

Age-related decrease in material strength of cortical bone has since been reported by a number of independent groups [80–83], though there is variance in the relationship between strength and age (some elderly donors have strong bones). Material strength can be measured as the stress at yielding when permanent damage begins to form or as the peak stress endured by bone during failure. A primary determinant of this decrease in material strength is an age-related decrease in apparent bone density (wet mass divided by the specimen volume or radiation absorption), but it is not the sole determinant [80] as the rate of decrease in strength is greater than that of density [81]. Of course, apparent bone density is product of intracortical porosity and degree of mineralization (or ash fraction). Both characteristics inversely or directly correlate, respectively, with strength [84, 85]. Therefore, if diabetes causes an increase in porosity or decrease in mineralization, then it would decrease the material strength of bone, thereby increasing fracture risk.

Collagen cross-links via lysyl oxidase are another important determinant of bone strength. When female rats were administered the toxin β-amino-propionitrile (BAPN) to inhibit lysyl oxidase, there was a decrease in material strength of bone that accompanied a decrease in mature enzymatic collagen cross-links (pyridinolines) without affecting mineral density [86]. Recently, treatment of growing male mice with BAPN resulted in reduced cortical bone strength, reduced fracture toughness, and reduced mature-to-immature cross-link ratio (these properties were positively correlated) without affecting tissue mineral density as determined by μCT [87]. Diabetes could potentially alter enzymatic collagen cross-linking. The B6 vitamer known as pyridoxamine is a cofactor of lysly oxidase, and diabetes via an oxidative stress pathway could potentially decrease enzymatic activity. There is at least one study reporting a decrease in pyridoxamine and enzymatic collagen cross-links with the progression of T2D in the WBN/Kob rat (material properties of bone were not directly reported) [88]. More information on the contribution of collagen cross-link to bone strength can be found in several review articles [89–91].

With aging, both advanced glycation end products (AGEs) [92, 93] and microdamage [94–98] increase in bone despite continual turnover throughout life.

Fatigue loading of cortical bone in vitro to generate microdamage reduces material strength [99, 100]. Conceivably, diabetes alters the matrix of bone in way that favors damage accumulation, especially if remodeling is impaired (turnover marker are generally down in diabetics).

As is the case with material strength, several independent groups have reported that toughness decreases with age [82, 83, 93]. In general, the collagen phase is primarily responsible for bone toughness or the ability of bone to dissipate energy during failure. Much of this energy occurs after yielding such that "brittle" bone has very low post-yield toughness. There are a number of collagen characteristics that partially explain the age-related decrease in human bone toughness: increase in porosity (i.e., loss of collagen) [82], decrease in collagen strength [92], a decrease in isometric shrinkage temperature or the peak rate of contraction of the collagen (i.e., collagen integrity) [101], an increase in secondary osteonal area [96], a decrease in collagen content relative to dry mass of bone [93], an increase in pentosidine [93], and a decrease in bound water [102, 103]. As such, the hyperglycemia that occurs with diabetes can possibly cause bone to become brittle through modifications to the collagen phase. For example, the accumulation of AGEs like pentosidine over the duration of diabetes could decrease the ability of collagen to deform and slide relative to the mineral phase, thereby decreasing toughness (for a review of bone quality in diabetes, see [104]).

The evidence for this possible mechanism of AGE accumulation reducing bone toughness comes from the documented association between pentosidine or AGEs and bone toughness [92] or fracture toughness [105] and in vitro riboslyation experiments [106]. When bovine cortical bone (18–24 months old) was incubated in high concentrations of sugar, namely 0.6 M ribose, for 14 or 38 days to induce nonenzymatic cross-linking, there was a reduction in fracture properties related to post-yield energy dissipation mechanisms, not strength [106, 107]. However, when fetal bovine bone was incubated in 0.2 M ribose for 15 days, there were no differences in material properties, as determined by three-point bending tests, between control and ribosylated bone, despite ribose significantly increasing pentosidine [108]. This suggests that sugar concentration and mineralization status of the tissue influence whether inducing AGEs lowers the material properties of bone. With respect to trabecular bone, which is likely more susceptible to nonenzymatic, glycation-mediated collagen cross-linking than cortical bone [109, 110], in vitro incubation in 0.6 M ribose for 7 days has been reported to lower the difference between strain at maximum stress and strain at yielding (post-yield energy) as determined by compression tests of human trabecular bone [111, 112]. Adding an AGE blocker such as aminoguanidine [108] or *n*-phenacylthiazolium bromide [112] partially prevents ribose-induced AGEs. However, in vivo demonstration that inhibiting diabetes-related increase in AGE prevents a loss of bone toughness has yet to be demonstrated.

As previously mentioned, there are multiple ways to characterize the fracture toughness of human cortical bone. Whether determined as the critical stress state beyond which the crack begins to grow (stress intensity factor K), the nonlinear elastic strain energy dissipated prior to and during fracture (J-integral), or the toughness evolution with crack extension (crack resistance curve or R-curve), fracture

toughness of human bone decreases with advancing age [73, 83, 92, 113, 114]. Moreover, there are many factors that correlate with fracture toughness of human cortical bone: apparent bone density [115], porosity [96, 116], collagen integrity [101], microdamage [96, 117], bound water [118], and pentosidine [92, 105]. Diabetes could potentially lower fracture toughness and overall fatigue resistance by increasing intracortical porosity and increasing AGEs such that the matrix has less capacity to resist damage.

Differences in Material Properties of Bone Between Nondiabetic and Diabetic Rodents

Different strains of rodents have been used to study the effects of both T1D and T2D on the material properties of the bone, although there are more studies reporting diabetes-related differences in structural properties such as stiffness and peak force [4]. Rodent models of T1D involve impairing pancreatic or β cell function [119]. This can be achieved by injecting a toxin such as alloxan or STZ, infecting transgenic mice with a virus specifically designed to disrupt β cells, and breeding mice that cannot adequately produce insulin (the non-obese diabetic or NOD mouse) [120]. Type 2 diabetic models involve single gene or polygenic mutations [15, 119, 121]. Rodents with single gene mutations become spontaneously diabetic. Examples include mutations in the leptin gene (db/db mouse or ZDF rat), the leptin receptor (ob/ob mouse), and in the IGF-1 receptor gene of muscle cells (MKR mouse). Note that the loss of function in these genes may affect the skeletal phenotype in ways that are independent of diabetic effects. Diabetes in polygenic models of T2D can also spontaneous, but it typically involves diets with moderate to high fat, thereby enuring that frank diabetes occurs. These models vary in the severity of diabetes, and age of diabetic onset often occurs before skeletal maturity. As such, more preclinical and clinical studies are needed to determine which rodents models best represent how diabetes affects bone in humans.

In general, material strength and toughness of bone are lower for rodents with T1D than without diabetes [18, 19, 122–126]. Table 9.1 summarizes some recent studies reporting material properties of bone in primarily STZ-induced models of T1D. Nonfasting glucose levels reproducibly exceed 350 mg/dL in this model with normal levels being less than 100 mg/dL (severity depends on strain of mouse however). There is a lack of information on the material properties of bone in other non-toxin models such as the NOD mouse. Difference in bone toughness between T1D rodents and nondiabetic controls depends on the duration of diabetes [19, 126], which could explain why not all T1D studies report a difference in this material property [18, 123].

As previously mentioned, a recent review by Fajardo et al. [15] comprehensively summarizes the skeletal phenotype of multiple rodent models of T2D. With respect to material properties of bone, material strength and toughness are typically lower, on average, for rodents with diabetes than for the nondiabetic controls [16, 127–129], but the differences are not always statistically significant (Table 9.2).

Table 9.1 Summary of the recent literature reporting differences in material properties of bone between normal rodents and rodents with type 1 diabetes

Strain of rodent	Age when diabetes started (weeks)	Duration of diabetes (weeks)	Bone tested in bending	Material strength (%)	Toughness (%)	Comments on toughness assessment	Reference
SD[a] rat	10	7	Femur	NR[b]	**−34**	Work-to-failure in the plastic range ($n=12$ per group)	[122]
			Tibia		**−40**		
F344 rat	12	12	Ulna	**−7.8**	−19 (NS)	Work-to-failurec ($n=9$–12 per group)	[18]
			Ulna	−1.6 (NS)	**−33**		
SD rat	12	12	Femur	NR	+2.6 (NS)		
			Femur	NR	−26 (NS)		
Wistar rat[d]	10	6	Femur	−5.9 (NS)	−8.9 (NS)	Area under the stress vs. strain curve ($n=16$ per group)	[126]
		12		−5.9 (NS)	−17.1 (NS)		
Wistar rat	13	8	Femur[e]	**−36.7**	**−58.1**	Area under the stress vs. strain curve ($n=7$ per group)	[124]
BALB/c mouse	14	5.7	Femur	**−3**	NR	Femur cross section assumed to be an ellipsoid ($n=5$–9 per group)	[123]
CD-1 mouse	10	5	Femur	**−16.8**	6 (NS)	Post-yield deflection ($n=7$–9 per group)	[125]
DBA/2 J mouse	11	10	Femur	−0.2 (NS)	−11.9 (NS)	Post-yield toughness[f] ($n=6$–11 per group)	[19]
		15		**−9.2**	−33.4 (NS)		
		18		**−10.8**	**−57.9**		

Unless otherwise noted, rodents were injected with streptozotocin to induce diabetes. Significant differences indicated in **bold**. Otherwise, the difference (100* (Mean-T1D − Mean-Control)/Mean-Control) was not statistically significant (NS)

[a]*SD* Sprague–Dawley

[b]*NR* not reported

[c]Area under force vs. displacement curve (not independent of structure)

[d]Injection of nicotinamide prior to STZ administration

[e]Bones were tested in tension

[f]Span-adjusted area-under-the-curve after yielding divided by bone cross-sectional area

Table 9.2 Summary of the recent literature reporting differences in material properties of bone between normal rodents and rodents with type 2 diabetes

