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Lower Bone Mineral Content in Children with Type 1 Diabetes Mellitus Is Linked to Female Sex, Low Insulin-Like Growth Factor Type I Levels, and High Insulin Requirement

Juliane Léger, Daniela Marinovic, Corinne Alberti, Sophie Dorgeret, Didier Chevenne, Claire Lévy Marchal, Nadia Tubiana-Rufi, Guy Sebag, and Paul Czernichow

Department of Pediatric Endocrinology and Diabetology and Institut National de la Santé et de la Recherche Médicale Unit 690 (J.L., D.M., C.L.M., N.T.-R., P.C.) and Departments of Biostatistics (C.A.), Radiology (S.D., G.S.), and Biochemistry (D.C.), Assistance Publique-Hôpitaux de Paris, University Paris VII, Hôpital Robert Debré, 75019 Paris, France

Context: Studies on bone mineral characteristics in children with type 1 diabetes mellitus (T1DM) have generated conflicting results.

Objective: Our objective was to investigate bone mineral characteristics in children with T1DM and to analyze their associations with bone metabolism and the IGF-I system.

Design: We recruited a cohort of Caucasian patients with T1DM for at least 3 yr and healthy children between January 2003 and June 2004.

Setting: This was a university hospital-based study.

Participants: A total of 127 patients and 319 controls aged 6 to 20 yr participated.

Methods: Dual-energy x-ray absorptiometry was performed in patients and controls. Serum bone alkaline phosphatase, CrossLaps, IGF-I, and IGF-binding protein 3 levels were determined in patients with values analyzed using our normative data from 1150 healthy children.

Results: After adjustment for age, sex, pubertal stage, and body mass index SD score, total body bone mineral content (BMC)/lean body mass was significantly lower in patients than in controls ($P < 0.04$). This difference was a result of the differences between the girls of the two groups. Girls with T1DM had significantly lower lumbar spine and total body BMC than control girls ($P = 0.002$), whereas no such difference was observed in boys. Serum bone alkaline phosphatase level was significantly lower in girls than in boys ($P = 0.04$). Low serum IGF-I levels and the administration of large amounts of insulin were found to have independent deleterious effects on BMC for children of all ages and both sexes, whereas disease duration and glycosylated hemoglobin levels did not.

Conclusions: A sex-related difference in the impairment of bone mineral characteristics was identified in children with T1DM. Longitudinal studies are required to investigate whether boys may gain slightly less bone mass during skeletal growth. **(*J Clin Endocrinol Metab* 91: 3947–3953, 2006)**

MANY STUDIES HAVE shown that children with type 1 (insulin-dependent) diabetes mellitus (T1DM) are at risk of having a decrease in bone mass (1–13). However, there is still some debate about the impact of diabetes on bone mass during childhood, because other studies found no bone mineralization abnormalities in diabetic children (14–19). It has been suggested that poor metabolic control has a negative effect on bone mineral characteristics and acquisition (5, 7, 11–13), but the relative importance of disease duration, insulin regimen, and metabolic control on bone mineral and bone metabolism remains unclear (20, 21). In adult patients, it has been postulated that the low serum IGF-I levels observed in patients with T1DM may be one of the pathogenic factors responsible for reduced bone mineral density (BMD) (22). However, the relationships between bone mineral characteristics, biochemical parameters of bone metabolism, and the IGF-I system have not been thoroughly investigated in children with T1DM. Previous studies have been limited by the small number of subjects and the restricted range of ages, from adolescence to young adulthood. Therefore, the purpose of this study was to further characterize bone turnover and bone mineral and body composition in a large group of children with T1DM.

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Abbreviations: BMAD, Apparent volumetric bone mineral density; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; DEXA, dual-energy x-ray absorptiometry; HbA1c, glycosylated hemoglobin; IGFBP3, IGF-binding protein 3; LBM, lean body mass; LS, lumbar spine; SDS, SD score(s); TB, total body; T1DM, type 1 (insulin-dependent) diabetes mellitus.

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Subjects and Methods

For this cross-sectional study, we consecutively enrolled, over an 18-month period, 127 children (73 boys and 54 girls) with T1DM (medical history consistent with T1DM and presence of a type 1 diabetes-associated autoantibody) who had been followed up in our department. Patients had to satisfy the following criteria to be eligible for the study: 6–20 yr old, diabetic for at least 3 yr, and of Caucasian origin. The exclusion criteria were secondary or genetic types of diabetes mellitus, type 2 diabetes mellitus, another simultaneous treatment or chronic disease such as thyroid, celiac, renal (all our patients were yearly screened for thyroid function and antibodies, presence of anti-gliadine IgA antibodies, and renal function), liver, or cardiac disease, and genetic

syndromes or pregnancy (n = 35). Patients were treated with two daily injections of a mixture of short- and long-acting insulin (n = 99), three preprandial injections of short-acting analog insulin, and long-acting analog insulin given at bedtime (n = 24), or an insulin pump (n = 4). Only one patient displayed persistent microalbuminuria in two consecutive overnight urine collections in the preceding year.

The study population was representative of the entire eligible diabetic population followed in our department, as shown by the median chronological age [12.9 (10.2–15.2) yr] and duration of diabetes [5.6 (4.6–8.8) yr] of the 91 (49 males) nonparticipating patients. Patients were compared with a large group of healthy Caucasian children (n = 319) aged 6–20 yr, with no history of chronic disease or any current disease or drug therapy and who were investigated for bone mineral and body composition measurements during the same period at Robert Debré Hospital.

All subjects (patients and controls) underwent bone mineral and body composition assessments, and their age, weight, height, pubertal status, calcium intake, and physical activity were also recorded. Bone age, insulin dose (units of insulin per kilogram body weight per day), biochemical markers of calcium metabolism and bone turnover, and serum IGF-I, IGF-binding protein 3 (IGFBP3), and glycosylated hemoglobin (HbA1c) concentrations were determined in patients. The biochemical markers of bone turnover used were serum bone alkaline phosphatase levels (a marker of bone formation) and serum CrossLaps levels (a marker of bone resorption). HbA1c values over the last 2 yr were collected from clinical records of each patient (three to four determinations per year), and a yearly average was calculated. No blood samples were taken from the controls, for whom only bone characteristics and body composition measurements were analyzed.

