The relationship between insulin resistance and osteoporosis in elderly male type 2 diabetes mellitus and diabetic nephropathy

Rapport entre la résistance à l’insuline et l’ostéoporose chez les hommes âgés ayant un diabète de type 2 et une néphropathie diabétique

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Abstract

Objective. – Diabetes is often associated with complications and comorbidities. Diabetic osteoporosis (OP) is increasingly recognized as a significant comorbidity of type 2 diabetes mellitus (T2-DM). In this study, we intended to determine whether type 2 diabetes was associated with a higher bone mineral density (BMD) in older males, and investigate the related risk factors of diabetes mellitus accompanied with OP. Materials and methods. – To assess the effects of diabetes and its complications on the risk of bone fractures, the daily glycemia, insulin and HbAlc of T2-DM patients were determined. At the same time, Dual Energy X-ray Absorptiometry (DEXA) was used to measure the BMD of whole body, lumbar spine and proximal femoral at 2-year intervals. Results. – The BMD in elderly male patients suffering from T2-DM was associated with kidney function. The decrease of BMD in elderly male patients with T2-DM was related to HbAlc and body mass index (BMI). The BMD in group of renal function insufficiency or clinical albuminuria which are closely related with insulin insufficiency were much lower than that in the group of normal control. Additionally, a significantly lower (P<0.05) T-score was found in patients with nephropathy as compared with those without the complications. Conclusion. – The elderly patients with type 2 diabetes mellitus are prone to develop OP. The insufficiency of insulin, the decreased insulin sensitivity and diabetic nephropathy are important causes for OP in the patients with type 2 diabetes.

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Résumé

Objectif. – Les complications et les comorbidités sont souvent associées au diabète. L’ostéoporose diabétique est une comorbidité de plus en plus fréquente du diabète de type 2. Dans cette étude, nous voulons savoir si le diabète de type 2 est associé à une densité minérale osseuse (DMO) plus élevée chez les hommes âgés et examiner les facteurs de risque d’ostéoporose associés au diabète de type 2. Matériels et méthodes. – Afin d’évaluer les effets du diabète et ses complications sur le risque de fracture, nous avons mesuré quotidiennement la glycémie, l’insulinémie et l’HbAlc, de patients atteints de diabète de type 2. De même, une densitométrie (DEXA) était réalisée tous les deux ans pour mesurer la DMO du corps entier, du rachis lombaire et du fémur proximal. Résultats. – La DMO des hommes âgés ayant un diabète de type 2 était liée à la fonction rénale. La diminution de la DMO des hommes âgés ayant un diabète de type 2 était liée à l’HbAlc et à l’indice de masse corporelle. La DMO des patients ayant une insuffisance rénale ou une albuminurie clinique, deux paramètres étroitement liés à une insuffisance en insuline, était très inférieure à celle du groupe de témoins normaux. De plus, une diminution significative du T-score (p<0.05) était trouvée chez les patients présentant une néphropathie comparés aux patients sans cette complication. Conclusion. – Les patients âgés ayant un diabète de type 2 sont susceptibles de développer une ostéoporose. Une insuffisance en insuline, une sensibilité à l’insuline et une néphropathie diabétique sont des causes importantes d’ostéoporose chez les patients atteints de diabète de type 2.

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1. Introduction

The number of adults diagnosed as type 2 diabetes worldwide is expected to grow from 135 million in 1995 to approximately 300 million in 2025 [1]. People with type 2 diabetes constitute ...
90% of the diabetic population [2]. Diabetes is often associated with complications and comorbidities, the top four chronic complications that influence these total expenditures for diabetes management are peripheral vascular, renal, cardiovascular, and neurologic comorbidities.