Strain of rodent	Age when diabetes started (weeks)	Duration of diabetes (weeks)	Bone tested in bending	Material strength (%)	Toughness (%)	Comments on toughness assessment	References
ZDF[a] rat	9–11	2–4	Femur	−10.2 (NS)	−2 (NS)	Post-yield displacement (n = 9–10 per group)	[16]
		9–11	Femur	−6 (NS)	+6 (NS)		
		2–4	Tibia	+6.9 (NS)	−31.1 (NS)		
		9–11	Tibia	+8.1 (NS)	−50.6 (NS)		
ZDF rat[b]	9–11	12–14	Femur[c]	**−22.8**	−15.8 (NS)	Area under the stress vs. strain curve (n = 3–7 per group)	[155]
ZDF rat[d]	9–11	22–24	Femur	+7.8 (NS)	−10.1 (NS)	Area under the stress vs. strain curve (n = 12 per group)	[17]
CD vs. ZDSD[e] rat[d]	21	10–12	Femur	+1 (NS)	+10.8 (NS)	Area under the stress vs. strain curve (n = 12–17 per group)	[17]
CD vs. ZDSD rat[d]	20	10–12	Femur	**−14.9**	**−50**	Area under the stress vs. strain curve (n = 7–11 per group)	[131]
CD vs. ZDSD rat[f]	20	8–10	Femur	−0.1 (NS)	−6.6 (NS)	Area under the stress vs. strain curve (n = 4–8 per group)	[129]
C57BL mouse[g]	3	16[h]	Femur	**−26**	+8 (NS)	Tested notched bone to determine fracture toughness (n = 14–15 per group)	[128]
	15			**−15**	**−21**		
ob-ob mouse		10	Femur	NR	**−31**	Work-to-failurei (n = 10 per group)	[127]
db-db mouse		10			**−43.1**		
WBN/Kob rat vs. Wistar	43–52	0–9	Femur	NR	**−38.4**	Work-to-failure (n = 7 per group)	[88]
		8–15			**−42.3**		
		16–25			**−48.1**		
		24–33			**−48**		
TallyHo/JngJ	4	4	Femur	NR	**−59**	Post-yield displacement (n = 8–10 per group)	[132]
vs. SWR/J		13			**−48**		

(continued)

Table 9.2 (continued)

Unless otherwise noted, rodents spontaneously develop diabetes. Significant differences indicated in **bold**. Otherwise, the difference (100* (Mean-T2D – Mean-Control)/Mean-Control) was not statistically significant (NS)

[a]*ZDF* Zucker Diabetic fatty

[b]Animals were fed a high fat diet (Purina 5008) starting at 11 weeks of age for the study duration

[c]This was a study investigating the effect of anti-Sclerostin antibody on bone in a diabetic model and included defect in contralateral femur with a slide plate

[d]Male rats were fed a high fat diet (Purina 5008) starting at 20 or 21 weeks of age for the entire duration

[e]*ZDSD* Zucker Diabetic Sprague Dawley

[f]Female rats were fed a high fat diet (Purina 5SCA) starting at 20 weeks of age for the study duration

[g]Male mice were fed a high fat diet (Research Diets) for 16 weeks

[h]Blood glucose levels indicated the obese group was likely diabetic

[i]Area under force vs. displacement curve (not independent of structure)

In the study by Hill-Gallant et al. [129], there was a difference in the estimated compressive strength of cancellous bone (femur metaphysis), but no significant differences in material strength were observed for the femoral mid-shaft when subjected to three-point bending. Using an indentation technique (described in the next section) to assess tissue properties, Hammond et al. [130] reported that the cortical tissue of the tibia mid-shaft from diabetic ZDSD rats had a higher resistance to indentation than the cortical tissue from nondiabetic CD rats, whereas Gallant et al. [131] reported the opposite effect of diabetes on indentation resistance when indenting the femur mid-shaft from similar CD and ZDSD rats. Besides the bone that was tested, the maximum indenting force differed between the studies (5 N vs. 10 N). Also, the rats became diabetic on a high fat diet at approximately 20 weeks of age, but the special diet was only given for 1 week in Hammond et al. study and for entire 12 weeks of diabetes in the Gallant et al. study. In a mouse model of juvenile T2D, Devlin et al. [132] performed a similar indentation test on tibia mid-shafts from 17-week-old, nondiabetic mice (SWR/J strain) and age-matched diabetic mice (TallyHo/JngJ) but using a maximum target force of 2 N (average of three sites). There was lower resistance to indentation with diabetes though method of indentation resistance was not exactly the same as the aforementioned studies. Note that in these indentation studies, the control animals are not littermates to the diabetic animals. Overall, there is a need for more biomechanical studies with sufficient sample size to establish the effect of diabetes on the material- and tissue-level properties of bone. Of the multiple models of T2D, the bone phenotype has only been reported for the Zucker Diabetic Fatty (ZDF), Zucker Diabetic Sprague Dawley (ZDSD), and WBN/Kob rats (Table 9.2).

Assessing Tissue Material Properties with Indentation Techniques

Another way to assess material properties of bone is to use indentation techniques. To avoid confusion, mechanical properties from microindentation or nanoindentation are referred to as intrinsic material properties, as opposed to apparent material properties that were previously described, or simply tissue properties. There are several reviews on the application of microindentation and nanoindentation to bone [133, 134]. Briefly, the techniques involve pressing a very hard tip (e.g., diamond) into a relatively smooth surface of bone tissue and measuring force (P) as a function of penetration depth (h) (Fig. 9.9) such that the contact area of the tip is a known function of h (via calibration using a standard like fused silica). Either the maximum depth that the indenter tip penetrates or the maximum force generated is specified. There are multiple tip geometries in microindentation (Brinell, Kopp, Rockwell, or Vickers) and nanoindentation (Berkovich, Cube corner, Flat punch, Spherical) with Vickers (four-sided pyramid) and Berkovich (three-sided pyramid) being the most commonly used in bone studies. Microindenters measure tissue hardness at a scale of ~50 to ~200 μm as they are designed to record maximum depth for a given indentation weight (15–300 g). Being more sophisticated with respect to the control of the actuator and isolation of vibration, nanoindenters can provide both elastic and viscoelastic properties at a scale of ~0.1 to ~10 μm. The elastic properties include tissue hardness and tissue modulus as typically determined by the Oliver-Pharr method [135]. The viscoelastic properties include loss modulus (E'), storage modulus (E''), and loss factor ($\tan \delta = E''/E'$) in which the latter characterizes the viscous dampening behavior of a material [136]. Unlike the elastic properties that are derived from the P vs. h curve, the viscoelastic properties are derived from oscillatory loading at a specified frequency (or frequency sweep), displacement amplitude, and depth level in which there is a phase shift (δ) between force oscillation (input)

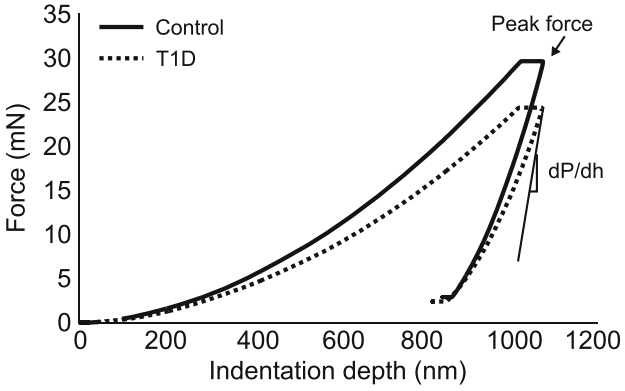

Fig. 9.9 The tissue hardness and modulus is determined from the peak force and the unloading slope generated during nanoindentation

and the responding displacement oscillation (output) [137]. Note that accurately measuring δ requires proper calibration and proper assumptions about the frame stiffness of the instrument. Other properties such as relaxation time constants and indentation work can be measured using nanoindentation, but hardness, modulus, and loss factor are the most widely reported for bone.

To the best of our knowledge, there are only two studies reporting elastic tissue properties of diabetic bone (none reporting viscoelastic properties). Acquiring femurs from wild-type and leptin-deficient (db/db) mice at 12 weeks of age, Williams et al. [138] cut embedded cross sections of the mid-shaft and polished the surface for nanoindentation. They observed that the dry tissue modulus was 9 % lower for the diabetic db/db mice than for the wild-type mice. In a study by Nyman et al. [19], tibia from normal mice and mice injected with STZ to induce T1D were embedded, sectioned, and polished for nanoindentation. In doing so, they found a 12 % reduction in dry tissue modulus after 18 weeks of diabetes with no significant differences between normal and diabetic bone tissue at 10 or 15 weeks postinjection.

Lower tissue modulus, or lower hardness for that matter, does not mean that the bone is more susceptible to fracture. Nanoindentation properties do not strongly correlate, if at all, with apparent-level modulus of human cortical bone [139] and modulus and strength of mouse bone [140]. Moreover, nanoindentation properties of human bone at the tissue level do not vary with age [139, 141], and for the most part, microindentation properties of bone do not vary with age either, though this has not been extensively analyzed with tissue from donors that span 20–100 years of life [142, 143]. The usefulness of microindentation and nanoindentation is the characterization of tissue at the organizational level of the lamella and then relating the tissue mechanical properties to other tissue properties of composition (degree of bone mineralization [142] or mineral-to-matrix ratio [144]) and organization (collagen orientation [145]). In doing so, there is insight into ultrastructural–mechanical relationships at the length scale in which bone cells interact with the matrix. By performing multiple indents throughout a bone section, an assessment of tissue heterogeneity can also be acquired. In general, a decrease in tissue modulus heterogeneity (i.e., at the nanoscale) causes a decrease in bone toughness [146]. If T2D reduces turnover and increases tissue age in humans, then the tissue modulus and hardness of diabetic bone would increase while the heterogeneity in these properties would decrease, thereby leading to a reduction in fracture resistance.