The study protocol was reviewed and approved by the faculty ethics committee. It was explained to all subjects and their parents, who signed a written consent form for participation.

Clinical assessment

Height and weight were expressed as an SD score (SDS) for sex and chronological age (23). We also calculated body mass index (BMI) (kg/m^2 = weight/height^2) in SDS for sex and chronological age (24). Pubertal development was assessed according to Tanner stage (25). Bone age was determined under blind conditions by a single investigator (J.L.) according to the method of Greulich and Pyle (26).

Questionnaires

Questionnaires were used to determine current dietary calcium intake and physical activity. Dietary calcium intake (mg/d) was assessed by means of a semiquantitative food frequency questionnaire (27). Weekly physical activity was determined according to three categories: no sport at all (A); physical education classes only, with an average of 3 h/wk (B); and physical education classes and organized extracurricular sports (C).

BMD measurements

Bone mineral content (BMC) and BMD (BMC divided by bone area) measurements of lumbar spine (LS) (L2–L4) and total body (TB) were obtained by dual-energy x-ray absorptiometry (DEXA, GE Lunar Prodigy Corp., Madison, WI). We corrected for bone size by calculating the apparent volumetric BMD (BMAD) of the LS with the BMAD LS = BMC/A$\sqrt{}$A (volume = A$\sqrt{}$A, in which A = area) model (28). TB DEXA was also used to estimate body composition as lean body mass (LBM) and fat tissue mass (in grams) and percent body fat mass. As recommended for the interpretation of DEXA in children, TB BMC for LBM (BMC/LBM) and LBM for height were calculated (29). The results were compared with those from our control population. SDS were calculated for bone mineral characteristics (LS and TB), based on values from a reference pediatric population provided by the manufacturer (GE Lunar) (30). The coefficient of variation (CV) was 1% for L2–L4 BMD and 0.64% for TB BMD. The CV was 1% for lean tissue mass, 1.2% for fat mass, and 4% for percent fat mass.

Biochemical parameters

Blood samples were obtained from all patients after an overnight fast for the assessment of calcium, phosphorus, magnesium, alkaline phosphatase, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, PTH, bone alkaline phosphatase, CrossLaps, IGF-I, and IGFBP3 levels. Urine samples were collected in the morning, in fasting conditions, after discarding the first void, for the measurement of urinary calcium and creatinine levels. Samples were stored at −20 C until assayed.

Plasma and urine creatinine, calcium, phosphorus, magnesium, and alkaline phosphatase concentrations were determined with an ADVIA analyzer (Bayer Diagnostics, Puteaux, France). HbA1c was determined by HPLC (VARIANT; Bio-Rad, Marnes-la-Coquette, France), with an interassay CV of less than 5.8%. Serum IGF-I and IGFBP3 concentrations were determined by fully automated two-site chemiluminescence immunoassays (Nichols Advantage; Nichols Institute Diagnostics, Paris, France), with interassay CV of less than 6.4 and 10%, respectively. Serum bone alkaline phosphatase and PTH concentrations were determined by radioimmunometric assays (Tandem-R Ostase from Beckman Coulter, Roissy, France, and IRMA intact PTH from Nichols Institute Diagnostics), with interassay CV of less than 9.2% and less than 10%, respectively. The serum CrossLaps assay is an enzyme-linked immunoassay (serum CrossLaps ELISA; Nordic Bioscience Diagnostics A/S, Herlev, Denmark) specific for a b-aspartate form of the EKAHD-b-GGR epitope derived from the cross-linked degradation products of C-terminal telopeptides of type I collagen (31). The interassay CV was less than 5%. Serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations were determined by RIAs (γ-B 25-hydroxy vitamin D RIA and γ-B 1,25-hydroxy vitamin D RIA; IDS, Boldon, UK) with interassay CV of less than 11% and less than 13%, respectively.

Statistical analysis

Because of some nonnormally distributed variables, results are expressed as medians (25–75th percentiles) for quantitative variables and absolute numbers for qualitative variables. We assessed the significance of differences in clinical characteristics between controls and patients by means of nonparametric tests: χ^2 or Fisher's exact test for categorical variables and Wilcoxon's test for continuous variables. SDS for serum bone alkaline phosphatase, CrossLaps, IGF-I, and IGFBP3 levels were calculated from our normative data from 1150 healthy Caucasian children with no history of chronic disease and with no current disease or drug treatment, and for whom blood samples were collected in the morning in fasting conditions (unpublished data). SDS for bone mineral characteristic (LS and TB) values were calculated from the data for a reference pediatric population supplied by the equipment manufacturer (GE Lunar) (30). We compared biochemical markers, bone mineral characteristics, and body composition between patients and controls by means of a linear regression model. This method was also used to investigate relationships among disease-related factors (duration of diabetes, HbA1c levels, and insulin regimen) and bone mineral characteristics (LS and TB), body composition measurements (LBM, fat body mass, and percent body fat mass), serum bone alkaline phosphatase, CrossLaps, IGF-I, and IGFBP3 levels and the relationship between biochemical markers and bone mineral characteristics. All models were adjusted for age, sex, pubertal stage, and physical activity, if appropriate. Obesity in otherwise healthy children is known to be associated with increased BMC (32). T1DM patients tend to have a higher BMI than controls (33). Models were therefore also adjusted for BMI SDS. We checked the normality of residuals and homoscedasticity. Dependent variables were log-transformed when appropriate.

All tests were two tailed. Statistical analyses were performed with the SAS 8.2 (SAS Institute Inc., Cary, NC) software package on a PC.

