Peripheral Bone Mineral Density and Bone Turnover in Postmenopausal Women with Type 2 Diabetes

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Abstract

Objective: Several studies have suggested that diabetes affects Bone Mineral Density (BMD). In this study, we investigated the effect of type 2 diabetes mellitus (T2DM) on BMD and the rate of bone turnover in postmenopausal women.

Methods: This is a cross-sectional study, in which we measured peripheral bone density and markers of bone turnover in 60 postmenopausal women with T2DM and 48 age, alcohol intake and physical activity - matched control postmenopausal women without diabetes.

Results: BMD was significantly greater in subjects with T2DM than controls (0.51 gm/cm^2 vs. 0.47 gm/cm^2, p<0.01). Women with T2DM also had higher Body Mass Index (BMI) than the control group (mean: 33.7 kg/m^2 vs. 26.7 kg/m^2, p<0.0001). The difference in BMD between the two groups became non-significant after adjusting for the effect of BMI by multiple regression analysis (p=0.091). Osteocalcin, a marker of bone formation and three markers of bone resorption (cross-linked-N-telopeptides [NTX], C-telopeptides of type 1 collagen [CTX], and Helical peptide) were significantly reduced in T2DM compared with controls. However, the difference in the three bone resorption markers also became insignificant after adjusting for BMI.

Conclusion: This study has shown that postmenopausal women with type 2 Diabetes Mellitus apparently have higher BMD and slow bone turnover when compared with matched controls. However, the difference in BMD between the two groups became non-significant after adjusting for the effect of BMI. This study therefore does not provide evidence that T2DM per se affects bone mineral density in postmenopausal women.

Keywords: Bone density; Diabetes; Bone Turnover; Osteoporosis; Menopause

Abbreviations: T2DM: Type 2 Diabetes Mellitus; T1DM: Type 1 Diabetes Mellitus; NTX: cross-linked-N-Telopeptides; CTX: C-Telopeptides of Type 1 Collagen; DpD: Deoxypyridinoline; OC: Osteocalcin; b-ALP: Bone-specific Alkaline Phosphatase

Introduction

Menopause is associated with increased bone loss due to estrogen deficiency, which results in reduced bone strength [1]. Low BMD is a strong predictor of fracture [2]. In menopausal women, each standard deviation (SD) decrease in BMD approximately doubles the fracture risk [3].

Diabetes is a chronic disease, which has widely been reported to influence bone metabolism. However, the effect of diabetes on bone density and bone metabolism is still controversial; although most studies in type 1 Diabetes Mellitus (T1DM) have shown low BMD values [4-7]. The controversy is greater for T2DM. BMD at the vertebrae or femoral neck was not significantly different [8-10], increased [11-12] or decreased [15] in T2DM compared to non-diabetic control subjects. There are fewer studies which measured BMD at the forearm, and they reported either increased [8,16] or no significant difference [17] or decreased [18] BMD in T2DM when compared with controls.

Many previous studies investigated bone turnover in diabetes but many early studies utilized non-specific markers like urinary calcium [19-21] and hydroxyproline [21,22]. Although there are now more specific markers for bone turnover, they tend to exhibit large individual variability, this is the case for urinary markers which need to be adjusted to urinary creatinine [23,24]. The few reports on the status of bone turnover in T2DM mostly reported low bone turnover [14,25] with some exceptions [22]. The previous studies in people with diabetes included predominantly either women or both women and men. The attention to type of diabetes mellitus was not uniform. With some exceptions [26], BMI was either not measured or not included in the analysis in most studies.

To compensate for the effect of the wide variability of bone marker levels, we measured a combination of some of the most specific bone turnover markers available. The bone formation markers studied were: osteocalcin (OC) and bone specific alkaline phosphatase (b-ALP). The bone resorption markers were: cross-linked-N-telopeptides (NTX) measured as osteomark, C-telopeptides of type 1 collagen (CTX) measured as β-crosslaps, deoxypyridinoline (DpD) and helical peptide. Helical peptide is a relatively new bone marker based on measurement of residues 620 - 633 derived from the helical peptide of the α1 chain of type 1 collagen [27]. It has never been measured in subjects with diabetes before. The other bone resorption markers (NTX, CTX and...
DpD) have been barely studied before in postmenopausal patients with T2DM [10,14]. It has been known for some time that obesity protects against osteoporosis [11,28-30]. Thus we hypothesised that in women with T2DM, bone turnover is reduced resulting in increased BMD. Those findings are likely to be related to higher BMI commonly seen in T2DM.

