Objectives: This Investigation on a Homogenous Cohort of Young Adult Caucasian Type 1 Diabetic (IDDM)

Priscille G. Masse  
University of Moncton, New Brunswick, Canada, priscille.masse@umoncton.ca

Maisha B. Pacifique  
University of Moncton, New Brunswick, Canada

Carole C. Trachant  
University of Moncton, New Brunswick, Canada

Barham H. Arjmansi  
Florida State University

Karen L. Ericson  
Indiana University - Purdue University Fort Wayne, ericsonk@ipfw.edu

See next page for additional authors

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Author(s)
Bone Metabolic Abnormalities Associated with Well-Controlled Type 1 Diabetes (IDDM) in Young Adult Women: A Disease Complication Often Ignored or Neglected

Priscilla G. Massé, PhD, Maïsha B. Pacifique, MS, Carole C. Tranchant, PhD, Barham H. Arjmandi, PhD, Karen L. Ericson, PhD, Sharon M. Donovan, PhD, Edgard Delvin, PhD, Marcel Caissie, MD

Department of Human Nutrition, University of Moncton (P.G.M., M.B.P., C.C.T.), Georges-L.-Dumont Hospital (M.C.), Moncton, New Brunswick, Department of Biochemistry, Sainte-Justine Hospital, Montréal, Québec, CANADA (E.D.), Department of Nutrition and Food, Florida State University, Tallahassee, Florida (B.H.A.), Department of Chemistry, Indiana-Purdue University, Indianapolis, Indiana (K.L.E.), Department of Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, Illinois (S.M.D.)
Original Research

Bone Metabolic Abnormalities Associated with Well-Controlled Type 1 Diabetes (IDDM) in Young Adult Women: A Disease Complication Often Ignored or Neglected

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Key words: type 1 diabetes, osteoporosis, bone mineral density, bone turnover markers, serum calcium, vitamin D, PTH, IGF-I

Objectives: This investigation on a homogenous cohort of young adult Caucasian type 1 diabetic (IDDM) patients (1) aimed at studying the occurrence of low bone mineral density (BMD) at an early stage prior to menopause (i.e., during the first decade after peak bone mass) and (2) elucidating the possible mechanisms underlying IDDM-induced bone complication.

Methods: Twenty-seven female patients with insulin-treated and well-controlled diabetes, without renal complications, and 32 well-matched healthy controls, aged between 30 and 40 years and fulfilling rigorous inclusion criteria to minimize bone-confounding factors, were enrolled. Areal BMD was evaluated by dual energy X-ray absorptiometry at axial (lumbar spine) and appendicular (femur) sites, using diagnostic WHO reference (T-scores). Osteoblast functions, bone metabolism, related key minerals, and 2 osteoclast-stimulating calciotropic hormones regulating their serum levels were assessed biochemically.

Results: The number of cases with low BMD (T-score below –1.1 SD) was almost 2-fold greater \((p < 0.01)\) in the IDDM group. BMD was significantly lower in this group for 3 lumbar sites \((p < 0.01)\) and femur Ward’s triangle \((p < 0.05)\). Bone formation was reduced, as evidenced by the suppressions of osteocalcin \((OC; p < 0.01)\) and IGF-I \((p < 0.001)\). However, bone alkaline phosphatase \((bALP)\) was induced \((p < 0.01)\), in contrast to what is usually observed in cases of reduced bone formation. Correlated total ALP activity was also significantly increased. There was no change in the specific marker of bone resorption \((\text{urinary deoxyypyridinoline})\). Serum calcium was significantly elevated, particularly after adjustment for albumin \((p < 0.001)\), despite lower \(25(OH)D_3\) \((p < 0.001)\) and no elevation of PTH. All significant bone-related biochemical changes were significantly correlated with glycosylated hemoglobin, a clinical indicator of long-term glycemic control, indicating a direct effect of the disease.

Conclusions: Bone loss in the IDDM group results from a decrease in bone formation rather than an increase of bone resorption. The induction of bALP is indicative of impaired osteoblast differentiation and maturation, which delayed (down-regulated) later stages of matrix mineralization, as evidenced by lower OC and BMD.

Address reprint requests to: Priscilla Massé, PhD, Department of Human Nutrition, University of Moncton, Moncton, NB, E1A 3E9 CANADA. E-mail: priscille.masse@umoncton.ca

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Abbreviations: 25(OH)D_3 = 25-hydroxycholecalciferol, 1,25(OH)_2D_3 = 1,25-dihydroxycholecalciferol, ACR = urinary albumin/creatinine ratio, ALP = alkaline phosphatase, bALP = bone-specific alkaline phosphatase, BMD = bone mineral density, BMI = body mass index, Dpd = free deoxypyridinoline, HbA1c = glycosylated hemoglobin, IGF-I = insulin-like growth factor I, iPTH = intact parathyroid hormone, OC = osteocalcin.


