Osteoporosis in older adults with non-insulin-dependent diabetes mellitus: Is it sex related?

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Abstract

Objective

To assess the association of non-insulin-dependent diabetes mellitus (NIDDM) with bone mineral density (BMD) and its effect on bone turnover.

Methods

BMD (measured by osteo C.T. of the lumbar spine) and bone resorption (deoxypyridinoline) and formation (alkaline phosphatase bone isoenzyme) markers were measured in 40 female postmenopausal patients with NIDDM and in 40 non-diabetic females of the same age. The same investigations were carried out in 20 males with NIDDM and in 20 normal non-diabetic males.

Results

Women with diabetes had significantly ($p < 0.01$) higher BMD levels than women with normal glucose tolerance. Diabetic females were also significantly overweight ($p < 0.001$) and had a longer duration after menopause ($p < 0.02$). Bone resorption markers and bone formation markers were significantly ($p < 0.001$) higher in the control group compared with the diabetic group. Men with diabetes had BMD levels similar to those men with normal glucose tolerance. Also there was no significant difference on comparing bone resorption and formation markers in the group of diabetic men to the control male group.

Conclusion

Older women with NIDDM had better BMD than normal women. No difference in bone density by diabetic status were obeserved in men. That sex difference may be explained by the obesity and the greater androgenecity reported in women with hyperglycemic and hyperinsulinemic conditions.

Key words

Diabetes mellitus, osteoporosis.

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Introduction

Several studies have suggested the existence of altered bone mineral metabolism in patients with diabetes mellitus (DM). Osteopenia has been reported as an established complication in insulin-dependent diabetics (1-4), especially in the first few years from the time of diagnosis (5), in patients with poor control and in patients who have been treated with large doses of insulin (6). On the other hand, the issue is controversial in patients with non-insulin-dependent diabetes mellitus (NIDDM) who showed evidence of decreased, normal or increased skeletal mass (1,7, 8). These contradictory results could be attributed to differences in the patients studied (age, sex, type of diabetes), differing methods of measurement of bone mass, failure to consider body weight, use of thiazide diuretics, and other determinants of bone density. In addition most investigators studied only one sex or failed to analyze data from men and women separately.

The mechanism of altered bone metabolism in diabetics is as yet unresolved (9). The suggestion that variations in calcitropic hormones are responsible is debated and in most cases remains a somewhat unsatisfactory explanation (10, 11). Disturbed calcium homeostasis has been reported in both short- and long-term animal models of diabetes (11, 12). However, it has been found that results obtained in experimental models in rats cannot be extrapolated to humans (13), which means that the effect of diabetes mellitus on bone turnover should be studied in humans.

Current interest in biochemical markers include their potential value in predicting changes in bone mass. The determination of bone marker concentrations has the advantages of being a specific, sensitive and non-invasive approach for monitoring bone metabolism. Bone-specific alkaline phosphatase and osteocalcin are osteoblast-derived, non-collagenous proteins that are released into the circulation during bone synthesis, and therefore have been used as markers of bone formation. Pyridinoline and deoxypyridinoline are two non-reducible trivalent crosslinks that stabilize type I collagen chains and are released during the degradation of mature collagen fibrils. Total pyridinoline and deoxypyridinoline can be measured in urine samples and are considered markers of bone resorption (14).

This controlled study was performed to determine the association of NIDDM with bone mineral density (BMD) in both men and women and to investigate the effect of the disease on bone turnover in these patients.

Material and methods

Patients

Forty postmenopausal Egyptian female and 20 Egyptian male patients diagnosed with NIDDM were included in this study. They were randomly collected from those attending the diabetic care outpatient clinic at Ain Shams University. They were all on oral hypoglycemic drugs and were currently ketosis-resistant. A full medical history including the duration of diabetes mellitus, history of back pain whether acute or chronic, history of falls or inability to bear weight was taken. A thorough clinical examination was made of each patient, with particular stress on localised tenderness over the vertebrae and the lower end of the radius, free range of motion of the hip joints, and paraspinal muscle spasm in addition to kyphosis and/or scoliosis. At initial evaluation, postmenopausal duration and number of pregnancies were recorded for the female patients. The height and weight of each patient was measured and the body mass index (BMI) (wt/height^2) was calculated as an estimate of obesity. A dietary history, especially calcium intake, was also recorded.

