The Impact of Diabetes and Diabetes Medications on Bone Health

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Patients with type 2 diabetes mellitus (T2DM) have an increased risk of fragility fractures despite increased body weight and normal or higher bone mineral density. The mechanisms by which T2DM increases skeletal fragility are unclear. It is likely that a combination of factors, including a greater risk of falling, regional osteopenia, and impaired bone quality, contributes to the increased fracture risk. Drugs for the treatment of T2DM may also impact on the risk for fractures. For example, thiazolidinediones accelerate bone loss and increase the risk of fractures, particularly in older women. In contrast, metformin and sulfonylureas do not appear to have a negative effect on bone health and may, in fact, protect against fragility fracture. Animal models indicate a potential role for incretin hormones in bone metabolism, but there are only limited data on the impact of dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 agonists on bone health in humans. Animal models also have demonstrated a role for amylin in bone metabolism, but clinical trials in patients with type 1 diabetes with an amylin analog (pramlintide) have not shown a significant impact on bone metabolism. The effects of insulin treatment on fracture risk are inconsistent with some studies showing an increased risk and others showing no effect. Finally, although there is limited information on the latest class of medications for the treatment of T2DM, the sodium-glucose co-transporter-2 inhibitors, these drugs do not seem to increase fracture risk. Because diabetes is an increasingly common chronic condition that can affect patients for many decades, further research into the effects of agents for the treatment of T2DM on bone metabolism is warranted. In this review, the physiological mechanisms and clinical impact of diabetes treatments on bone health and fracture risk in patients with T2DM are described. (Endocrine Reviews 36: 194–213, 2015)

I. Introduction

Epidemiological studies have shown an increase in fracture risk in patients with T2DM, despite their increased body weight. The mechanisms by which T2DM increases skeletal fragility are unclear. Axial bone mineral density (BMD) is typically normal or higher in patients with T2DM, thus removing low BMD as a mechanism for increased fragility (1). Complications of T2DM (such as sensory neuropathy and visual impairment) increase the risk of falling and may contribute to the increased risk of fracture. In addition, it has been hypothesized that neuropathy and chronic kidney disease may adversely impact regional bone mass and quality and therefore increase fragility. The addition of other factors, such as treatment-
induced bone loss, may accentuate the underlying skeletal fragility, increasing the risk for fractures in patients with T2DM. Thus, T2DM is associated with increased risk of fracture, which is likely due to a combination of greater risk of falling, regional osteopenia, and impaired bone quality and treatment effects. Unfortunately, little is known about the impact of most diabetes treatments on bone quality and fracture risk.

II. Epidemiological Evidence Linking Type 2 Diabetes Mellitus and Fracture Risk

A. Diabetes and overall fracture risk

A number of recent epidemiological studies have demonstrated an increased risk of fractures among patients with T2DM (Table 1). The Study of Osteoporotic Fractures (SOF) prospectively characterized the epidemiology of fractures in older American women. Participants in the SOF with T2DM (n = 657) had a 22% higher risk of nonspine fractures than those without T2DM (n = 8997) (2). Adjustment for diabetes complication-related variables did not attenuate the increased risk of fracture. The Women’s Health Initiative Observational Study included 93,000 postmenopausal women, of whom 5285 subjects had T2DM. During 7 years of follow-up, women with diabetes had a significantly higher risk of fracture after controlling for multiple risk factors, including a previous history of falls (relative risk [RR], 1.20; 95% confidence interval [CI], 1.11–1.30) (1). In this study, an increased risk of fracture was found at several sites, including the hip, foot, and spine (1). The Nurses’ Health Study, a study of 109,983 women with a mean follow-up of 22 years, demonstrated that subjects with both type 1 diabetes mellitus (T1DM) (n = 292) and T2DM (n = 8348) had an increased risk of fractures. After adjustment for other risk factors, the RR was 2.2 (95% CI, 1.87–2.7) for those with T2DM compared to those without (3). The Rotterdam Study, which included 6655 men and women over the age of 55, demonstrated that subjects with T2DM (n = 792) had higher BMD than subjects without diabetes. Nevertheless, the study revealed an increased risk of nonvertebral fractures in subjects with T2DM (RR, 1.33; 95% CI, 1.00–1.77) (4). Vestergaard et al (5) conducted a case-control study using a Danish national database that included 124,655 subjects who experienced a fracture in the year 2000 to determine the risk of fracture in patients with T1DM and T2DM. After adjustment for the use of antidiabetic agents (including insulin and oral agents), diabetes carried an increased risk of fracture in patients with both T1DM and T2DM, and, adjusting for complications of diabetes (except for kidney disease), added little to the overall risk of fractures (5).

In the Health, Aging and Body Composition Study, a higher fracture rate was observed in the diabetic population compared to adults without diabetes. After adjustments for hip BMD and additional risk factors for fracture, the RR of fracture was 1.64 (95% CI, 1.07–2.51) (6) in those with diabetes compared to those without.

Yamamoto et al (7) examined T2DM patients (161 men > 50 y old and 137 postmenopausal women) and nondiabetic controls (76 men and 622 postmenopausal women) using lateral spine radiography and dual-energy x-ray absorptiometry. Logistic regression analysis adjusted for age, body mass index (BMI), and BMD showed that the presence of T2DM was an independent risk factor for vertebral fracture in women (odds ratio [OR] = 1.86; P = .019) and men (OR = 4.73; P < .001). Comparison of T2DM patients with and without vertebral fractures showed no significant differences in BMD, markers of bone metabolism or glycemic control (7).

B. Diabetes and hip fracture risk

The Iowa Women’s Health Study, a prospective study of approximately 32,000 postmenopausal women in which diabetes status was self-reported, demonstrated that women with T2DM had an increased risk of hip fracture (RR, 1.7; 95% CI, 1.14–2.25) after adjusting for age, smoking status, estrogen use, BMI, and waist-to-hip ratio (8). Interestingly, an increased risk of hip fracture was also noted in subjects who did not have diabetes at baseline but developed diabetes during the follow-up period (RR, 1.60; 95% CI, 1.14–2.25) (8). Lipscombe et al (9) performed a retrospective cohort analysis using population-based Ontario, Canada, health care data on 197,412 residents age > 66 years of age with a mean follow-up of 6.1 years. Compared to individuals without diabetes, both men (hazard ratio [HR], 1.18; 95% CI, 1.12–1.24; P < .0001) and women (HR, 1.11; 95% CI, 1.08–1.15; P < .0001) with diabetes had an increased risk of hip fractures (9). Individuals in the cohort with diabetes had greater comorbidity, were less likely to have had a BMD evaluation, and were more likely to have been treated with medications that increase the risk of falling and decrease BMD (9).