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INTRODUCTION

Bone studies on the effect of insulin-dependent type 1 diabetes mellitus (IDDM) in young adult women at a time in life following the attainment of peak bone mass are limited. In most cases, diabetic patients under investigation were already affected by complications of the disease or investigated at a time when peak bone mass was not attained (e.g., during childhood and adolescence). Although there seems to be a consensus in the literature on IDDM-induced low bone mineral density (BMD) [1,2], published results on the narrower age and gender spectrums of interest in the present study are conflicting. An increased incidence of low BMD has been associated with IDDM [3–6], but some investigators have not found significant reductions [7–9], including Rozadilla et al. [10], who studied young IDDM patients at an age of interest, that is, after adolescence, when peak bone mass was almost theoretically achieved. This controversy could be explained, among other factors, by the heterogeneity of the study populations (age, [pre]menopause, gender, mixed ethnicity, duration and metabolic control of the disease, i.e., presence of complications, type of diabetes) as revealed by a recent meta-analysis and literature review studies [1,2].

The fundamental feature of bone in IDDM patients seems to be the retardation of remodeling, primarily due to a reduced bone formation [11–13]. This outcome could derive from a decrease in the number and functions of osteoblasts (bone formation cells). Osteoblasts have receptors for insulin and insulin-like growth factor (IGF-I; insulin dependent), both of which stimulate the activity and replication of these cells [14]. Although the levels of insulin necessary for this effect are unknown, the below-normal concentrations at the preclinical stage of IDDM are likely inadequate. Data from Kemink et al. [12] suggested that low osteoblast-stimulating IGF-I may be responsible for osteopenia in IDDM and indicative of a cellular maturation defect and lowered bone formation. Reduced serum IGF-I values have been reported in poorly controlled patients [15]. According to Bouillon et al. [15], the most mature osteoblast marker, osteocalcin (OC), is decreased in all types of diabetes, irrespective of age. A reduction in the renal synthesis of 1,25-dihydroxycholecalciferol [1,25(OH)\(_2\)D\(_3\)] has been found in diabetic patients with poor glycemic control and a tendency for acidosis [16,17]. A decline in this vitamin hormone has a notable repercussion on bone metabolism and integrity.

Since the involvement of bone in diabetes is of ambiguous pathogenicity and factors involved are not well defined and remain controversial, bone studies must be designed to examine this tissue at both the cellular and matrix (collagen) levels and to analyze concomitantly serum mineral concentrations, their pertinent regulatory hormones, and related factors. Among these factors, iron is certainly of particular interest. In bone metabolism, iron has a double role as the cofactor in reactions of the hydroxylation of prolyl/lysyl amino acid residues of bone collagen and of the renal hydroxylation of 25-hydroxycholecalciferol [25(OH)\(_2\)D\(_3\)] to its active form [1,25(OH)\(_2\)D\(_3\)] [18]. Pair feeding an iron-deficient diet to rats, in a recent histomorphometric study, Katsumata et al. [19] found significant reductions in serum concentrations of 1,25(OH)\(_2\)D\(_3\), IGF-I, and OC.

The need to better understand the effects of IDDM on bone stems in part from the risk of osteoporosis and related fractures later in life. In a 12-year prospective study, Krakauer et al. [20] showed that radial bone loss continued at the expected rate in IDDM patients. But there is disagreement on this issue. From their prospective study, Kayath et al. [6] concluded that diabetic osteopenia, despite being a complication of high prevalence in IDDM, was nonprogressive in most patients after the attainment of peak bone mass.

The present study targeted young adult female IDDM patients during the first decade after the achievement of peak bone mass (age category 30–40 years). Its first objective was to evaluate the occurrence of bone loss at an early stage, prior to menopause. The second objective was to shed light on the underlying mechanisms by studying bone density and metabolism using densitometry combined with diverse specific biochemical markers that aimed at assessing osteoblast functions, bone turnover markers, key bone minerals, and 2 osteoclast-stimulating calciotropic hormones regulating their serum levels. The study protocol was designed to recruit rigorously IDDM patients and well-matched healthy controls to form a homogenous cohort of Caucasian women to minimize important bone confounders. Women were targeted because they are more at risk of osteoporosis than men because of high nutritional needs during pregnancy and lactation.

SUBJECTS AND METHODS

Caucasian women with insulin-treated and well-controlled type 1 diabetes, aged between 30 and 40 years, were referred by Diabetes Clinics located in New Brunswick, Canada. Age- and ethnicity-matched healthy women volunteered to participate as controls in response to public advertising. Eligibility criteria were specified. An initial screening of all respondents was conducted over the phone to exclude noneligible subjects, smokers and vegetarians. Only eumenorrheic women (non-pregnant, nonlactating, and not taking oral contraceptives) were eligible. The other criteria were to be nonobese (body mass index [BMI] <30 kg/m\(^2\)), without a history of bone fracture [21] or renal complication (in the case of diabetic subjects), and not using regularly nutritive supplement and medication (statins, corticosteroids, thiazide diuretics, anticonvulsive drugs, calcetionin, and diphosphonates) known to interfere with bone metabolism.
Women who met the inclusion criteria attended an initial information meeting and signed a consent form. The research protocol was approved by the Ethics Committee on Human Research of the Université de Moncton and by the review boards of hospitals involved in the study. Subjects who consented to enroll filled out a general questionnaire, which included items on socioeconomic and reproductive characteristics and medical history. Physical activity was estimated using the scores from Elders et al. [22].

**Anthropometric Measurements**

Height was determined using a wall-mounted stadiometer. Body weight was determined to within 100 g using a standard beam platform balance scale detector (Bionetics, St-Laurent, Québec, Canada). Subjects were weighed with indoor clothing without shoes. BMI was calculated from measured body weight (kg) and height (m) as weight/(height)$^2$. Body frame size was assessed by measuring wrist circumference [23]. Waist and hip circumferences (cm) were measured to calculate the waist-hip ratio [24], used as a general health status indicator.

