Pediatric Diabetes

Pediatric Diabetes 2011
doi: 10.1111/j.1399-5448.2010.00739.x

Pediatric Diabetes

Original Article

Parathormone – 25(OH)-vitamin D axis and bone status in children and adolescents with type 1 diabetes mellitus

Hamed EA, Abu Faddan NH, Adb Elhafeez HA, Sayed D.
Parathormone – 25(OH)-vitamin D axis and bone status in children and adolescents with type 1 diabetes mellitus.
Pediatric Diabetes 2011.

Background: Skeletal involvement in patients with type 1 diabetes mellitus (T1DM) has complex pathogenesis and despite numerous researches on this problem, many questions remain unanswered.
Objective: This study aimed to assess bone status by measurement parathormone (PTH), 25-hydroxy vitamin D [25(OH)D] serum levels in children and adolescents with T1DM and its relation to insulin-like growth factor-1 (IGF-1), disease duration, puberty stage, and metabolic control.
Patients and methods: This study included 36 children and adolescents with T1DM and 15 apparently healthy controls. Serum levels of 25(OH)D, PTH, IGF-1 measured using enzyme-linked immunosorbent assay (ELISA), while glycosylated hemoglobin (HbA1c), calcium (Ca), inorganic phosphorus (PO_4) using autoanalyzer. Bone quality assessed using dual energy X-ray absorptiometry (DEXA).
Results: Diabetic patients showed significant increase in PO_4 and PTH levels, while significant decrease in Ca, IGF-1, and 25(OH)D serum levels. As much as 52.8% of patients showed reduced 25(OH)D, and 30.65% showed elevated PTH serum levels. In diabetic patients, abnormal bone status (osteopenia-osteoporosis) found mostly in total body (94.40%) then lumber-spine (88.90%), ribs (88.90%), pelvis (86.10%), thoracic-spine (80.60%), arms (80.60%) and legs (77.80%), while head bones showed no abnormalities. Long diabetic duration had negative; meanwhile PTH, onset age, and puberty age had positive impact on bone status.
Conclusions: Children and adolescent with T1DM have abnormal bone status mostly in axial skeleton which may be contributed to impairment of formation of 25(OH)D and IGF-1. Physical activity, calcium and vitamin D supplement seem important in T1DM. Elevated serum PTH level in diabetic patients is not uncommon and its positive correlation with bone status needs further investigations.

Enas A Hamed[a], Nagla H Abu Faddan[b], Hebh A Adb Elhafeez[c] and Douaa Sayed[d]

[a]Department of Physiology, Faculty of Medicine, Assiut University, Assiut, P. O. Box 71526, Egypt; [b]Department of Pediatrics, Pediatric Assiut University Hospital, Assiut, P. O. Box 71526, Egypt; [c]Department of Clinical Pathology, Assiut University Hospital, Assiut, P. O. Box 71526, Egypt; and [d]Department of Clinical Pathology, South Egypt Cancer Institute, Assiut University, Assiut, P. O. Box 71526, Egypt

Key words: bone status – 25(OH)D – IGF-1 – PTH – TIDM

Corresponding author:
Assist. Prof. Enas A Hamed, MD,
Department of Physiology,
Faculty of Medicine,
Assiut University,
Assiut, P. O. Box 71526, Egypt.
Tel: +2 0164743592;
fax: +2 088 2333327;
e-mail: eah3a2010@yahoo.com

Submitted 22 July 2010.
Accepted for publication 6 October 2010

The prevalence of type 1 diabetes mellitus (T1DM) in childhood is increasing with a worldwide annual increase estimated at 3% (range 2–5%) (1). T1DM has negative effects on bone health and leads to an increase in fracture risk among middle aged and older individuals (2). However, there is still some debate about the effect of diabetes on bone status during childhood and adolescence (3, 4). Moreover, there is no general agreement on the relative importance of several diabetes – specific characteristics, such as age of onset, diabetic duration, glycemic control, and insulin regimen on bone health (5). Puberty has a key role in bone development. Skeletal mass approximately doubles at the end of adolescence (6). The pubertal phase is characteristically associated with a reduction in insulin sensitivity, which is known to be more severe in patients with T1DM, and might negatively influence growth and height gain (7). Identifying risk factors that predispose to a low bone mineral density (BMD) in diabetic patients is therefore desirable.

