Future studies using histomorphometry in type 1 diabetes mellitus

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Purpose of review
This article reviews the current state of research in type 1 diabetes and bone, focusing on human bone turnover markers and histomorphometry.

Recent findings
Bone turnover markers have been used for decades to document static bone turnover status in a variety of diseases but especially in diabetes. Two new studies focus on dynamic testing conditions to examine the acute effects of insulin and exercise on bone turnover. Publications of human bone histomorphometry in type 1 diabetes are few but there are several new studies currently underway.

Summary
Here, we review the most recent literature on human bone turnover markers and histomorphometry. Low bone turnover is thought to be a major underlying factor in bone fragility in T1DM. Further studies in human transilial bone biopsies will be helpful in determining the mechanisms.

Keywords
bone histomorphometry, bone quality, bone turnover markers, diabetes

INTRODUCTION

Diabetes mellitus is associated with many well characterized complications including retinopathy, neuropathy, nephropathy and cardiovascular disease. Bone fragility and fractures are increasingly recognized as further complications of diabetes that affect patients with diabetes at all ages. This is true for both type 1 diabetes (T1DM) and type 2 diabetes (T2DM), but these may have different underlying mechanisms. The increased risk of fracture does not appear to be only a result of decreased bone mass. Although patients with type 1 diabetes generally have lower bone mass than age-matched and sex-matched controls, this does not solely account for the increased fracture risk in these patients. Patients with type 2 diabetes also have a higher risk for fracture despite having higher bone mass than age-matched and sex-matched controls [1]. This indicates that the fracture risk of diabetes is predicated by something other than bone mass, leading us to the largely unknown area of 'bone quality', all the factors that contribute to bone strength other than bone mass. Bone turnover is one facet of bone quality. It can be measured by histomorphometry examination of transilial bone biopsies or with bone turnover markers.

TYPE 1 DIABETES MELLITUS AND FRACTURE

The focus of this review is type 1 diabetes and bone. The ultimate outcome of poor bone quality, whatever the underlying mechanism, is fracture. Fracture risk is clearly higher in the T1DM population across all age groups. Reviewed thoroughly by Vestergaard [1] and Shah *et al.* [2]. In typical postmenopausal osteoporosis, low bone mass has been clinically accepted as one of the most predictive factors for fracture risk, although fracture-predictive algorithms have incorporated other important predictors, such as age and history of fracture. Although studies of bone mass in T1DM have generally found lower bone mineral density (BMD) than controls, this does not fully account for the increased risk of fracture in T1DM [1]. Other mechanisms have been

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KEY POINTS

- Fracture risk is three to seven times higher in T1DM of all ages compared with those without diabetes.
- Low bone turnover is thought to be a major underlying factor in bone pathophysiology in T1DM.
- Dynamic studies of acute effects of glucose, insulin and exercise on bone turnover in T1DM are helpful in distinguishing underlying mechanisms.
- Although transilial bone biopsy remains the gold standard for documenting bone turnover, very few have been done thus far in T1DM.

proposed for the underlying disease leading to increased fracture risk. One widely accepted mechanism is low bone turnover in patients with diabetes. This is in sharp contrast to postmenopausal osteoporosis, which is characterized by high levels of remodeling. Low remodeling rates could lead to fracture by not repairing small damaged areas in the bone. This damage could accumulate until the bone becomes fragile enough to fracture. Low bone turnover has been explored through studies of serum and urine bone turnover markers and a few reports of histomorphometry of bone biopsies.

BONE REMODELING MARKERS IN TYPE 1 DIABETES MELLITUS

Low remodeling rates are a tenable cause for the increase in fracture in diabetic humans. Many studies of diabetic bone disease have used serum or urine biomarkers of bone remodeling (BTM) as a noninvasive way of assessing remodeling. There have been no studies reported that compared static BTM to bone turnover documented by histomorphometry.

Markers that are specific to bone formation include: bone-specific alkaline phosphatase (BSAP), osteocalcin, and N-terminal propeptide of type 1 procollagen (P1NP). C-terminal telopeptide of type 1 collagen (CTX) and N-terminal telopeptide or amino-terminal collagen crosslinks (NTX) are markers specific for bone resorption. BTMs are currently used in clinical practice mainly to monitor response to osteoporosis therapy, to identify nonresponders or to monitor bone turnover in poorly healing fractures or atypical fractures.