**Evaluation of Self-selected Diets**

Subjects were asked to record their food intakes for 3 nonconsecutive days (including 1 weekend day) preceding blood collection. Written instructions were provided to all of them and explained by a registered dietitian to maximize completeness and accuracy of recording. Subjects were asked not to modify their regular food intakes while recording and to record all foods and beverages immediately after consumption.

Each dietary record was checked for completeness by the same dietitian in the presence of each subject. Daily energy and pertinent nutrient intakes were determined using Food Processor SQL® (version 9.5, 2004, Esha Research, Salem, OR). Estimated intakes of each nutrient were averaged ($n = 3 \text{ d}$) to compare the 2 groups of women and to assess the nutritional adequacy of their diets with respect to current dietary reference intakes [25]. Retrospective dietary intakes of calcium during periods of high calcium nutritional needs (adolescence, pregnancy, and lactation) and caffeine consumption were estimated using a frequency questionnaire based on the best food sources of calcium and most common caffeine-containing beverages consumed, respectively, assuming standard portions explained by the dietitian.

**Determination of BMD**

BMD of the anteroposterior lumbar spine and standard femoral sites was determined at the Georges-L.-Dumont Hospital by dual energy X-ray absorptiometry (DEXA; Lunar Corp., Madison, WI), after blood and urine collection. The instrument was calibrated daily according to the manufacturer’s instructions. All measurements were carried out on the same machine by the same operator, and the results were analyzed on the same software. Areal BMD (g/cm$^2$) was measured at diverse lumbar (L) and femoral sites (L1 to L4, L1–L2, L1–L3, L1–L4, L2–L3, L2–L4, L3–L4, femur neck, Ward’s triangle, trochanter, and diaphysis). The BMD of the femoral diaphysis, a predominantly cortical bone, was determined by scanning the upper shaft of the proximal femur below the intertrochanteric line. To take bone size into account [26], apparent volumetric density (BMAD, in g/cm$^3$) was calculated for vertebrae (L) using the following equation: $\text{BMAD} = \text{BMC}/(A_p \times w)$, where BMC is the bone mineral content, $A_p$ is the projected spinal area, and $w$ is the vertebral width [26]. Typical precision, expressed as coefficient of variation, with our DEXA method in normal subjects was less than 2% for all sites studied. Its accuracy (0.5%) was assessed by bone simulation phantoms. According to the definition established by World Health Organization (WHO) experts [27], bone loss is diagnosed when the BMD value is between 1.0 SD and 2.5 SD below the average value (T-score) of the peak bone mass of 30-year-old healthy adults, in the case of osteopenia, and when the T-score is less than $-2.5 \text{ SD}$ in the case of osteoporosis.

**Biological Sample Collection and Biochemical Analyses**

Antecubital venous blood and urine samples were collected from all subjects, in the morning and after an overnight fast, and sent immediately to routine hospital laboratories according to their procedures or kept frozen at $-70 \degree$C in our laboratory until analyses in cases of specialized tests. Blood used for serum analyses was allowed to clot for 1 hour at 37°C and then centrifuged at 2000 × g for 10 minutes at 4°C. The metabolic control of diabetes was evaluated by the determination of glycosylated hemoglobin (HbA1c), a clinical indicator of long-term glucose control. Kits from Stanbio Laboratory (Boerne, TX) were used to assay urinary albumin and creatinine. Their ratio (ACR) was calculated and used to assess renal status [28]. Blood hemoglobin level and serum concentrations of albumin, calcium, inorganic phosphorus, iron, magnesium, intact parathyroid hormone (iPTH), and total alkaline phosphatase (ALP) were determined by routine automated procedures. Values for serum calcium were adjusted for albumin according to Berry et al. [29].

Plasma OC and bone-specific ALP (bALP), used as markers of bone formation (to assess osteoblastic functions), were analyzed using kits from Quidel Corporation (Mountain View, CA). The osteoblast-stimulating insulin growth factor (IGF-I) in circulation was also determined, using the method of Donovan et al. [30]. Free deoxypyridinoline (Dpd), a collagen end-metabolite commonly used as a marker of bone resorption, was measured on nonhydrolyzed urine samples using a competitive enzyme immunoassay (Metra Dpd, Quidel Corporation, Mountain View, CA). Values were corrected for the urinary concentration of creatinine in the sample. Plasma
Type 1 Diabetes–Induced Bone Complication

Table 1. Descriptive Characteristics of Subject Groups Pertinent to General Health and Nutritional Protein Status in Fulfillment of Selection Criteria