Bone remodeling is regulated by systemic hormones and locally produced factors acting to maintain bone mass (8). Vitamin D, through its active form, 1,25-dihydroxyvitamin D [1,25(OH)2D], is essential for intestinal calcium absorption and plays a central role in maintaining calcium homeostasis and skeletal integrity (9). Vitamin D stores are derived from either dietary intake or cutaneous synthesis following ultraviolet irradiation. Vitamin D from either source undergoes 25-hydroxylation in the liver to form 25-hydroxy vitamin D [25(OH)D]. Optimal vitamin D status is important to human health, and there is a consensus that serum or plasma 25(OH)D should be used to assess vitamin D status because it reflects combined dietary supply and dermal production (10). Age, gender, lifestyle, geographical areas, sunlight, and vitamin D supplementation are important determinants of vitamin D levels (11). Hypovitaminosis D is highly prevalent in Middle Eastern countries (12). Limited sunlight exposure has been blamed on cultural practices, such as clothing and veiling in Muslim women, spending a great deal of time indoors, and exclusive or prolonged breast-feeding without vitamin D supplements (13, 14). Severe clinical vitamin D deficiency [serum/plasma 25(OH)D $< 10–25$ nmol/L] leads to rickets in children and osteomalacia in adults. Less severe deficiency causes secondary hyperparathyroidism and increases bone turnover and bone loss (15). Environmental factors play an important role in the development and progression of systemic autoimmune diseases along with susceptible genetic and hormonal background. TIDM is an autoimmune disease and environmental factors contribute to its development (16). The parallel rise in incidence of both T1DM and vitamin D deficiency raises the possibility that vitamin D may play a role in the pathogenesis of T1DM (17). Evidence from basic, clinical, and epidemiological studies provides a rationale for this hypothesis (18–20). Given the negative effect of vitamin D inadequacy and T1DM on bone health, patients with both conditions have multiple risk factors for increased skeletal fragility.

Endogenous human parathyroid hormone (PTH) has contrasting effects on bone, depending upon the length of exposure. The 'anabolic' or bone-building effects require brief exposures to higher than average PTH concentrations. The 'catabolic' effects result from pathological conditions in which one or more parathyroid glands secrete too much hormone continuously at a sustained level. Such continuous secretion of PTH can lead to bone destruction (21). The anabolic effect of PTH on bone has led to its indication in treatment of osteoporosis (22). Insulin-like growth factor-1 (IGF-1) is an important anabolic regulator of bone cell function (8). Circulating IGF-1 is produced by the liver. It mediates skeletal growth promoting actions of growth hormone (GH). IGF-1 is also produced locally by muscle and bone tissues, where it is acting in a paracrine manner (23). In the osseous tissue, production of IGF-1 is stimulated by PTH, which also stimulates the transcription of insulin growth factor binding protein-1 (IGFBP-1) in osteoblasts and thus amplifies the osteotrophic effect of IGF-1 (24). The production of IGF-1 in human osteoblasts is also stimulated by 1,25(OH)2D (25). Thus, the IGF must receive considerable attention as a mechanism for inadequate bone formation in T1DM.

The aim of the present work is to assess bone status especially by measuring the serum levels of PTH and 25(OH)D in children and adolescents with T1DM and its relation to IGF-1 as well as disease duration, puberty stage, and metabolic control.