Patients and Methods

Consecutive female patients were recruited from Annual Review and Follow up Diabetic Clinics. T2DM was diagnosed according to the criteria of the National Diabetes data Group [31]. Controls were recruited either as relatives of patients with diabetes or members of Gateshead Health Trust who responded to invitation by poster or Hospital Newsletter. They did not have any illness or were on any medication which might affect bone turnover. Controls have normal fasting glucose and HbA1c levels. The BMD measurements were done by the same personnel. Postmenopausal status of participants was confirmed by interview and measurement of follicle stimulating hormone levels. The study was approved by the local ethics committee and all study subjects have given their written consent.

The characteristics of patients and controls are detailed in Table 1. Nine diabetic patients were treated with diet alone, 9 patients with insulin alone, 16 patients were treated with a combination of insulin and oral hypoglycemic agents and 27 patients were treated with oral hypoglycemic agents.

Waist hip measurement was recorded at midpoint between lower rib margin and anterior iliac crest and hip at its widest aspect by single observer on one occasion. Physical activity was estimated by patients themselves. Menopausal status was confirmed by interview and FSH levels. Blood pressure was measured in fasting state after 5 minutes sitting using OMRON automatic digital blood pressure monitor HEM-705CP. We used patient's right arm with appropriate cuff size. During the measurement, patients were not speaking and remained in relaxed position with uncrossed legs.

Peripheral BMD was measured in the distal non-dominant radius using a peripheral osteometer DTX 2000, (Osteometer MediTech, Inc, Hawthorne, CA, USA). Absolute values were recorded, together with T and Z scores.

Bone formation markers (osteocalcin and bone specific alkaline phosphatase) were measured in a fasting serum sample by EIA (MetraBiosystems Inc., Mountainview, CA). Serum β-cross laps were measured on a Roche Elecsys 2010 Analyzer, Lewes, UK. NTX were measured on a Roche Elecsys 2010 Analyzer, Lewes, UK. NTX were measured as urinary osteomark (Ostex, Seattle, WA, USA), helical peptide and osteomark. Serum and urinary resorption markers (DpD, β-crosslaps, helical peptide and osteomark) were significantly lower in the diabetic group than the control group. Osteocalcin, a marker of bone formation was also lower in the diabetic group (Table 2). Bone–specific alkaline phosphatase and DpD levels were similar in both groups.

The effect of BMI on BMD was investigated by multiple regression analysis. The results showed that after adjusting for BMI, the difference in BMD levels between the diabetic and the non-diabetic group became non-significant. Adjustment for BMI also abolished the significant difference in the levels of the bone resorption markers. The difference between the two groups in osteocalcin levels remained significant even after adjusting for BMI (Table 3 and 4).

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that women with diabetes had significantly higher BMD, which was
and proximal femur [40]. More recently it has been shown
this result is in keeping with previous studies, which demonstrated
the effect of BMI on BMD difference between the diabetic patients
protects against osteoporosis [11,28-30]. We therefore adjusted for
bone turnover [2,35-37]. It is therefore possible that the true BMD in
Markers of bone formation and resorption [27,32,33] and studies on
osteocalcin* (p=0.0175)
Table 2: Comparison of the Bone mineral density (BMD) and concentrations of bone turnover markers in the two study groups.

| Independent variable | Coefficient | p value |
|----------------------|-------------|---------|
| Body Mass Index | -0.6987 | <0.00010 |
| Group | -3.799 | 0.091 |

Bone mineral density (gm/cm²) was dependent variable. The Independent vari-
able were Body mass index and group allocation. The results show that the dif-
fERENCE in bone mineral density between the diabetic and the control group was no
longer significant after adjustment for body mass index (p=0.0901)

Table 3: Use of multiple regression analysis to adjust for the effects of Body mass
index on concentrations of metabolic bone markers.

| Dependent variable | Coefficient for Group allocation p value (diabetic or non diabetic control) |
|--------------------|---------------------------------------------------------------------|
| Osteocalcin         | 1.7799 0.0175*                                                      |
| b-Cross Laps        | -0.0898 0.4618                                                     |
| Helical peptide     | 16.553 0.1666                                                      |
| Osteomark           | 10.1525 0.4054                                                     |

In each occasion, a metabolic bone marker was dependent variable. The Inde-
pendent variables were Body mass index and group allocation. The results show that the dif-
ference in metabolic bone markers between the diabetic and the control group was no
longer significant after adjustment for body mass index, except for osteocalcin* (p=0.0175)

Table 4: Use of multiple regression analysis to adjust for the effects of Body mass
index on concentrations of metabolic bone markers.

In this study we found a significantly higher BMD at the forearm in postmenopausal women with T2DM when compared with controls. Rates of smoking were significantly higher among patients with diabetes. Smoking is associated with decreased BMD and increased bone turnover [2,35-37]. It is therefore possible that the true BMD in the diabetic group is slightly higher than that reported in this study.