Koh et al (10) recently described the association between diabetes and hip fracture in a population-based prospective cohort study of 63,257 Chinese men and women aged 45–74 years enrolled in the Singapore Chinese Health Study who were followed for a mean duration of 12 years. The diagnosis of diabetes was self-reported during the baseline interview, and postenrollment hip fractures were identified using a nationwide database of hospital discharges. After adjustment for other risk factors including BMI, physical activity, smoking, and self-reported cal-
Cium consumption, the risk of hip fracture was significantly increased among people with diabetes compared to people without diabetes (RR, 1.98; 95% CI, 1.71–2.29) (10). The study also illustrated a strong dose-dependent relationship between duration of diabetes and risk of hip fracture. The authors were unable to evaluate certain potential confounders, including previous history of falls, use of corticosteroids, and vitamin D levels. In addition, the authors did not have information regarding antidiabetic treatment, glycemic control, or the presence of micro- or macrovascular complications, all of which have been implicated as potential causes for increased fragility fractures in patients with diabetes. Nevertheless, this prospective cohort study, which was the first to examine the

Table 1. Summary of Clinical Studies Regarding Risk of Fracture at Various Sites in Patients With T2DM

<table>
<thead>
<tr>
<th>First Author (Ref.)</th>
<th>Study Design</th>
<th>Duration of Follow-up, y</th>
<th>Type of Fracture</th>
<th>Patient Population</th>
<th>No. of Participants by Gender</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forsen (173)</td>
<td>Prospective cohort</td>
<td>9</td>
<td>Hip</td>
<td>n = 1416, age &gt;50 y</td>
<td>581 men, 835 women</td>
<td>Men 1.2 (0.4–3.2), women 1.8 (1.1–2.9)</td>
</tr>
<tr>
<td>Ivers (145)</td>
<td>Prospective cohort</td>
<td>5</td>
<td>Any nontraumatic</td>
<td>The Blue Mountains Eye Study, n = 216, age &gt;49 y</td>
<td>NA</td>
<td>0.9 (0.7–1.2)</td>
</tr>
<tr>
<td>Nicodemus (8)</td>
<td>Prospective cohort</td>
<td>11</td>
<td>Hip</td>
<td>The Iowa Women’s Health Study, n = 1682 postmenopausal women, mean age 62 y</td>
<td>NA</td>
<td>1.7 (1.2–2.38)</td>
</tr>
<tr>
<td>Schwartz (2)</td>
<td>Population-based cohort</td>
<td>9.4</td>
<td>Hip</td>
<td>The Study of Osteoporotic Fractures, n = 657 women, age &gt; 65 y, mean age 72</td>
<td>NA</td>
<td>1.82 (1.24–2.69)</td>
</tr>
<tr>
<td>Ottenbacher (174)</td>
<td>Prospective cohort</td>
<td>7</td>
<td>Hip</td>
<td>Hispanic Established Population for the Epidemiologic Study of the Elderly, n = 690, age &gt; 65 y</td>
<td>NA</td>
<td>1.5 (1.03–2.39)</td>
</tr>
<tr>
<td>de Liefde (4)</td>
<td>Population cohort</td>
<td>6.8</td>
<td>Nonvertebral</td>
<td>n = 792, age &gt;55 y, mean age 74</td>
<td>309 men, 483 women, 276 men, 166 women</td>
<td>1.33 (1.00–1.76)</td>
</tr>
<tr>
<td>Holmberg (175)</td>
<td>Population cohort</td>
<td>17</td>
<td>Any nontraumatic</td>
<td>Malmo Prevention Project, n = 442, mean age 46 y</td>
<td>Men 2.4 (1.7–3.4), women 1.9 (1.3–2.8)</td>
<td>2.2 (1.8–2.7)</td>
</tr>
<tr>
<td>Janghorbani (3)</td>
<td>Prospective cohort</td>
<td>26</td>
<td>Hip</td>
<td>The Nurses’ Health Study, n = 8348, mean age 62 y</td>
<td>NA</td>
<td>1.20 (1.11-1.30)</td>
</tr>
<tr>
<td>Bonds (1)</td>
<td>Prospective cohort</td>
<td>7</td>
<td>Hip, foot, and spine</td>
<td>The Women’s Health Initiative Observational Study, postmenopausal women, n = 5285, mean age 65 y</td>
<td>NA</td>
<td>1.64 (1.07-2.51)</td>
</tr>
<tr>
<td>Strotmeyer (6)</td>
<td>Prospective cohort</td>
<td>4.5</td>
<td>Any nontraumatic</td>
<td>Health, Aging and Body Composition Study, n = 566, age 70–79 y</td>
<td>100 322 men, 97 090 women</td>
<td>Men 1.18 (1.12–1.24), women 1.11 (1.08–1.15)</td>
</tr>
<tr>
<td>Lipscombe (9)</td>
<td>Population-based, retrospective cohort</td>
<td>6.1</td>
<td>Hip</td>
<td>n = 197 412, age &gt; 66 y</td>
<td>100 322 men, 97 090 women</td>
<td>Men 1.18 (1.12–1.24), women 1.11 (1.08–1.15)</td>
</tr>
<tr>
<td>Vestergaard (5)</td>
<td>Case control</td>
<td>NA</td>
<td>Any nontraumatic</td>
<td>n = 124 655, mean age 43 y</td>
<td>NA</td>
<td>1.19 (1.11–1.27)</td>
</tr>
<tr>
<td>Ahmed (176)</td>
<td>Population-based cohort</td>
<td>6</td>
<td>Hip</td>
<td>Tromso Study, n = 373, mean age 64 y</td>
<td>175 men, 198 women</td>
<td>Men 1.63 (0.59–4.5), women 1.9 (1.04–3.49)</td>
</tr>
<tr>
<td>Koh (10)</td>
<td>Prospective cohort</td>
<td>12</td>
<td>Hip</td>
<td>The Singapore Chinese Health Study, n = 5668, age 47–74 y</td>
<td>2415 men, 3253 women</td>
<td>Men 1.77 (1.29–2.43), women 2.06 (1.75–2.43)</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available.
association between diabetes and hip fracture in a non-white population living in Asia, demonstrated results comparable to those in Western populations.

A meta-analysis of these and other studies by Janghorbani et al (11) has confirmed an increased fracture risk, particularly in the hip, foot, and proximal humerus, in patients with T2DM. Eight of the 12 studies included in the analysis found a statistically significant positive association between T2DM and incident hip fracture. The individual RRs ranged from 0.62–9.2, whereas the summary RR for all 12 studies was 1.7 (95% CI, 1.3–2.2) (11). In summary, most, but not all (12, 13), studies have demonstrated an association between fragility fractures and T2DM.

C. Assessing fracture risk in patients with T2DM

As noted above, T2DM is typically associated with obesity and a higher BMD, but paradoxically a higher risk for fractures (2, 12, 14, 145, 169). Thus, established methods for predicting fractures such as BMD and the fracture risk algorithm (FRAX) may not adequately reflect the risk in patients with T2DM. Schwartz et al (18) used data from three prospective observational studies with fracture outcomes—the Study of Osteoporotic Fractures (15), the Osteoporotic Fractures in Men Study (16), and the Health, Aging, and Body Composition Study (17)—to assess the association of BMD, T-score, and FRAX score with fractures in older adults with T2DM. The combined data included older, community-dwelling, adult men (n = 7436) and women (n = 9449) living in the United States. All fractures were self-reported. The study showed that among older adults with T2DM, femoral neck BMD T-score and FRAX score were associated with hip and nonspine fracture risk. However, the fracture risk in patients with T2DM was higher for a given T-score and age or for a given FRAX score compared to patients without diabetes (18). These results indicate that the increase in bone fragility in patients with T2DM is not predicted by low BMD and is more closely related to bone quality than bone quantity. It is therefore necessary to take diabetes into account when predicting fracture risk.

III. Bone Metabolism in Patients With T2DM

Bone remodeling is an ongoing cyclic process normally characterized by tight coupling between bone formation by osteoblasts and bone resorption by osteoclasts. Abnormalities in either process or an imbalance between the two are thought to account for metabolic bone disease. Bone resorption and formation can be determined indirectly by measurement of serum and/or urinary concentrations of a number of biomarkers. These markers are either enzymes involved in bone remodeling or bone matrix components released into the circulation during bone formation or resorption (19, 20).