Patients with a history of renal involvement were excluded from this study. Also patients with a history of other disease [e.g., malignancy, thyrotoxicosis, hypercorticoidism (endogenous or iatrogenic), hyperparathyroidism) or medications (steroids, thyroid hormone, diuretics, antihypertensive drugs containing thiazides, antimitotics, heparin and anticonvulsants) that could alter bone metabolism] were also excluded from the study. None of our postmenopausal patients were on hormone replacement therapy or any other form of treatment for osteoporosis. None of our patients were smokers or alcoholics.

Radiological assessment

Each patient was subjected to plain x-ray (anteroposterior and lateral views) for the dorsal and lumbar spines. X-rays of the forearm and/or pelvis were taken if fracture was suspected. Bone mineral content (BMC) was measured by quantitative CT (Siemens) of the L1-L3 lumbar vertebrae. The procedure was carried out according to the method of Cann and Gennant (15). Briefly, a phantom (QCT Bone Minderal Analysis System, San Francisco, CA) containing potassium phosphate standard was positioned under the patient. Cursors were placed on the vertebral image to define a 100 mm thick transverse section through the center of L1, L2, L3. Cross-sectional images of each vertebra were obtained and used to position elliptical cursors in the trabecular area of each vertebral body. CT counts were then obtained for the selected vertebrae. Spinal measurements were referenced to a calibration curve obtained from the standard and were expressed in mg/cc. The results were expressed as a Z score (number of standard deviations above or below the normal mean after comparison with age- and sex-matched normal control values supplied by the manufacturer).

The T score for the women was calculated according to the equation provided by the manufacturer: BMC (trabecular) - 159.1 (BMC of an average 35-year-old female) / 27.6 (SD)

The T score for men was calculated according to the equation: BMC (trabecular) - 175.5 (BMC of an average 20-year-old male) / 26.5 (SD).

Laboratory investigations

Each patient underwent blood analyses (to measure serum creatinine and glycosylated haemoglobin) and urinalysis. Insulin hormone was measured in 27 female and 20 male patients by an enzyme immunoassay using kits and instrumentation (Axsym) from Abbott Laboratories (Wiesbaden, Germany).

Bone mineral profile

The following parameters were assayed: serum calcium, phosphorous, alkaline phosphatase, urinary deoxypridinoline (bone resorption marker) and bone-specific alkaline phosphatase (bone formation marker). Aliquots of urine were stored frozen at -70°C until each batch assay was completed. Venous blood samples were obtained, centrifuged and stored in a similar manner.

Deoxypyridinoline was measured in the urine by estimating Pyrilinks-D using a competitive EIA (monoclonal antideoxypyridinoline antibody coated on monitor strips with deoxypyridinoline-AP conjugate to quantitate the free deoxypyridinoline) kit obtained from Metrabiosystems Ltd. (California, USA).

Bone specific alkaline phosphatase was assessed using an EIA (Metrabiosystems) based on monoclonal anti-bone alkaline phosphatase antibody coated on microtitre strips to capture and quantitate bone alkaline phosphatase activity.

Control group

Forty Egyptian postmenopausal females and 20 Egyptian males matched for age were randomly collected and included as a control group. They had no history of any disease or medication known to affect bone metabolism. They underwent the same laboratory and radiological assessments. In addition, their blood was tested for fasting and 2-hour plasma glucose levels and glycosylated haemoglobin, which were all found to be within the normal range.

All patients and controls provided their informed consent to participate in this study.

Statistical analysis

Data was analysed using the Statistical Package for Social Sciences (SPSS) Software on an IBM-PC. The different variables were expressed as the means ± SD. Student's t-test was used to compare the data between cases and controls. The Mann-Whitney test was used to compare the variables which were not uniformly distributed. Spearman's correlation coefficient was used to assess the correlations between age, BMI, glycosylated haemoglobin, the duration of diabetes mellitus, insulin hormone, and the T and Z scores in the diabetic males and females. The effect of obesity was removed by stratified analysis.

Results

Table I shows a comparison of the characteristics of the 40 women with NIDDM versus the controls. It was found that postmenopausal females with NIDDM had a better BMD than the control group, while among the males (Table II) there was no significant difference between patients and controls (although BMD

Table I. Mean ± SD of the variable assessed in the female diabetic and control groups.

