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BONE MINERAL LOSS
IN INSULIN-TREATED DIABETES MELLITUS:
STUDIES ON PATHOGENESIS

By

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ABSTRACT

To elucidate pathogenetic factors of bone mineral loss in diabetes mellitus, bone mineral content (BMC), glucose and calcium homeostasis were evaluated in a cross-sectional study of 215 insulin-treated diabetics. BMC declined 10% during the first 5 years of diabetes. This coincided with cessation of insulin secretion, deterioration of metabolic control and raising urinary excretion rates of calcium and phosphorus. BMC was inversely correlated to fasting blood glucose ($P < 0.02$), to glycosuria ($P < 0.02$) and to insulin requirement ($P < 0.002$), and positively to the glucagon-stimulated serum C-peptide levels ($P < 0.005$). Urinary excretion rates of calcium and phosphorus correlated positively with the degree of hyperglycaemia ($P < 0.001$) and glycosuria ($P < 0.001$). The skeletal calcium loss corresponded to the excess of urinary calcium excretion during the phase of BMC reduction. There was no evidence of secondary hyperparathyroidism.

The relationship between bone loss and disturbed glucose homeostasis indicates that diabetic bone loss is secondary to the metabolic abnormalities, possibly acting directly on bone.

Decreased bone mineral content, has recently been recognized as a general feature of insulin-treated diabetes mellitus (Levin et al. 1976; Ringe et al. 1976; Rosenbloom et al. 1977; McNair et al. 1978), while conflicting observations have been made in patients treated exclusively with diet or sulphonyl-
ureas (Levin et al. 1976; De Leeuw & Abs 1977; McNair et al. 1977). The reduction in bone mass develops during the first 3 to 5 years of clinical diabetes and becomes most severe in patients with diabetes onset before 21 years of age (McNair et al. 1978). The degree of osteopenia certainly depends on the quality of diabetic control (McNair et al., sub. for publ.), but the way in which defective glucose homeostasis interferes with calcium metabolism remains to be established. One possible mechanism is that hyperglycaemia and/or glycosuria decreases the tubular re-absorption of calcium (Lemann et al. 1970), leading to secondary hyperparathyroidism and loss of bone mineral. Alternatively, changes in metabolism induced by lack of insulin may act directly upon bone, causing bone loss with a secondary rise in the urinary excretion of calcium and phosphorus and a suppression of parathyroid hormone (PTH) secretion.

In order to elucidate the pathogenesis of the bone mineral loss we have measured various indices of glucose and mineral metabolism in 215 insulin-treated diabetic out-patients.

**Patients**

A cross-sectional study was undertaken of 215 insulin-treated diabetic out-patients, aged 7–70 years with clinical diabetes for 0–29 (mean 10) years. All patients had a normal serum creatinine (<115 μmol/l) and none had other diseases or received drugs known to interfere with calcium metabolism. Details of patient selection have been presented elsewhere (McNair et al. 1978). The patients were prescribed a diet fixed on calories and with a relative content of 40–45% fat, of 35–40% carbohydrate and 15% protein.

**Methods**

All investigations were carried out at the out-patient clinic of calcium metabolism outside the diabetes hospital (see Discussion). Blood samples were drawn at 8–9 a.m. after an overnight abstinence from food and tobacco before administration of insulin. A 24-h urine collection was completed in the morning.

**Bone mineral content** (BMC) was measured in both forearms by photon absorptiometry (Christiansen et al. 1975b). The method has a high precision, coefficient of variation 1.2% (Christiansen & Rodbro 1977), but the inter-individual variation is considerable: 16% (1 sd) in normal subjects (Christiansen & Rodbro 1975b).

**Endogenous insulin secretion capacity** was estimated by measuring the serum concentration of immunoreactive C-peptide (by radioimmunoassay using antibody M 1230 (Faber et al. 1978)) 6 min after iv injection of 1 mg glucagon (Faber & Binder 1977). A detectable C-peptide level (>0.05 nmol/l) signifies residual B-cell function.