There was however, significant difference in the BMD measurements between the two groups. It has been known for some time that obesity protects against osteoporosis [11,28-30]. We therefore adjusted for the effect of BMI on BMD difference between the diabetic patients and controls, by multiple regression analysis. The results showed that after adjusting for BMI, the difference in BMD levels between the diabetic and the non-diabetic group became non-significant (Table 3). This result is in keeping with previous studies, which demonstrated a protective effect of body weight on the bone mass of axial skeleton [11,38,39] and proximal femur [40]. More recently it has been shown that women with diabetes had significantly higher BMD, which was
significantly correlated to BMI at forearm and spine but not at hip site [41]. It has been suggested that varying BMD measurements have been noted at different anatomic regions however no reference was made to BMI of the studied subjects [42]. The possible protective effect of T2DM on bone mass was presumed to be due to a mechanical effect, increased circulating estrogen caused by conversion of androgens to estrogen or hyperinsulinemia [43-45]. The association between obesity and increased BMD was also thought to be caused by high leptin concentrations in obese people [46]. Leptin plays a direct role in stimulating osteoblast number and activity in cell culture [47]. However, in a recent study on postmenopausal women with T2DM, There was no correlation between low BMD and leptin, adiponectin and insulin resistance [48]. In our study we have not shown any increase in the bone formation markers in diabetes but on the contrary a significant reduction was seen in osteocalcin levels.

The difference in osteocalcin levels between the two groups remained statistically significant after adjusting for BMI, although less significant than before adjustment for BMI. It is possible that bone formation, as reflected by osteocalcin levels, is reduced in the diabetic group, irrespective of BMI. There is also evidence that hyperglycemia inhibits osteocalcin release from osteoblasts and can therefore contribute to the reduced levels in diabetic patients [49].

The two bone formation markers studied behaved differently; while OC levels were significantly reduced in postmenopausal women with T2DM, b-ALP levels were comparable between the two groups. The discrepancy of bone formation markers has been reported from several studies. One possible explanation for such discrepancy could be that different bone formation markers reflect different stages of osteoblastic differentiation. While alkaline phosphatase activity is produced by mature cells, OC is produced during the mineralization phase [50,51]. Previous studies in T2DM showed a more consistent decrease in OC levels in diabetic patients compared to non-diabetic controls [52-56].

Most of the resorption markers levels measured were significantly lower in women with diabetes, suggesting a slow bone turnover in this group. Most previous studies reported decreased bone resorption in T2DM based on low DpD and β-cross laps levels [10,14], while other studies which measured urinary DpD levels only, found no difference between diabetic patients and controls [55,56].

The difference in the bone resorption markers between diabetic and nondiabetic control group became insignificant after adjusting for BMI by multiple regression analysis. This suggests that the effect of BMI on preservation of bone in the diabetic group was caused by slowing down bone turnover as was also shown by others [14,57].

This is the largest to date prospective study of postmenopausal women with type 2 diabetes that measured BMD and a combination of several highly specific bone markers.

This is the first report on helical peptide in relation to BMD measurements, which proved the usefulness of helical peptide in the assessment of bone turnover. Whether the increased BMD in diabetic patients will protect them from fractures still requires further investigation. Although BMD is a strong predictor of fracture susceptibility in postmenopausal women, it is not the only one. Factors like bone size and skeletal architecture are also important predictors of fracture [2,58,59]. The overall susceptibility to fracture in this population will likely depend on the interplay of several factors, which may vary in different populations. This may explain the controversy
surrounding the previous studies on the association between diabetes and fractures, which produced conflicting results [4,7,12,60]. Although increased BMD in T2DM is seen as an advantage this does not necessarily translate in this group into a reduced incidence of fractures as has been suggested before [61]. Recently, a large study demonstrated that despite having higher bone density, diabetic women have lower indices of femoral neck strength relative to load, consistent with higher fracture risk [62]. Another multi-centre study showed that obesity is not protective against fracture in postmenopausal women and is associated with increased risk of ankle and upper leg fractures [63]. The association between obesity and fracture in postmenopausal women was also found to be site-dependent, obesity being protective against hip and pelvis fractures but associated with an almost 30% increase in risk for proximal humerus fractures when compared with normal/underweight women [64].

This indicates that other factors, including co-morbidities associated with diabetes such as neuropathy or postural hypotension may increase the risk of fracture regardless of higher BMD. Diabetic polyneuropathy is associated with high bone turnover in postmenopausal women [65]. Insulin resistance appears to play an important role in bone strength reduction in diabetes [62].

In conclusion, we have shown that in patients with type 2 DM bone turnover is reduced resulting in increased BMD. Those findings are related to higher BMI in comparison to healthy controls. Further work is required to clarify the relationship between BMD in postmenopausal women and T2DM and susceptibility to fracture.

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