<table>
<thead>
<tr>
<th></th>
<th>Reference Values$^2$</th>
<th>Control Group</th>
<th>Diabetic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>—</td>
<td>34.8 ± 4.1</td>
<td>36.9 ± 3.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>—</td>
<td>68.3 ± 8.3</td>
<td>68.4 ± 12.6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>—</td>
<td>161 ± 6</td>
<td>162 ± 5</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>&lt;30$^3$</td>
<td>24.3 ± 2.6</td>
<td>26.1 ± 4.2</td>
</tr>
<tr>
<td>Waist–hip ratio, cm/cm</td>
<td>&lt;0.84$^4$</td>
<td>0.78 ± 0.05</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td>Wrist circumference (cm)</td>
<td>—</td>
<td>15.2 ± 0.9</td>
<td>15.6 ± 1.2</td>
</tr>
<tr>
<td>Body frame size</td>
<td>9.9–10.9$^4$</td>
<td>10.6 ± 0.6</td>
<td>10.4 ± 0.7</td>
</tr>
<tr>
<td>Diabetes duration, y</td>
<td>—</td>
<td>—</td>
<td>19.8 ± 9.0</td>
</tr>
<tr>
<td>Glycosylated hemoglobin, %</td>
<td>&lt;7.5</td>
<td>5.5 ± 1.6</td>
<td>7.8 ± 1.4$^{***}$</td>
</tr>
<tr>
<td>Hemoglobin, g/100 mL</td>
<td>&gt;11.7</td>
<td>13.4 ± 1.2</td>
<td>13.6 ± 1.0</td>
</tr>
<tr>
<td>Serum albumin, g/100 mL</td>
<td>&gt;3.5</td>
<td>4.39 ± 0.27</td>
<td>4.25 ± 0.27</td>
</tr>
<tr>
<td>Urinary albumin, mg/L</td>
<td>—</td>
<td>37.5 ± 4.0</td>
<td>37.6 ± 5.1</td>
</tr>
<tr>
<td>Urinary creatinine, mmol/L</td>
<td>—</td>
<td>9.71 ± 6.03</td>
<td>8.61 ± 7.33</td>
</tr>
<tr>
<td>Urinary albumin/creatinine, mg/mmol</td>
<td>&lt;2.8$^5$</td>
<td>0.46–1.16</td>
<td>0.51–1.40</td>
</tr>
<tr>
<td>Menarche, y</td>
<td>—</td>
<td>13.0 ± 1.4</td>
<td>13.4 ± 1.7</td>
</tr>
<tr>
<td>Number of full-term pregnancies</td>
<td>—</td>
<td>1.0 ± 1.0</td>
<td>1.1 ± 0.9</td>
</tr>
<tr>
<td>Number of years since last pregnancy</td>
<td>—</td>
<td>6.3 ± 4.3</td>
<td>9.4 ± 6.3</td>
</tr>
<tr>
<td>Caffeine intake, mg/d</td>
<td>—</td>
<td>96–261</td>
<td>163–377</td>
</tr>
<tr>
<td>Physical activity (score)$^6$</td>
<td>—</td>
<td>10.8 ± 2.8</td>
<td>9.3 ± 3.0</td>
</tr>
</tbody>
</table>

$^1$ Mean ± standard deviation, except for urinary albumin/creatinine ratio and caffeine intake (95% confidence intervals).

$^2$ Reference values from our laboratories, except where otherwise specified.

$^3$ Gibson, 2005 [24].

$^4$ Range values for medium body frame size (Lee and Nieman, 2007 [23]).

$^5$ Canadian Diabetes Association, 2008 [28].

$^6$ Elders et al., 1989 [22].

**** $p < 0.0001$.

25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ concentrations were measured by a radioimmunoassay (ImmunoDiagnostic Systems, Boldon, UK) and compared with reference values [31,32]. Iron, involved as the cofactor in the hydroxylation of 25(OH)D$_3$ to 1,25(OH)$_2$D$_3$, was determined using an automated colorimetric technique (Vitros Fusion 5.1 Microslides, Ortho-Clinical Diagnostics, Rochester, NY).

The interassay coefficient of variation for all assays was less than 8%.

Statistical Analyses

Data were analyzed using InStat (version 2.0, 1998, GraphPad Software, San Diego, CA). Descriptive statistics were calculated, and normality was assessed. Variables were analyzed accordingly, using Student 2-tailed unpaired t test or nonparametric Mann-Whitney test. Relationships between pertinent biochemical variables were analyzed using Pearson or Spearman correlation coefficient ($r$) as appropriate. A value of $p \leq 0.05$ was considered significant.

RESULTS

Among the 50 healthy women who responded to public advertisings and the lists of IDDM patients who were referred by the Diabetes Clinics, 32 and 27 subjects, respectively, met all eligibility criteria and completed the study. The mean age of each group, 34.8 and 36.9 years, respectively, was comparable. Using HbA$_1c$ to confirm the diagnosis of diabetes and its metabolic control revealed that the only marked difference ($p < 0.0001$) between the 2 groups was the presence of this disease, as expected (Table 1). IDDM was well controlled according to this clinical indicator (only slightly above the reference value of 7.5%), a prerequisite to participate in the study. HbA$_1c$ values below 6.0% are found in healthy, euglycemic persons and may be difficult to achieve for persons with diabetes, even with intense insulin therapy. Diabetic patients were diagnosed with IDDM since 19.8 years on average. They were otherwise healthy, another inclusion criteria, with normal serum albumin and iron concentrations, and apparently without renal complications (as assessed by ACR) and no diagnosis of anemia (a sign of a more advanced stage of the disease and presence of irregular menstruations) as assessed by hemoglobin level. The 2 groups were also comparable for age at menarche, parity, and physical activity (Table 1), as well as ethnicity, geographic region (latitude 46$^\circ$N), and socioeconomic background (data not shown). Height, body weight, and frame did not differ significantly.