The **insulin requirement** was calculated for each patient as the dose of insulin given at the time of investigation (IU/kg body weight/day).

Serum iPTH was measured by a mainly C-terminal specific double antibody radioimmunoassay (Christiansen et al. 1978). Glucose was measured by an enzymatic ana-
ysis, creatinine and protein on the SMA 6/60 autoanalyzer and phosphorus by colorimetry. Calcium was determined by atomic absorption spectrophotometry (Perkin Elmer 403), and the serum levels were corrected for individual variation in serum protein concentration to a constant protein level (Christiansen et al. 1975a). The coefficients of variation were 4–5 % for measurements of glucose, creatinine, C-peptide and iPTH, and below 2 % for the other chemical measurements. The BMC and serum phosphorus were expressed in per cent of mean values from sex- and age-matched controls as described in detail elsewhere (McNair et al. 1978). Urinary excretion rates were calculated per gram creatinine excreted, in order to eliminate the considerable variation inherent in quantitative 24-h urine collections. Furthermore, the sex- and age-dependency of calcium and phosphorus excretion rates are minimized by this calculation (Nordin 1976).

Adult reference values were obtained in blood donors aged 21–70 years: 194 served as reference for serum calcium, 99 for serum phosphorus and 102 for iPTH. Reference values for subjects below 21 years were obtained in healthy school children aged 7–20 years: 230 subjects for serum calcium and iPTH, and 145 children and adolescents for reference values of serum phosphorus.

Statistical evaluation. – Student’s t-test was used for evaluation of differences between mean values. Furthermore, analysis of variance (F-test) and the Spearman rank correlation test were used. Multivariate rank correlation analysis was performed according to principles described by Puri & Sen (1971).

RESULTS

Within the first 5 years of clinical diabetes bone mineral content declined by about 10 % (Fig. 1 A) and insulin secretion ceased in about 85 % of the patients (Fig. 1 B). Simultaneously, the hyperglycaemia (Fig. 1 C), and the urinary excretion rates of calcium (Fig. 1 D), of phosphorus (not shown) and of glucose (not shown) rose to a maximum.

After about 5 years of diabetes the bone mineral content and the proportion of the patients with residual B-cell function remained fairly stable (Fig. 1 A and 1 B), while the hyperglycaemia gradually declined from a maximum of 2.5 to 2.2 g/l (Fig. 1 C). The urinary excretion rates of calcium fell from 250 to 210 mg/g creatinine (Fig. 1 D), renal phosphorus excretion declined from a maximum of 800 to 650 mg/g creatinine and the glycosuria from 90 to 70 g/g creatinine.

Statistically, the bone mineral content was associated with serum C-peptide (R = 0.19, P < 0.005), with the dosage of insulin (R = -0.22, P < 0.002), with the fasting blood glucose concentration (R = -0.17, P < 0.02) as shown in Fig. 2, and with the glycosuria (Table 1). In order to eliminate the influence of diabetic duration, the R values concern triple correlations with the duration of diabetes as the third variable. Similar correlations between bone mineral content and insulin dosage and blood glucose were found in the subgroup of 145 patients with ceased insulin secretion.
The urinary excretion rates of calcium and phosphorus were related to the glycosuria (Table 1) and to the degree of hyperglycaemia ($R = 0.24$, $P < 0.001$ and $R = 0.28$, $P < 0.001$, respectively) (Fig. 3), but not to serum C-peptide, insulin dosage or bone mineral content. These relations were virtually the same whether the excretion rates were corrected for differences in clearance of creatinine or expressed per gram creatinine excreted.

![Graphs](image)

**Fig. 1.**

Bone mineral content (BMC) in per cent of age- and sex-matched controls (A), per cent of patients with preserved B-cell function (insulin secretors) (B), fasting blood glucose (C) and urinary calcium excretion rate (D) in 215 insulin treated diabetic patients stratified according to duration of disease. Each point presents mean ± 1 SEM. n: number of patients.
Bone mineral content in per cent of sex- and age-matched controls in 215 insulin treated diabetic patients stratified according to serum C-peptide concentration after 1 mg glucagon iv (A), according to insulin dosage (B) and according to fasting blood glucose (C). Each point presents mean ± 1 SEM. n: number of patients.