There is some evidence that impaired bone turnover in T2DM results from decreased bone formation because osteoblast number and possibly function are decreased (21). Verhaeghe et al (22) showed that serum osteocalcin (a marker of osteoblast function) levels were decreased in diabetic rats compared with those of nondiabetic controls. Recently, Achemlal et al (23) investigated biochemical markers of bone turnover in male patients with poorly controlled T2DM receiving treatment with metformin, or a sulfonylurea, or both. They found serum osteocalcin levels were lower in diabetic patients than in controls, whereas markers of bone resorption (C-terminal telopeptides from type I collagen [CTX] levels) were not significantly different (23). Other data have suggested that alterations in bone resorption may also be present in T2DM. Suzuki et al (24) reported that concentrations of serum and urine markers of osteoclastic activity (tartrate-resistant acid phosphatase, CTX, and N-telopeptide of type I collagen) were all significantly elevated in men with T2DM. Alterations in bone formation and resorption in patients with T2DM can slow the rate of bone loss and cause a higher BMD than expected for their age (25–27). The reduction in bone turnover can also increase bone fragility, independent of BMD.

Petit et al (28) used peripheral quantitative computed tomography to compare tibial and radial bone volumetric BMD (vBMD), total and cortical bone area, and estimates of bone compressive and bending strength in a subset (n = 1171) of older men (> 65 y of age) who participated in the multisite Osteoporotic Fractures in Men Study. At both the distal tibia and radius, patients with T2DM had greater vBMD and a smaller bone area (28). At the mostly cortical bone midshaft sites of the radius and tibia, men with T2DM had a lower bone area resulting in lower bone bending strength at both sites after adjusting for body weight, despite the lack of difference in cortical vBMD at these sites. These data demonstrate that older men with T2DM have bone strength that is low relative to body weight at the cortical-rich midshaft of the radius despite having no difference in cortical vBMD (28).

Recent advances have established that bone is an endocrine organ regulating, among other functions, energy expenditure and glucose metabolism (29–31). Osteocytes have been shown to secrete a bone-specific glycoprotein called sclerostin that inhibits osteoblast differentiation (32, 33). By binding to low-density lipoprotein receptor-related protein 5 or 6 on the osteoblastic cells, sclerostin acts as an antagonist for the Wnt/β-catenin canonical sig-
naling pathway (34–36). Decreased Wnt/β-catenin results in decreased osteoblastogenesis and bone formation (37, 38). Recently, two cohort studies demonstrated that women with elevated sclerostin levels were at increased risk of fracture after adjusting for BMD (39, 40). Researchers have also demonstrated that in patients with T2DM, higher levels of circulating sclerostin were associated with disease duration and glycated hemoglobin (HbA1c) but were inversely related to bone turnover markers (41, 42). Yamamoto et al (43) conducted a cross-sectional observational study in 146 postmenopausal women and 175 men over 50 years old that examined the relationship between sclerostin levels and the presence of vertebral fractures in patients with T2DM. They found that elevated sclerostin levels were associated with an increased risk of vertebral fracture in male patients with BMD T-scores > -1 (OR = 1.85; 95% CI, 1.12–3.07) and female patients with T-scores < -1 (OR = 3.23; 95% CI, 1.42–7.34) after adjusting for multiple variables including BMD and markers of bone metabolism (43). Ardawi et al (44) also showed that increased serum sclerostin levels were positively associated with vertebral fractures (P = .006) in postmenopausal women with T2DM. These findings suggest that elevated sclerostin levels may increase bone fragility by worsening bone quality independent of BMD in patients with T2DM.

Recent advancements in genetic engineering and use of mouse models have identified a crucial role for osteocalcin in regulating insulin metabolism and energy expenditure (45). Osteocalcin is produced by osteoblasts and is γ-carboxylated to enhance attachment to hydroxyapatite (46, 47). Further investigations have shown that osteocalcin is also found within the skeletal matrix and is released into the circulation, in both the carboxylated and uncarboxylated form, during bone resorption (45). An examination of osteocalcin-null mice showed increased adiposity and dysfunction in glucose metabolism. Lee and colleagues (48) demonstrated that the relatively strong effects of loss of osteocalcin on glucose metabolism were due to changes in an enzyme that binds to the insulin receptor. Ferron et al (49) found that intermittent administration of osteocalcin significantly improved glucose tolerance and insulin sensitivity in mice fed a normal diet. The improvement was attributed to an increase in both β-cell mass and insulin secretion (49).

IV. Impact of Diabetes Treatments on Bone Health and Fracture Risk

In recent years, a large number of new oral and injectable medications have become available for the treatment of diabetes. Each class of medications operates through distinct biological pathways, and each has certain advantages as well as disadvantages. In the following section, we review the data on the effects of the most commonly prescribed medications for diabetes. In general, little is known about the bone effects of other, infrequently prescribed, drugs that are not covered below.

A. Thiazolidinediones

The thiazolidinedione (TZD) class of oral hypoglycemic agents, including rosiglitazone and pioglitazone, improves insulin sensitivity and is widely prescribed for the treatment of T2DM. In addition to an established role in the management of T2DM, TZDs have potential applications in other disorders characterized by insulin resistance such as polycystic ovary syndrome (50). The effects of TZDs are mediated by activation of the nuclear hormone receptor peroxisome proliferator-activated receptor (PPAR)-γ, particularly in adipocytes where expression of PPAR-γ is high. In addition to improving insulin sensitivity in adipose tissue, they promote differentiation of preadipocytes into mature adipocytes and accumulation of triglyceride into lipid droplets.

1. Results from cellular and animal studies

Activation of the PPAR-γ receptor has been shown to influence the lineage allocation of mesenchymal stem cells in the bone marrow (Figure 1). In rodent models, treatment with rosiglitazone increased the allocation of mesenchymal stem cells toward adipocytes and decreased differentiation toward osteoblasts while decreasing osteoblast function, leading to bone loss (51, 52). In addition to bone loss, high doses of rosiglitazone resulted in greater marrow adiposity in animal models (51, 52). The extent of fat deposition in vertebral marrow correlates negatively with bone mass and indicates a greater fracture propensity (53). Clinical studies are needed to investigate whether TZDs cause substantial changes in human bone marrow adipocytes. If marrow adipocytes proliferate at the expense of osteoblast formation in humans treated with TZDs, this may explain how these drugs decrease bone formation and increase fragility.

Recent animal studies have also shown that activation of PPAR-γ by TZDs promotes differentiation of osteoclasts. Wan et al (54) used a mouse model to specifically delete the PPAR-γ gene in the hematopoietic and endothelial cells in order to separate the effect of PPAR-γ deletion on the hematopoietic and mesenchymal lineage. Thus, PPAR-γ was deleted in osteoclasts but not osteoblasts. The mice developed increased bone mass as a result of impaired osteoclast differentiation. Subsequent ligand activation of PPAR-γ by rosiglitazone increased osteo-
blast differentiation in a receptor-dependent manner (54). These data suggest that PPAR-γ functions as a regulator of genes that mediate osteoclast differentiation (55) and impact the skeleton through increased osteoclast activity and bone resorption.