None of the differences between groups in terms of dietary intakes of interest were significant, including proline and lysine, the 2 specific amino acids of the helical collagen
polypeptide chains (Table 2). Energy intakes were below the estimated energy requirement for their age category, and consequently, both groups did not meet their nutritional needs for several nutrients, including those crucial for bone health, such as calcium, vitamin D, and magnesium.

Fig. 1 shows the occurrence of combined cases of osteopenia/osteoporosis (based on T-scores) in both groups. Depending on bone site, the proportions varied markedly from 9%–45% in the diabetic group, compared with 4%–25% in healthy controls. As shown in Fig. 1, the proportion of cases was greater in the diabetic group for all sites, except for L2–L4 and L3–L4 for unknown reasons. When averaging all sites, the number of cases (%) was almost 2-fold greater ($p < 0.01$) in the diabetic group (23.4 vs. 12.1; 95% confidence intervals: 16.1–30.9 vs. 8.4–15.9). As shown in Fig. 2, the BMD of all sites was lower in this group, and differences between groups reached a significance level for 3 lumbar sites (L1, L2, and L1–L2; $p < 0.01$) and femur Ward’s triangle ($p < 0.05$). IDDM patients were 4 times more likely ($p < 0.01$) to be osteopenic/osteoporotic (based on T-scores) at these apparently most sensitive sites as compared with healthy controls. It is also worth noting that significant differences in BMD at lumbar sites L1 and L1–L2 represent the least dense vertebra (L1), as described by Mylonakis et al. [33].

No significant difference between groups was found for femur diaphysis, predominantly cortical bone. Volumetric BMAD (data not shown), which take into consideration the size of vertebrae, showed similar results but did not add more precision (no change in significance level) to the areal measurements, in contrast to the findings of a previous study on menopausal women [34] and the findings of Jergas et al. [35].

A striking biochemical finding, directly related to bone metabolism, was observed in the diabetic group, that is, the alteration in serum calcium homeostasis occurring independently of the effect of the major calcitropic hormones, 1,25(OH)$_2$D$_3$ and PTH (Table 3). In effect, the elevation of serum calcium, highly significant after adjusting for albumin ($p < 0.001$), occurred despite a significantly lower plasma 1,25(OH)$_2$D$_3$ ($p < 0.001$) and no elevation of serum PTH which was found to be inversely correlated with serum calcium ($p < 0.001$) and with HbA$_1$C ($p < 0.01$; Table 4). Hepatic precursor 25(OH)D$_3$ was unchanged and above the cutoff of 50 nmol/L established by Calvo and Whiting [31], indicating adequate nutritional vitamin D status despite low dietary intakes (Table 2). The reduction in plasma 1,25(OH)$_2$D$_3$

### Table 2. Average Usual Intakes (n = 3 d) of Energy and Nutrients Pertinent to the Study$^{1,2}$

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>DRI$^3$</th>
<th>Control Group</th>
<th>Diabetic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>2400</td>
<td>1950 ± 480</td>
<td>1798 ± 427</td>
</tr>
<tr>
<td>Protein, g</td>
<td>46</td>
<td>79.2 ± 18.6</td>
<td>79.2 ± 15.4</td>
</tr>
<tr>
<td>Lysine, g</td>
<td>2.6$^4$</td>
<td>2.3 ± 1.4</td>
<td>2.9 ± 1.4</td>
</tr>
<tr>
<td>Proline, g</td>
<td>—</td>
<td>2.2 ± 1.2</td>
<td>2.4 ± 1.1</td>
</tr>
<tr>
<td>Vitamin D, µg</td>
<td>5</td>
<td>2.1 ± 2.8</td>
<td>1.8 ± 2.3</td>
</tr>
<tr>
<td>Vitamin B$_6$, mg</td>
<td>1.3</td>
<td>1.2 ± 0.7</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>75</td>
<td>87 ± 68</td>
<td>114 ± 88</td>
</tr>
<tr>
<td>Vitamin A, µg RAE</td>
<td>700</td>
<td>485 ± 413</td>
<td>577 ± 249</td>
</tr>
<tr>
<td>Vitamin K, µg</td>
<td>90</td>
<td>21.1 ± 19</td>
<td>16.5 ± 10.1</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>320</td>
<td>178 ± 93</td>
<td>169 ± 60</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>1000</td>
<td>883 ± 328</td>
<td>830 ± 338</td>
</tr>
<tr>
<td>Past intakes$^5$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation</td>
<td>1000</td>
<td>762 ± 183</td>
<td>633 ± 204</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>1000</td>
<td>731 ± 311</td>
<td>608 ± 167</td>
</tr>
<tr>
<td>Adolescence</td>
<td>1300</td>
<td>595 ± 260</td>
<td>566 ± 250</td>
</tr>
<tr>
<td>Phosphorus, mg</td>
<td>700</td>
<td>769 ± 306</td>
<td>756 ± 279</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>18</td>
<td>13.3 ± 4.3</td>
<td>12.5 ± 4.9</td>
</tr>
<tr>
<td>Manganese, mg</td>
<td>1.8</td>
<td>1.9 ± 0.9</td>
<td>2 ± 1.3</td>
</tr>
<tr>
<td>Copper, µg</td>
<td>900</td>
<td>804 ± 575</td>
<td>762 ± 345</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>8</td>
<td>6.7 ± 3.1</td>
<td>5.8 ± 2.8</td>
</tr>
</tbody>
</table>

$^1$ Mean ± standard deviation. RAE, retinol activity equivalent.