Results

Clinical and biological characteristics

The clinical characteristics of the study population are indicated in Table 1. The T1DM patients were generally older and at a more advanced pubertal stage than the control subjects. Median BMI was significantly higher in the T1DM group than in the control subjects ($P < 0.01$). When analyzed

TABLE 1. Clinical characteristics of the study population by sex

	Patient group (n = 127)		Control group (n = 319)	
	Boys (n = 73)	Girls (n = 54)	Boys (n = 162)	Girls (n = 157)
Chronological age (yr)	13.0 (10.1 to 16.2)	14.4 (11.8 to 16.5)	11.3 (8.8 to 13.8)[a]	11.7 (9.5 to 14.4)[a]
Pubertal stage				
1	31 (42)	9 (17)	87 (54)	54 (34)[a]
2	6 (8)	8 (15)	17 (10)	23 (15)
3	7 (10)	5 (9)	18 (11)	25 (16)
4	11 (15)	6 (11)	17 (10)	13 (8)
5	18 (25)	26 (48)	23 (14)	42 (27)
Bone age (yr)	13.0 (10.0 to 16.5)	15.0 (11.8 to 16.5)		
Height (SDS)	0.8 (0.3 to 1.7)	0.7 (−0.2 to 1.3)	1.1 (0.4 to 1.7)	1.0 (0.2 to 1.8)
Weight (SDS)	1.1 (0.1 to 1.7)	1.2 (0.1 to 2.3)	0.9 (0.2 to 1.9)	0.9 (0.2 to 1.8)
BMI (SDS)	0.7 (−0.2 to 1.5)	1.0 (0.0 to 1.7)	0.6 (−0.2 to 1.3)	0.5 (−0.2 to 1.3)[a]
Disease duration (yr)	6.3 (4.1 to 8.6)	5.8 (4.1 to 8.4)		
Insulin requirement (U/kg·d)	0.9 (0.7 to 1.1)	0.9 (0.8 to 1.1)		
HbA1c (%)	8.3 (7.9 to 9.4)	8.9 (7.9 to 9.7)		
Categories of physical activity				
A	1 (1)	1 (2)	6 (4)	6 (4)
B	28 (39)	18 (34)	37 (23)	47 (30)
C	42 (59)	34 (64)	118 (73)[a]	104 (66)
Calcium intake (mg/d)	910 (711 to 1063)	850 (691 to 1063)	878 (676 to 1045)	786 (599 to 965)

Values are expressed as median (25 to 75th percentile) or number (%).
[a] $P < 0.01$, patients *vs.* controls.

by sex and by pubertal stage, the only significant difference in BMI SDS observed was between girls with T1DM at pubertal stage 5 and control girls [1.36 (1.03–2.41) SDS, n = 26, *vs.* 0.64 (0.05–1.15) SDS, n = 42; $P < 0.0001$]. Disease duration, insulin requirements, and HbA1c levels did not differ between the sexes in T1DM patients. Median HbA1c levels during each of the preceding 2 yr [8.43% (7.77–9.13%) and 8.55% (7.90–9.23%)] did not differ from current HbA1c levels [8.40% (7.90–9.50%)]. We therefore used only current HbA1c levels for subsequent analysis. Calcium intake was similar in patients and control subjects. A difference in physical activity was observed only in boys with T1DM compared with control boys.

Table 2 shows the markers of calcium metabolism according to sex. Most values were within the normal range, but girls with T1DM had lower serum calcium, magnesium, and alkaline phosphatase and higher PTH levels than boys with T1DM. They also had higher median urinary calcium/creatinine ratios than boys with T1DM. Interestingly, serum magnesium concentration was negatively correlated with HbA1c levels, with a regression coefficient [95% confidence interval (CI)] of −0.018 (−0.036 to 0.000) ($P = 0.05$) in girls but not in boys.

All patients had normal median serum bone alkaline phosphatase and CrossLaps levels. However, median serum bone alkaline phosphatase levels were significantly lower in girls than in boys with T1DM ($P = 0.04$). Median serum IGF-I and IGFBP3 levels were low in all patients (median values, −1.3 SDS for IGF-I and IGFBP3, respectively), with no significant difference between male and female patients.

Bone mineral and body composition

After controlling for age, sex, pubertal stage, and BMI SDS, median TB BMC/LBM was significantly lower in patients with T1DM than in controls ($P < 0.04$). The observed dif-

TABLE 2. Biochemical markers of calcium metabolism and serum concentrations of bone alkaline phosphatase, CrossLaps, IGF-I, and IGFBP3 in 127 treated children with T1DM

	T1DM boys	T1DM girls	*P* values, T1DM boys *vs.* girls	All patients, median (25–75th percentile)	Normal range
Calcium (mmol/liter)	2.26 (2.22 to 2.31)	2.22 (2.16 to 2.26)	0.002	2.24 (2.20 to 2.29)	2.2–2.6
Phosphorus (mmol/liter)	1.6 (1.4 to 1.7)	1.5 (1.4 to 1.6)	0.13	1.5 (1.4 to 1.7)	1.3–1.8
Magnesium (mmol/liter)	0.82 (0.78 to 0.87)	0.79 (0.78 to 0.85)	0.03	0.81 (0.76 to 0.87)	0.75–0.95
Alkaline phosphatase (IU/liter)	224 (275 to 282)	124 (71 to 227)	0.0001	192 (93 to 252)	120–400
PTH (ng/liter)	22 (15 to 33)	26 (21 to 36)	0.04	24 (17 to 34)	10–55
25-Hydroxyvitamin D (ng/liter)	16 (12 to 20)	16 (12 to 20)	0.61	16 (12 to 20)	10–40
1,25-Dihydroxyvitamin D (ng/liter)	43 (35 to 52)	42 (30 to 53)	0.41	43 (34 to 53)	20–80
Urinary calcium/creatinine concentration (mmol/mmol)	0.22 (0.12 to 0.39)	0.29 (0.19 to 0.51)	0.03	0.27 (0.15 to 0.47)	<0.5
Bone alkaline phosphatase (SDS)	0.5 (−0.1 to 1.2)	0.1 (−0.8 to 0.9)	0.04	0.3 (−0.5 to 1.1)	
CrossLaps (SDS)	0.2 (−0.7 to 1.1)	0.1 (−0.8 to 0.9)	0.48	0.1 (−0.8 to 0.9)	
IGF-I (SDS)	−1.2 (−2.0 to −0.3)	−1.6 (−2.4 to −0.7)	0.19	−1.3 (−2.1 to −0.3)	
IGFBP3 (SDS)	−1.3 (−2.1 to −0.7)	−1.8 (−2.6 to −0.1)	0.49	−1.3 (−2.3 to −0.6)	