Variable	Diabetic Group N = 40	Control Group N = 40	P
Age (yrs.)	52.9 ± 6.16	53.6 ± 5.54	N.S.
Weight (kg.)	83.2 ± 13.216	68.6 ± 10.558	< 0.001
BMI (weight/height^2)	35.03 ± 6.328	29.35 ± 3.491	< 0.001
Parity	8.58 ± 3.98	8.9 ± 3.622	N.S.
Glycosylated haemoglobin (%)	8.56 ± 1.41	4.92 ± 0.47	< 0.01
Duration aftter menopause (yrs.)	8.16 ± 6.203	5.44 ± 4.454	< 0.02
Insulin H (uU/ml)	26.4 ± 29.98		
Cortical BMC (mg/cm^3)	238.36 ± 41.376	234.84 ± 42.031	N.S.
Trabecular BMC (mg/cm^3)	96.53 ± 28.58	79.99 ± 32.288	< 0.01
Z-score	-0.68 ± 0.923	-1.33 ± 0.935	< 0.002
T-score	-2.27 ± 1.175	-2.87 ± 1.026	< 0.01
Serum Ca (mmol/L)	2.19 ± 0.12	2.23 ± 0.14	N.S.
Pphosphorous (mmol/L)	1.19 ± 0.15	1.21 ± 0.2	N.S.
Alkaline phosphatase (mmol/L)	16.37 ± 8.14	17.83 ± 6.17	N.S.
Deoxypyridinoline (nmol/mmol)	4.82 ± 2.19	7.68 ± 2.24	< 0.001
Bone alkaline phosphatase isoenzyme (U/L)	17.14 ± 6.011	22.48 ± 5.720	< 0.001

BMI: Body mass index, BMC: Bone mineral content.

Table II. Mean ± SD of the variable assessed in the male diabetic and control groups.

Variable	Diabetic group N = 40	Control group N = 40	P
Age (yrs.)	51.6 ± 5.433	52.35 ± 6.434	N.S.
Weight (kg)	89.85 ± 11.310	86.5 ± 10.836	N.S.
BMI (weight/height^2)	31.64 ± 3.559	29.56 ± 3.141	< 0.05
Glycosylated haemoglobin %	8.08 ± 1.27	4.8 ± 0.53	< 0.001
Insulin H (uU/ml)	18.99 ± 10.41		
Cortical BMC (mg/cm^3)	314.9 ± 43.814	300 ± 58.03	N.S.
Trabecular BMC (mg/cm^3)	149.3 ± 27.211	146.3 ± 31.302	N.S.
Z-score	0.85 ± 0.70	0.83 ± 0.69	N.S.
T-score	-0.95 ± 1.024	-1.07 ± 1.185	N.S.
Serum Ca (mmol/L)	2.18 ± 0.093	2.16 ± 0.110	N.S.
Phosphorous (mmol/L)	1.17 ± 0.12	1.19 ± 0.17	N.S.
Alkaline phosphatase (mmol/L)	18.21 ± 5.59	13.96 ± 3.89	< 0.001
Deoxypyridinoline (nmol/mmol)	4.41 ± 1.741	5.09 ± 1.898	N.S.
Bone alkaline phosphatase isoenzyme (U/L)	16.26 ± 4.044	17.45 ± 3.663	N.S.

BMI: Body mass index, BMC: Bone mineral content.

was slightly better in the diabetic group). Furthermore, the bone resorption and formation markers were significantly lower in the diabetic females compared with the females who had normal glucose tolerance. In contrast there were no significant differences in the bone resorption and formation markers between the male patient and control groups (although they were lower in the diabetic male group). The diabetic patients (both male and female) were overweight and their BMI differed significantly from the respective control groups.