Endocrine and metabolic alterations in diabetes mellitus can trigger disorders of calcium homeostasis, skeletal metabolism and bone mass [3]. It is reported that more than of 50% type 1 diabetes have osteoporosis (OP), which is called diabetic osteoporosis (DO), a reduced bone mass and an increased fracture risk shown to occur in type 1 diabetes mellitus [4–6]. On the contrary, in type 2 diabetes, several but not all cross-sectional studies have found normal [7,8] or elevated [9–12] bone mass, and these results are surprising given the increased fracture risk associated with type 2 diabetes [13–15]. In T2DM patients complicated with OP, more decrease of bone formation than bone resorption in comparison with that postmenopausal OP, it mainly influences the indexes of bone formation and may be a lower turnover ratio type.

In this study, we intended to determine whether type 2 diabetes was associated with a higher bone mineral density (BMD) in older males, and investigate the related risk factors of diabetes mellitus accompanied with OP.

2. Methods and materials

2.1. Overall test of patients

Dual Energy X-ray Absorptiometry (DEXA) was used to measure the BMD of lumbar spine and proximal femoral in 70 males with 2-DM, who were over 60 years old, including 25 cases of normal buminuria, 25 cases of microalbuminuria, and 20 cases of clinical albuminuria or renal inadequacy. Seventy diabetes as well as 40 normal controls were examined, including data of bone metabolism, markers of bone formation (serum hormone, calcitonin, large dosage of calcium or bisphosphonate, and so on. All patients did not use glucocorticoids, androgenic hormones, calcitonin, large dosage of calcium or bisphosphonate, and so on.

2.2. Divide different groups

Diabetes was defined by published standards, using of hypoglycemic medications or fasting glucose greater or equal to 126 mg/dl (≥ 7.0 mmol/l) and a casual plasma glucose greater or equal to 200 mg/dl (≥ 11.1 mmol/l) in accordance with the American Diabetes Association criteria [16]. After the exclusion of two patients with hyperthyroidism, four patients with renal failure and four patients with primary hyperparathyroidism, 50 patients aged 71 to 84 years (mean ± SD, 73.92 ± 6.0 years) were studied. The duration of diabetes varied from 0 to 240 months (57.47 ± 59.35 months). The treatment of the patients consisted of diet in 11 patients, oral hypoglycemic agent in six patients, diet plus oral hypoglycemic agent in 26 patients, and seven patients were newly diagnosed.

2.3. Type 2 diabetes group (DG)

Seventy male volunteers were divided into three groups with result of inspection:

- A group: normal albuminuria (MA < 30 mg/l);
- B group: minute albuminuria (MA: 30∼300 mg/l);
- C group: mass albuminuria or renal inadequacy (MA > 300 mg/l or Blood Cr > 178 μmol/l).

Type 2 DG were divided into two groups with result of Bone densitometry:

- diabetes with OP group (DOG);
- diabetes without OP group (nDOG)

2.4. Control Group (CG)

Forty male volunteers (inpatients) with normal functions of liver and kidney and normal level of blood sugar, excluding chronic bronchitis, hyperthyroidism, hypothyroidism, prostatic carcinoma, hepatocirrhosis, paralysis and long usage of cortical hormone.

The measurements of the CG were carried out in parallel to those in diabetic patients. The study protocol was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People’s Hospital, and was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki as revised in 2000. All the subjects involved in the study were drawn by the department of OP and geratology from a local population of Asian. Every subject signed informed consent documents before entering the project.

2.5. Anthropometric and biochemical measurements

During the clinic visit, a medical history was obtained by a standard questionnaire. Weight was measured with a calibrated balance-beam scale with participants without shoes, and height was measured using a stadiometer. BMI – weight divided by square height (kg/m²) – was calculated as a measure of obesity.

After an overnight fast (8 hours), blood was drawn to measure the levels of calcium, phosphorus, total alkaline phosphatase (ALP), glucose, creatinine, bone gla protein (BGP), intact parathyroid hormone (PTH). Twenty-four-hour urine samples were analyzed for urinary calcium and creatinine measurements.
(U-Ca and U-Cr) and spot urine samples for deoxy PIR measurements (DPD). Serum BGP and urinary DPD were tested by ELISA, the test reagents were provided by America Metra Biological System Company. Urinary DPD was revised by creatinine (Cr) at the same time. ALP was tested with Abbott-Aerose automatic biochemical analyzer. Serum BGP and ALP were the markers for bone formation and DPD/Cr for bone resorption.