There is a relatively new microindentation technique called reference point indentation (RPI). Unlike the traditional indentation techniques, RPI does not require any special processing to create a smooth bone surface. Moreover, it can be performed through the periosteum of the tibia mid-shaft of patients in a minimally invasive way (local anesthetic), thereby providing a clinical assessment of indentation properties. Two versions of RPI currently exist: (1) a bench-top instrument (BioDent™, Active Life Scientific, Inc.) uses an annular reference probe to engage the bone surface so that an inner, stainless steel, test probe with a spherical tip can indent the bone surface over multiple cycles of load–dwell–unload (2 Hz) at a user-specified maximum force (typically 10 N for human bone) [147] and (2) a handheld

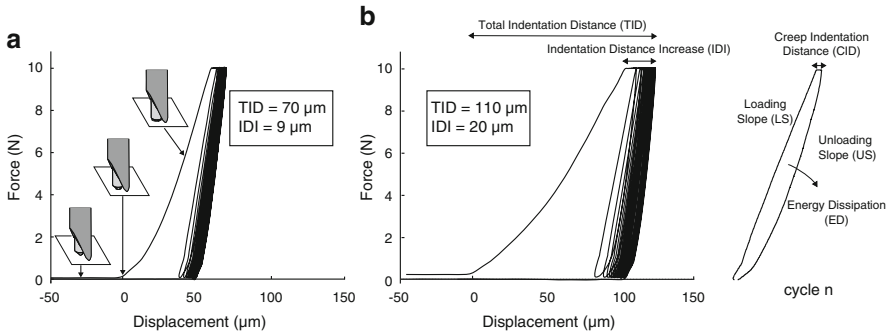

Fig. 9.10 In reference point indentation, the depth of the probe tip is measured over multiple cycles of load–dwell–unload (**a**). Properties related to tissue stiffness, resistance to creep, and energy dissipation can also be measured (**b**)

device (OsteoProbe®, Active Life Scientific, Inc.) with a similar spherical, stainless steel tip imparts a one-time impact force of ~45 N into the bone [148]. The BioDent records the force vs. displacement curve from which a number of properties can be recorded (Fig. 9.10) including those related to the depth that the indenter tip travels into the bone (e.g., indentation distance increase or IDI and total indentation distance or TID) [149]. In addition, properties can be calculated from each cycle of load–dwell–unload and include the loading stiffness, unloading stiffness, creep during the dwell portion, and the energy dissipated (i.e., area under the force vs. displacement curve). These properties vary over the first 4 cycles of load–dwell–unload and then asymptotically approach a constant value over the remaining 20 cycles [150, 151]. The OsteoProbe on the other hand provides only one measurement, which is the distance that the tip travels into the bone. This indentation distance increase (IDI-Bone) from the impact is then divided into the average distance that the same test probe tip penetrates a plastic standard (PMMA) to give a property called bone material strength index (BMSi = 100 * IDI-PMMA/IDI-Bone). Because the BioDent indents the bone at a length scale of ~120 μm (diameter) (depth of ~70 μm at 10 N) in dynamic manner while the OsteoProbe indents the bone with impact force at a length scale of 300 μm (depth of ~150 μm), the two instruments do not necessarily measure the same tissue properties. That is, their assessment of resistance to indentation may differ.

In the initial experiments using the BioDent, Hansma et al. [147] found that IDI was different between non-irradiated and gamma irradiated bovine cortical bone and between a tibia from a 17-year-old and 79-year-old female donor. These tests involved approximately 100 indents, and IDI was normalized to mean IDI acquired from indenting a PMMA standard block. Subsequent work found that baking bone at 150 °C profoundly increased IDI (lower resistance to indentation), but freeze–thaw cycles had minimal effect on IDI (within biological variance) [152]. At radiation levels used in high-resolution μCT, there is no effect of irradiation on RPI properties [151]. Also, in a sample of bovine cortical bone, sodium-fluoride

treatment, which affects the collagen–mineral interface, increased normalized IDI and the material strength at the apparent level [153].

Several concepts or "best practices" have emerged with the growing use of the BioDent: (1) acquire the average of multiple indents per bone sample (at least five sites), (2) perform at least ten cycles of loading, (3) indent PMMA standard regularly and possibly normalize data to this reference in order to minimize variance in tip geometry, especially since the probe wears during repeated use, (4) grind irregular surfaces to minimize variance in probe contact, (5) remove data from the average calculation when a pore has been indented (apparent in force vs. displacement curve), (6) apply the same preload according to manufacturer's recommendation, and (7) maintain the same hydration conditions across all bones. Other issues to keep in mind when performing RPI include: differences in properties among groups may depend on selected target force, preconditioning may not be necessary if there is no soft tissue present, and the reference probe may "skip" if not properly anchored into the bone tissue.

In recent studies from independent groups, RPI properties of bone have been found to exhibit anisotropy (dependent on direction of indentation relative to osteonal axis) [149], especially for stiffness (unloading and loading slopes) and average creep indentation distance for human cortical bone [150]. Correlations between IDI (not normalized) and apparent-level materials of bone have also been reported for a target maximum force of 10 N. IDI explained 56 % of the variance in toughness as determined from three-point bending tests of rat femurs acquired from 7 nondiabetics CD male rats (Charles River Labs) and 11 age-matched, diabetic, ZDSD male rats (PreClinOmics, Indianapolis, IN) [131]. Indenting the left femur opposite of the one subjected to whole-bone bending, IDI was an average of five sites on the anterior side. IDI explained 27 % of the variance in the toughness of human cortical bone as determined by three-point bending of machine samples acquired from 34 donors spanning 21–99 years of age [150]. In both studies, IDI was inversely proportional to toughness. IDI and other RPI properties however cannot be interpreted as surrogates of material properties of bone. RPI and bending tests assess bone at different length scales and under different modes of deformation. Thus, factors dictating resistance of bone tissue to indentation may be different than those influencing bone strength, toughness, or fracture toughness. Conceivably, the relationship between IDI and toughness (or strength) could be negative or positive depending on the disease or genetic model. As an example, Carriero et al. [154] found no relationship between normalized IDI and fracture toughness of mouse cortical bone (femur mid-shaft) when bones were collected from several mouse strains including one with osteogenesis imperfecta (OI) and one with defective mineralization (mice lacking a gene critical to phosphate production). In effect, IDI can be high because collagen structure is defective, as in the case of OI relative to normal mice, or high because mineralization was reduced, as in the case of *Phospho1*−/− relative to *Phospho1*+/+ mice. Fracture toughness, on the other hand, decreases when collagen integrity is poor (OI) but increases when mineralization decreases (hypomineralization) conferring more ductility to the bone.

Reported differences in RPI properties from clinical studies can be found in Chap. 10. The more recent studies involved the OsteoProbe, and because it is easier to use in a clinical setting than the BiodDent, the expectation is that emerging studies will also use the hand-held device that provides only one measurement. As is this the case with apparent-level material properties, additional preclinical studies are needed to understand what tissue-level properties RPI assess.

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Chapter 10
Bone Quality in Type 2 Diabetes Mellitus

Joshua N. Farr and Sundeep Khosla

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common chronic diseases worldwide [1]. As changing lifestyles lead to increased obesity [2], the prevalence of T2DM will continue to grow and the economic public health burden will worsen significantly [1, 3]. Indeed, the direct medical costs of T2DM are estimated at over $116 billion annually in the USA alone [3]. Globally, 285 million people have T2DM, and this number is predicted to increase to 439 million by 2030 [1].

While there is abundant evidence that patients with T2DM are at significant risk for premature mortality and morbidity from macrovascular disease, retinopathy, nephropathy, and neuropathy [3], emerging evidence suggests that T2DM also has adverse skeletal effects. Indeed, numerous studies have established T2DM as an independent risk factor for fragility fractures at skeletal sites such as the hip, spine, and distal forearm (see Chap. 2) [4–10]. For example, based on existing data, T2DM is associated with a 50–80 % increased extremity fracture risk [4, 8], and a meta-analysis of 12 studies found a relative risk of 1.7 (95 % CI: 1.3–2.2) for hip fracture [10]. These findings are perhaps surprising given that patients with T2DM often have normal or increased dual-energy X-ray absorptiometry (DXA)-derived areal bone mineral density (aBMD) [11], even when normalized for body mass index (BMI) [12]. Nonetheless, data from several large, prospective studies in the USA [13] and Canada [14] have recently demonstrated that T2DM patients have a higher fracture risk (see Chap. 3) for either a given femoral neck aBMD *T*-score and age or a given FRAX probability (defined by the World Health Organization's Fracture

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Risk Algorithm [FRAX®] score [15]), suggesting that other factors, independent of aBMD, are likely responsible.

Since the risk for developing T2DM increases with advancing age and therefore frequently coexists with age-related bone loss [16], established risk factors for fragility fractures that occur with normal aging also contribute to fracture risk in patients with T2DM. Nevertheless, there is now considerable evidence that specific fracture risk factors are either exacerbated in patients with T2DM (e.g., obesity, reduced muscle quality, poor balance, and falls [17, 18]) or T2DM specific (e.g., poor glycemic control, T2DM duration, and diabetic medications and complications [4–10]). However, available evidence suggests that these risk factors do not sufficiently explain the increased fracture risk in T2DM patients [19]. Collectively, these findings point to the likelihood that skeletal factors, not captured by DXA, may contribute toward increased fracture risk in T2DM patients, which has led to the hypothesis that an important factor underlying fragility fractures in patients with T2DM is deteriorated "bone quality."

Measuring Aspects of Bone Quality in Patients with T2DM

Historically, progress in understanding how aspects of bone quality might be altered in patients with T2DM has been hampered by methodological barriers. Importantly, however, recent technological advances now allow for the safe quantification of previously inaccessible, but theoretically vital [20], components of bone quality (i.e., structural and material properties that determine skeletal fragility [21]), advances which may enhance our ability to stratify fracture risk in patients with T2DM beyond that provided by DXA.