$^2$ None of the differences between groups were significant.

$^3$ Dietary reference intakes (Food and Nutrition Board, Institute of Medicine, 2006 [25]).

$^4$ On the basis of 38 mg/kg of body weight.

$^5$ From frequency questionnaire.

![Fig. 1.](image-url) Number of cases (%) of low bone mineral density (T-score < -1.1) in healthy controls and in the diabetic group for the 10 lumbar and 3 proximal femur sites under investigation. When averaged over all sites, the proportion for each group differed significantly ($p < 0.01$). L = lumbar vertebrae, Neck = femur neck, Ward’s = femur Ward’s triangle, Troch = femur trochanter.
triggered an examination of possible explanatory factors. Iron, a cofactor in the hydroxylation of 25(OH)D$_3$ to 1,25(OH)$_2$D$_3$ in the kidneys, was not deficient, although its serum concentration was significantly reduced ($p < 0.05$) in the diabetic group (Table 3). As expected, a positive association was found between 1,25(OH)$_2$D$_3$ and serum iron, but it was not significant (Table 4). Reduction in the synthesis of this hormone did not seem to result from defective renal function either, since ACR was similar in both groups and well below the threshold used to assess normal renal functioning [28]. In addition, the correlation between 1,25(OH)$_2$D$_3$ and ACR was not significant (Table 4).

Fig. 3 depicts data relative to biomarkers of osteoblast functions and bone turnover. It shows, first of all, another important biochemical finding: bone loss associated with type 1 diabetes was not the result of bone resorption (enhanced osteoclastic activity), as evidenced by no significant increase in urinary Dpd excretion (Fig. 3C). This finding is in line with less osteoclastic activity, evidenced in the IDDM group by the significant reduction of 1,25(OH)$_2$D$_3$ and with that of PTH (although not significant), the 2 major calcitropic hormones having osteoclastic activity (Table 3). However, there was less bone formation, as it usually occurs when activity and/or functions of osteoblasts are reduced. This cellular impairment was confirmed by significant suppressions in OC ($p < 0.01$; Fig. 3B) and IGF-I ($p < 0.001$; Table 3). In contrast, the expression of bALP (Fig. 3A), another protein by-product of osteoblasts, normally reduced when there is less bone formation (at least in nondiabetic subjects), was induced ($p < 0.01$) in the diabetic group. This finding makes sense considering that total ALP activity (from all cells, i.e., from liver, intestine, and bone) was also significantly increased (Table 3). The correlation between total ALP and bALP was highly significant ($p < 0.001$; Table 4). No significant correlation was found between this ALP isoenzyme and serum calcium. Circulating IGF-I levels were not significantly correlated with the two bone formation markers.

Fig. 2. Bone mineral density at the spine and femur in healthy controls and the diabetic group. Mean ± standard deviation. L = lumbar vertebrae, Neck = femur neck, Ward’s = femur Ward’s triangle, Troch = femur trochanter, Dia = femur diaphysis. ** $p < 0.01$, * $p < 0.05$.

Table 3. Biochemical Data Relevant to Bone Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Reference Values</th>
<th>Control Group</th>
<th>Diabetic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium, mmol/L</td>
<td>2.02–2.60</td>
<td>2.27 ± 0.07</td>
<td>2.32 ± 0.09*</td>
</tr>
<tr>
<td>Serum adjusted calcium, mmol/L</td>
<td>—</td>
<td>2.32 ± 0.07</td>
<td>2.40 ± 0.80***</td>
</tr>
<tr>
<td>Serum phosphorus, mmol/L</td>
<td>0.9–1.81</td>
<td>1.15 ± 0.16</td>
<td>1.07 ± 0.16</td>
</tr>
<tr>
<td>Serum iron, μmol/L</td>
<td>&gt;5</td>
<td>21.6 ± 7.8</td>
<td>17.1 ± 6.6**</td>
</tr>
<tr>
<td>Serum iPTH, pmol/L</td>
<td>1.5–7.6</td>
<td>4.66 ± 1.85</td>
<td>4.00 ± 1.88</td>
</tr>
<tr>
<td>Plasma 25(OH)D$_3$, nmol/L</td>
<td>&gt;50$^4$</td>
<td>78.6 ± 30.2</td>
<td>72.8 ± 26.6</td>
</tr>
<tr>
<td>Plasma 1,25(OH)$_2$D$_3$, pmol/L</td>
<td>&gt;70$^5$</td>
<td>132 ± 48</td>
<td>94 ± 23***</td>
</tr>
<tr>
<td>Plasma IGF-I, μg/L</td>
<td>&gt;70$^6$</td>
<td>120 ± 47</td>
<td>79 ± 31***</td>
</tr>
<tr>
<td>Serum total ALP, U/L</td>
<td>37–112</td>
<td>64.0 ± 9.3</td>
<td>74.6 ± 18.8***</td>
</tr>
</tbody>
</table>

1 Mean ± standard deviation. iPTH = intact parathyroid hormone, IGF-I = insulin-like growth factor-I, ALP = alkaline phosphatase.
2 Reference values from our laboratories, except where otherwise specified.
3 Adjusted for albumin according to Berry et al., 1973 [29].
4 Calvo and Whiting, 2003 [31].
5 Konradsen et al., 2008 [32].
6 Donovan et al., 1991 [30].
*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$.
Interestingly, all significant biochemical changes found in the diabetic group that were directly related to bone metabolism were significantly correlated with HbA1c, indicating a direct effect of the disease.