Hemoglobin A1c (A1C) was measured in serum specimens using standard laboratory procedures at baseline. The serum levels of glucose, calcium, phosphorus, creatinine and ALP were determined by automated techniques (Roche Modular System); BGP by microenzyme, linked immunosorbent assay (micro-ELISA, Tc2an); intact PTH and urinary deoxy PIR by electro-chemiluminescence immunoassay (ECLIA, Immulate 2000).

The homeostasis model assessment of insulin resistance was calculated from fasting insulin and glucose by the following equation: HOMA-IR = FPI × FPG/22.5, where FPI is fasting serum insulin concentration (uU/ml) and FPG is fasting plasma glucose (mmol/l).

Renal function of diabetic patients was evaluated with measurements of 24 hours albuminuria by RIA (30–300 mg/24 hour was considered as microalbuminuria and > 300 mg/24 hour as macroalbuminuria). Glomerular filtration rate (GFR) was estimated using the abbreviated Modification of Diet in Renal Disease.

BMD was measured by dual-energy x-ray absorptiometry technique (DXA) on a Hologic QDR 2000 (Hologic, Bedford, MA, USA). The DXA scanner was on fan-beam mode. DXA is the preferred technique for the evaluation of BMD because of its low radiation dose, accuracy and rapid performance [17]. BMD was measured at lumbar L1-L4 anteroposterior, femoral (neck, trochanter, intertrochanteric region, total) and forearm (one-third, mid and ultradistal [UD]) levels.

Bone mass is expressed as BMD resulting from BMC/area ratio (g/cm²), T score (SD, standard deviation from the mean value obtained in 30-year-old normal subjects) and Z score (SD from the mean value obtained in subjects of the same age and sex). The scanner was recalibrated daily using the manufacturer’s software and phantom. The Tscores that were −1 SD or greater were considered normal, between −1 and −2.5 SD osteopenia and less than −2.5 SD OP. BMD examinations were done in 70 diabetic and 40 control subjects at lumbar, femoral and forearm levels.

Height and body weight were measured using standard equipment. BMI was defined as the weight/height² in the units of kilogram/meter².

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BMD(g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lumbar total</td>
</tr>
<tr>
<td>Diabetic</td>
<td>70</td>
<td>0.88 ± 0.31*</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>1.09 ± 0.25</td>
</tr>
</tbody>
</table>

The comparison between diabetic group and control group (CG). *: *P < 0.05; **: *P < 0.01.

#### 2.6. Statistics

All statistical analyses were performed with SPSS statistical software package (version 11.0). Numerical data are expressed as mean ± SD and as a percentage in the qualitative variable.

The distribution of variables was analyzed with the Kolmogorov-Smirnov test. To test the differences between quantitative variables in diabetic patients and control subjects, independent sample Student’s t test is used for variables that are normally distributed. Non-normally distributed numerical data were analyzed with Mann-Whitney U test.

χ² tests were used to compare the rates and the analysis of risk factor was performed with ANOVA. P values for comparisons between the three mutually exclusive cohorts were conducted using the Tukey-Kramer method. Cost comparisons among the cohorts were conducted using a stepwise multivariate regression that controlled for patient characteristics and comorbid conditions. For all comparisons, P values < 0.05 were considered as statistically significant.

### 3. Results

The incidence of OP was 65.70% in the T2DM group and 32.50% in CG. The BMDs of patients with T2DM were lower than those of CG and the difference was significant (P < 0.05) (Table 1). After investigated different tissues of the individuals, compared to CG we found diabetic nephropathy was significantly related with bone density (Fig. 1).