To test whether bone microarchitecture, an important component of bone quality [20, 21], is altered in patients with T2DM, several studies have used novel imaging techniques (see Chap. 11) such as high-resolution peripheral quantitative computed tomography (HRpQCT), which provides a noninvasive "virtual bone biopsy" of the distal radius and tibia. Because the skeleton is approximately 80 % cortical bone [22], and cortical porosity has emerged as a potentially critical determinant of bone quality and a strong independent predictor of fracture risk [20, 21], there has been considerable interest in assessing cortical bone porosity by HRpQCT in patients with T2DM. In the first such study, Burghardt et al. [23] found that 19 postmenopausal women with T2DM tended to have better trabecular microarchitecture but worse cortical microarchitecture (i.e., higher cortical porosity) at the distal radius, and similar changes at the distal tibia, when compared to 19 nondiabetic postmenopausal control subjects. By contrast, Shu et al. [24] reported no significant differences in trabecular or cortical bone parameters (assessed by HRpQCT) at the radius or tibia in postmenopausal women with T2DM vs. nondiabetic postmenopausal control subjects, although only 14 subjects per group underwent HRpQCT scanning and cortical porosity was not reported. Consistent with the findings of Burghardt and colleagues [23], we found that radial cortical porosity tended to be higher in 30 postmenopausal women with T2DM as compared to 30 age-matched nondiabetic

postmenopausal control subjects after adjustment for BMI, although this difference did not reach statistical significance [25]. Finally, in the only study thus far of postmenopausal women with T2DM and fragility fractures, Patsch et al. [26] found significantly higher cortical porosity at the distal radius in 20 T2DM facture patients as compared to 20 T2DM patients without fracture. Collectively, these relatively small studies in postmenopausal women suggest that cortical bone may be preferentially compromised in patients with T2DM, and that cortical porosity in particular may be of relevance for understanding fracture risk in diabetic patients.

In addition to cortical microarchitecture, the material composition of bone is another important component of bone quality [20, 21]. For many years, however, our understanding of how human bone material properties are affected by aging or disease was limited to cadaveric studies. Despite the inherent limitations of studying cadaveric bone specimens, these early studies were pivotal in identifying deterioration of bone material properties (e.g., increased crack growth and reduced fracture toughness) with advancing age [27, 28], suggesting that worsening bone material properties may contribute to fracture risk. Nonetheless, because of the invasive measures required to assess bone material properties at the time, it was not possible to confirm such findings in human subjects. The recent advent of technology for noninvasively measuring the material properties of bone in vivo in humans, however, has begun to fill this void. To do so, Hansma and colleagues sought to develop an instrument capable of measuring human bone material properties in vivo, a process which required the development, extensive validation, and iterative improvement of a series of instruments [29–33]. The first of these microindentation devices to be utilized in human subjects for the measurement of indices of bone material properties in vivo was the "reference point indentation (RPI) instrument" (Fig. 10.1); other versions were later called the "bone diagnostic instrument" [29–31] and the "tissue diagnostic instrument" [32]. Using this technology, small

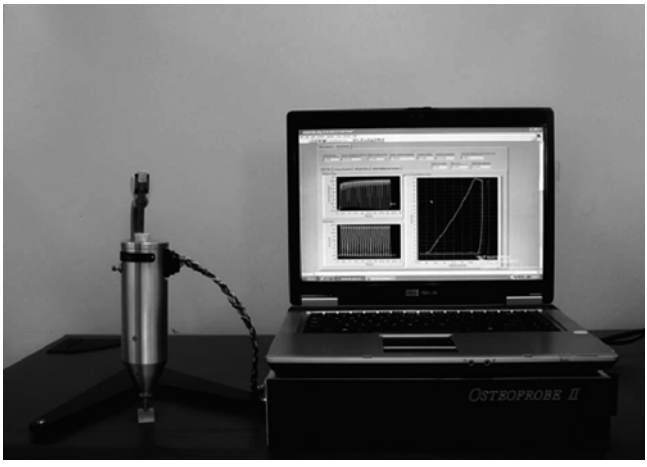

Fig. 10.1 Image of the Reference Point Indentation (RPI) Instrument, the first microindentation device to be utilized in human subjects for the measurement of indices of bone material properties in vivo. Reproduced from Hansma et al. (Rev Sci Instrum 2008;79(6):064303) with permission

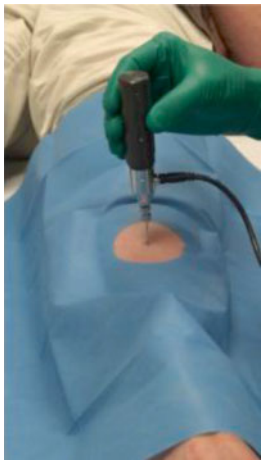

Fig. 10.2 Image of the OsteoProbe® (Active Life Scientific Inc., Santa Barbara, CA), a handheld microindentation instrument designed for in vivo measurements of the material properties of bone (i.e., bone material strength index [BMSI]) in humans at the midshaft of the non-dominant anterior tibia. Reproduced from Farr et al. (J Bone Miner Res 2014;29:787–95) with permission

studies found that in vivo indices of bone material properties may be worse in hip fracture [34] and atypical femoral fracture [35] patients. While implementation of this technology represented a noteworthy advance, the RPI procedure unfortunately required complete displacement of the periosteum from the bone surface at the site of measurement, a factor which rendered routine clinical assessments of bone material properties using the RPI prototype impractical.

To address these issues, these same investigators developed a new microindentation device that allows for the safe quantification of an index of bone material properties (without the need for a reference probe or displacement of the periosteum) in humans with minimal discomfort. This measure of bone material properties, later coined the "bone material strength index" or BMSI, can now be obtained using the OsteoProbe® (Active Life Scientific Inc., Santa Barbara, CA, USA), a small handheld microindentation instrument designed for in vivo bone material property measurements [33, 36]. Figure 10.2 shows the device positioned over the midshaft of the anterior tibia, the optimal testing site (determined by calculating the midpoint from the proximal end of the medial border of the tibial plateau to the distal edge of the medial malleolus). Indeed, this site provides a viable flat surface, away from tendons, ligaments, blood vessels, and nerves, for performing cortical bone material property measurements.

Primary components of the OsteoProbe® include an impact mechanism, a displacement transducer, and a sterilized stainless steel disposable probe with a 90° conical tip (375 μm diameter; <10 μm tip sharpness radius). After administration of local anesthesia (1 % lidocaine), the probe is inserted through the skin, soft tissue, and periosteum until resting on the bone surface. While keeping the device perpendicular to the bone surface (within 10°), the measurement is started by slowly depressing the outer housing unit of the instrument, which in turn compresses the internal primary spring until the trigger mechanism initiates an impact. The impact mechanism creates a force (peak of 40 N) to drive the probe a minimal distance into

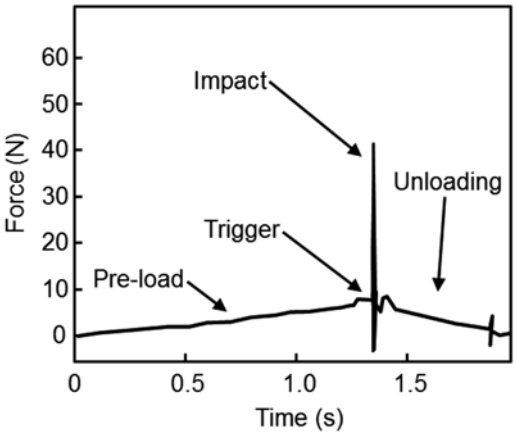

Fig. 10.3 Time (s; seconds) vs. force (N; newtons) plot showing a single OsteoProbe® measurement cycle including the four basic operations of the device: (1) pre-load, (2) trigger, (3) impact, (4) unloading. Adapted and reproduced from Bridges et al. (Rev Sci Instrum 2012;83:044301) with permission

the bone cortex, while the displacement transducer measures the indentation distance increase (μm) from impact. Thus, the BMSI is a direct in vivo measure of how well bone resists deformation in response to microindentation.

Accurate and precise in vivo measurements of BMSI are due to four basic operations: (1) pre-load, (2) trigger, (3) impact, and (4) unloading, as described previously [33]. Each operation occurs at different rates as indicated in the time (s; seconds) vs. force (N; newtons) graph shown in Fig. 10.3, which displays the forces imposed on the bone during each measurement cycle. Pre-load (operation 1) occurs over ~1 s when the operator slowly depresses the device's outer housing unit to compress the internal primary spring. This ensures that the test probe is securely anchored to the bone before the primary indentation occurs. Once the pre-load force reaches 10 N, the trigger (operation 2) mechanism is automatically initiated, generating an impact (operation 3) lasting ~0.25 ms (with a peak force of 40 N and a constant impulse rate at 0.01 N s). After impact, unloading (operation 4) results in return of the force to 0 N and completion of the measurement cycle. A displacement transducer measures the indentation distance increase (μm) from impact, which is converted by computer to BMSI, defined as 100 times the ratio of the harmonic mean indentation distance increase from impact from five separate impacts into a polymethyl-methacrylate phantom relative to the indentation distance increase from impact into bone [36]. For each subject, BMSI is calculated as the average of 5–10 measurements, generally performed in a circular, rather than linear, fashion at different midshaft tibial sites (separated by >2 mm). It should be noted that the procedure causes minimal discomfort (only during the local anesthesia injection) and no complications have been observed to date. In fact, subjects report feeling only light pressure on the bone, but no pain, during the procedure, which typically takes less than 5 min to perform. Although minimally invasive, it should be noted that this procedure is not intended for patients who have a significant skin disorder, bruising, local edema, or infection, as well as those undergoing treatment for blood clots or severe coagulation defects.

The indentations are very small (on average, <200 μm), but do create minimal microcracks in the bone surface that are clinically insignificant and only detectable by scanning electron microscopy [33, 36]. Thus, BMSI is a direct measure of fracture resistance because the farther the probe indents the bone (higher the indentation distance from impact), the more easily bone is fractured (lower the BMSI). This technology has now been used in a number of relatively small clinical studies [25, 33, 36–39], and similar microindentation approaches have been utilized in previous studies involving animals [40–42] and humans [34, 35].