Serum alkaline phosphatase levels and urinary calcium/creatinine ratio were adjusted for age and pubertal stage (transformed log values for comparison). Serum levels of bone alkaline phosphatase, CrossLaps, IGF-I, and IGFBP3 are expressed as z-scores (SDS). Bone alkaline phosphatase and CrossLaps were adjusted for pubertal stage. IGF-I and IGFBP3 levels were adjusted for pubertal stage and BMI SDS.

TABLE 3. Comparison of absolute bone mineral characteristics and body composition values in 127 children with T1DM (n = 73 boys) *vs.* 319 controls (n = 162 boys), adjusted for age, sex, pubertal stage, and BMI SDS

	Boys		Girls		All subjects	
	Regression coefficient (95% CI)	*P* value	Regression coefficient (95% CI)	*P* value	Regression coefficient (95% CI)	*P* value
LS BMD (g/cm^2)	0.013 (−0.016 to 0.041)	0.38	−0.030 (−0.061 to 0.002)	0.07	−0.006 (−0.027 to 0.015)	0.61
LS BMAD (g/cm^3)	0.001 (−0.003 to 0.006)	0.58	−0.004 (−0.009 to 0.002)	0.17	−0.001 (−0.004 to 0.003)	0.69
LS BMC (g)[a]	0.03 (−0.03 to 0.08)	0.30	−0.07 (−0.13 to 0.00)	0.04	−0.01 (−0.06 to 0.03)	0.52
TB BMD (g/cm^2)	0.002 (−0.017 to 0.020)	0.86	−0.014 (−0.032 to 0.003)	0.10	−0.006 (−0.018 to 0.007)	0.37
TB BMC (g/cm^2)[a]	0.01(−0.03 to 0.06)	0.48	−0.05 (−0.10 to −0.01)	0.03	−0.01 (−0.05 to 0.02)	0.37
TB BMC/LBM	−0.0006 (−0.0017 to 0.0006)	0.36	−0.0020 (−0.0035 to −0.0005)	0.01	−0.0011 (−0.0021 to −0.0001)	0.04
LBM (g)[a,b]	0.02 (−0.01 to 0.06)	0.19	−0.03 (−0.07 to 0.01)	0.12	0.00 (−0.03 to 0.03)	0.94
LBM/height[a,b]	0.02 (0.00 to 0.05)	0.10	−0.02 (−0.04 to 0.01)	0.22	0.00 (−0.01 to 0.02)	0.63
Fat body mass (g)[a,b]	−0.03 (−0.12 to 0.07)	0.60	0.00 (−0.08 to 0.07)	0.95	−0.01 (−0.08 to 0.05)	0.71
Percent body fat mass[b]	−0.01 (−0.02 to 0.00)	0.14	0.01 (−0.01 to 0.02)	0.42	0.00 (−0.01 to 0.01)	0.58

Results are expressed as β -coefficient value (95% CI) from a linear regression model.
[a] Transformed log values for comparison.
[b] Also adjusted for physical activity.

ference in TB BMC/LBM resulted primarily from the difference between girls with T1DM and control girls [regression coefficient (95% CI) = −0.0020 (−0.0035 to −0.0005); P = 0.01]. In boys, TB BMC/LBM was similar for the T1DM and control groups (P = 0.36). Girls with T1DM also had significantly lower LS BMC (P = 0.04) and TB BMC (P = 0.03) than control girls (Table 3), whereas no such difference was observed for boys.

When BMC measurements were expressed as SDS for age and sex with respect to a reference pediatric population, a significant difference in median LS BMC and TB BMC SDS was also found between girls with T1DM and control girls, whereas no such difference was observed for boys (Table 4 and Fig. 1). When data were corrected for bone age instead of chronological age, similar patterns were seen (data not shown).

LBM, LBM/height, fat body mass, and percent body fat mass did not differ between the T1DM and control groups (Table 3).

Effect of disease duration, insulin regimen, and HbA1c levels

We explored the effects of disease-related factors, such as disease duration, insulin regimen, and HbA1c levels, on bone mineral and body composition and serum bone alkaline phosphatase, CrossLaps, IGF-I, and IGFBP3 concentrations by carrying out multiple regression analysis including age, sex, pubertal stage, BMI, physical activity, and disease-related factors. Insulin requirement was significantly associated with TB BMC/LBM, percent body fat mass, LBM/height, and serum IGF-I and IGFBP3 concentrations. HbA1c concentration was associated only with percent body fat mass. We detected no effect of disease duration on any of the factors studied (Table 5).

In univariate analysis, we observed no difference in the factors studied between patients treated with two injections per day, three or more injections per day, or an insulin pump (data not shown).

Relationship between serum biochemical marker SDS and bone mineral characteristics

After controlling for age, sex, pubertal stage, BMI SDS, and insulin regimen, no significant relationship was observed between serum bone alkaline phosphatase, CrossLaps, or IGFBP3 SDS and any of the bone mineral characteristics measured. However, serum IGF-I SDS were positively related to TB BMC (log) values [regression coefficient (95% CI) = 0.037 (0.011–0.063); P = 0.006], LS BMC (log) values [regression coefficient (95% CI) = 0.061 (0.027–0.095); P = 0.001], and BMC/LBM [regression coefficient (95% CI) = 0.002 (0.001–0.002); P = 0.001].

No association was observed between bone formation (bone alkaline phosphatase levels) and resorption (CrossLaps) markers and serum IGF-I or IGFBP3 SDS.