Multivariate regression analysis showed that BMD of diabetic patients negatively correlated with age, course, HbA1c, LDL-C and positively correlated with BMI. The decrease of BMD in elderly male patients with 2-DM was significantly related to HbA1c and BMI (P < 0.05) (Table 2).

Based on many values of bone metabolism related indexes, it proved that the BMD in group of renal function insufficiency or

Fig. 1. Diabetic nephropathy was significantly related with bone density. Bone mineral density (BMD) of male diabetic nephropathy compared different groups with control group. Compared A, B and C groups with control group (CG), *P < 0.05, **P < 0.01.
Bone metabolism indexes compare diabetic nephropathy groups with control group (x±s) (Table 2).

Table 2
Characteristics of study participants was significantly related with bone density clinic character compare of DOG and nDOG (x±s).

<table>
<thead>
<tr>
<th>Subject</th>
<th>DOG</th>
<th>nDOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>46</td>
<td>24</td>
</tr>
<tr>
<td>Age (years)</td>
<td>78.07±14.2</td>
<td>74.26±11.3</td>
</tr>
<tr>
<td>Course of diseases (years)</td>
<td>10.3±7.56**</td>
<td>7.33±5.51</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>14.2±4.51**</td>
<td>10.9±2.24</td>
</tr>
<tr>
<td>2hBG (mmol/L)</td>
<td>17.6±3.43*</td>
<td>14.5±5.17</td>
</tr>
<tr>
<td>24 h Albuminuria (g)</td>
<td>1.08±0.37**</td>
<td>0.54±0.52</td>
</tr>
<tr>
<td>Insulin sensitivity index</td>
<td>0.005±0.011**</td>
<td>0.018±0.010</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.67±1.38**</td>
<td>7.06±1.24</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2±2.37**</td>
<td>25.2±1.85</td>
</tr>
</tbody>
</table>

Table 3
Bone metabolism indexes compare diabetic nephropathy groups with control group (x±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>BMI (kg/m²)</th>
<th>FBG (mmol/L)</th>
<th>2hBG (mmol/L)</th>
<th>HbA1c (%)</th>
<th>Ca (mmol/L)</th>
<th>P (mmol/L)</th>
<th>ALP (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>20.5±4.3</td>
<td>5.82±0.51</td>
<td>–</td>
<td>–</td>
<td>2.32±0.13</td>
<td>1.07±0.15</td>
<td>74.2±19.7</td>
</tr>
<tr>
<td>A</td>
<td>25.5±4.1</td>
<td>11.4±5.2**</td>
<td>15.4±7.3</td>
<td>7.5±2.5</td>
<td>2.34±0.15</td>
<td>1.09±0.21</td>
<td>72.5±16.8</td>
</tr>
<tr>
<td>B</td>
<td>26.1±3.9</td>
<td>11.2±4.5**</td>
<td>17.5±5.2</td>
<td>9.8±2.4</td>
<td>2.35±0.13</td>
<td>1.06±0.18</td>
<td>74.7±18.3</td>
</tr>
<tr>
<td>C</td>
<td>23.9±4.5</td>
<td>12.1±6.4**</td>
<td>18.0±8.5</td>
<td>11.4±3.2</td>
<td>2.31±0.23</td>
<td>1.21±0.22</td>
<td>73.3±17.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>PTH (pg/ml)</th>
<th>BGP (ng/ml)</th>
<th>BUN (mmol/L)</th>
<th>Cr (μmol/L)</th>
<th>Urine Ca/Cr</th>
<th>DPD/Cre/CG</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>62.5±16.7</td>
<td>5.6±2.42</td>
<td>4.6±1.3</td>
<td>72.4±21.3</td>
<td>0.39±0.12</td>
<td>4.73±1.82</td>
</tr>
<tr>
<td>A</td>
<td>70.1±20.3*</td>
<td>4.9±2.35*</td>
<td>4.8±1.7</td>
<td>68.1±23.6</td>
<td>0.31±0.15</td>
<td>5.06±2.42*</td>
</tr>
<tr>
<td>B</td>
<td>73.6±25.5*</td>
<td>4.2±3.52*</td>
<td>4.7±1.6</td>
<td>75.7±28.5</td>
<td>0.65±0.24*</td>
<td>5.22±2.84*</td>
</tr>
<tr>
<td>C</td>
<td>107.2±68.5**</td>
<td>3.8±4.91**</td>
<td>9.3±7.921**</td>
<td>93.5±76.4**</td>
<td>0.82±0.42**</td>
<td>7.85±3.61**</td>
</tr>
</tbody>
</table>