**DISCUSSION**

**BMD and Occurrence of Low BMD**

The present study showed, first, that the IDDM group had significantly lower BMD at the spine, particularly at the least dense vertebra [33], and femur Ward’s triangle. The occurrence of osteopenia/osteoporosis was almost double among subjects with diabetes. Despite its small sample size, this study has reached the same conclusion as heterogeneous larger studies because of its rigorous design in terms of subjects’ recruitment and consequent homogeneity within and between groups, except for the presence of the disease. This investigation of a well-defined cohort of IDDM women with diabetes and healthy controls is of particular interest because of its focus on a specific and significant time point in the life cycle, that is, the first decade following peak bone mass. Participants were eumenorrheic and not taking estrogen (oral contraceptives); they were not too young to ensure that peak bone mass had been reached and not premenopausal to avoid irregular menstrual cycle. The study was also designed to minimize other important BMD confounders such as body frame and weight, physical activity, and parity. The disease in the patient group was well monitored at the time of the study, without known renal complication, as self-reported and documented on the basis of ACR. There was no indication of anemia (a sign of an advanced stage of the disease) in both groups; protein intakes and nutritional status were adequate.

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**Table 4. Pertinent Correlations between Blood Biochemical Data in the Diabetic Group**

<table>
<thead>
<tr>
<th></th>
<th>HbA1c</th>
<th>IGF-I</th>
<th>bALP</th>
<th>Calcium</th>
<th>1,25(OH)2D3</th>
<th>PTH</th>
<th>ACR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td>-0.3989**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ALP</td>
<td>+0.5038**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bALP</td>
<td>+0.3784**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>-0.4214**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>-0.5693**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iPTH</td>
<td>-0.4683**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>+0.3962**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>-0.2972**</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values are correlation coefficients (r). HbA1c = glycosylated hemoglobin, IGF-I = insulin-like growth factor-I, bALP = bone-specific alkaline phosphatase (ALP), Calcium = serum calcium adjusted for albumin, iPTH = intact parathyroid hormone, ACR = urinary albumin/creatinine ratio.

*** p < 0.001.
** p < 0.01.
* p < 0.05.

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**Fig. 3.** Biomarkers of osteoblast functions and bone turnover in healthy controls and the diabetic group: (A) osteoblast functions, assessed by plasma bone alkaline phosphatase (bALP); (B) bone formation, assessed by plasma concentration of osteocalcin; and (C) bone resorption, assessed by urinary excretion of deoxypyridinoline (Dpd). The reference range for each biomarker is indicated by the shaded area. Mean ± standard deviation. ** p < 0.01.
Regarding the prevalence of bone complication in IDDM, contradictory data have been reported in the literature, depending on the criteria used, age, gender, and clinical profile of patients. The number of cases (31%) with low BMD (osteopenia and osteoporosis combined) in the present study was lower than that reported by Kayath et al. [6]. Their cohort was heterogeneous (as is often the case in bone studies) and included young black and white people, of both genders, who did not have full closure of long bone epiphyses and had not reached their peak bone mass. The age of their female patients varied widely (21–55 years) as did their hormonal status: some were near or at menopause (estrogen deficiency) or on oral (estrogen) contraceptives. In the study of Kemink et al. [12], a high proportion (57%) of diabetic women were diagnosed with osteopenia. In the study by Munoz-Torres et al. [36] composed of 97 female and male IDDM patients with complications and risk factors of osteoporosis, and whose age varied between 20 and 56 years, 20% of all diabetic subjects met the WHO diagnostic criteria for osteoporosis.

**Cellular Development and Functions of Osteoblasts**

Although there seems to be a consensus on the negative effect of IDDM on BMD, human studies on the coexistence of potential mechanism(s) involving both cellular and circulating levels are limited and conflicting. Serum total ALP, whose activity includes bone and other cells, namely, from the liver and intestine, is often increased in bone disorders. It was found to be elevated in the present study, as shown by other investigators [8,37]. With hepatic and renal functions being similar in both groups, based on blood and urine tests, we could presume that this observation referred to a bone origin. As reported by Kemink et al. [12], bALP in the present study was closely correlated with total ALP.

The significant coupled reductions of OC and IGF-I observed in our study strongly confirmed a cellular bone abnormality in IDDM patients. The reduction in IGF-I, an important factor responsible for the final maturation of osteoblasts, is in agreement with other reports [7,12,15,38]. Chronic hyperglycemia may indeed alter osteoblast differentiation and maturation [39,40]. Using a diabetic rat model, Verhaeghe et al. [41] found that osteoblast activity was decreased.