Bone remodeling (the entire process of bone resorption and bone formation) is highly regulated and resorption and formation are tightly linked making it difficult to separate single direct effects on either osteoblasts or osteoclasts (generally effects on one will change the other; Fig. 1).

Insulin and insulin-like growth factor (IGF-1) are generally considered to be anabolic for bone. In cell culture, osteoblasts respond to insulin by increasing collagen production and increasing osteocalcin, which in turn has a 'feed-forward' role in stimulating pancreatic beta cell proliferation and insulin secretion. When dysregulation of insulin signaling in bone occurs, which would be especially pronounced in T1DM with little endogenous insulin who rely on exogenous insulin, there is a reduction in osteocalcin levels indicating low bone formation [3]. Serum osteocalcin has been consistently low in multiple studies of humans with T1DM [4–6].

Glucose is a necessary nutrient for most cells including osteoblasts and osteoclasts. *In vitro*, osteoclasts and osteoclasts are inhibited by excess glucose in the media. *In vivo*, the effects of glucose are more complicated with either a direct effect of glucose or an indirect effect through insulin release or inflammation. Glucose (in a meal, orally or intravenous) suppresses bone turnover in healthy individuals (thoroughly reviewed by Sherk *et al.* [7▪]. Hyperglycemia also appears to suppress bone turnover in children and adolescents with T1DM [8]. Average glucose control (HgA1c or glycated hemoglobin) and concurrent glucose levels drawn with the BTM have shown hyperglycemia is associated with decrease in bone turnover [9,10].

There have been several dozen studies of BTMs in both T1DM and T2DM. The results vary widely depending on which turnover marker is used and the age of the population and the stage of their diabetes. Adolescents and children with T1DM have the bone formation marker osteocalcin decreased, but not P1NP (formation) or CTX (resorption) in a review of 26 studies [9] whereas all BTMs were decreased in a recent study of children and adolescents [11] Although adults have had various findings, ranging from decreased turnover to no effect on turnover, large metanalyses point to overall decreased bone turnover in adults with T1D [4] with stability over 10 years of follow-up [12]. Stage of disease seems important as those in the first year of diagnosis of T1DM had decreases in bone formation markers and increases in bone resorption markers [6].

Although there is much heterogeneity among studies, as a whole, both markers of resorption and formation are generally lower in T1DM when compared with healthy controls (reviewed in [4]).

Dynamic testing of bone turnover markers in type 1 diabetes mellitus

In the past, studies using BTMs have assumed that bone turnover is rather static. However, new studies done with dynamic testing have given insight into