There is also considerable interest in how bone material properties relate to other measures of skeletal properties such as whole-bone mechanical properties and bone microarchitecture (see Chap. 9). For example, studies utilizing an animal model of T2DM found strong correlations between impaired bone material properties derived from an in vitro microindentation testing instrument, called the BioDent® (Active Life Scientific Inc., Santa Barbara, CA, USA), and reduced bone strength assessed by traditional ex vivo bone mechanical testing techniques (three-point bending and axial compression) at both appendicular and axial skeletal sites [42]. However, given the different loading patterns utilized by the OsteoProbe® (single-impact) and BioDent® (cyclic loading), these instruments likely provide different measures of bone material properties. Nonetheless, understanding how the outputs of these instruments relate to bone quality and to one another is of great importance toward their application in predicting fracture risk in both preclinical and clinical settings.

Although additional work is needed to answer these questions, some data are starting to emerge. For example, Granke et al. [43] recently tested the extent to which bone parameters derived from the BioDent® and OsteoProbe® were related to other measures of cortical bone quality in human cadaveric bone specimens. Based on measurements performed by both instruments at the same site, BMSI (derived from the OsteoProbe®) was inversely correlated with local cortical porosity ($r = -0.69$, $P < 0.001$), whereas none of the BioDent® parameters were related to any bone microarchitectural parameters. The authors concluded from these findings that the OsteoProbe® may be more sensitive than the BioDent® for measurement of aspects of cortical bone microarchitecture, in addition to its ability to assess bone tissue material properties [43]. This may reflect the greater force (~40 N) and depth (on average, ~200 μm) of the single-impact loading mechanism used by the OsteoProbe® vs. the BioDent® (force of ~20 N, depth of ~70 μm). Further, because fractures typically result from a single insult, it is possible that a one-time impact load more accurately reflects how bone responds to trauma as compared to a cyclic loading pattern of modeling. Future studies are needed to test these hypotheses experimentally.

There is also interest in how in vivo measures of bone material properties (e.g., obtained using the OsteoProbe®) relate to bone imaging parameters in humans, although this issue has received little attention to date. Interestingly, in T2DM patients [25], BMSI was not significantly (all P values >0.05) correlated with any radial or tibial cortical bone parameters (derived from HRpQCT) or any regional aBMD parameters (derived from DXA), suggesting that in patients with T2DM,

bone material properties may be a major predictor of skeletal fragility independent of cortical bone microarchitecture and aBMD. Notwithstanding these preliminary findings, additional studies will be required to test this possibility in larger cohorts of both diabetic and nondiabetic women and men.

Bone Material Properties in Patients with T2DM

Whether patients with T2DM have compromised bone material properties as compared to nondiabetic persons has been a question of long-standing interest [16, 44]. To address this, we recently performed in vivo microindentation testing (using the OsteoProbe®) of the tibia to directly measure BMSI in 60 postmenopausal women (age range, 50–80 years): 30 patients diagnosed with T2DM for >10 years and 30 age-matched, nondiabetic women [25]. We also assessed bone microarchitecture of the distal radius and tibia by HRpQCT and regional aBMD by DXA. In addition, we examined the associations of BMSI with circulating glycated hemoglobin A1c levels. Compared to controls, T2DM patients had significantly lower BMSI: unadjusted (−11.7 %; $P<0.001$; Fig. 10.4a) and following adjustment for BMI (−10.5 %; $P<0.001$; Fig. 10.4b). These differences remained significant following additional adjustments for potential confounders, including hypertension and diabetic complications (data not shown). Interestingly, the mean glycated hemoglobin level over the previous 10 years was negatively correlated with BMSI ($r=-0.41$; $P=0.026$) in patients with T2DM. By contrast, BMSI was not associated with a cross-sectional glycated hemoglobin level in nondiabetic control subjects ($r=-0.09$, $P=0.630$). These findings thus represent the first demonstration of compromised bone material properties in patients with T2DM. In addition, our results highlight the potential detrimental effects of prolonged hyperglycemia on bone quality. We infer from these findings that the skeleton warrants recognition as another important target tissue subject to diabetic complications [25].

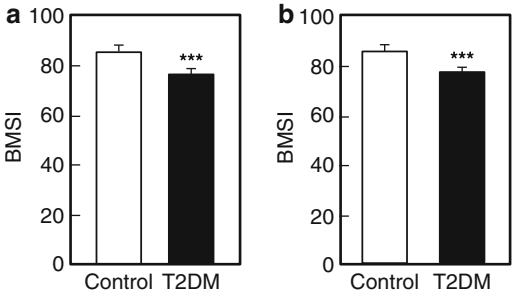

Fig. 10.4 Unadjusted (**a**) and body mass index (BMI)-adjusted (**b**) comparisons of bone material strength index (BMSI) between patients with type 2 diabetes mellitus (T2DM) and age-matched, nondiabetic controls. Values are shown as mean ± SE. ***$P<0.001$. Adapted and reproduced from Farr et al. (J Bone Miner Res 2014;29:787–95) with permission

A valid concern of the OsteoProbe® instrument is safety. Notably, our group [25] and others have extensive experience using the device [25, 33, 36–39] and a closely related prototype [34, 35] in humans. Importantly, no complications have been reported at either the time of measurement or in follow-up. Nevertheless, as noted previously, out of an abundance of caution, patients with a significant skin disorder, bruising, local edema, or infection, as well as those undergoing treatment for blood clots or severe coagulation defects should not undergo this procedure. In addition, although the indentations are very small (on average <200 μm) and separated by at least 2 mm, it is likely preferable to perform the indentation in a circle rather than linearly down the tibia in order to minimize the extremely improbable, but theoretical [44], risk of stress fracture.

Pathogenesis of Reduced Bone Quality in T2DM

Because patients with T2DM have a higher fracture risk than nondiabetic persons despite having normal or even increased aBMD [4, 13, 14], they likely have impairments in bone quality. Our finding of reduced bone material properties in patients with T2DM [25] is consistent with this hypothesis, and points to important unanswered questions regarding the mechanism(s) of human diabetic bone disease. Studies in T2DM rodent models have identified advanced glycation end products (AGEs) among the primary proximal culprits [45]. Unfortunately, however, there are currently no T2DM animal models that sufficiently recapitulate the skeletal phenotype observed in human patients with adult-onset T2DM [46]. Thus, humans currently serve as the best model system for studying the pathogenesis of diabetic bone disease.

Although additional studies in living human subjects with T2DM are direly needed, as noted previously, most of our knowledge to date of diabetic bone disease in humans has been derived from cadaveric bone specimens. Nevertheless, findings from such studies have been critical in demonstrating that the increased risk of fragility fractures in patients with T2DM may stem from non-enzymatic glycation and collagen cross-links in diabetic bone, partially resulting from the increased production of AGEs. Because AGEs are recognized for their central role in mediating diabetic complications [45], these "bad" intermediate protein products have emerged as potentially viable therapeutic targets for preventing or perhaps reversing diabetic bone disease in humans [47].

AGEs are intermediate protein products that undergo undesirable chemical modifications after excessive glucose exposure [48]. Thus, prolonged high circulating glucose concentrations in T2DM may lead not only to higher glycated hemoglobin levels, but also to the accumulation of AGEs both in the circulation and in bone tissue itself [49]. Increases in AGEs negatively impact type I collagen as well as the generation and survival of osteoblasts in bone [16, 49, 50]. While enzymatic processes normally produce pyridinium crosslinks necessary for the mechanical integrity of collagen, non-enzymatic glycation creates undesired crosslinks that modify the extracellular matrix in bone and decrease bone formation [51–53]. Further, in vitro experiments have demonstrated that AGEs impair bone formation

by interfering with osteoblast differentiation, attachment to bone surfaces, function, and survival [54–56]. Thus, the increased production of AGEs in T2DM likely leads to defects in bone formation and ultimately, due to coupling between bone formation and resorption, to low bone turnover.

While many different AGEs have been identified in human tissues [48], the most studied is pentosidine, circulating levels of which have been associated with clinical fractures in T2DM patients [57, 58]. N^{ε}-carboxy-methyl-lysine (CML) is another dominant component of total AGEs [59], and its level in circulation has been related to osteoporosis [60] and hip fracture [61]. While commercial immunoassays have been developed to measure pentosidine in human serum, concerns exist regarding assay validity due to numerous factors in blood and urine that interfere with immunoassay standardization [62]. Thus, reverse phase high performance liquid chromatography is currently perhaps the most reliable method for the quantification of pentosidine in the circulation [63, 64].

To date, few studies have measured AGEs in human bone tissue, with most limited to cadaveric specimens obtained from nondiabetic human subjects. Although bone pentosidine is increased in nondiabetic fracture patients relative to non-fracture controls [65, 66] and has been shown to predict vertebral biomechanical properties (independent of BMD) in nondiabetic subjects [67], bone pentosidine levels have not been directly measured in vivo in T2DM patients. Furthermore, while pentosidine has conventionally been used as a surrogate biomarker of total AGE accumulation, it is only a single component of the total AGE content in bone [49]. Indeed, recent evidence suggests that pentosidine may not fully account for the overall influence of glycation on bone tissue [53].

To address this issue, the Vashishth laboratory has developed techniques to directly quantify total AGEs (ng quinine/mg collagen) via a fluorometric assay [68] and pentosidine (mmol/mol collagen) using ultra-high performance liquid chromatography (UPLC) methods [69] in human bone tissue specimens. Using these techniques, they showed that, in nondiabetic human cadaveric bone specimens, total bone AGEs are associated with bone mechanical properties, including yield strain ($r=0.54$; $P<0.01$), ultimate strain ($r=0.038$; $P=0.09$), and toughness ($r=–0.39$; $P=0.08$). In addition, pentosidine is also associated with yield strain ($r=0.46$; $P<0.05$) and ultimate strain ($r=0.44$; $P<0.05$), but not toughness ($r=–0.25$; $P>0.05$) [52]. While these findings provide potential insights into human diabetic bone disease, to our knowledge, no study has examined AGEs in bone tissue specimens obtained from living subjects with T2DM. Such studies may be pivotal toward unveiling the pathogenesis of skeletal fragility in patients with T2DM.