2. Results from clinical trials

Because animal models indicate that TZDs may cause reduced bone formation and bone loss, a number of studies have been conducted to examine the clinical effects of these antihyperglycemic agents on the skeleton. Grey et al (56) conducted a randomized, placebo-controlled trial indicating that rosiglitazone may cause bone loss in humans. The trial included healthy, nondiabetic, postmenopausal women without osteoporosis who were treated with rosiglitazone. After 14 weeks of treatment, a significant decrease in BMD (−1.9% rosiglitazone vs −0.2% placebo) was observed at the hip, accompanied by a modest reduction in markers of bone formation (56). The Health, Aging and Body Composition observational study conducted by Schwartz et al (57) also reported increased bone loss in diabetic women, but not men, taking any TZD medication (rosiglitazone, pioglitazone and troglitazone combined). The ADOPT (A Diabetes Outcome Progression Trial) study results showed a higher risk of fracture in diabetic women, but not men, on rosiglitazone monotherapy compared with metformin or glyburide (58). The increased risk was evident for both lower limb and upper limb fractures. Providing further evidence of a possible association between long-term use of TZDs and fractures, Meier et al (59) conducted a nested, case-control analysis using the UK General Practice Research Database to examine the use of TZDs or other oral antidiabetic drugs and the risk of fracture. Patients with T2DM who had used TZDs for approximately 12–18 months had an increased risk of fracture, particularly of the hip and wrist, compared to matched controls (59). The association was independent of patient age and sex and tended to increase with TZD dose. Loke et al (60) conducted a meta-analysis of the impact of TZDs on the risk of fracture and bone loss among patients with diabetes. The analysis, which included 10 randomized controlled trials that lasted at least 12 months and involved approximately 13 000 patients, found that TZDs were associated with a 45% increase in fracture (60). The risk appeared to be limited to women. The authors estimate that for every 55 women at low risk of fracture who take TZDs for 1 year, one fracture would occur. Among women at high risk, one fracture would occur for every 21 women who take TZDs for 1 year. A retrospective cohort study in a large health system in southeast Michigan examined the time-dependent relationship between TZD use and fracture risk (61). The primary outcome measure of the study was time to fracture, and further analyses were conducted to assess subgroups defined by age and sex. TZD use was associated with an increased risk of fracture in the overall cohort (adjusted HR, 1.35; 95% CI, 1.05–1.71) and in women (adjusted HR, 1.57; 95% CI, 1.16–2.14), but there was no increased risk of fracture in men alone. Women over the age of 65 had the greatest risk of fracture (61). Recent data from Bilik et al (62) found a statistically significant increased risk of fracture in postmenopausal women taking TZDs and a subset of men taking both loop diuretics and TZDs. The study used data from Translating Research into Action for Diabetes, a multicenter prospective observational study of diabetes care in the managed care setting. In a matched, case-control analysis, a total of 786 cases were identified, and up to four controls were matched to each case based on age, sex, race/ethnicity, and BMI. The study found that women over the age of 50 with fracture were more likely to have been exposed to TZDs (OR, 1.71; 95% CI, 1.13–2.58). A higher dose of TZD was associated

Figure 1. Pathophysiology of the increased risk of fragility fracture in patients with T2DM. AGES, advanced glycation end-products.
with a greater risk of fracture. There was no difference in fracture risk with the use of rosiglitazone or pioglitazone, indicating a class effect for the TZDs. A potential weakness of this study included the use of administrative data from a number of sources including surveys and medical records. In addition, the matched case controls did not control for all potential variables that may impact fracture risk, and this could have confounded the results. Further analyses of nonrandomized or observational studies have suggested that fracture risk may also be elevated in men and in the axial skeleton (63).

B. Metformin

Metformin is the most commonly prescribed medication for the treatment of T2DM. Although the exact mechanism is not fully understood, metformin likely improves glucose metabolism via activation of AMP-activated protein kinase (AMPK) (Figure 2) (64, 65). AMPK is ubiquitously expressed throughout the body, including in bone (66). AMPK subunits have been shown to have differential tissue-specific expression and activation (67). The AMPK α1-subunit is highly expressed in bone tissue, primary osteoblasts, and osteoclasts as well as in a number of bone cell lines (68–71).

1. Results from cellular and animal studies

Metformin has been shown to be a potent stimulator of AMPK activation in bone marrow progenitor cells (72), primary osteoblasts (71), and primary bone marrow macrophages (73). Stimulation of AMPK results in the differentiation and mineralization of osteoblasts in cell culture (74). Thus, activation of AMPK by metformin could directly influence bone metabolism. Kanazawa et al (75) demonstrated that metformin induced the differentiation and mineralization of osteoblastic MC3T3-E1 cells via activation of the AMPK signaling pathway and subsequent enhancement of endothelial nitric oxide synthase and bone morphogenetic protein-2 production. Cortizo et al (76) also investigated the effects of metformin on growth and differentiation of two osteoblast-like cell lines (MC3T3-E1 and UMR106) and found that metformin increased a marker of osteoblastic differentiation (alkaline phosphatase) in the MC3T3-E1 cells. In addition, treatment with metformin stimulated type 1 collagen production in both cell lines in a dose-dependent manner and, after 3 weeks of culture, strongly induced the formation of nodules of mineralization in MC3T3-E1 cells (76). These data suggest a direct osteogenic effect of metformin on osteoblasts in culture.

Metformin has also been shown to increase the osteogenic induction of bone marrow progenitor cells both in vitro and in vivo and to enhance the process of bone repair in diabetic and nondiabetic rat models (72). The osteogenic actions of metformin were associated with an increase in osteoblast-specific transcription factor Runx2/Cbfa1 and with an increase in the phosphorylation of AMPK (72). Zhen et al (77) investigated whether metformin could abrogate the deleterious effects induced by hyperglycemia in primary rat osteoblast cell cultures. They concluded that treatment with metformin significantly decreased intracellular reactive oxidative species production and osteoblast apoptosis. Treatment with metformin was also associated with a direct osteogenic effect on osteoblasts that was partially mediated by promotion of Runx2 and IGF-1 gene expression (77). Gao et al (78) investigated the action of metformin on bone mass in ovariectomized rats. The investigators found that the impaired bone density and bone quality induced by ovariectomy were improved by treatment with metformin. Experimental studies have also shown that treatment with metformin prevents bone loss in ovariectomized rats by reducing receptor activator of nuclear factor-κB ligand expression in osteoblasts and inhibiting osteoclast activity (79). Thus, the evidence sug-
gests that metformin has a direct effect to inhibit bone loss. It is also possible that metformin has beneficial effects on bone through augmentation of incretin and other gut hormone secretion (Section V.D).

2. Results from clinical trials

In the ADOPT study, the risk for fractures in newly diagnosed men or women with T2DM was lower among those randomized to metformin treatment compared to TZD treatment (58). Vestergaard et al (80) conducted a population-based case-control study on the RR of fractures in patients with T1DM and T2DM to assess the fracture risk associated with different treatment modalities. After adjustment for multiple covariates including prior fracture, use of drugs with potential effects on bone, and socioeconomic indices and others, treatment with metformin was associated with a 19% reduced risk of any fracture (OR, 0.81; 95% CI, 0.6–2.7; P < .01) (80). Treatment with metformin did not affect the risk of fracture at sites most often associated with significant morbidity including the forearm, spine, or hip (80), however. Monami et al (81) found no association between metformin and incident bone fractures in their case-control study of 1945 patients with T2DM. Recently, Borge et al (82) performed a randomized, parallel group, double-blind, multicenter study comparing the efficacy and safety of the combination of rosiglitazone/metformin with metformin monotherapy on long-term (80 wk) glycemic control and BMD in drug-naive patients with T2DM. At the conclusion of the study, the group treated with metformin had a modest increase in BMD, whereas those treated with rosiglitazone/metformin had a decrease in BMD (adjusted mean treatment difference between groups, −2.2%; 95% CI, −3.5, −0.9; P = .0012; and total hip, −1.5%; 95% CI, −2.3, −0.7; P < .05) (82). Mannucci et al (83) reported increased active GLP-1 concentrations after an oral glucose load in patients with T2DM treated with metformin. Metformin has been shown to increase cumulative 4-hour postmeal active GLP-1 levels by 1.7-fold (171). These studies highlight another potential mechanism by which metformin may enhance or preserve bone health. In summary, these data suggest that metformin may have a beneficial effect on bone.