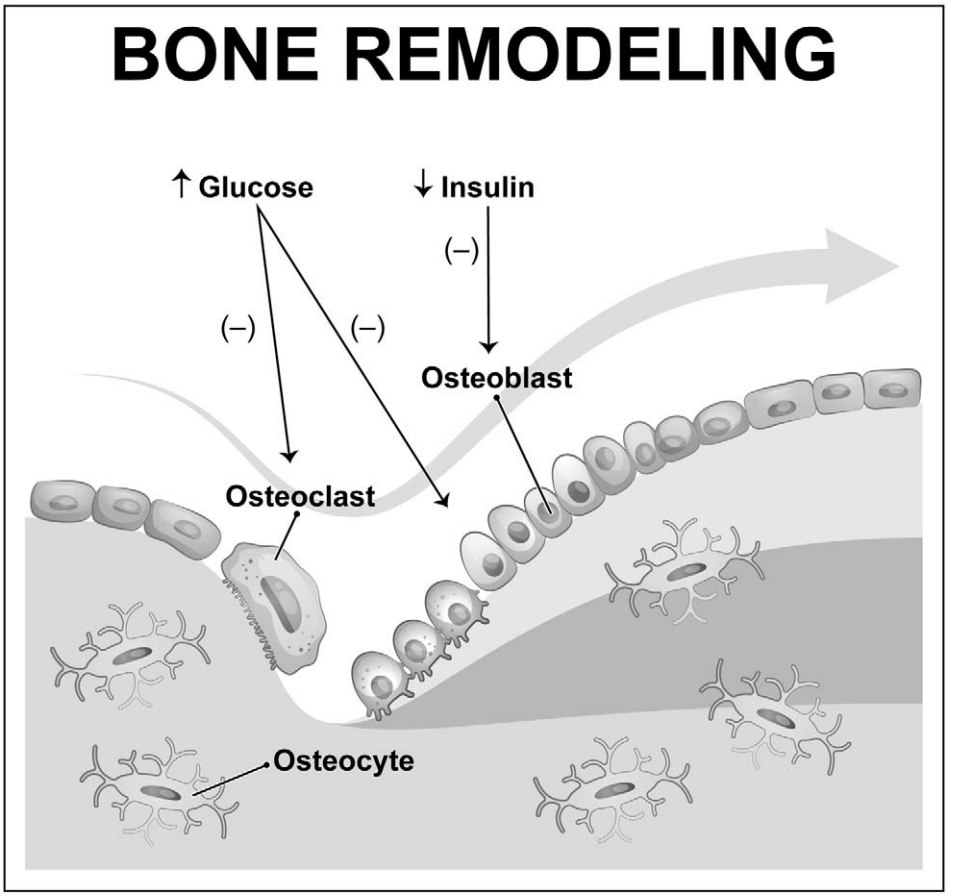

FIGURE 1. Illustration of bone remodeling (resorption and formation) and specific effects of type 1 diabetes mellitus.

the separate and combined acute effects of glucose, exogenous insulin and exercise on bone turnover. In an elegant study designed to illicit the distinct effects of glucose and insulin on bone turnover, Sherk *et al.* reported the effect of acute hyperinsulinemia during a hyperinsulinemic–euglycemic clamp on bone turnover in people with T1DM. There were many BTMs measured in this study but two were emphasized. Although the effect was small, P1NP acutely decreased and osteonectin acutely increased with insulin. If this acute effect was extrapolated to a chronic effect, this could lead to more brittle, less tough bone. These changes were dependent on insulin but not glucose as glucose levels were maintained in the normal range [13▪▪].

Exercise also has an acute effect on bone turnover. What the effect is depends on many factors including type, duration and intensity of exercise, and if the person is fed or fasting. In a study by Taylor *et al.* examining moderate exercise with a preexercise snack in T1DM vs. controls, there was no change in bone formation (assessed by P1NP) and a decrease in bone resorption (assessed by CTX) in both T1DM and controls. Those with diabetes had higher CTX than controls after exercise, indicating they may have more bone resorption with exercise than those without diabetes [14▪].

HISTOMORPHOMETRY

Histomorphometry of fluorochrome double-labeled (typically with tetracycline or demeclocycline) transilial bone biopsy is still considered the gold standard for measuring bone turnover. Although invasive, these procedures are well tolerated and give more opportunity for ex-vivo study of bone histomorphometry and remodeling. The bony specimen is about 7 mm in width and spans both cortices as the sample is taken from below the iliac crest. After being embedded in plastic, sections of the bone are examined under the microscope and areas of interest are measured (i.e. osteoclast resorption sites, sections of osteoid and surfaces covered with osteoblasts, etc.). If the patient has taken a prescribed regimen of fluorochrome (which deposits at the sites of active bone mineralization), measurements can be taken of the rate of bone formation during the time between labeling (Fig. 2). The dynamic measures typically reported are calculated from these labeling sites. The most common reported derived dynamic variables are bone