Fig. 2.
**Table 1.**
Bone mineral content (BMC) in per cent of controls matched for sex and age, urinary calcium excretion (U-Ca/Cr) and urinary phosphorus excretion (U-P/Cr) in 215 diabetic patients grouped according to glycosuria.

<table>
<thead>
<tr>
<th>Glycosuria (g/g creatinine)</th>
<th>Number of patients</th>
<th>BMC (%) mean ± 1 SEM</th>
<th>U-Ca/Cr (mg/g creatinine) mean ± 1 SEM</th>
<th>U-P/Cr (mg/g creatinine) mean ± 1 SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–9</td>
<td>42</td>
<td>96.1 ± 2.6</td>
<td>171 ± 12</td>
<td>545 ± 22</td>
</tr>
<tr>
<td>10–49</td>
<td>69</td>
<td>88.9 ± 1.4</td>
<td>197 ± 10</td>
<td>589 ± 21</td>
</tr>
<tr>
<td>50–99</td>
<td>53</td>
<td>90.6 ± 1.8</td>
<td>227 ± 15</td>
<td>707 ± 25</td>
</tr>
<tr>
<td>100–199</td>
<td>33</td>
<td>87.6 ± 2.6</td>
<td>270 ± 17</td>
<td>831 ± 42</td>
</tr>
<tr>
<td>200–</td>
<td>18</td>
<td>84.9 ± 2.4</td>
<td>246 ± 28</td>
<td>874 ± 72</td>
</tr>
</tbody>
</table>

Significance of difference*:  \( P < 0.02 \) \( P < 0.001 \) \( P < 0.001 \)

* Analyses of variance.

**Fig. 3.**
Urinary excretion rates of calcium (A) and phosphorus (B) in 215 insulin treated diabetic patients grouped according to fasting blood glucose. Each point presents mean ± 1 SEM. n: number of patients.
Table 2.
Serum protein-corrected calcium (Ca), serum phosphate (P), and immunoreactive parathyroid hormone (iPTH) in 215 patients with insulin treated diabetes mellitus and in control subjects.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Patients mean ± SEM</th>
<th>Controls mean ± SEM</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg/l)</td>
<td>98.0 ± 0.2</td>
<td>98.8 ± 0.2</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>P (per cent)*</td>
<td>106.7 ± 1.1</td>
<td>100.0 ± 0.7</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>iPTH (μg/l)</td>
<td>0.31 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

SEM: Standard error of the mean.
* Of sex- and age-matched control values.

The clearance of creatinine averaged 97 ± 2 ml/min/1.73 m² (mean ± 1 sem) and did not vary as a function of diabetes duration.

Just minimal deviations from normal were observed in the mean values of serum calcium, iPTH and phosphorus (Table 2). Serum calcium and iPTH were slightly lower than average control values, while serum phosphorus slightly exceeded the mean values of sex- and age-matched controls. The

Table 3.
Serum protein-corrected calcium (Ca), serum immunoreactive parathyroid hormone (iPTH), and phosphate (P) in per cent of sex- and age-matched controls, in 215 diabetic patients grouped according to fasting blood glucose.

<table>
<thead>
<tr>
<th>Blood glucose (g/l)</th>
<th>Number of patients</th>
<th>Ca (mg/l) mean ± 1 SEM</th>
<th>P (% of normal) mean ± 1 SEM</th>
<th>iPTH (μg/l) mean ± 1 SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.49</td>
<td>49</td>
<td>98.2 ± 0.4</td>
<td>114 ± 3</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>1.50–1.99</td>
<td>34</td>
<td>97.5 ± 0.5</td>
<td>107 ± 3</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>2.00–2.49</td>
<td>40</td>
<td>98.1 ± 0.4</td>
<td>100 ± 2</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>2.50–2.99</td>
<td>40</td>
<td>97.7 ± 0.4</td>
<td>104 ± 2</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>3.00–3.49</td>
<td>36</td>
<td>98.1 ± 0.4</td>
<td>106 ± 2</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>3.50–</td>
<td>16</td>
<td>98.9 ± 0.6</td>
<td>101 ± 3</td>
<td>0.28 ± 0.01</td>
</tr>
</tbody>
</table>

Significance of difference*: N.S. P < 0.001 N.S.