C. Sulfonylureas

Despite the fact that sulfonylureas are among the most commonly prescribed drugs for T2DM and have been in use for over 50 years, surprisingly little clinical data exist on the effects of sulfonylureas on bone health. On balance, the available data suggest that sulfonylureas are at least neutral in regard to bone health. Vestergaard et al (80) reported that treatment with a sulfonylurea was associated with a significant reduction in risk of all-type fractures (adjusted OR, 0.88; 95% CI, 0.80–0.96) and hip fracture (adjusted OR, 0.77; 95% CI, 0.63–0.95). In addition, Monami et al (81) found no significant association between bone fracture and treatment with sulfonylureas, although fracture risk tended to be lower in patients on current sulfonylurea therapy (adjusted OR, 0.77; 95% CI, 0.44–1.37) as well as with prior therapy (adjusted OR, 0.67; 95% CI, 0.33–1.34) when compared to controls. Kanazawa et al (84) examined whether various treatment options for diabetes were associated with the presence of vertebral fracture in a study of 494 men and 344 postmenopausal women with T2DM. The study found that in postmenopausal women, treatment with a sulfonylurea was significantly and inversely associated with vertebral fracture (OR, 0.48; P = .018). Recent analysis of circulating bone biomarkers in a subset of patients from the ADOPT trial demonstrated that serum CTX (s-CTX), a marker of osteoclast activity, was reduced in both male and female patients treated for 52 weeks with metformin and glyburide (85). However, another analysis of patients with T2DM treated with sulfonylureas has demonstrated lower plasma levels of osteocalcin when compared to healthy controls (21).

D. Incretin-based treatments: dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 receptor agonists (GLP-1RA)

In addition to insulin resistance and islet cell dysfunction (86), significant impairments in the incretin effect contribute to hyperglycemia in patients with T2DM (87, 88). The incretin effect is defined as the enhancement in insulin secretion in response to an oral glucose challenge compared to an isoglycemic iv glucose challenge. The incretin effect is mediated by glucose-dependent insulino tropic polypeptide (GIP) released from enteroendocrine K-cells in the duodenum and proximal jejunum (89) and glucagon-like peptide (GLP)-1 secreted from L-cells located in the distal ileum and colon (89). GIP and GLP-1 are secreted into the circulation as active hormones after nutrient ingestion and bind to specific G protein-coupled receptors present on β-cells and other target tissues. Both hormones are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4), a ubiquitous serine peptidase (88, 90). In patients with T2DM, the incretin effect is markedly reduced to approximately 25% of the level observed in people without diabetes (87). Plasma levels of GIP are normal to increased in patients with T2DM, whereas postprandial levels of GLP-1 are decreased in patients with T2DM in some, but not all studies (91). Despite the normal to increased levels of GIP in patients with T2DM, the insulinotropic response to native GIP is diminished (92).
Insulinotropic responses to GLP-1 are also somewhat impaired in patients with T2DM, but at pharmacological levels GLP-1 can produce robust insulin secretory responses. DPP-4 inhibitors represent a novel class of oral medications for the treatment of T2DM that act as incretin enhancers by inhibiting the inactivation of endogenous incretin hormones by DPP-4. DPP-4 inhibitors increase active incretin hormone levels 2- to 3-fold by providing up to 90% inhibition of plasma DPP-4 activity over 24 hours in vivo (93, 94). Drugs in this class are gaining popularity as treatments for T2DM.

In addition to their role to enhance insulin secretion in response to ingested nutrients, there is accumulating evidence that the incretin hormones play an important role in bone homeostasis. Calcium homeostasis is maintained through a reversible demineralization of the skeleton. During times of need, the skeleton acts as a reservoir for mobilization of calcium. In times of acute excess, for instance after a meal, or chronic excess, such as in obese patients, mechanisms are in place to restore the important balance of serum calcium levels (95). The acute change in bone turnover in response to the ingestion of nutrients is part of a system designed to adapt to changes in energy and nutrient uptake. In times of energy and nutrient excess, the balance is tipped in favor of bone formation, whereas in times of energy and nutrient insufficiency, bone resorption increases (95). One or more hormones, released by the gut during the ingestion of nutrients, may integrate nutrient uptake and bone turnover. In particular, GIP and possibly GLP-1 and GLP-2 may play a role in linking nutrient ingestion to suppression of bone resorption and stimulation of bone formation (96).

### 1. Results from cellular and animal studies

Supporting a potential role of incretin hormones in modulating bone metabolism, Bollegaard et al (97) demonstrated that GIP receptors (GIPRs) are present in bone cells, including osteoblasts and osteocytes, as well as in human osteoblast-like osteosarcoma cell lines. These investigators showed that human osteoblast-like cell lines respond to physiological levels of GIP stimulation, resulting in an increase in intracellular calcium levels, cellular cAMP content, alkaline phosphatase activity, and collagen type 1 expression. GIP may also have an anabolic effect on bone by protecting the osteoblasts against apoptosis (98). The GIPR is also present on osteoclasts and has been shown to inhibit bone resorption through the inhibition of PTH (99). In vivo, GIPR knockout (Gipr −/−) mice exhibit low bone density secondary to increased bone resorption and decreased bone formation. Histological analysis of 8-week-old male mice showed thinner trabeculae in Gipr −/− mice compared to Gipr +/+ controls (98). Histochemical analysis showed a similar number of osteoblasts in the knockout and control mice, but the number of osteoclasts was increased in the knockout animals (98). Gipr −/− mice were also found to have increased urinary levels of pyridinoline cross-link, a marker of bone resorption (98). Xie et al (100) investigated the role of GIP in modulating bone turnover by evaluating serum markers of bone metabolism, BMD, and changes in biomechanical bone strength in GIPR knockout mice. As in earlier studies, GIPR knockout mice had decreased bone size, lower bone mass, and altered microarchitecture. In addition, knockout animals had decreased levels of serum markers of bone formation including osteocalcin (53.8%) and alkaline phosphatase (16.7%) as compared to wild-type controls. Serum levels of a marker of bone resorption (pyridinoline cross-links) were not significantly different between the GIPR knockout and wild-type animals (100). Further supporting the role of GIP in bone formation, GIP-overexpressing transgenic mice were found to have increased bone mass (96). In contrast to the consistent data in rodents, there are limited data on the effects of GIP in humans. Serum markers for bone resorption and bone formation were unaffected by the infusion of iv administration of GIP in a small number of human participants (101). However, the study was difficult to interpret due to the lack of a control group, short infusion time, and very short duration of follow-up.

The physiological role of GLP-1 on bone is less clear. Using a transgenic mouse model, Yamada et al (102) have shown that mice with a homozygous deletion for the pancreatic GLP-1 receptor have osteopenia and increased bone fragility as a result of increased bone resorption by osteoclasts. The effect on osteoclasts was thought to be related to reduced thyroid calcitonin expression. Yamada et al (102) suggest that GLP-1 may target calcitonin to modulate bone turnover because GLP-1 does not appear to have a direct effect on osteoblasts or osteoclasts in vitro. Nuche-Berenguer et al (103) demonstrated that treatment with GLP-1 had an insulin-independent bone anabolic effect in rat models of insulin resistance and T2DM. In a follow-up study, the same group (Nuche-Berenguer et al [103]) reported that GLP-1 can directly and functionally interact with the MC3T3-E1 osteoblastic mouse cell line. The binding is further evidence for the presence of a GLP-1 receptor that displays both high- and low-affinity binding capacity for the gut hormone. The study details evidence for a GLP-1 binding membrane protein in MC3T3-E1 cells that is similar to that found in other extrapancreatic tissues (104). Recent work by Pacheco-Pantoja et al (105) confirmed the presence of a GLP-1 receptor in two human osteoblastic cell lines (MG-63 and TE-85). Other preclinical evidence suggests that GLP-1 receptor antagonism in-
increases bone formation, decreases bone resorption, and increases BMD (106). Collectively, these data suggest that GLP-1 may play a role in either direct or indirect modulation of postprandial bone metabolism.