Discussion

This cross-sectional study demonstrates that bone mineral characteristics are slightly impaired in children with T1DM at a median age of 13.8 yr and after a median duration of the disease of 6 yr. The difference in BMC between children with T1DM and controls was significant only after correcting

TABLE 4. Bone mineral characteristics expressed as z-scores (SDS) for age and sex, based on a pediatric reference population (30), in T1DM patients and controls

	Patients		Controls		*P* values	
	Boys	Girls	Boys	Girls	Boys with T1DM *vs.* controls	Girls with T1DM *vs.* controls
LS BMC (SDS)	−0.02 (−0.44 to 0.57)	−0.37 (−1.29 to 0.53)	0.08 (−0.34 to 0.64)	−0.08 (−0.61 to 0.73)	0.19	0.002
TB BMC (SDS)	−0.20 (−0.82 to 0.58)	−0.34 (−0.92 to 0.54)	−0.04 (−0.60 to 0.38)	0.02 (−0.55 to 0.49)	0.13	0.002

Values are expressed as median (25–75th percentile). The data have been adjusted for pubertal stage and BMI SDS.

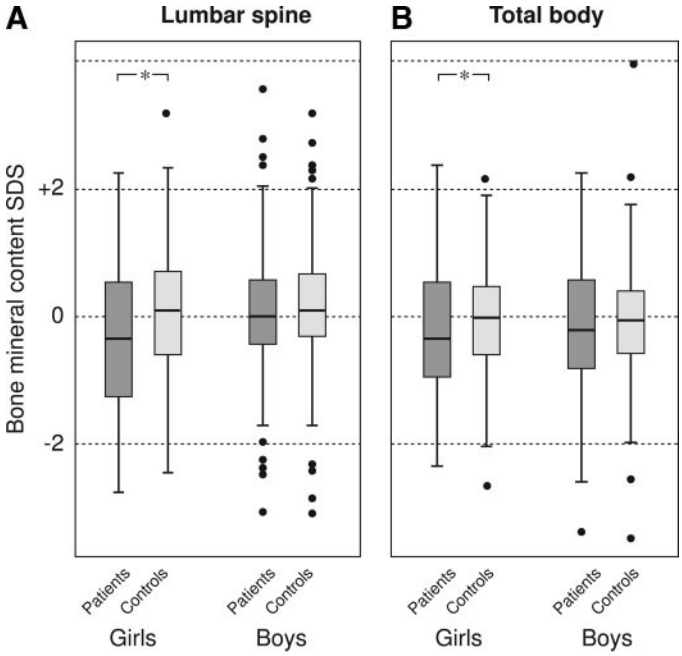

FIG. 1. Box and whisker plots of LS (A) and TB (B) BMC expressed as SDS for age and sex based on a pediatric reference population (30) in T1DM patients and controls. The *horizontal line* represents the median, the *box* indicates the interquartile range, and the *whiskers* show the range of the data. The data have been adjusted for pubertal stage and BMI SDS. *, $P = 0.002$ for T1DM girls *vs.* controls.

whole BMC for LBM, confirming the importance of lean tissue mass in the interpretation of TB bone mineral characteristics in children (29). This study demonstrates for the first time that the difference in BMC between children with T1DM and controls resulted primarily from a difference between girls with and without T1DM. Although the patient and control groups differed in age and pubertal stage distributions, patients with T1DM were compared with a carefully selected healthy control group, controlled for age, sex, BMI, and pubertal stage. These analyses demonstrate the importance of using an appropriate comparison group of healthy subjects for the assessment of bone health in children to adjust for differences in bone and body composition characteristics. Conflicting data on bone mineral characteristics have been published for children with T1DM; some studies have reported normal bone mineral characteristics (14–19), whereas others have reported poorer bone mineral characteristics in children with T1DM than in controls (1–13). Our study was specifically designed to address the drawbacks associated with previous studies. By restricting the study to Caucasian patients who had had diabetes for at least 3 yr, with no other simultaneous treatment or chronic disease, we were able to take into account most of the confounding factors. The larger size of this study population than of the populations studied in previous cross-sectional studies of children also made it possible to obtain more precise estimates of the effect of T1DM on bone mineral and bone metabolism characteristics.

TABLE 5. Effect of metabolic control, insulin regimen, and disease duration on bone mineral and body composition and serum IGF-I and IGFBP 3 levels in children with T1DM

	Regression coefficient (95% CI)	*P* values
TB BMC/LBM		
Disease duration	−0.00003 (−0.0004 to 0.00034)	0.88
Insulin requirement	−0.005 (−0.009 to −0.001)	0.02
HbA1c	0.0001 (−0.0008 to 0.0009)	0.84
% Body fat mass		
Disease duration	−0.0001 (−0.0032 to 0.0029)	0.93
Insulin requirement	−0.040 (−0.074 to −0.006)	0.02
HbA1c	0.012 (0.005–0.019)	0.001
LBM/height		
Disease duration	0.001 (−0.006 to 0.008)	0.76
Insulin requirement	0.083 (0.006–0.159)	0.03
HbA1c	−0.012 (−0.028 to 0.003)	0.12
IGF-I SDS		
Disease duration	−0.04 (−0.12 to 0.03)	0.27
Insulin requirement	0.97 (0.11–1.83)	0.03
HbA1c	−0.17 (−0.35 to 0.02)	0.08
IGFBP3 SDS		
Disease duration	0.01 (−0.07 to 0.09)	0.84
Insulin requirement	1.069 (0.128–2.010)	0.03
HbA1c	0.03 (−0.16 to 0.22)	0.75

Bone mineral and body composition values were adjusted for sex, age, pubertal stage, BMI, physical activity. IGF-I and IGFBP3 SDS were adjusted for BMI.