Patients and methods

Patients and study protocol

This study was approved by the Ethical Committee of Faculty of Medicine, Assuit University Hospital according to the latest revision of *Declaration of Helsinki* and informed consent was obtained from participant's parent/legal guardian. This study consisted of 36 children and adolescent with T1DM recruited from Diabetes Outpatient Clinic, Pediatric Hospital, Assiut University, Assiut, Egypt, during the period from March 2008 to December 2009. There were 13 boys and 23 girls, with ages ranging from 3 to 15 yr (mean ± SD, 10.38 ± 3.17 yr). Fifteen apparently healthy children and adolescent (seven boys and eight girls), their ages ranging from 3 to 14 yr (mean ± SD, 8.47 ± 4.17 yr) matched with patient's age, pubertal stage, and physical activity recruited from patient's relevant were consider as controls. At the time of the study, the puberty stages in patients and controls were prepubertal (Tanner stage I) in 44.40, 40.00%, respectively; early pubertal (Tanner stages II and III) in 50.00, 60.00%, respectively, and adolescent (Tanner stage IV) only in 5.60% of patients (Table 1). Inclusion criteria consisted of: (i) first diagnosis of T1DM made before 18 yr of age; (ii) no evidence of diabetic retinopathy (assessed via fundoscopy examination), neuropathy (assessed using clinical history and physical examinations), or nephropathy (determined using 24-h urine albumin >30 mg/d) as these complications may affect bone status (26); (iii) no intake of medications in the preceding 6 months other than insulin; (iv) no history of other chronic diseases including autoimmune diseases; (v) no history of hospitalization or ketoacidosis in the preceding 6 months; (vi) no restriction of physical activity; (vii) no history of bone fractures during the last year. All the patients were on two daily doses of mixed type of insulin at a dosage of 0.86 ± 0.14 (0.70–1.00)

Table 1. Demographic and clinical characteristics of studied population

Parameters	Patients (n = 36)	Control (n = 15)	Significance (p-value)
Age (yr)	10.38 ± 3.17 (3.00–15.00)	8.47 ± 4.17 (3.00–14.00)	0.081
Sex (M/F)	13 /23 36.10%/63.90%	7/8 46.70%/53.30%	0.346
Puberty stage	1.69 ± 0.79 (1.00–3.00)	1.87 ± 0.83 (1.00–3.00)	0.487
I	16 (44.40%)	6 (40%)	
II	17 (47.20%)	5 (33.30%)	
III	1 (2.80%)	4 (26.70%)	
IV	2 (5.60%)	—	
BMI (kg/m^2)	17.83 ± 7.07 (9.51–38.27)	16.42 ± 2.88 (12.19–21.75)	0.461
Age at diagnosis (yr)	7.68 ± 3.70 (1.00–14.00)	—	—
Diseased duration (yr)	2.67 ± 3.52 (0.10–12.75)	—	—
Insulin dose (IU/kg/d)	0.86 ± 0.14 (0.70–1.00)	—	—
HbA1c (%)	9.14 ± 1.90 (6.20–13.00)	5.33 ± 0.61 (4.20–6.00)	0.001
Poorly controlled (≥8.00%)	22 (61.10%)	—	0.182
Serum glucose (mg/dL)	377.64 ± 149.19 (114.00–596.00)	91.87 ± 7.21 (80.00–105.00)	0.001
Family history	11 (30.65 %)	—	—
Bone pain	3 (8.30%)	—	—

BMI, body mass index; HbA1c, glycosylated hemoglobin.
Data are presented as the mean ± SD (range) or number (%) as appropriate; p: significance vs. control.

IU/kg/d. All the participants were subjected to a thorough clinical history and examination. Height, weight, and body mass index (BMI) were measured and recorded. Pubertal stage was assessed according to Tanner–Whitehouse (27).

Measurements

Morning fasting venous blood samples were collected. Serum levels of glucose, calcium (Ca), inorganic phosphorus (PO_4), total alkaline phosphatase, and liver and kidney functions tests were measured using Synchron CX Pro autoanalyzer, Beckman Counter (Tokyo, Japan). Circulating levels of glycosylated hemoglobin (HbA1c), a clinical indicator of blood glucose control, was measured by Hitachi 911 autoanalyzer (Hitachi Co. Ltd., Tokyo, Japan). HbA1c determination is based on turbidimetric inhibition immunoassay for hemolyzed whole blood from Roche/Hitachi 911, Tokyo, Japan. Normal values of HbA1c according to Heap et al. (28) ranged from 4.0 to 6.0%. Our control children laid in this range. HbA1c values were recorded for the previous 12-month period from the participants' clinic record and then averaged. Poor metabolic control was considered when HbA1c reached >8.0% (29). Serum 25(OH)D was determined using competitive binding protein assay (Immundiagnostik AG, Blenheim, Germany). The interassay coefficient of variation was 8%. 25(OH)D level was considered as insufficient if <50 nmol/L (<20 ng/mL) (3). Serum PTH concentration was estimated using immunoenzymetric assay (ELISA) (BioSource, Nivelles, Belgium). The interassay coefficient of variation was 10%. PTH level was considered elevated if >65 pg/mL (30). Serum IGF-1 levels were measured by ELISA, based on the principle of competitive binding (DRG-IGF-1600, Bensheim, Germany). Intra- and interassay variabilities were less than 10%.