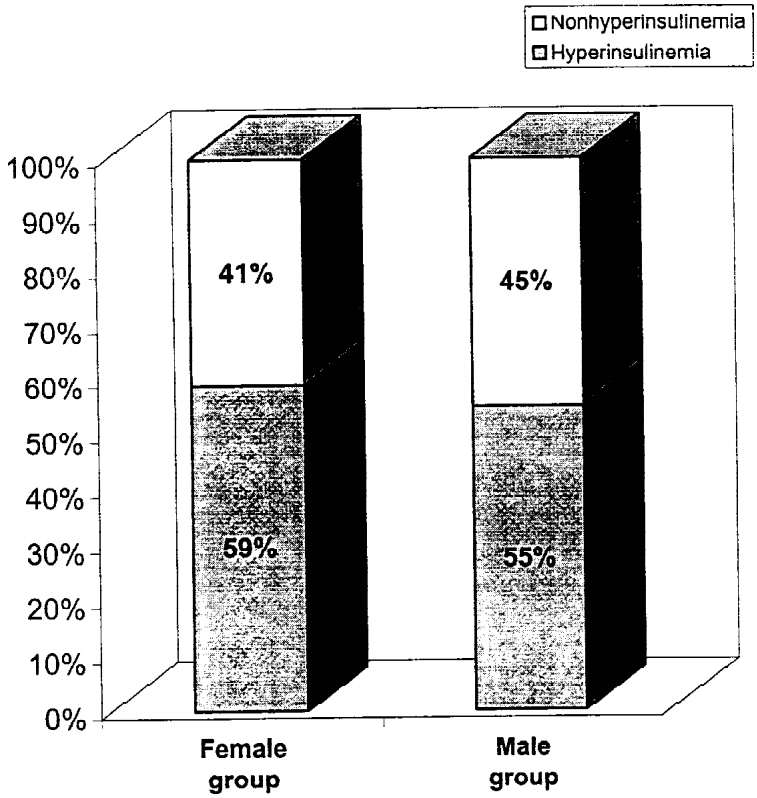

Fig. 1. Prevalence of hyperinsulinemia in both diabetic female and males groups studied.

Glycosylated haemoglobin was significantly higher in the diabetic male and female groups compared to the control groups. This may reflect the difficulty of establishing a correct diet-drug balance, which is a relatively common problem among Egyptian diabetics.

Plain x-rays showed reduced bone density in 10% (4/40) of the female patients, while no osteopenia was seen in the male patients. No fractures were observable on x-ray in any of the patients included in this study.

Hyperinsulinemia (fasting level > 24 mcU/ml) was found in 59% (16/27) of the diabetic females and in 55% (11/20) of the diabetic males (Fig. 1).

Table III shows the correlation coefficients for the variables tested in the diabetic female group. It shows that there was no significant correlation between the T and Z-scores and the duration of the disease, the body mass index, or glycosylated haemoglobin. On the other hand there was significant correlation between the insulin hormone level in the blood of the female diabetic patients and their T and Z-scores.

Table IV shows the correlation coefficients for same variables in the diabetic male group. As in the female group, there was no correlation between the Z-score and the body mass index nor the glycosylated haemoglobin level. Insulin hormone correlated significantly with the Z-score ($p < 0.01$).

Table V shows a comparison of the Z-scores between obese diabetic females (BMI > 30) and obese non-diabetic postmenopausal females, and between non-obese diabetic females (BMI < 30) and non-obese non-diabetic females. In both cases there was a significant difference ($p < 0.01$ and $p < 0.04$, respectively).

Discussion

Diabetes mellitus has been variously reported to be associated with an increased, a normal or a decreased bone mass when compared with controls and there is no consensus in the literature as to what abnormalities, if any, generally exist. Moreover, it is relevant to notice that most investigators studied only one sex or failed to analyze data on men and women separately.

The results of our study show that post-

menopausal women with NIDDM have a higher cortical and trabecular ($p < 0.01$) bone mineral content in the axial spine than women with normal glucose tolerance. Also the Z and T-score are significantly better ($p < 0.002$ and $p < 0.01$, respectively) in the diabetic group. These differences cannot be explained by obesity, cigarette smoking, dietary calcium or the use of medications that are known to increase bone mineral density.