Future Directions

For many years, the lack of appropriate tools for noninvasively assessing critical aspects of bone quality significantly limited our understanding of skeletal deterioration in patients with T2DM. However, recent technological advances in high-resolution image acquisition and analysis, as well as assessment of bone material

properties in vivo offer great potential for improving prediction of fragility fractures in this population. Despite the success and increasingly widespread use of novel tools such as HRpQCT and bone microindentation testing, additional work is needed to understand how the outputs of these instruments improve fracture prediction in patients. Furthermore, although our group previously demonstrated in patients with T2DM that cortical porosity is increased and that BMSI is reduced and correlates inversely with chronic glycemic control as assessed cross-sectionally [25], whether cortical porosity and BMSI deteriorate over time to a greater extent in diabetic vs. nondiabetic subjects remains unknown and are important issues that need to be resolved. This will require concurrently evaluating biomechanically relevant components of bone structure and quality in both cross-sectional and longitudinal studies of large numbers of women and men with and without T2DM using identical methodology. In addition, while it is plausible that glycemic control (as assessed by glycated hemoglobin) or AGEs (as measured in the circulation or in bone tissue) are associated with changes in bone material properties over time in patients with T2DM, this remains untested experimentally. Finally, further work is needed to establish the potential causal role of AGEs in mediating diabetic bone disease.

Conclusions and Working Model

In conclusion, considerable evidence indicates that the skeleton needs to be recognized as another important target tissue subject to diabetic complications. Our current understanding of the pathogenesis of skeletal fragility in diabetes suggests a working model (Fig. 10.5), whereby poor glucose control in patients with T2DM leads to increases in AGEs that have negative effects on osteoblasts, which in turn causes a reduction in bone formation. This defect in bone formation subsequently results in low bone turnover in T2DM patients, which prolongs the lifespan of type

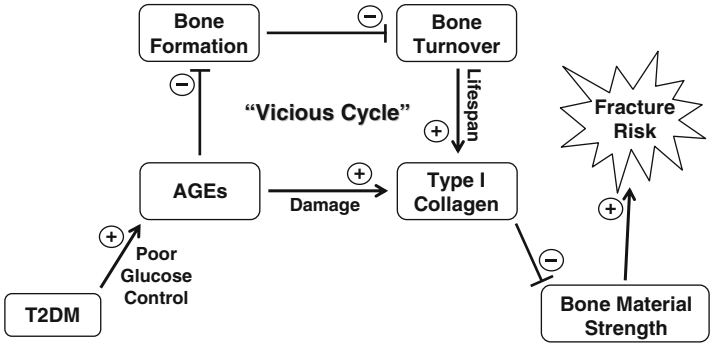

Fig. 10.5 Working model of the pathogenesis of fragility fractures in patients with type 2 diabetes mellitus (T2DM). *AGEs* advance glycation endproducts

I collagen in bone, thereby leaving it particularly vulnerable to damage from increased AGEs. Ultimately, this creates a "vicious cycle" that may contribute to reduced bone quality and increased fracture risk in patients with T2DM.

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Chapter 11
Imaging of Diabetic Bone Structure

Thomas M. Link and Ursula Heilmeier

Introduction and Background

A number of cohort studies and a comprehensive meta-analysis have found an increased risk of fragility fractures in patients with type 2 diabetes (T2DM) [1–3]. In particular, hip fractures are a cause of substantial morbidity and mortality in older adults and nearly always result in hospitalization. They are fatal in about 20 %, and also produce permanent disability in approximately 50 % [4]. In patients with diabetes, the morbidity and mortality associated with fractures is likely to be even higher related to diabetic complications, frequently found obesity, and possibly also slower fracture healing. Hip fractures are the worst-case scenario as a complication of diabetic bone disease, however, low energy spine, proximal humerus, ankle and feet fractures associated with diabetes are also a cause of substantial morbidity. Schwartz et al. [3] found that insulin-treated diabetics had more than double the risk of foot fractures (multivariate adjusted relative risk (RR) 2.66) compared with non-diabetics and these fractures are a serious and well-recognized complication of diabetes mellitus which may impair the clinical outcome of the patients remarkably.

So far, the most reliable and best validated techniques to assess fracture risk and biomechanical stability of bone are those focusing on *bone mass*, i.e., dual X-ray absorptiometry (DXA) and quantitative computed tomography (QCT). However, for diabetes bone mass, respectively bone mineral density (BMD) measurements have limitations. Previous studies have found average, or increased [2, 5–7] BMD in type 2 diabetes, even after statistically correcting data for effects of larger body size. This paradox of higher BMD but increased fracture risk in type 2 diabetes is not fully explained by more frequent falls or other traditional risk factors for fracture

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in those subjects with diabetes. Type 2 diabetes is associated with a moderate increase in the risk of falling [8], with some evidence that type 2 diabetic women using insulin have two to three times greater risk of falling than those without diabetes. However, in observational studies that have considered reported falls and risk factors for falls and injurious falls, these factors do not account for the association between diabetes and fracture [3]. This suggests that there are additional factors beyond the traditional risk factors of low BMD and more frequent falls that are contributing to fracture risk in older diabetic adults. These include *bone structure and composition* that may be compromised in patients with type 2 diabetes, which may explain the combination of increased fracture risk with higher BMD. The paradox of higher BMD and increased fracture risk in diabetes provides an opportunity to assess bone properties/biomarkers beyond BMD that may contribute to fracture risk. A better understanding of the impact of diabetes on bone strength could provide insights that would guide efforts to prevent fractures in those with diabetes and in older adults generally.

Recently, a number of studies have analyzed cortical bone structure assessed with high-resolution quantitative computed tomography (HR-pQCT) [9, 10], trabecular bone structure and bone marrow composition using magnetic resonance (MR)-based techniques [11–13], quantitative ultrasound (QUS) [14] and trabecular bone score derived from DXA to study diabetic bone disease [15, 16]. This chapter will provide an overview of recent studies characterizing bone structure and composition in an effort to explain increased bone fragility in T2 diabetes.

Imaging Technologies

High-Resolution Quantitative Computed Tomography

HR-pQCT is a new technology to investigate bone architecture analyzing cortical and trabecular bone structure. The dedicated extremity imaging system designed for imaging of trabecular and cortical bone architecture is currently available from a single manufacturer (XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) and was developed based on experimental MicroCT technology (Scanner and patient set up with a representative distal tibia scan shown in Fig. 11.1). This device has the advantage of substantially higher signal-to-noise ratio (SNR) and spatial resolution compared to Multidetector-CT (MD-CT) and MRI. The nominal isotropic voxel dimension in clinical patients is 82 μm in the older (generation 1) and 61 μm in the newer scanners (generation 2) [17]. Furthermore, the effective radiation dose is substantially lower compared to whole body MD-CT, and primarily does not involve critical, radiosensitive organs (effective dose <3 microSv) [18]. The scan time for HR-pQCT is approximately 3 min for each scan of the distal tibia and radius.

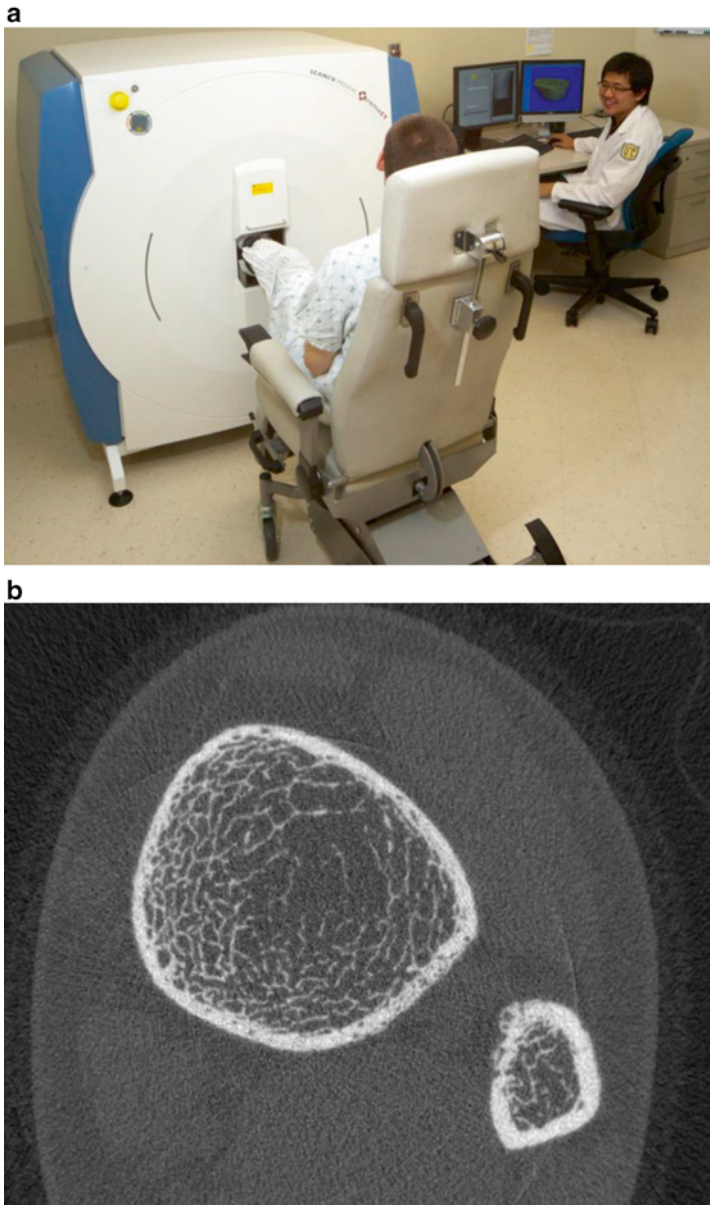

Fig. 11.1 HR-pQCT. (**a**) Demonstrates the correct patient positioning for a lower extremity scan. The patient's lower extremity is immobilized through a carbon fiber cast and is anchored in the scanner to minimize motion. For each scan, the effective patient dose amounts to approximately 3 microSv. Average time per scan is 2.8 min. (**b**) Displays a representative mid-stack HR-pQCT image of the lower extremity cross section in a healthy patient

Using HR-pQCT, three studies investigated bone architecture in patients with and without diabetes [9, 10, 19]. Shu et al. [19] analyzed a cohort of 14 T2DM postmenopausal women and 14 control postmenopausal women. Standard trabecular and cortical bone parameters were calculated from the HR-pQCT images, which included cortical thickness and density but not cortical porosity. None of the investigated parameters showed significant differences. It should be noted that information on fragility fractures in this cohort was not available.