BMD values depended upon bone mineral content (BMC) and cross-sectional area (CSA). In this study, the assessment was carried out using calibrated dual energy X-ray absorption (DEXA) method (Hologic Model Delphi, CT, USA). BMD (g/cm^2 and *Z*-score), BMC (g), and CSA (cm^2) of head, arms, ribs, thoracic (T) spine, lumbar (L) spine, pelvis, legs, and total body were measured. Values of arms, ribs, and legs were calculated as the mean values of right and left sides. BMD values were expressed as *z*-score [number of standard deviation (SD) adjusted by age and sex] in comparison with a reference healthy age- and sex-matched Egyptian population. According to the World Health Organization (31), a *Z-score* of −2 standard deviations (SDs) below the mean in relation to the patient's age is defined as osteoporosis and between −1.0 and −2.0 SDs as osteopenia.

Data analysis

Statistical Science for Social Package (SPSS V12, SPSS Inc., Chicago, IL, USA) was used for data analysis. Data were presented as mean (SD) or number (%) as appropriate. For comparison of two groups, the parametric 'Student's *t* test' and non-parametric 'chi-squared test' for independent variables were used, while comparisons of multiple groups were performed using analysis of variance (ANOVA) and Kruskall Wallis tests for parametric and non-parametric variables, respectively. Spearman's and Pearson's correlation tests were used for correlating non-parametric and parametric variables. For all tests, a probability (p) <0.05 was considered significant.

Results

Demographic and clinical characteristics of studied children were listed in Table 1. At the time of assessment, blood glucose and HbA1c levels were higher in patients than in controls ($p < 0.0001$ for each). Most of our patients (61.10%) showed poor metabolic control (HbA1c $\geq$8.0%). Positive family history of diabetes and history of bone pain were found in 30.65 and 8.30% of the patients, respectively.

Table 2 showed that mean serum levels of calcium, IGF-1, 25(OH)D were significantly lower ($p < 0.006$, $p < 0.018$, $p < 0.001$), while inorganic phosphorus, PTH were significantly higher ($p < 0.007$, $p < 0.007$) in T1DM patients than in controls. 25(OH)D was reduced (<50 nmol/L) in 52.80% of patients, while PTH (>65 pg/mL) was elevated in 30.65%.

In patients with sufficient and insufficient 25(OH)D, serum levels of inorganic phosphorus ($p < 0.009$, $p < 0.028$) and PTH ($p < 0.033$, $p < 0.011$) were significantly higher, while IGF-1 ($p < 0.014$, $p < 0.025$) and 25(OH)D ($p < 0.000$, $p < 0.000$) were significantly lower than controls. Compared with the control group, patients with insufficient 25(OH)D showed lower Ca levels ($p < 0.001$), while patients with sufficient 25(OH)D showed lower total alkaline phosphatase than controls ($p < 0.039$) (Table 3).

Table 4 showed the DEXA finding of different measured areas. Abnormal bone status was highest in total body (94.9%) followed by L-spine (88.9%), ribs (88.9%), pelvis (86.1%), arms (80.6%), T-spine (80.6%), and legs (77.8%). Meanwhile, no abnormal bone status was found in the head.

Table 5 compares bone status in patients with reduced (<50 nmol/L) and satisfactory ($\geq$50 nmol/L) 25(OH)D. BMC and abnormal bone status of arms abnormal bone status of the ribs were higher ($p < 0.049$, $p < 0.028$, $p < 0.013$), while *Z*-score of the arms ($p < 0.0001$) and BMD and *Z*-score of the ribs ($p < 0.007$, $p < 0.0001$) were significantly decreased in patients with sufficient compared to those with insufficient 25(OH)D.