On the other hand, the bone mineral density in men with NIDDM did not differ significantly from that in males with normal glucose tolerance. As noted earlier, bone mass in diabetics has been found to be elevated, normal or decreased (1-9). It seems likely that most type I diabetics have decreased bone mass (1-4). Type II diabetics may constitute a different subgroup. This study suggests that postmenopausal women with type II diabetes generally have increased bone mineral density. These results are in concordance with what has been reported in previous studies by Meema and Meema (7), Johnston and colleagues (16) and Weinstock *et al.* (17), who reported less osteopenia in older women with diabetes. Moreover, in another study Conner and Holbrook (18) reported that older women with NIDDM had better bone mineral density than women with normal glucose tolerance, independently of differences in obesity. Furthermore, they reported no differences in bone density in men with NIDDM compared with controls.

Our diabetic postmenopausal women had a significantly higher body weight and BMI ($p < 0.001$) compared with the control group. Obesity has been reported to protect against osteoporosis (19). Endogenous estrogen produced in the subcutaneous fat of heavier women due to the aromatization of androstenedione to estrone could account for this difference (20). However, obesity alone may not be the sole explanation for this difference. Comparing the obese (BMI > 30) and non-obese (BMI < 30) diabetic females with the control group revealed significant differences in the bone mineral density (Z-score) (Table V). In addition, there was no significant correlation between BMI and bone mineral density (Table III). Moreover, women with NIDDM were less overweight than the diabetic men. Despite this fact, there was a significant difference in BMD between patients and controls in the female group while there was no difference on the male side. An interesting finding was reported in the study by Johnston *et al.* (16), who observed that in diabetics and non-diabetics of similar weight the estrone level was nevertheless higher in the diabetic group.

Alternatively, hyperinsulinemia has been suggested as a possible explanation for the better BMD seen in patients with NIDDM (17). However, when the insulin hormone levels were measured in our two groups of patients, we found hyperinsulinemia in 59% of the females and in 55% of the males. That makes this hypothesis seems less likely, as less osteoporosis was found only in women with NIDDM and no difference in BMD was found in the men. Reduced levels of insulin-like growth factors has been reported in patients with diabetes or hyperinsulinemia (21). However, a reduction of insulin-like growth factors would cause osteopenia and not the reverse, as seen in our patients.

Dietary calcium cannot explain the sex

Table III. Correlation coefficient of the variables tested in the diabetic female group.

	Age	BMI	Glycosyl. Hgb.	Duration of illness	Insulin Hormone	T
Z-score	-0.2280	-0.0391	-0.0981	-0.0350	0.6566 P < 0.001	0.7847 P < 0.001
T-score	-0.5444 P < 0.001	0.0841	-0.0872	0.1851	0.4637 P < 0.01	
Insulin hormone	-0.0716	0.0780	-0.0303	-0.2857		
Duration of illness	-0.1450	0.0917	0.2929			
Glycosylated Hgb.	-0.0632	0.0613				
BMI	-0.1576					

Table IV. Correlation coefficient of the variables tested in the diabetic male group.

	Age	BMI	Duration of illness	Glycosyl. Hgb.	Insulin hormone
Z-score	-0.1586	-0.1724	0.6770 p < 0.001	0.1447	0.4295 p < 0.005
Insulin hormone	0.1338	-0.0851	0.8046 p < 0.001	-0.2228	
Glycosylated Hgb.	-0.0908	-0.0083	-0.0221		
Duration of illness	0.1952	0.0101			
BMI	-0.4118				

BMI: Body Mass Index

Table V. Comparison of Z-score between obese and non-obese diabetic and control female groups.

	Obese diabetic	Obese control	P	Non-obese diabetic	Non-obese control	P
Z-score	-7.5 ± 0.92	-1.3 ± 0.66	P < 0.01	0.00 ± 0.68	-1.31 ± 1.13	P < 0.05

difference, since both the men and women with diabetes reported a somewhat lower calcium intake than non-diabetic subjects, which would have been expected to cause reduced bone density in the diabetic women and not to increase it. Our findings are in concordance with the results of Connor and Holbrook (18), who reported that separate analysis adjustment for calcium intake did not explain the sex difference in BMD.