Moderately reduced BMD has been observed in adult patients with T1DM (20). Sexual dimorphism in the impact of T1DM on BMD has been reported in only one study, in which the difference in BMD was significant only for the female subgroup (22). In children, a larger bone mass deficit in girls than in boys has been suggested in some studies (2, 5, 14), whereas others reported no sex-specific effect on BMD (11, 15, 17, 18). Conversely, BMD and BMC/LBM ratio have been reported to be higher in girls with T1DM than in boys with T1DM (3, 10, 12). This finding may be attributed to the markedly higher BMI of many of the girls, as also found in this study. Because obesity is known to induce an increase in whole-body BMC during childhood and adolescence (32), the apparently preserved BMC/LBM found in girls with T1DM in this previous study may reflect a lack of adjustment of the data for BMI.

Bone mineral characteristics are determined by many genetic, demographic, and lifestyle factors, such as sex, height, weight, dietary calcium intake, and physical activity. Bone mass increases with age, and its peak value is achieved after puberty (34). We cannot exclude the possibility that children, and particularly boys, with T1DM display a slight impairment in bone mass accumulation during skeletal growth, as recently suggested for a limited group of adolescent patients followed for 1 yr, by comparison with a cross-sectional reference population (13). Other longitudinal studies are therefore required to investigate the rate of bone mineral accumulation throughout childhood and puberty, in patients with T1DM, comparing it with that in a group of healthy control children. However, abnormalities in bone mineral characteristics may become more evident in both sexes later in life, as shown in two recent studies (19, 35), perhaps with the long-term onset of progressive microvascular complications (36–39). The physiopathological mechanisms involved in the development of bone loss are unknown. In our study, girls with T1DM had higher urinary calcium/creatinine concentrations and lower serum calcium concentrations, resulting in higher levels of PTH secretion and lower serum magnesium concentrations than in boys. These metabolic

disturbances, which have also been reported in other studies, regardless of the sex of the patients considered (4, 6, 20), may contribute to the development of bone loss. In our study, serum bone alkaline phosphatase levels were also found to be significantly lower in girls than in boys with T1DM, reflecting sex-specific differences in the pattern of bone formation in these patients. This mechanism may result in a lower level of bone accumulation during the period of skeletal growth (20, 40), which, although modest, may become evident in girls earlier than in boys. Our results are consistent with the hypothesis that androgens protect the bone mass by promoting periosteal bone formation, whereas estrogens either inhibit or have no effect on periosteal bone formation (41, 42).

The combined effects of chronic hyperglycemia, insulin deficiency, and low IGF-I concentrations may also reduce osteoblast activity, leading in turn to a decrease in bone formation (21, 43, 44). Most previous studies have been limited by the lack of simultaneous information on bone mineral characteristics and biochemical markers of bone turnover, including measurements of markers of formation and resorption and serum IGF-I and IGFBP3 concentrations. Some studies have suggested that bone formation rates are high (45) or normal (19) in patients with T1DM, but most have reported lower levels of osteoblast function (35, 36, 40, 44, 46). We found that serum bone alkaline phosphatase concentration (a marker of bone formation) and serum CrossLaps levels (a marker of bone resorption) were normal when expressed as SDS for age and sex. Longitudinal studies of this population are required to determine whether bone turnover rate is affected later in life. As in our study, the possible adverse effects on bone of poor metabolic control or of disease duration have not been confirmed in children or adult patients (5, 9, 10, 14, 15, 17–19, 45). A longitudinal study of our population is also required to investigate the possible later contribution of disease-related factors. However, we demonstrated, for the first time, that insulin requirement is negatively correlated with BMC/LBM. It remains unclear how the effects of exogenous and endogenous insulin levels differ in terms of their impact on the skeleton (21). Low serum total and free IGF-I and IGFBP3 levels, despite high circulating GH levels as well as relatively high IGFBP1 levels in patients with T1DM, are thought to arise because of relative GH resistance and portal hypoinsulinemia (47). The elevated GH levels lead to a decrease in insulin sensitivity. Elevated IGFBP1 levels could also contribute to the decrease in insulin sensitivity in these patients by altering levels of free IGF-I (47). Whether alteration in circulating IGFBPs may influence the bioavailability of the IGFs for target tissue such as bone remains to be explored. Insulin requirement has been shown to be linked to body composition abnormalities, with excessive pubertal weight gain, mostly in girls with T1DM (33, 48–50). Serum IGF-I concentration is low in patients with T1DM and is also related to insulin regimen; our findings in this respect are similar to those reported by other studies (51–53). Insulin treatment may therefore affect serum IGF-I concentration, body composition, and bone mineral characteristics. In adult diabetic patients, serum IGF-I concentration is significantly lower in patients with osteopenia than in those without osteopenia (54). Like this previous study in adult patients, our study demonstrates that in children, serum IGF-I SDS is correlated with bone mineral characteristics that were unrelated to resorption or formation markers. In animal models, a threshold serum IGF-I concentration has been demonstrated to be required for normal bone growth and density, suggesting that serum IGF-I concentration plays a prominent role in the pathophysiology of osteoporosis (55).

In conclusion, this study is the first prospective population-based study to identify a sex-specific difference in the impairment of bone mineral characteristics during childhood and adolescence in patients with T1DM. High doses of insulin and low serum IGF-I levels were also found to have independent deleterious effects on bone in patients of all ages and both sexes in this population.

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Address all correspondence and requests for reprints to: Juliane Léger, M.D., Pediatric Endocrinology Unit and INSERM U 690, Hôpital Robert Debré, 48 Boulevard Sérurier, 75019 Paris, France. E-mail: juliane.leger@rdb.ap-hop-paris.fr.