A, B and C groups were compared with control group (CG), *P<0.05, **P<0.01.

4. Discussion and conclusion

With the development of economy, conversion of life style and trend of population aging, the incidence of T2DM is increasing year after year and the prevention of its chronic complication in heart, brain and kidney has been widely recognized. The effect of diabetes on BMD is still not clear. BMD in patients with T2DM was reported to be decreased [18], unchanged [19] or even increased [7,11].

There have been conflicting reports about the skeletal involvement in patients with diabetes mellitus. T2DM and OP are both common senile diseases, which can occur at the same time or sequentially. DM is often accompanied by disturbances of bone, calcium and phosphorus metabolism; OP is severe complication of body bone system in DM.

Although there have been conflicting studies on the relationship between T2DM and BMD, most of the studies carried out on T2DM revealed a normal or high-normal bone mass at both appendicular and axial skeletal sites [11,13]. The Rotterdam Study [9], however, which examined the association between diabetes and BMD in elderly people, displayed that diabetes was associated with a 3% increase in hip and spine BMD both in men and women. In theory, the increased BMD in T2DM should be associated with a decreased risk of fractures, but the opposite is seen [20,21]. Since the early onset of T2DM, insulin is generally higher, often after the decrease, so insulin in patients with type 2 diabetes may be caused by fluctuations, inconsistent with one of the reasons.

Our findings indicate that OP may be a chronic complication of T2DM, an increased risk for OP in T2DM patients with a statistical significant 2.08 risk. Our study provides evidence for an association between noninsulin-dependent diabetes and depressed bone density. A decrease in BMD values at all examined levels was also present in diabetic older men. These results suggest that examining BMDs may be very important in elderly T2DM patients, especially in diabetic nephropathy.

Based on our results, it also indicated that body weight is one of important index affecting BMD, while keeping healthy weight has become an important measure in diabetic control. However, low prevalence of DO in T2DM patients with higher BMI is observed and so is contrary to patients with lower BMI, which indicated that BMI is significantly related with BMD.
Increasing BMI is associated with a decreased risk of fractures in the general population [22], so it may show that the increased BMI in T2DM is protective against fractures.

DCCP and UKPDS suggest that the occurrence of diabetic chronic complications is associated with poor control of state of diabetes, especially with poor control of blood glucose [23]. To point at a common risk factor linked to the high blood glucose levels, which may weaken bone strength, our results show that HbA1c is related with BMD and is positively related with the occurrence of OP. Therefore, it is implied that better control of blood glucose can stabilize BMD, which is consistent with other reports. With the prolongation of course of diabetes the loss of bone mass has been gradually advancing in severity and the prevalence of OP has increased in elderly T2DM men.

The bone metabolic is the process that osteoblasts form new bone and osteoclasts absorb old bone. Bone metabolic markers include bone formation markers, which reflect osteoblasts activity, and bone resorption markers, which reflect osteoclasts activity. Inconsistencies also exist in the available reports on biochemical markers of bone metabolism in diabetes [24–28]. In our present study, among all the parameters that have been looked for, significant changes were confined to serum ALP, BGP and DPD/Cr in diabetic patients compared to the CG. In T2DM complicated with OP patients, more decrease of bone formation than bone resorption, so it may be a lower turnover ratio type.