The sex difference in BMD is compatible with diabetes-related differences in endogenous sex hormones. Older males with diabetes often have lower levels of androgens than men without diabetes (22, 23). Plasma insulin and glucose levels were found to be positively correlated with androgenecity in women (24, 25), whereas in men a significant inverse correlation was seen (26). We did not measure the levels of sex hormones in our two groups of patients. However, our group of diabetic females had a significantly longer duration after menopause ($p < 0.02$). In a study by Scheidt-Nave *et al*. (27) significantly ($p < 0.001$) higher levels of unbound testosterone were found in menopausal women with diabetes mellitus than in control women without diabetes. It may be of value here to add the finding reported by Horton and Tait (28), who stated that androstenedione is not only the major source of estrone in postmenopausal women but also of testosterone in women of all ages. Taken together, these data are compatible with the hypothesis that higher androgen levels in hyperglycemic women could protect them from some of the bone loss that occurs after menopause. Reduced androgens in diabetic men may have less impact because of their higher baseline androgen levels and higher bone mass.

Glycation and glycoxidative reactions have been recognized to play a major pathogenetic role in the biochemical and biophysical alterations of proteins in diabetes mellitus (29). Glycation of proteins is associated with the formation of high molecular weight aggregates that are stabilized by non-reducible cross-linking components (30). The formation of cross links has an impact on collagen triple helix stability, as has been shown previously for collagen II from the intervertebral discs of elderly patients (31). That raises the possibility that the non-enzymatic glycosylation of extracellular proteins could alter turnover at the level of the bone. This is supported in part by our finding of a significantly elevated glycosylated haemoglobin percentage ($p < 0.001$) in both the diabetic males and the diabetic males females compared to the control groups. However, there was no significant correlation between the glycosylated haemoglobin percentage and bone mineral density in the diabetic patients (Tables III and IV).

Another interesting point is the possible link between non-insulin diabetes mellitus and osteoarthritis (32). The impact of this link on bone density is questionable in view of the consistent evidence supporting the theory of an inverse relationship between bone density and osteoarthritis (33).

Studies of bone mineral metabolism in diabetic patients have focused on osteopenia in insulin dependent diabetic subjects. Earlier studies reported that calcium homeostasis is disturbed in both short- and long-term animal models of diabetes (11, 12). In another study, Pedrazzoni *et al*. (34) reported that calcium homeostasis is altered in diabetic patients, leading to slightly elevated calcium levels and subsequent parathyroid hormone suppression associated with increased calcitonin levels. Rico *et al*. (35) correlated impaired bone formation, manifested by low osteocalcin levels in IDDM patients, to deficient osteoblastic function rather than to insulin deficiency as the cause of the diminished bone formation.

To the best of our knowledge, the present study is the first to assess markers of bone resorption and formation in patients with NIDDM and to correlate them with BMD. Our results reveal a significant decrease in both resorption and formation markers in postmenopausal women with NIDDM. This suggests that such women have lower rate of bone turnover than women with normal glucose tolerance. On the other hand, postmenopausal non-diabetic women showed a significant increase in bone resorption and formation markers, indicating accelerated bone loss.

The lower rate of bone turnover in diabetic females may reflect the impact of elevated sex hormones on bone in this group of patients. Our finding of lower levels of the bone formation marker bone alkaline phosphatase isoenzyme agrees with earlier studies measuring osteocalcin in diabetic patients (34, 35). However, these studies correlated this lower level of the bone formation marker to a deficiency of osteoblastic function rather than using it as assessment of bone turnover in their group of patients. Moreover, in their study Rico *et al.* (35) observed a marked and significant reduction in the bone formation marker in insulin dependent diabetics, while it was relatively higher (although still significantly lower than the control group) in patients with NIDDM. This was correlated to the effect of insulin hormone which was found to stimulate osteoblasts directly (36). On the other hand, sex steroids (both estrogen and testosterone) have been repeatedly reported to inhibit bone resorption and to increase bone mineral density (37, 38). This may confirm our previous observation that type I diabetics decreases bone mass, a finding which could be attributed to deficient osteoblastic function, while type II diabetic patients represent a different subgroup showing reduced bone turnover by combined reductions in bone resorption and formation markers.

In conclusion, postmenopausal women with NIDDM were found to have a higher bone mineral density together with a decreased rate of bone turnover than non-diabetic women. On the contrary, no difference in bone density as a function of diabetic status were observed in males. The reason for this sex-related difference in bone metabolism is not clear; most probably the levels of endogenous sex steroids and to some extent obesity could explain in part the greater peak bone mass and slower bone loss with age found in postmenopausal women with NIDDM.

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