Bone-specific ALP, also synthesized by osteoblasts, was found to be significantly elevated in the IDDM group. Bouillon et al. [15], on the other hand, reported normal bALP levels in adult patients with IDDM. The imbalance of bone formation markers (increased ALP and decreased OC) observed in the present study is consistent with Botolin and McCabe’s [42] findings from their animal model of diabetes. These investigators demonstrated that the osteoblast response to chronic hyperglycemia was expressed by an induction of ALP and suppression of OC. This imbalance appears to be characteristic of the bone complication of IDDM and reflects an impairment in osteoblast differentiation and maturation. During development, osteoblasts undergo a series of differentiation stages, such as proliferation, matrix development, and matrix mineralization. bALP activity is expressed early in the development of osteoblasts, reaches a maximum during the phase of matrix maturation, and is down-regulated during late stages of matrix mineralization [43]. In their *in vitro* study, Balint et al. [44] observed a significantly higher level of ALP in cells incubated in the presence of high glucose concentration. At the end of the experimental period, the total amount of calcium uptake into the glucose-treated cells was almost half of control cell cultures; in other words, it took significantly longer to deposit the same amount of calcium.

In the present study, a defective collagen network was ruled out on the basis of adequate plasma supply of vitamin B<sub>6</sub>, a coenzyme in extracellular matrix maturation (data not shown). However, there was certainly inefficiency of the bone matrix to mineralize as a result of incompetent osteoblasts to synthesize OC and collagen involved in the process, as shown by an experimental animal model [39]. Using the SRXRF micro-probe technique and a rat model of diabetic osteoporosis, Fei et al. [45] demonstrated that calcium content in bone (femur) was reduced. These findings are in accordance with bone densitometry studies in IDDM patients and the *in vitro* study by Balint et al. [44].

**Biomarkers of Bone Turnover**

Although Miazowski and Czekalski [13] failed to demonstrate that diabetic osteopenia was influenced by the duration of the disease or its metabolic control, poor glycemic control has metabolic effects, such as acidosis and hypercalciuria, that can lead to bone resorption, according to Bouillon [16]. In the present study, the IDDM group did not exhibit accrued bone resorption compared with healthy controls; urinary Dpd excretion, used as the specific marker of bone resorption, was not significantly greater. There are only a few reports on pyridinoline and Dpd in IDDM; these collagen cross-links are formed during the final stage of extracellular collagen maturation. Their measurements in urine have been shown to provide the most valid clinical markers of collagen degradation [46]. Since Dpd has a much more restricted tissue distribution and is primarily located in bone collagen, it seems to be a more specific and sensitive marker of bone resorption than pyridinoline [46].

First, the lack of biochemical evidence of bone resorption in our IDDM patient group confirmed good glycemic control (HbA<sub>1c</sub> close to 7.5%), a selection criterion specified in the study design. Second, in agreement with animal models [39,47] and human studies [11–13], this finding confirmed that IDDM-induced bone loss results from a defect in formation in contrast to menopausal bone loss, characterized
Serum Calcium Homeostasis and Vitamin D Metabolism

A biochemical abnormality characterizing IDDM-induced bone disorder in the present study was the highly significant elevation of serum calcium concentration, particularly after adjustment for albumin. This alteration in calcium homeostasis seems to be contradicting in a metabolic context of lower intestinal calcium absorption due to renal reduction of 1,25(OH)₂D₃. Furthermore, plasma concentration of the hepatic precursor [25(OH)D₃], used as an indicator of vitamin D nutritional status, and current and past dietary calcium intakes during adolescence, pregnancy, and lactation were similar in both groups. Serum iron, which may have contributed to the reduction of 1,25(OH)₂D₃ owing to its role as a cofactor in the hydroxylation of 25(OH)D₃, was significantly reduced in the IDDM group. However, the absence of a significant correlation between these 2 parameters makes this explanation unlikely. This finding should not be interpreted to mean that the association between IDDM and iron is not relevant. Quite the contrary, in severe iron deficiency, not only does 1,25(OH)₂D₃ concentration decrease, but there is also less bone formation through reductions of OC and IGF-I [49].

Serum calcium concentration is closely controlled by hormones, and an elevation would inhibit PTH release, perhaps explaining why serum PTH level was lower (although not significantly) in our IDDM group. However, the inverse correlation was found to be significant. A state of functional hypoparathyroidism has been described in patients with IDDM [50]. This could be another indirect skeletal sequela of the disease. In the oral glucose load study on healthy women by D'Erasmo et al. [51], PTH was suppressed. Balint et al. [44] postulated, from their in vitro model of the inhibitory effect of glucose on osteoblastic calcium deposition, that improvement of osteoblast mineralization function could lower serum calcium concentration, and thus ameliorate the renal hypercalcuria status found in IDDM patients with poor glycemic control.

In brief, the present study highlighted the persistence and magnitude of IDDM-induced bone disorder despite good glycemic control and apparent absence of renal complications. Both lumbar vertebrae and femur were affected. The underlying pathogenic mechanisms that were evidenced are as follows: (1) low bone formation due to diminished activity/functions of osteoblasts and uncoupled bone formation and resorption and (2) independent alterations in serum calcium homeostasis and vitamin D metabolism. The interpretation of bALP as a marker of bone formation in investigations on IDDM-induced bone complication warrants caution. Other rigorous large studies are needed to address this issue. Although the complete picture of the pathogenesis of bone disease in IDDM has not been revealed, the present study identified a real need to examine and follow up diabetic patients for the risk of osteoporosis and related fractures at an early stage. It also underlined the importance of a well-designed protocol that will allow a minimum of confounding variables and include the clinical profile combined with nutritional status evaluation in bone investigations.

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