Serum ALP plays a very important role in bone formation and bone mineralization and it is one of the important markers for the appraisal of bone formation and bone transformation. This article showed that ALP concentration had no obviously difference among the people at different groups. Because serum ALP comes from different tissue such as liver, bone, spleen, kidney and placenta, it exists in serum with different homotype-dimer. All of them are isoenzyme, so they lack specificity and sensitivity for the bone. For old person, the bone mineral mass changes very slow, so the change of ALP is not outstanding. To test bone specific ALP will contribute a lot to the osteoblasts function.

PIR is a kind of collagen hydroxylation, existing in the bone and cartilage and as an interactive chain in collagen molecule of bone and cartilage. PIR forms from the maturity of collagen fiber cell and it is released after collagen degradation. After PIR mixes in blood and become circulation in blood, it will not synthesize collagen, but discharge by urine. The PIR in urine exists in the form of educt (nearly 40%) and peptide combination (nearly 60%), because it will not affect by diet and the degradation of new synthesized collagen, it has highly specificity in bone tissue.

From the above, to test the concentration of urinary PIR can directly appraise the loss rate of bone. Presently, DPD is the best marker to evaluate the bone respiration and the concentration of DPD should be revised by Cr. In our study, the urinary DPD/Cr of OP group was noticeable higher than the normal group. This meant urinary DPD/Cr is a sensitive marker for the early diagnosis of OP and evaluation of bone loss. Meanwhile, we found that increased blood glucose levels were associated with an increased calcium loss in the urine and thus a negative calcium balance.

BGP was regarded as the specific marker of osteoblasts function. It plays a very important role in bone mineralization, controlling the formation of abnormal hydroxylapatite crystalization and cartilage mineralization. Delmas [29] believed that when bone formation coupled with bone resorption, BGP was the specific marker for bone formation. The reports on bone resorption in diabetic patients are also limited with conflicting results [7,30–32]. Our present findings also indicate that elderly men’s BGP lowered down and the PYD/Cr raising with growth of age. BGP of elderly men OP group was lower than that of normal group while the PYD/Cr was higher than that of normal group. Our results show that serum BGP and PYD/Cr are specific and sensitive markers for giving expression to bone formation and bone resorption, prior to the change of BMD.

The secretion of parathyroid hormone (PTH) is stimulated by a decrease in the free calciumion (Ca2+) concentration of the blood perfusing the parathyroid glands. In our subjects, circulating PTH levels were comparable to non-diabetic control values. We found that hyperplasia of the parathyroid glands with increased secretion of PTH occurring in patients especially with diabetic nephropathy. Both in type-1 and type-2 diabetes, high levels of circulating PTH have been observed and correlated to the severity and duration of the disease [33–35]. Furthermore, our results show that the serum insulin levels were correlated with BMD. Insulin may act either directly on bone or by binding to the receptor of insulin-like growth factor in several epidemiologic studies [36–38]. In our studies, the diabetic patients were divided into OP and non-OP, the levels of fasting, postprandial 2-hour insulin and BMI in the group of non-OP were significantly higher than those of the group of OP.

In conclusion, our experiments indicate that:

- OP may be a chronic complication of T2DM, there is a higher morbidity of OP in elderly male type 2 DM patients. An increased risk for OP in T2DM patients with a statistical significant 2.08 risk; with the prolongation of course of diabetes the loss of bone mass has been gradually advancing in severity and the prevalence of OP has increased;
- body weight is one of important index affecting BMD, while keeping healthy weight has become an important measure in diabetic control;
- HbA1c is related with BMD and is positively related with the occurrence of OP. Therefore it is implied that better control of blood glucose can stabilize BMD, which is consistent with other reports;
- an important role is also played by chronic diabetes complications, especially diabetic nephropathy, it has become a vital risk factor for DO;
- IR contributes to the development of OP in insulin-dependent diabetes mellitus. The bone change is characterized by high bone absorption rate and low bone formation rate.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.
References