Burghardt et al. [9] applied HR-pQCT to characterize cortical and trabecular microarchitecture and biomechanics in the peripheral skeleton of female patients with T2DM using a cross-sectional study design. Elderly female patients (age, 62.9 ± 7.7 years) with a history of T2DM ($n = 19$) and age- and height-matched controls ($n = 19$) were recruited and imaged using HR-pQCT at the distal radius and tibia. The T2DM cohort included patients with and without fragility fractures. Quantitative measures of volumetric (BMD), cross-sectional geometry, trabecular and cortical microarchitecture were calculated. Additionally, compressive mechanical properties were determined by microfinite element analysis. These investigators found that compared to the controls, the T2DM cohort had 10 % higher trabecular volumetric BMD ($P < 0.05$) adjacent to the cortex and higher trabecular thickness in the tibia (13.8 %; $P < 0.05$). Cortical porosity differences, however, were consistent with impaired bone strength in T2DM patients and were significant in the radius (>+50 %; $P < 0.05$), whereas pore volume approached significance in the tibia (+118 %; $P = 0.1$). They concluded that, in T2DM patients, the impaired resistance to bending loads may be due to inefficient redistribution of bone mass, characterized by loss of intracortical bone offset by an elevation in trabecular bone density. These results may provide a potential explanation for the inability of standard BMD measures to explain the elevated fracture incidence in patients with T2DM.

In a more recent study comparing T2DM postmenopausal women and age-matched control women differences were found in cortical porosity at the distal radius and tibia between T2DM patients with and without fragility fractures [10]. This study provided evidence for the key importance of cortical abnormalities in the increased prevalence of fragility fractures in T2DM patients and the potential role of HR-pQCT as a novel diagnostic imaging test measuring cortical porosity. For this study 80 women (mean age 61.3 ± 5.7 years) were recruited into four groups ($n = 20$ per group) with and without diabetes and with and without fragility fractures. Participants underwent DXA and HR-pQCT of the ultradistal and distal radius and tibia. In the HR-pQCT images volumetric bone mineral density, cortical and trabecular structure measures, including cortical porosity, were calculated. Bone strength was estimated using microfinite element analysis (microFEA). At the ultradistal and distal tibia, women with diabetic fractures had substantially greater intracortical pore volume (+52.6 %, $p = 0.009$; +95.4 %, $p = 0.020$), relative porosity (+58.1 %; $p = 0.005$; +87.9 %, $p = 0.011$) and endocortical bone surface (+10.9 %, $p = 0.031$; +11.5 %, 0.019) compared to diabetic women without fracture. At the distal radius T2DM women with fractures had a 4.7-fold greater relative porosity ($p < 0.01$) than women without fractures. At the ultradistal radius, intracortical pore volume was significantly higher in T2DM women with fractures compared to those

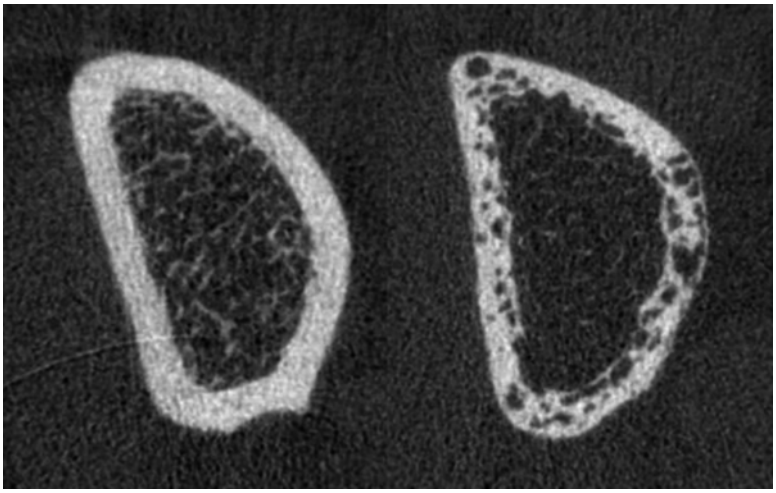

Fig. 11.2 Representative HR-pQCT images of the distal radius in Type 2 diabetic postmenopausal women with and without fragility fracture. Shown are the mid-stack tomograms for a Type 2 diabetic postmenopausal woman without history of fragility fracture (*left*) and a Type 2 diabetic woman with a positive history of fragility fracture (*right*). Note significant differences in cortical porosity between the 2 patients

without fractures (+67.8 %, $p=0.018$). T2DM women with fractures also displayed larger trabecular heterogeneity (ultradistal radius; +36.8 %, $p=0.035$), and lower total and cortical BMD (ultradistal tibia: −12.6 %, $p=0.031$; −6.8 %, $p=0.011$) than women without fractures. T2DM women with fractures also exhibited significantly higher pore-related deficits in stiffness, failure load and cortical load fraction at the ultradistal and distal tibia, and the distal radius than women with diabetes and no fractures. Figure 11.2 illustrates the findings of increased cortical porosity in a woman with type 2 diabetes and fragility fracture in relation to a diabetic woman without fracture. Interestingly, comparing nondiabetic fracture and control women, only a nonsignificant trend was found with increase in pore volume (+38.9 %, $p=0.060$) at the ultradistal radius. Overall, similar to the previous study, the results of this study suggested that severe deficits in cortical bone quality may be responsible for fragility fractures in postmenopausal diabetic women.

Magnetic Resonance Based Techniques

A small number of recent studies used magnetic resonance based techniques to investigate bone architecture and bone marrow composition in patients with diabetes [11–13]. Magnetic resonance spectroscopy (MRS) provides information on the biochemical composition of tissues and Li et al. introduced a method to quantify

vertebral bone marrow adiposity with proton MR spectroscopy ((1)H-MRS) at 3 T [20]. In their study, they showed high reproducibility of the technique with an average coefficient of variation of vertebral marrow fat content quantification of 1.7 %. They showed variation of marrow adiposity at different vertebral levels and feasibility for identifying patients with low bone density. In addition to quantifying overall bone marrow fat at 3 T, they were also able to selectively quantify the unsaturation level of the bone marrow fat [21].

Specifically using this technology in patients with T2DM, it was shown that vertebral bone marrow fat content correlated significantly with HbA1c and visceral adipose tissue in T2DM patients [22] and that decreased unsaturated bone marrow lipids were found to be associated with T2DM and fragility fractures [10]. Baum et al. [22] compared vertebral bone marrow fat content quantified with proton MR spectroscopy ((1)H-MRS) with the volume of abdominal adipose tissue, lumbar spine volumetric bone mineral density (vBMD), and blood biomarkers in postmenopausal women with and without T2DM. Thirteen postmenopausal women with T2DM and 13 age- and body mass index-matched healthy controls were included in this study. All subjects underwent (1)H-MRS of L1–L3 to quantify vertebral bone marrow fat content (FC) and unsaturated lipid fraction. QCT was performed to assess vBMD of L1–L3. The volumes of abdominal subcutaneous (SAT), visceral (VAT) and total adipose tissue (TAT) were determined from the QCT images and adjusted for abdominal body volume (SAT(adj)/VAT(adj)/TAT(adj)). Fasting blood tests were also obtained and included plasma glucose and HbA1c. Mean FC showed an inverse correlation with vBMD ($r=-0.452$; $P<0.05$) in the whole study population. While mean FC was similar in the diabetic women and healthy controls (69.3 ± 7.5 % vs. 67.5 ± 6.1 %; $P>0.05$), mean unsaturated lipid fraction was significantly lower in the diabetic group (6.7 ± 1.0 % vs. 7.9 ± 1.6 %; $P<0.05$). SAT(adj) and TAT(adj) correlated significantly with mean FC in the whole study population ($r=0.538$ and $r=0.466$; $P<0.05$). In contrast to the control group, significant correlations of mean FC with VAT(adj) and HbA1c were observed in the diabetic group ($r=0.642$ and $r=0.825$; $P<0.05$). This study demonstrated that vertebral bone marrow fat content correlates significantly with SAT(adj), TAT(adj), and lumbar spine vBMD in postmenopausal women with and without T2DM, but with VAT(adj) and HbA1c only in women with T2DM.

Patsch et al. [11] quantified vertebral bone marrow fat content and composition in diabetic and nondiabetic postmenopausal women with fragility fractures and compared these measurements with those of non-fracture controls with and without type 2 diabetes mellitus. Sixty-nine postmenopausal women (mean age 63 ± 5 years) were recruited. Thirty-six patients had spinal and/or peripheral fragility fractures. Seventeen of the fracture patients were diabetic. Thirty-three women were controls without fractures and 16 of these were diabetic. To quantify vertebral bone marrow fat content and composition, patients underwent MR spectroscopy (MRS) of the lumbar spine at 3 T. BMD was determined by DXA of the hip and lumbar spine and QCT of the lumbar spine. To evaluate associations of vertebral marrow fat content and composition with spinal and/or peripheral fragility fractures and diabetes, linear regression models adjusted for age, race, and spine volumetric bone mineral density

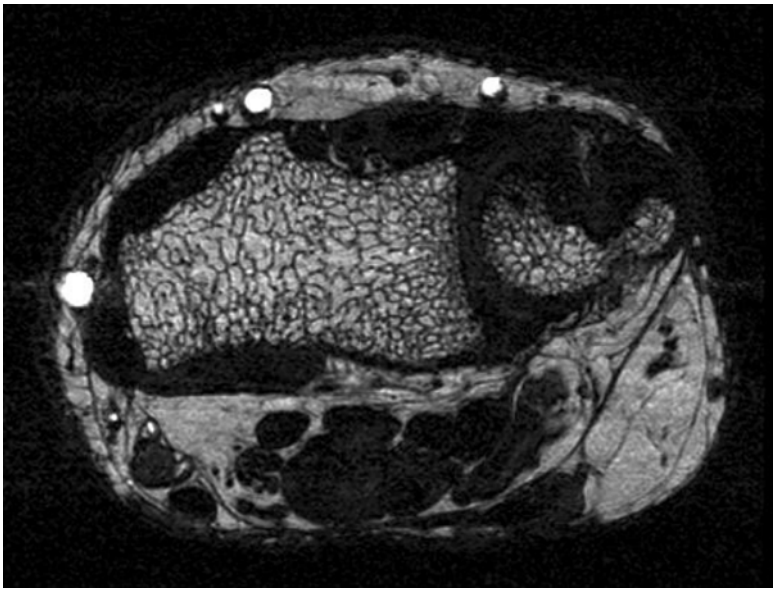

Fig. 11.3 *High resolution axial 3 T Magnetic Resonance Image of the distal radius* obtained with a 3D steady state free precession sequence with a spatial resolution of 156 µm × 208 µm × 500 µm. The image shows trabecular bone architecture with bone marrow interlay