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References

1. **Levin ME, Boisseau VC, Avioli LV** 1976 Effects of diabetes mellitus on bone mass in juvenile and adult-onset diabetes. N Engl J Med 294:241–245
2. **Rosenbloom AL, Lezotte DC, Weber FT, Gudat J, Heller DR, Weber ML, Klein S, Kennedy BB** 1977 Diminution of bone mass in childhood diabetes. Diabetes 26:1052–1055
3. **Santiago JV, McAlister WH, Ratzan SK, Bussman Y, Haymond MW, Shackelford G, Weldon VV** 1977 Decreased cortical thickness and osteopenia in children with diabetes mellitus. J Clin Endocrinol Metab 45:845–848
4. **Wiske PS, Wentworth SM, Norton JA, Epstein S, Johnston CC** 1982 Evaluation of bone mass and growth in young diabetics. Metabolism 31:848–854
5. **Hui SL, Epstein S, Johnston CC** 1985 A prospective study of bone mass in patients with type I diabetes. J Clin Endocrinol Metab 60:74–81
6. **Saggese G, Bertelloni S, Baroncelli GI, Federico G, Calisti L, Fusaro C** 1989 Bone demineralization and impaired mineral metabolism in insulin-dependent diabetes mellitus: a possible role of magnesium deficiency. Helv Paediatr Acta 43:405–414
7. **Lettgen B, Hauffa B, Mohlmann C, Jeken C, Reiners C** 1995 Bone mineral density in children and adolescents with juvenile diabetes: selective measurement of bone mineral density of trabecular and cortical bone using peripheral quantitative computed tomography. Horm Res 43:173–175
8. **Gunczler P, Lanes R, Paoli M, Martinez V, Villarel O, Weisinger JR** 2001 Decreased bone mineral density and bone formation markers shortly after diagnosis of clinical type 1 diabetes mellitus. J Pediatr Endocrinol Metab 14:525–528
9. **Ersoy B, Goksen D, Darcan S, Mavi E, Ozturk C** 1999 Evaluation of bone mineral density in children with diabetes mellitus. Indian J Pediatr 66:375–379
10. **Vina Simon EV, Lozano GB, Maresca MLA, Francisco JLR, Perez CF, Tonkin CL, de Frias EC** 2000 Bone mineral density in juvenile-onset diabetes mellitus. Ann Esp Pediatr 52:507–515
11. **Valerio G, del Puente A, Esposito-del Puente A, Buono P, Mozzillo E, Franzese A** 2002 The lumbar bone mineral density is affected by long term poor metabolic control in adolescents with type 1 diabetes mellitus. Horm Res 58:266–272
12. **Heap J, Murray MA, Miller SC, Jalili T, Moyer-Mileur LJ** 2004 Alterations in bone characteristics associated with glycemic control in adolescents with type 1 diabetes mellitus. J Pediatr 144:56–62
13. **Moyer-Mileur LJ, Dixon SB, Quick JL, Askew EW, Murray MA** 2004 Bone mineral acquisition in adolescents with type 1 diabetes. J Pediatr 145:662–669
14. **Weber G, Beccaria L, de Angelis M, Mora S, Galli L, Cazzufi MA, Turba F,**