Like GLP-1, GLP-2 arises from posttranslational processing of proglucagon, is synthesized in the L cells of the intestinal mucosa, and is secreted in response to the presence of nutrients in the intestinal lumen (107). GLP-2 is also rapidly inactivated by DPP-4 with a biological half-life of approximately 7 minutes (108). GLP-2 is not considered an incretin hormone because it does not stimulate insulin secretion by the pancreatic β-cells. Rather, GLP-2 is thought to have a primary role maintaining the integrity of the gut epithelium. Similar to other proglucagon-derived peptides, GLP-2 is involved in the regulation of absorption and disposal of nutrients in the postprandial period (109). The presence of a GLP-2 receptor has not been confirmed in osteoblastic or osteogenic cells; however, two studies provide some evidence for its existence in bone-related cells (105). Small studies in humans have shown that GLP-2 may also play a role in inhibiting bone resorption after nutrient ingestion. In healthy volunteers and postmenopausal women, a single-dose, sc injection of GLP-2 has been shown to reduce markers of bone resorption (s-CTX) in an acute, dose-dependent manner (110). In addition to acute reductions in markers of bone resorption, a sustained reduction in s-CTX was found after 14 days of treatment with SC, once daily GLP-2 in 60 postmenopausal women (111). Neither short-term nor longer term treatment with GLP-2 resulted in changes in markers of bone formation. Thus, GLP-2 may affect bone remodeling by disassociating bone resorption and bone formation (111). Shifting the delicate balance of bone metabolism toward bone formation may lead to increased bone mass. It is not known whether the effects of GIP, GLP-1, and GLP-2 on bone are important in humans, whether they are diminished in patients with T2DM, and whether this contributes to bone fragility in this population.

2. Results from clinical trials

Because of their effects to stabilize active forms of GIP, GLP-1, and GLP-2, DPP-4 inhibitors might, in theory, be expected to improve postprandial calcium accretion through the effects of enhanced incretin hormone activity and thereby have beneficial effects on bone health. Bunck et al (112) conducted a study to assess whether a DPP-4 inhibitor, vildagliptin, had an impact on bone resorption and calcium homeostasis. Fifty-nine drug-naïve patients with T2DM were randomized to either 1-year treatment with the DPP-4 inhibitor vildagliptin 100 mg once daily or placebo. Before and after the 50-week treatment period, patients received a mixed meal test, after which concentra-

lations of s-CTX, a marker of bone resorption, were measured. Vildagliptin 100 mg, compared to placebo, did not change postprandial CTX concentrations from pretreatment (AUC0–4h, adjusted least squares mean ± SE ratio from pretreatment: vildagliptin, 1.00 ± 0.10; placebo, 0.87 ± 0.09; between group ratio, 1.15 ± 0.17; P = .320) (112). Additional markers of bone metabolism and calcium homeostasis, such as fasting serum alkaline phosphatase, calcium, and phosphate also remained unaffected after 1-year treatment. The authors concluded that 1 year of treatment with vildagliptin 100 mg was not associated with changes in markers of bone resorption and calcium homeostasis in drug-naïve patients with T2DM and mild hyperglycemia. Monami et al (113) recently published a meta-analysis of randomized clinical trials of DPP-4 inhibitors and bone fractures. The meta-analysis included all trials with a duration of > 24 weeks, enrolling patients with T2DM and comparing DPP-4 inhibitors with placebo or other active drugs. The principal outcome was the effect of DPP-4 inhibitors on the incidence of bone fractures reported as serious adverse events. Of the 54 available trials that were reviewed, 16 did not disclose bone fractures and 10 reported zero events. The meta-analysis was performed on 28 trials and included 11 880 patients on DPP-4 inhibitors and 9175 comparators. The mean duration of treatment was 35 weeks. Age, sex, BMI, duration of diabetes, and baseline Hba1c were similar in both groups. The study reported a total of 63 bone fractures, with 26 in the DPP-4 inhibitor group and 37 in the comparators. The Mantel-Haenszel OR for DPP-4 inhibitors was 0.60 (95% CI, 0.37–0.99; P = .045) (113). It is important to note that the trials used for this analysis did not include fractures as a principal endpoint. Fractures were reported as serious adverse events, which may have underestimated the total number of fractures. The study also noted the relatively short duration of the trials as a potential limitation. In addition, the authors were unable to discriminate between sexes, pre- and postmenopausal women, or fracture site. Despite the limitations of this meta-analysis, the available trial data suggest that DPP-4 inhibitors could have a protective effect on bone. An in-depth analysis and/or a prospective trial assessing the risk of fracture with use of DPP-4 inhibitors is needed.

GLP-1 receptor agonists are an alternative approach to leveraging the beneficial effects of the incretin system for the treatment of T2DM. There are four GLP-1 receptor agonists currently available in the United States for the treatment T2DM, and a number of products are in various stages of development. Exenatide is a synthetic, 39-amino acid peptide form of the exendin-4 molecule that shares 53% homology with mammalian GLP-1 but is resistant to enzymatic degradation by DPP-4 (114). A once-weekly
preparation of exenatide was approved by the Food and Drug Administration in 2012. Liraglutide is a once-daily human GLP-1 analog with 97% homology with human GLP-1. The addition of a fatty acid side chain accounts for the slower absorption and a longer duration of action of liraglutide (115). Albiglutide and dulaglutide are novel, once-weekly GLP-1 receptor agonists recently approved for the treatment of T2DM. In theory, treatment with a GLP-1 agonist could impact bone metabolism by mimicking the physiological actions of native GLP-1. To date there have been few clinical trials that have examined the impact of GLP-1 receptor agonists on bone metabolism.

Pratley et al (172) performed an analysis of total BMD in a subgroup of patients from the liraglutide vs glimepiride monotherapy for T2DM (LEAD-3 Mono) trial. LEAD-3 Mono was a double-blind, double-dummy, active-control, parallel-group study that investigated the safety and efficacy of two doses of liraglutide (1.2 and 1.8 mg/d) vs glimepiride for treatment of T2DM. The subgroup of patients included a total of 90 individuals divided into one of three treatment groups (liraglutide 1.2 or 1.8 mg/d or glimepiride 8 mg/d). Baseline characteristics including age, BMI, duration of diabetes, HbA1c and baseline total BMD were similar across treatment groups. There was no statistically significant difference in total BMD in patients who received liraglutide 1.8 or 1.2 mg/d or glimepiride 8 mg/d at 52 or 104 weeks. All relative changes in BMD were less than 1% of baseline. Bunck et al (116) presented similar findings after an assessment of the differential effects of exenatide and insulin glargine on BMD and markers of calcium homeostasis in patients with T2DM. A total of 69 metformin-treated patients (45 males, 24 females) were randomized to exenatide (n = 36) or insulin glargine (n = 33). The average HbA1c was 7.5 ± 0.8%. BMD was assessed at baseline and after 44 weeks. The results showed that at 44 weeks the exenatide group had a statistically significant reduction in body weight compared to the insulin glargine group (exenatide, −3.5 ± 0.9 kg; insulin glargine, +0.3 ± 0.9 kg). Despite the change in weight, no effect on total BMD was observed with either exenatide or insulin glargine. Adjustments for gender did not change the results. Markers of calcium homeostasis (serum alkaline phosphatase, calcium, and phosphate) did not change after 44 weeks of treatment (116). Two recent meta-analyses of fractures reported in clinical trials of GLP-1RA demonstrated the lack of available data, reporting only 19 and 48 fractures, respectively (117, 118).

These relatively small clinical trials did not show that GLP-1 receptor agonists had a significant impact on total BMD in patients with T2DM. However, the trials investigated changes in total BMD. It remains possible that treatment with a GLP-1 agonist caused regional changes in BMD that were not detected in these trials. Further studies are needed to extend these findings to a larger population of patients with T2DM and to determine whether treatment with a GLP-1 receptor agonist is associated with a reduced risk of fracture in patients with T2DM.