(vBMD) by QCT were used. At the lumbar spine, nondiabetic and diabetic fracture patients had lower vBMD than controls and diabetics without fractures ($p = 0.018$; $p = 0.005$). However, areal bone mineral density (aBMD) by DXA did not differ between fracture and non-fracture patients. After adjustment for age, race, and spinal vBMD, the prevalence of fragility fractures was associated with −1.7 % lower unsaturation levels (confidence interval [CI] −2.8 to −0.5 %, $p = 0.005$) and +2.9 % higher saturation levels (CI 0.5–5.3 %, $p = 0.017$). Diabetes was associated with −1.3 % (CI −2.3 to −0.2 %, $p = 0.018$) lower unsaturation and +3.3 % (CI 1.1–5.4 %, $p = 0.004$) higher saturation levels. Diabetics with fractures had the lowest marrow unsaturation and highest saturation levels. In summary, these results demonstrate that altered bone marrow fat composition (lower unsaturation level) is linked with fragility fractures and diabetes. The authors suggested that MRS of spinal bone marrow fat may therefore serve as a novel tool for BMD-independent fracture risk assessment.

In addition to using magnetic resonance based spectroscopy for quantifying tissue composition, magnetic resonance imaging (MRI) can also be used to assess trabecular bone quality. A number of previous studies have shown that high-resolution MRI can directly visualize trabecular bone architecture at the distal radius, tibia, and proximal femur [23–26] (Fig. 11.3). Pritchard et al. were the first to use high-resolution MRI of the distal radius to compare trabecular bone microarchitecture of postmenopausal women with and without type 2 DM [13].

They acquired axial images with a 1 T MRI system and a voxel size of $0.195 \times 0.195 \times 1$ mm^3. Image post-processing included geometric, topologic, and stereologic measures including number and size of trabecular bone network holes (marrow spaces), endosteal area, trabecular bone volume fraction, nodal and branch density, and apparent trabecular thickness, separation, and number. They also measured lumbar spine and proximal femur BMD with DXA. They found that T2DM women ($n = 30$, mean ± SD age 71.0 ± 4.8 years) had larger holes (+13.3 %; $P = 0.001$) within the trabecular bone network than women without T2DM ($n = 30$, mean ± SD age 70.7 ± 4.9 years). Interestingly after adjustment for body mass index, DXA-based lumbar spine BMD did not differ between the diabetes and nondiabetes groups. They concluded that in women with type 2 DM, the average hole size within the trabecular bone network at the distal radius is greater compared to controls and hypothesized that this may explain the elevated fracture risk in women with T2DM.

These investigators also performed follow-up MR studies in a subset of their patients [12]. The aim of this study was to compare 2-year changes in trabecular bone microarchitecture in women with and without T2DM. Using the same technology as in the other study, they analyzed the number and size of trabecular bone holes, bone volume fraction (BV/TV), trabecular thickness (Tb.Th), number (Tb.N) and separation (Tb.Sp), endosteal area, nodal and branch density in 37 women. After adjustment for ethnicity, women with diabetes had a higher percent increase in number of trabecular bone holes compared to controls (10[1] % vs. −7[2] %, $p = 0.010$), however, results were no longer significant after adjustment for multiple comparisons ($p = 0.090$). There were also no differences in the change of the other trabecular bone microarchitecture variables between the two groups. This study provides feasibility data but clearly larger longitudinal studies with longer time intervals are required to study the evolution of T2DM-related bone architecture changes.

In the future, combined MR and HR-pQCT approaches may be used to better study the detailed mechanisms that drive bone changes in T2DM patients, in particular those that drive cortical porosity by fusing high-resolution peripheral quantitative computed tomography images (HR-pQCT) with high-resolution MR images [27]. Recent work showed that it is possible to study bone microarchitectural parameters and characterize intracortical marrow and vascular content, that are otherwise accessible only through destructive procedures [27].

Quantitative Ultrasound

Quantitative ultrasound (QUS) has been shown to measure mechanical properties of bone related to elastic modulus and compressive strength which reflect bone architecture, density, and elasticity [28]. Previous studies have shown that QUS of the calcaneus is effective in differentiating subjects with and without fragility fractures,

that it can be used to monitor treatment in metabolic bone disease and that it provides additional information beyond bone mineral density [29–31]. Based on the results of these previous studies, Yamaguchi et al. [14] investigated the role of calcaneal QUS in assessing fracture risk in T2DM. To test this hypothesis, these investigators measured calcaneal QUS as well as BMD at the lumbar spine, femoral neck, and 1/3 radius in 96 women (mean age 66.6 years old) and 99 men (64.7 years old) with T2DM, and examined their associations with prevalent vertebral fractures. Calcaneal speed of sound measurements were obtained in all patients. In T2DM patients, vertebral fractures were found in 33 women and 45 men. When comparing patients with and without vertebral fractures, there were no significant differences in values of speed of sound or BMD at any site between the groups in either gender. Logistic regression analysis adjusted for age and BMI showed that both QUS and BMD values were not significantly associated with the presence of vertebral fractures in either gender. The authors concluded that these results showed that QUS and BMD are unable to discriminate T2DM patients with and without prevalent vertebral fractures.

Another study, however, suggested that QUS measurements of the calcaneus could be used to differentiate elderly T2DM women with and without low energy fragility fractures at multiple different sites [32]. Patel et al. reported that while the fracture group did not differ significantly from the non-fracture group by age, diabetes-related risk factors or DXA BMD *Z* scores QUS variables were lower in the fracture group ($P=0.04$). It should be noted, however, that the results were borderline significant. Clearly, larger studies are required in the future to comprehensively assess the role of QUS in assessing fracture risk in patients with T2DM.

Trabecular Bone Score

Trabecular bone score (TBS) is a relatively new texture measure, which is used to analyze DXA images of the lumbar spine [33, 34]. Though DXA of the lumbar spine images provide very limited visualization of bone structure, they are obtained under very standardized conditions, are highly reproducible and potentially provide a texture assessment of combined cortical and trabecular bone [34]. Promising results have been found using TBS in previous studies; in a large cohort of 29,407 women, Hans et al. were able to demonstrate that significantly lower spine TBS and BMD were found in women with major osteoporotic, spine, and hip fractures (all $p<0.0001$) [35]. Interestingly spine TBS and BMD predicted fractures equally well, and the combination was superior to either measurement alone ($p<0.001$). The authors concluded that spine TBS predicts osteoporotic fractures and provides information that is independent of spine and hip BMD.

Using this texture measure in patients with T2DM, Leslie et al. [16] found similar results in differentiating women with and without major osteoporotic fractures. These investigators performed a retrospective cohort study using BMD results from

a large clinical registry for the province of Manitoba, Canada. They included 29,407 women 50 years old and older with baseline DXA examinations, among whom 2356 had been diagnosed with diabetes. TBS was obtained in each patient and patient records were assessed for incident nontraumatic major osteoporotic fractures. They found that T2DM was associated with higher BMD at all sites but lower lumbar spine TBS in unadjusted and adjusted models (all $P<0.001$). Major osteoporotic fractures were identified in 175 women (7.4 %) with and 1493 (5.5 %) without diabetes ($P<0.001$). Lumbar spine TBS was a BMD-independent predictor of fracture and predicted fractures in those with diabetes (adjusted hazard ratio 1.27, 95 % CI 1.10–1.46) and without diabetes (hazard ratio 1.31, 95 % CI 1.24–1.38). The investigators concluded that lumbar spine TBS predicts osteoporotic fractures in those with diabetes and captures a larger portion of the diabetes-associated fracture risk than BMD.

In another recently published cross-sectional study, Dhaliwal et al. investigated TBS in a smaller cohort of women aged 30–90 years with T2DM [15]. They found that mean TBS was lower in T2DM (1.228 ± 0.140 vs. 1.298 ± 0.132, $p=0.013$), irrespective of age while mean BMD was higher in T2DM (1.150 ± 0.172 vs. 1.051 ± 0.125, $p=0.001$). Within the T2DM group, TBS was higher (1.254 ± 0.148) in subjects with good glycemic control (A1c ≤ 7.5 %) compared to those (1.166 ± 0.094; $p=0.01$) with poor glycemic control (A1c >7.5 %). They concluded that in T2DM, TBS is lower and associated with poor glycemic control. It should be noted, however, that while the results of both of these studies are encouraging TBS is a texture parameter obtained from DXA images, which does not directly measure microarchitecture [33]. It is not entirely clear how TBS works in characterizing fracture risk and the results have to be interpreted cautiously.

Conclusion

Given the paradox of increased BMD and higher fracture risk in T2DM-related bone disease, a number of studies were performed using novel measurements of bone architecture, composition, and quality to better characterize bone abnormalities and fracture risk in T2DM patients. Promising results have been found in particular for HR-pQCT based cortical porosity, which may serve as a potential imaging biomarker for fracture risk in diabetic bone disease. Diabetic fracture and non-fracture groups could also be differentiated with MRS-based quantification of unsaturated lipids and a DXA-based texture measure of the lumbar spine. It should be noted, however, that most of these studies were conducted in relatively small subject numbers and larger scale research studies in this new field are required to investigate new imaging biomarkers that could predict and potentially help to treat the particularly devastating fragility fractures in T2DM patients.

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