Frisone F, Guarneri MP, Chiumello G 1990 Bone mass in young patients with type I diabetes. Bone Miner 8:23–30
15. **Roe TF, Mora S, Costin G, Kaufman F, Carlson ME, Gilsanz V** 1991 Vertebral bone density in insulin-dependent diabetic children. Metabolism 40:967–971
16. **Ponder SW, McCormick DP, Fawcett D, Tran AD, Ogelsby GW, Brouthard BH, Travis LB** 1992 Bone mineral density of the lumbar vertebrae in children and adolescents with insulin-dependent diabetes mellitus. J Pediatr 120:541–545
17. **Pascual J, Argente J, Lopez MB, Munoz M, Martinez G, Vazquez MA, Jodar E, Rerez-Cano R, Hawkins F** 1998 Bone mineral density in children and adolescents with diabetes mellitus type 1 of recent onset. Calcif Tissue Int 62:31–35
18. **De Schepper J, Smitz J, Rosseseu S, Bollen P, Louis O** 1998 Lumbar spine bone mineral density in diabetic children with recent onset. Horm Res 50: 193–196
19. **Liu EY, Wactawski-Wende J, Donahue RP, Dmochowski J, Hovey KM, Quattrin T** 2003 Does low bone mineral density start in post-teenage years in women with type 1 diabetes? Diabetes Care 26:2365–2369
20. **Carnevale V, Romagnoli E, D'Erasmo E** 2004 Skeletal involvement in patients with diabetes mellitus. Diabetes Metab Res Rev 20:196–204
21. **Thrailkill KM, Lumpkin CK, Bunn RC, Kemp SF, Fowlkes JL** 2005 Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues? Am J Physiol Endocrinol Metab 289:E735–E745
22. **Jehle PM, Jehle DR, Mohan S, Böhm BO** 1998 Serum levels of insulin-like growth factor system components and relationship to bone metabolism in type 1 and type 2 diabetes mellitus patients. J Endocrinol 159:297–306
23. **Sempé M, Pedron G, Roy-Pernot MP** 1979 Auxologie, méthodes et séquences. Paris: Théraplix
24. **Rolland-Cachera MF, Cole TJ, Sempé M, Tichet J, Rossignol C, Charraud A** 1991 Body mass index variations: centiles from birth to 87 years. Eur J Clin Nutr 45:13–21
25. **Tanner JM** 1978 Growth at adolescence. Oxford, UK: Blackwell; 28–39
26. **Greulich WW, Pyle, SI** 1959 Radiographic atlas of skeletal development of the hand and the wrist. 2nd ed. Stanford, CA: Stanford University Press
27. **Tricopoulou A, Vassilakou T** 1990 Recommended dietary intakes in the European Community member states: an overview. Eur J Clin Nutr 44:51–125
28. **Carter DR, Bouxsein Ml, Marcus R** 1992 New approaches for interpreting projected bone densitometry data. J Bone Miner Res 7:137–145
29. **Hogler W, Briody J, Woodhead HJ, Chan A, Cowell CT** 2003 Importance of lean mass in the interpretation of total body densitometry in children and adolescents. J Pediatr 143:81–88
30. **Wacker W, Barden HS** 2001 Pediatric reference data for male and female total body and spine BMD and BMC. Madison, WI: GE Lunar Corp., and Dallas, TX: International Society of Clinical Densitometry
31. **Rosenquist C, Fledelius C, Christgau S, Pedersen B, Bonde M, Qvist P, Christiansen C** 1998 Serum CrossLaps one step ELISA: first application of monoclonal antibodies for measurement in serum of bone-related degradation products from C-terminal telopeptides of type I collagen. Clin Chem 44:2281–2289
32. **Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS** 2004 Obesity during childhood and adolescence augments bone mass and bone dimensions. Am J Clin Nutr 80:514–523
33. **Gregory JW, Wilson AC, Greene SA** 1992 Body fat and overweight among children and adolescents with diabetes mellitus. Diabet Med 9:344–348
34. **Bachrach L** 2001 Acquisition of optimal bone mass in children and adolescent. Trends Endocrinol Metab 12:22–28
35. **Krakauer JC, Mckenna MJ, Buderer NF, Sudhaker R, Whitehouse FW, Parfitt AM** 1995 Bone loss and bone turnover in diabetes. Diabetes 44:775–782
36. **Mathiassen B, Nielsen S, Johansen JS, Hartwell D, Ditzel J, Rodbro P, Christiansen C** 1990 Long term bone loss in insulin dependent diabetic patients with microvascular complications. J Diabetes Complications 4:145–149
37. **Munoz Torres M, Jodar E, Escobar-Jimenez F, Lopez-Ibarra PJ, Luna JD** 1996 Bone mineral density measured by dual x-ray absorptiometry in Spanish patients with insulin-dependent diabetes mellitus. Calcif Tissue Int 58:316–319
38. **Clausen P, Feldt-Rasmussen B, Jacobsen P, Rossing K, Parving HH, Nielsen PK, Feldt-Rasmussen U, Olgaard K** 1997 Microalbuminuria as an early indicator of osteopenia in insulin-dependent diabetic patients. Diabet Med 14: 1038–1043
39. **Campos Pastor MM, Lopez-Ibarra PJ, Escobar-Jimenez F, Serrano Pardo MD, Garcia-Cervigon A** 2000 Intensive insulin therapy and bone mineral density in type I diabetes mellitus: a prospective study. Osteoporos Int 11:455–459
40. **Bouillon R, Bex M, Van Herck E, Laureys J, Dooms L, Lesaffre E, Ravussin E** 1995 Influence of age, sex, and insulin on osteoblast function: osteoblast dysfunction in diabetes mellitus. J Clin Endocrinol Metab 80:1194–1202
41. **Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C** 2004 Androgens and bone. Endocr Rev 25:389–425
42. **Wang Q, Alen M, Nicholson PHF, Halleen JM, Alatato SL, Ohlsson C, Suominem H, Cheng S** 2006 Differential effects of sex hormones on peri- and endocortical bone surfaces in pubertal girls. J Clin Endocrinol Metab 91:277–282
43. **Terada M, Inaba M, Yano Y, Hasuma T, Nishizawa Y, Morii H, Otani S** 1998 Growth-inhibitory effect of a high glucose concentration on osteoblast-like cells. Bone 22:17–23
44. **Lu H, Kraut D, Gerstenfeld LC, Graves DT** 2003 Diabetes interferes with the bone formation by affecting the expression of transcription factors that regulate osteoblast differentiation. J Clin Endocrinol Metab 144:346–352
45. **Miazgowski T, Czekalski S** 1998 A 2-year follow-up study on bone mineral density and markers of bone turnover in patients with long-standing insulin-dependent diabetes mellitus. Osteoporos Int 8:399–403
46. **Olmos JM, Perez Castrillon JL, Garcia MT, Garrido JC, Amado JA, Gonzalez-Macias J** 1994 Bone density and biochemical markers in type I diabetes mellitus. Bone Miner 26:1–8
47. **Hedman CA, Frystyk J, Lindström T, Chen JW, Flyvbjerg A, Orskov H, Arnqvist HJ** 2004 Residual β-cell function more than glycemic control determines abnormalities of the insulin-like growth factor system in type 1 diabetes. J Clin Endocrinol Metab 89:6305–6309
48. **Danne T, Kordonouri O, Enders I, Weber B** 1997 Factors influencing height and weight development in children with diabetes. Diabetes Care 20:281–285
49. **Holl RW, Grabert M, Sorgo W, Debatin KM** 1998 Contribution of age, gender and insulin administration to weight gain in subjects with IDDM. Diabetologia 41:542–547
50. **Ahmed ML, Ong KKL, Watts AP, Morrell DJ, Preece MA, Dunger DB** 2001 Elevated leptin levels are associated with excess gains in fat mass in girls, but not boys, with type I diabetes: longitudinal study during adolescence. J Clin Endocrinol Metab 86:1188–1193
51. **Massa G, Dooms L, Bouillon R, Vanderschueren-Lodeweyckx M** 1993 Serum levels of growth hormone-binding protein and insulin-like growth factor I in children and adolescents with type I (insulin-dependent) diabetes mellitus. Diabetologia 36:239–243
52. **Clayton KL, Holly JMP, Carlsson LMS, Jones J, Cheetham TD, Taylor AM, Dunger DB** 1994 Loss of the normal relationships between growth hormone, growth hormone-binding protein and insulin-like growth factor I in adolescents with insulin-dependent diabetes mellitus. Clin Endocrinol (Oxf) 41:517–524
53. **Ahmed ML, Connors MH, Drayer NM, Jones JS, Dunger DB** 1998 Pubertal growth in IDDMIs determined by HbA1c levels, sex, and bone age. Diabetes Care 21:831–835
54. **Kemink SA, Hermus AR, Swinkels LM, Lutterman JA, Smals AG** 2000 Osteopenia in insulin-dependent diabetes mellitus: prevalence and aspects of pathophysiology. J Endocrinol Invest 23:295–303
55. **Yakar S, Rosen CJ, Beamer WG, Acken-Bicknell CL, Wu Y, Liu JL, Ooi G, Setser J, Frystyk J, Boisclair YR, LeRoith D** 2002 Circulating levels of IGFI directly regulate bone growth and density. J Clin Invest 110:771–781

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