E. Pramlintide

Amylin belongs to a family of peptides that includes calcitonin, calcitonin gene-related peptides, adrenomedullin, and intermedin. Amylin acts through a specific G protein-coupled receptor, the calcitonin receptor, in combination with a receptor activity-modifying protein (RAMP1 or RAMP3) (119). Amylin is cosecreted with insulin from the pancreatic β-cells. The effects of amylin on glucose metabolism and food intake are mediated by peripheral and central mechanisms. T1DM is associated with the total or near-total absence of amylin, whereas early in T2DM amylin is present in excess with a decline to low concentrations late in the disease. In addition, amylin concentrations do not seem to change in response to meals in patients with advanced T2DM (12). Like calcitonin, amylin has been implicated in bone physiology.

1. Results from cellular and animal studies

In vitro studies of the effects of amylin have shown that the hormone may influence the function of both osteoblasts and osteoclasts. Cornish et al (120) reported that physiological doses of amylin stimulated the proliferation of fetal rat osteoblasts in vitro. The researchers also noted a substantial increase in histomorphometric indices of bone formation and an increase in mineralized bone area after 5 days of injections of amylin over the calvariae of adult mice in vivo (120). The local injection of amylin over the calvariae of female OF-1 mice increased the mineral apposition rate to a similar degree as the potent bone anabolic peptide, PTH (121). In osteoblasts, amylin has been shown to increase cAMP and activate MAPK and protein kinase C (122). Further work conducted by Cornish et al (123) investigated the systemic administration of amylin on bone metabolism in adult male mice. Adult male mice were given daily sc injections of amylin or vehicle for a 4-week period. In the amylin-treated group histomorphometric indices of bone formation increased from 30–100%, and indices of bone resorption fell by approximately 70% (P < .005 for all indices) (123). Amylin has been shown to inhibit osteoclast activity through an increase in cAMP levels and reduction of osteoclast development (124, 125). Pietschmann et al (126) demonstrated that treatment with amylin resulted in the reduction of baseline and stimulated bone resorption through the stimulation of cAMP in a neonatal mouse model. Amylin de-
ficiency has been shown to lead to bone loss in mice, and treatment with amylin can partially reverse osteopenia in ovariektomized rats (127, 128). In rats made diabetic by streptozotocin, treatment with amylin, insulin, or both improved or normalized many of the indices of osteopenia present in untreated diabetic rats (129). Bronsky and Prsa (130) compared total and unreduced fasting plasma amylin levels in patients with osteoporosis (n = 28) and T2DM (n = 10) and in a control group (n = 24). Plasma levels were lower in patients with osteoporosis than in patients with T2DM (P < .01) or the control group (P < .001) (130).

2. Results from clinical trials

Pramlintide is a synthetic analog of amylin that is approved for the treatment of T1DM as an adjunct to mealtime insulin in patients who have failed to achieve glycemic targets (131). In addition, pramlintide is approved as an adjunct to mealtime insulin for use in patients with T2DM who have failed to achieve glucose control despite optimal insulin therapy, with or without the use of a sulfonylurea and/or metformin (131). A number of clinical trials have shown that the addition of pramlintide to insulin and/or oral agents in patients with T2DM resulted in a small, but statistically significant reduction in HbA1c and weight when compared to placebo (132–136). The impact of pramlintide on bone metabolism has not been extensively studied. Borm et al (137) conducted a small clinical trial in 23 patients (13 males and 10 females) with T1DM (age, 42.2 ± 10.3 y; duration of diabetes, 20.7 ± 9.8 y) that investigated the effect of 12 months of treatment with pramlintide on bone metabolism. BMD measurement of the lumbar spine by dual-energy x-ray absorptiometry and biochemical markers of bone metabolism, including calcium, PTH, osteocalcin, and urinary pyridinium cross-links, were obtained at baseline and after 1 year of treatment with pramlintide. There was no statistically significant change in BMD or markers of bone metabolism after 1 year. The subjects did not have osteopenia at baseline, and therefore the impact of pramlintide on bone metabolism in patients with T1DM and osteopenia is unclear (137).

Most of the available in vitro and animal study data suggest a role for amylin in the physiological regulation of bone metabolism; however, human clinical trials in patients with amylin deficiency (ie, T1DM) have not demonstrated a clear impact on bone metabolism with amylin treatment. The potential impact on patients with T2DM is unknown, but it is possible that reduced amylin levels late in the disease process and/or reduced physiological effectiveness of amylin is one of several factors that lead to the increased risk of fracture in these patients. Clinical trials are needed to further investigate the impact of treatment with amylin analogs on long-term bone health and fracture risk in patients with T2DM.

F. Insulin

To date, there have been no randomized controlled trials designed to evaluate the impact of insulin treatment on bone health in patients with T2DM. The clinical findings of osteopenia and osteoporosis in young patients with T1DM have led to the hypothesis that insulin is an anabolic agent in bone (138). Animal models have also supported a direct anabolic effect of insulin on bone. Streptozotocin-induced diabetic mice expressed reduced IGF-1, IGF-1 receptor, and insulin receptor levels within the skeletal growth centers (139). The animals also exhibited severe bone histological changes and growth retardation. In addition, in vivo murine models that lack insulin receptor substrate that mediates both insulin and IGF-1 signaling were found to have impaired bone formation and low bone turnover (140, 141). Insulin receptor substrate-1 knockout mice have impaired bone healing, whereas insulin receptor substrate-2 knockout mice appear to develop normally but have osteopenia with decreased indices of bone formation and evidence of increased bone resorption (140, 142). Clinical trials in humans with T1DM also support the concept that insulinopenia may impair bone formation. In a study of 57 patients with T1DM (mean age, 29 ± 9 y), intensive insulin treatment was associated with a stable BMD over a 7-year period and a 38% decrease in bone resorption determined by serum activity of tartrate-resistant acid phosphatase (143). However, the treatment group also had a significant improvement in their glycemic control during the study compared to controls.

In contrast to the hypoinsulinemia of T1DM, T2DM is characterized by insulin resistance, impaired insulin secretion, and a variable degree of hyperinsulinemia (144). A number of studies have shown that fracture risk is higher in insulin-treated patients than in non-insulin-treated patients with T2DM. In an analysis from the Study of Osteoporotic Fractures, Schwartz et al (2) compared risk of fracture in postmenopausal women with T2DM treated with and without insulin. The risk of foot fracture was increased in the insulin-treated group (RR, 2.54; 95% CI, 1.01–6.34) after multivariate adjustment. There was no statistically significant increased risk of fracture at other skeletal sites in women treated with insulin (2). Similar findings of an increased risk of fracture of the proximal humerus and all fractures combined in patients with T2DM treated with insulin were reported by Ivers et al (15) in the Blue Mountain Eye Study. However, the authors were unable to accurately determine what propor-
tion of subjects had T1DM vs T2DM. Nicodemus et al (8) showed that postmenopausal women with T2DM who used insulin were at the highest risk (RR, 2.66; 95% CI, 1.52–4.64) for hip fracture. A more recent case-control study by Vestergaard et al (80), insulin treatment (with varying doses) did not affect the incidence of all-type fracture (adjusted OR, 1.04; 95% CI, 0.92–1.18). Review of the published data shows a trend toward a reduction in the risk of fracture at the hip in insulin-treated patients; however, the difference was not statistically significant (80).

The lack of randomized, controlled trials regarding the impact of insulin treatment on fracture risk in patients with T2DM makes it difficult to draw conclusions on whether the association between fracture risk and insulin is related to the treatment with insulin or the patient’s disease state. Insulin-treated patients on average have a longer duration of disease and a higher prevalence of micro- and macrovascular complications. Thus, insulin use may be a surrogate for severity or duration of T2DM, presence of complications, risk of hypoglycemia, or increased fall risk—all of which likely play a role in the increased fracture risk of patients with T2DM.

