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/cm² vs. 0.47 gm/cm², p<0.01). Women with T2DM also had higher Body Mass Index (BMI) than the control group (mean: 33.7 kg/m² vs. 26.7 kg/m², p<0.001). 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] 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 intra-individual variability, this is specially 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) measured as osteomark, C-telopeptides of type 1 collagen (CTX) measured as β-crosslaps, deoxypyridinoline (DpD) and helical peptide.
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 patients 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 osterometer DTX 2000, (Osteometer Meditech, Inc, Hawthorner, 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 as urinary osteomark (Ostex, Seattle, WA, USA), helical peptide was measured by EIA (Quidel Corporation, Mountain view, CA, USA) and urinary DpD measured on a second, fasting, early morning urine sample, using the Pyrilinks-D assay (MetraBiosystems Inc., Mountainview, CA). Results for urinary markers were expressed as per creatinine. Samples were stored at -30°C until analysed.

HbA1c was measured on the Menarini HPLC system. Plasma glucose and serum creatinine were measured on a Roche Modular Analyser (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

Analyses were performed with the statistical package SPSS/PC + (SPSS Inc, Chicago, III). All tests used were two-tailed and p<0.05 was considered significant. The normality of distribution of any variable was assessed using the Kolmogorov-Smirnov test and skewed distributions were logarithmically transformed. Comparisons between groups (T2DM patients and controls) were performed using the unpaired t-test or the unpaired Wicoxon rank test for nonparametric values. Correlation analysis was performed using Pearson’s correlation coefficient.

Multiple regression analysis was used to adjust the effect of weight on BMD and bone markers. In the multiple regression analysis, BMD was used as a dependent variable and BMI or coded group allocation (either diabetic or non-diabetic) as independent variables. To adjust for the effect of BMI on the levels of metabolic bone markers, each bone marker was considered as a dependent variable and BMI or coded group allocation as independent variables. The difference between the two groups was considered non-significant when the p value for the group allocation was >0.05.

Results

The main characteristics of the study groups were detailed in Table 1. The groups were matched for age, activity levels, and alcohol intake. Patients with T2DM had significantly greater tobacco consumption than the non-diabetic group (Table 1). BMI was greater in patients with T2DM than in the controls (mean 33.7 vs. 26.7, p<0.0001).

BMD was significantly greater in women with T2DM than in controls (mean: 0.51 gm/cm² vs. 0.47, p<0.01). BMD did not correlate with HbA1c or duration of diabetes. There was no significant correlation between BMD and BMI. Three bone resorption markers (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).

Discussion

Postmenopausal bone loss is associated with increased bone...
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 non-diabetic 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 BMI 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 BMI 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

<table>
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<tr>
<th>Table 3: Use of multiple regression analysis to adjust for the effects of Body mass index on bone mineral density.</th>
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<tr>
<td><strong>Dependent variable</strong></td>
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<tr>
<td>Osteocalcin</td>
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<td>b-Cross Laps</td>
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<td>Helical peptide</td>
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In each occasion, a metabolic bone marker was dependent variable. The Independent variables were Body mass index and group allocation. The results show that the difference in metabolic bone markers between the diabetic and the control group was no longer significant after adjustment for body mass index (p=0.0901)
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 with T2DM and susceptibility to fracture.

References

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