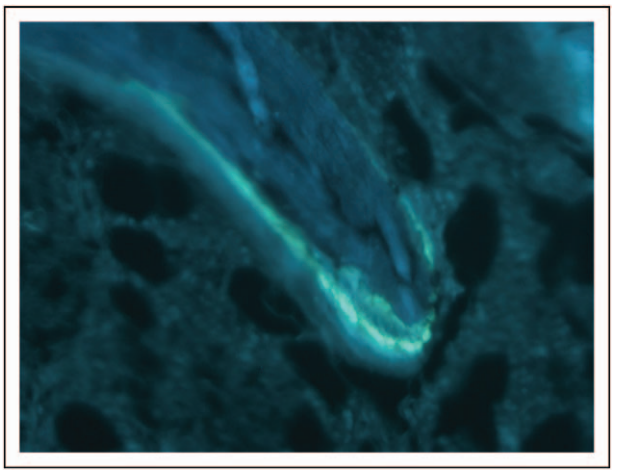

FIGURE 2. Unstained slide in type 1 diabetes mellitus. The unstained sections are used to measure formation. Orally administered fluorochromes are deposited in areas of bone formation/deposition and fluoresce (light green) under polarized light.

formation rates (BFR), estimates of bone volume and surface that are replaced every year, and activation frequency (AcF), which is the best index of bone remodeling as it reflects the probability that a new remodeling unit will be initiated [15]. Structural measures of bone volume, trabecular number, thickness and separation are also calculated (Fig. 3).

Histomorphometry studies in type 1 diabetes mellitus

In years past, the only available histomorphometric studies of diabetes included both T1DM and T2DM

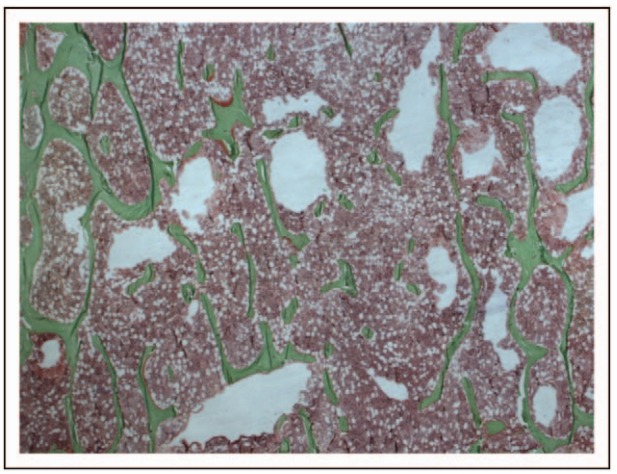

FIGURE 3. Histomorphometry in type 1 diabetes mellitus. The Goldner Trichrome stain distinguishes bone matrix (green) and osteoid seams (bright red) and bone marrow (pink and white). This stain is used to delineate structure for measurements of bone volume, bone surface area and/or osteoid surface area.

[16]. In this historic study, of the eight diabetic patients biopsied, only two had T1DM. From these eight bone biopsies, decreased bone formation rate and decreased osteoblasts were observed compared with existing histomorphometric data from premenopausal women. The authors concluded from these limited data that diabetic patients have low bone remodeling [16]. A limitation of applying this finding to T1DM patients, is that only two patients with T1DM were included. They had three decades of diabetic history and had some level of serious complications related to their diabetes at the time of the biopsy. Those patients also had low bone turnover but it is difficult to draw conclusions from these two patients alone.

More current histomorphometric studies have focused on well characterized T1DM patients and have excluded profound complications, which in themselves could compromise bone quality. Armas *et al.* completed a histomorphometric study of a healthy group of young (19–49 year)s T1DM patients without notable complications related to T1DM. The study had 84 patients who completed a bone biopsy, 29 with T1DM and 55 controls. A preliminary report of 18 T1DM and 18 age-matched and sex-matched controls' histomorphometry data showed no difference in the structural measures or bone turnover measures (BFR and AcF) between groups [17]. Further study of the complete cohort also showed no differences in structural measures or bone turnover measures between those with and without diabetes (author's unpublished data). These unexpected findings were thought to be a result of having relatively young diabetic patients who had good blood glucose control (average HgA1c 6.8%) and no diabetic complications, which have been associated with fracture in other studies. Interestingly, although the study did not recruit for fracturing diabetics there were five diabetic patients who had sustained previous fragility fractures. Although not enough patients with fracture were recruited to have adequate power to detect statistical differences, these patients with fracture had trends toward lower bone turnover (BFR and AcF). These individuals also tended to have poorer blood glucose control than the rest of the cohort (average HgA1c 8.1%).