G. Sodium-glucose co-transporter 2 inhibitors (SGLT2)

SGLT2 inhibitors are a new class of oral diabetes medications. Three SGLT2 inhibitors (dapagliflozin, canagliﬁlozin, and empagliflozin) are currently available in the United States for treatment of T2DM. SGLT2 is almost exclusively expressed in the renal proximal tubules and is responsible for 90% of glucose resorption by the kidneys (146). Preclinical and clinical trials have shown that SGLT2 inhibitors work independently of insulin and lead to negative energy balance through increased glucosuria (147). SGLT2 inhibitors have been shown to improve hyperglycemia without significant hypoglycemia and are typically associated with modest weight loss in patients with T2DM (148).

1. Results from clinical trials

Given the mechanism of action of SGLT2 inhibitors, there has been concern regarding the potential for alterations in calcium homeostasis and BMD. List et al (149) conducted a study in which 389 patients with T2DM were randomized to one of five dapagliflozin doses, metformin XR, or placebo for 12 weeks. The primary objective was to compare mean change in HbA1c, but other objectives included the evaluation of adverse events and laboratory measurements. Treatment with dapagliflozin resulted in no significant change from baseline in serum calcium, 1,25-dihydroxyvitamin D, and 25-hydroxyvitamin D levels. Mean changes in the 24-hour urinary calcium-to-creatinine ratio were similar to those with placebo. Small increases in mean PTH concentrations (range, 0.6–7.0 pg/mL above baseline of 31.1–35.0 pg/mL) were generally greater than the 0.8 pg/mL increase for placebo (149). Dapagliflozin has been shown to produce weight loss (148, 150–153) and may potentially reduce BMD (154). Recently, Ljunggren et al (155) reported no significant change in markers of bone formation and resorption, or in BMD at any investigated anatomical region with dapagliflozin treatment over 50 weeks vs placebo. The group also reported no significant change in calcium, 25-hydroxyvitamin D, or PTH compared to placebo. During 2 years of continued observation, only one fracture occurred in the treatment group (156). The safety of dapagliflozin was reviewed in a pooled analysis of phase IIb/III studies by Ptaszynska et al (157). Patients received comparator or dapagliflozin as monotherapy, as an add-on to antidiabetic therapy, or as initial combination with metformin. The principal analysis used data from 12 placebo-controlled studies. There was no evidence of increased fracture risk with dapagliflozin in the pooled analysis (157). A recent randomized controlled trial conducted by Kohan et al (158) investigated the use of dapagliflozin in patients with T2DM and moderate renal impairment. Through 104 weeks, 13 (7.7%) patients experienced a fracture in the dapagliflozin group vs no patients in the placebo group (158). All fractures occurred after trauma and were described as low impact. The authors reported that seven of 13 patients who sustained a fracture had a history of diabetic neuropathy or exhibited orthostatic hypotension and may, therefore, have been predisposed to falls. The significance of the increased number of fractures in this study is uncertain. All but one of the fractures (which was at the hip) were at sites not typically suggestive of an insufficiency fracture.

H. Drugs in development

Fibroblast growth factor (FGF) 21 was recently identified as a novel metabolic and endocrine hormone (159). There are a total of 22 members of the FGF family, and most possess broad mitogenic and cell survival-inducing effects. FGF21 is predominantly produced in the liver, but can also be found in white adipose tissue, pancreatic ß-cells and skeletal muscle (159). Kharitonenkov et al (160, 161) demonstrated that FGF21 induces glucose up-
take and decreased glucose and lipid concentrations in obese mice (160) and diabetic monkeys (161). FGF21 has also been shown to increase energy expenditure, promote weight loss, and up-regulate fatty acid oxidation (162, 163). A number of trials have demonstrated the effects of FGF21 on carbohydrate and lipid metabolism. The centrally mediated effects of FGF21 have been shown in rodents, obesity has been defined as a FGF21-resistant state, and hormonal regulation of FGF21 has been determined in both rodent and human studies (164). Expression of FGF21 is controlled by a number of transcriptional activators such as PPAR-α in the liver (165) and PPAR-γ in adipocytes (166). FGF21 is a powerful regulator of glucose homeostasis, lipid metabolism, and energy balance and is a potential drug for the treatment of diabetes and obesity.

Wei et al (167) examined the consequences of chronic FGF21 exposure on bone using a FGF21-transgenic (Tg) mouse, in which FGF21 concentrations are 5-fold higher than in fasted mice. Microcomputed tomography imaging of the tibias demonstrated a decrease in trabecular bone in the FGF21-Tg mice compared to wild-type controls. In addition, the bone formation marker N-terminal propeptide of type 1 procollagen was 40% lower, whereas the bone resorption marker CTX was 22% higher in the FGF21 mice. Bone histomorphometry showed that the osteoblast number and surface were significantly lower, whereas osteoclast number and surface were significantly higher (167). The results show that the lower bone mass in the FGF21-Tg mice was caused by a combination of decreased bone formation and increased bone resorption. Short-term treatment with FGF21 caused bone loss in diet-induced obese mice (167). FGF21-knockout mice developed high bone mass phenotype with more trabecular bone when compared to wild-type controls (167). Wei et al (167) also conducted ex vivo bone marrow differentiation assays under conditions that favored either adipocyte or osteoblast formation. FGF21 treatment was found to significantly increase the differentiation of marrow mesenchymal stem cells to adipocytes (167). The data show that FGF21 is a physiologically relevant regulator of skeletal development and favors the differentiation of adipocytes over osteoblast. These represent mechanisms by which treatment with FGF21 could alter bone metabolism and increase fracture risk. As drugs in this class progress through the various stages of development, further evaluation is needed to assess the long-term effects on bone metabolism and fracture.

V. Conclusions and Recommendations

Patients with T2DM have an increased risk of fragility fractures. The mechanism behind the increased risk of fracture is likely multifactorial. Patients with T2DM pose a diagnostic and prognostic dilemma due to the limitations of BMD measurement in predicting fractures. Evidenced-based recommendations are not available with regard to the diagnosis or treatment of osteoporosis in patients with T2DM. However, most osteoporosis treatment studies have included patients with impaired glucose tolerance and T2DM. Despite the increased risk of fractures in patients with T2DM there are no current recommendations regarding routine screening or initiation of preventative medications for osteoporosis in patients with diabetes.

We believe that good glycemic control is essential for reducing the risk of bone fragility and fracture in patients with diabetes. Adequate glycemic control prevents or reduces the risk of micro-and macrovascular complications. Diabetic vascular complications may directly contribute to bone fragility as well as increase an individual’s risk of falls. In addition, glycemic control may reduce the non-enzymatic glycosylation of collagen. Several studies have demonstrated the importance of implementation of established methods to reduce the risk of falls or fractures. These methods include regular exercise, improvement in muscle strength, balance training, withdrawal of psychotropic medication, and regular visual assessments (168). Clinicians should also ensure that their patients with diabetes are meeting the current recommendations for calcium intake and vitamin D levels.

Medications used in the treatment of T2DM may have an impact on bone metabolism. We believe that TZDs should be used with caution in older patients with T2DM who are risk for fall and/or fracture, particularly in post-menopausal women. A better understanding of the biological mechanisms and impact on bone metabolism of the various classes of diabetes medications is essential to aid clinicians in their decision-making regarding the different medications available for the treatment of T2DM.

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References


98. Tsukiyama K, Yamada Y, Yamada C, et al. Gastric inhibitory polypeptide as an endogenous factor promoting new


131. Singh-Franco D, Robles G, Gazze D. Pramlintide acetate...


139. Maor G, Karnieli E. The insulin-sensitive glucose transporter (GLUT4) is involved in early bone growth in control and diabetic mice, but is regulated through the insulin-like growth factor 1 receptor. Endocrinology. 1999;140(4):1841–1851.


158. Beenken A, Mohammadi M. The FGF family: biology,