* Analyses of variance: N.S.: not significant (P > 0.05).
serum levels of calcium and iPTH were not related to the degree of hyperglycaemia, while the serum concentration of phosphorus was inversely correlated to the fasting blood glucose level (Table 3).

**DISCUSSION**

In a cross-sectional study of 215 insulin treated diabetic out-patients cessation of insulin secretion and deterioration of glucose homeostasis occurred during the first few years simultaneous with declining bone mineral content and raised renal excretion of bone minerals. Low bone mineral content was clearly associated with poor metabolic control, indicating an influence of abnormal glucose homeostasis on the loss of bone mineral in diabetes. These data are consistent with the finding of normal bone mineral content in a previous study of diabetic patients treated exclusively with oral antidiabetics and selected to be in a good metabolic control (*De Leeuw & Abs 1977*).

The present study was performed outside the diabetes clinic at another hospital with the informed purpose of a calcium metabolic investigation. The blood and urine glucose values were on average 1.8 times higher at this occasion than the values measured at the immediately previous and following visit to the diabetes clinic, and may in fact be a relatively good estimate of "every day" diabetic control. The relatively low correlation coefficients between bone mineral content and indices of metabolic control are not surprising: bone mineral content varies in normal subjects between 68 and 132 % (Christiansen & Rodbro 1975b) and the metabolic state was evaluated by glucose homeostasis from only one 24-h period.

During the initial phase of bone loss the urinary excretions of calcium and phosphorus exceeded the more stable and approximately normal (*Jørgensen 1975; Mosekilde & Christensen 1977*) level attained later. Assuming the same relation between bone mineral content in the forearms and in the total body in diabetic patients as in normal subjects (Christiansen & Rodbro 1975a), a 10 % reduction of bone mineral in course of 3 to 5 years would imply a negative calcium balance of about 50–100 mg per 24 h. During the phase of bone loss the mean calcium excretion per gram creatinine was about 50 mg higher than later, corresponding to a daily calcium excess in urine of about 50–80 mg.

Previous studies have demonstrated that intravenous glucose increases urinary calcium excretion in normal subjects (*Lemmann et al. 1970*). In parallel to this we found a positive correlation between blood glucose levels and urinary excretions of both calcium and phosphorus. The tubular re-absorption of calcium and phosphorus must be decreased in the presence of hyperglycaemia, since the filtered load of the two quantities were unchanged.
Through which mechanisms abnormal glucose homeostasis causes bone mineral loss in diabetes was evaluated in the present study by measuring serum levels of calcium and iPTH. If the decreased tubular re-absorption of calcium and phosphorus seen with raising blood glucose levels was the primary pathogenic factor, the calcium loss would lead to low serum levels of calcium and a secondary increase in serum iPTH. In fact, a minute decrease in serum calcium of 1 mg/l was found, but serum iPTH values were low. Since secondary hyperparathyroidism is a sensitive indicator of calcium deficient states (Fisher et al. 1975), the low serum iPTH levels in the diabetic patients may indicate a direct action of the abnormal glucose homeostasis upon bone. Previous classification, by bone histomorphometry, of diabetes mellitus as a slow turn-over state (Klein et al. 1964) is also consistent with a primary osseous action of changes in insulin-dependent metabolism (Canalis et al. 1977).

This study therefore suggests poor metabolic control of diabetes in the pathogenesis of diabetic bone loss, probably caused by a direct action on bone metabolism and mediated through increased renal excretion of bone minerals.

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