Structural data from transilial bone biopsies correlate with other methods of measurement. The Armas *et al.* study included 3D micro-computed tomography (CT) data scanned at 16 μm resolution, which correlated with the findings of the 2D structural measures by classic histomorphometry. Structural histomorphometry measures from this study also correlated with Trabecular Bone Score of the spine measured by DEXA [18]. These bone samples have great potential for providing information

beyond classic histomorphometry. The bone was also examined for Pentosidine, a surrogate for advanced glycation end products, microhardness and intrinsic material properties. Interestingly, the fracturing diabetic patients had significantly more Pentosidine in the trabecular bone than either the nonfracturing age-matched and sex-matched diabetic patients or the age-matched and sex-matched controls [19]. Having bone biopsy tissue available for these types of ex-vivo studies in bone is invaluable.

Histomorphometry studies in type 2 diabetes mellitus

In contrast to T1DM, there have been several studies of histomorphometry in T2DM. This population has the effects of high glucose without the effects of insulin deficiency (at least at the beginning of the disease). Krakauer *et al.* [16] found decreased BFR in a group of diabetic patients that included six with T2DM. Leite Duarte and da Silva [20] found lower trabecular bone volume and cortical thickness, fewer osteoblasts and less unmineralized bone in 26 patients with T2DM (age 50–89 years). Manavalan *et al.* [21] found similar results in five postmenopausal women with T2DM, with decreased BFR, osteoblasts and cortical width compared with controls. Another group studied premenopausal women with T2DM and examined the effects of glucose control and complications. In contrast to the other studies, they found higher trabecular bone volume in T2DM compared with controls but they did find less unmineralized bone in those with poorer glucose control similar to the other studies [22]. Raman spectroscopy of these samples revealed higher advanced glycation endproducts (AGEs) in those with poor glucose control and chronic diabetes complications [23].

The histomorphometry data in T2DM is consistent across studies and age groups revealing decreased bone formation rate and varying effects on structure. The current T1DM histomorphometry data is limited to a healthy young cohort without complications. There is a gap in histomorphometric studies of T1DM, which include older patients with longer duration of disease and those with diabetes complications.

Future of histomorphometry in type 1 diabetes mellitus

There is much still to be learned about T1DM and bone. In recognition of this problem, the National Institutes of Health called for a Request for Application for Diabetes and bone health studies and has funded several new studies focused on T1DM and bone including three new studies that will use transilial bone biopsies. These are ongoing and will be using transilial bone biopsies to obtain tissue for further study. Shah *et al.* and Recker *et al.* are recruiting postmenopausal women with T1DM for transilial biopsies and comparing them with age-matched controls. The outcomes will be slightly different. Recker's group will be measuring AGEs, mechanical properties and classic histomorphometry and Shah's group will be using Raman spectroscopy, nanoindentation, and backscattered electron microscopy to determine bone tissue composition and tissue material properties. Both of these studies will focus on postmenopausal women with T1DM, a group that is at high fracture risk because of their diabetes duration, age and postmenopausal status. Another study under Schafer, is obtaining bone biopsies from a subset of the PERL trial (A Multicenter Clinical Trial of Allopurinol to Prevent GFR Loss in T1D), which gives the unique opportunity to examine the dual impact of T1D and diabetic kidney disease of varied severity. As kidney disease has its own impact on bone fragility, it is important to examine the combined effects of diabetes and kidney disease on bone.

CONCLUSION

Clinically, fractures in diabetes are an ongoing problem and while there are several studies exploring potential mechanisms, there is little known about which treatment options are effective. For example, if low bone remodeling is the main underlying issue, then our typical antiresorptive treatments may not be helpful and may further decrease remodeling. If this is the case, anabolic bone therapy may be more useful. Fortunately, there are several studies with bone biopsies currently underway, which show promise to reveal more about the underlying pathophysiology of T1DM and bone fragility.

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Conflicts of interest

E.S. and L.G.A. report no conflicts of interest. R.R. reports grant funding from Amgen, Inc.

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- ■■ of outstanding interest

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