In diabetic patients, the duration of disease correlated negatively with total body BMC and CSA ($r = -0.346$, $p < 0.039$; $r = -0.366$, $p < 0.028$) (Fig. 1). Age of disease onset correlated positively with BMD of arms and ribs ($r = 0.431$, $p < 0.009$; $r = 0.521$, $p < 0.001$) and *Z*-score of arm and ribs ($r = 0.412$, $p < 0.012$; $r = 0.436$, $p < 0.008$) (Fig. 2). Puberty stage correlated positively with BMD of ribs ($r = 0.380$, $p < 0.022$); BMC ($r = 0.405$, $p < 0.014$) and CSA of L-spine ($r = 0.470$, $p < 0.004$) and *Z*-score of arm ($r = 0.361$, $p < 0.031$). Serum PTH correlated positively with BMD of ribs, L-spine ($r = 0.359$, $p < 0.031$; $r = 0.362$, $p < 0.030$) and *Z*-score of arms and ribs ($r = 0.358$, $p < 0.032$; $r = 0.343$, $p < 0.041$) (Fig. 3).

Table 2. Serum concentrations of measured parameters in all participants

Parameters	Patients (n = 36)	Controls (n = 15)	Significance (p-value)
Calcium (mg/dL)	8.81 ± 1.22 (6.60–12.50)	9.85 ± 1.01 (7.90–11.50)	0.006
Inorganic phosphorus (mg/dL)	5.08 ± 1.35 (0.78–8.72)	3.99 ± 0.94 (2.51–5.01)	0.007
Total alkaline phosphatase (IU/L)	204.44 ± 111.60 (47.00–515.00)	247.33 ± 95.58 (140.00–400.00)	0.199
IGF-1 (ng/mL)	141.08 ± 112.70 (25.00–380.00)	220.00 ± 81.95 (130.00–400.00)	0.018
25(OH)D (nmol/L)	46.75 ± 12.74 (29.20–69.72)	85.42 ± 5.77 (71.93–94.17)	0.001
Low vitamin D (<50 nmol/L)	19 (52.8%)	—	0.001
PTH (pg/mL)	47.75 ± 25.00 (16.00–90.00)	29.03 ± 10.21 (17.00–47.00)	0.007
Hyper PTH (>65 pg/mL)	11 (30.65%)	—	0.001

IGF-1, insulin-like growth factor-1; 25(OH)D, 25-hydroxy cholecalciferol; PTH, parathormone.
Data are presented as the mean ± SD (range) or number (%) as appropriate; p: significance vs. control.

Table 3. Serum concentrations of measured parameters in subgroup of patients according to 25 hydroxy cholecalciferol [25(OH)D] serum levels status

Parameters	Patients: Sufficient (25(OH)D ≥50 nmol/L) (n = 17)	Patients: Insufficient (25(OH)D <50 nmol/L) (n = 19)	Controls (n = 15)
Calcium (mg/dL)	8.51 ± 0.99 (6.60–10.80)	9.15 ± 1.39 (6.90–12.50)	9.85 ± 1.01 (7.90–11.50)
Significance	p < 0.091	p < 0.001 *p < 0.104	
Inorganic phosphorus (mg/dL)	4.98 ± 1.59 (0.78–8.72)	5.20 ± 1.06 (3.56–7.49)	3.99 ± 0.94 (2.51–5.01)
Significance	p < 0.009	p < 0.028 *p < 0.599	
Total alkaline phosphatase (IU/L)	236.22 ± 122.31 (47.00–515.00)	168.94 ± 88.75 (58.00–391.00)	247.33 ± 95.58 (140.00–400.00)
Significance	p < 0.039	p < 0.759 *p < 0.060	
IGF-1 (ng/mL)	147.89 ± 108.62 (32.00–380.00)	133.47 ± 119.97 (25.00–338.00)	220.00 ± 81.95 (130.00–400.00)
Significance	p < 0.014	p < 0.025 *p < 0.802	
25(OH)D (nmol/L)	58.82 ± 5.12 (50.36–69.72)	35.94 ± 5.59 (29.20–46.81)	85.42 ± 5.77 (71.93–94.17)
Significance	p < 0.0001	p < 0.0001 *p < 0.0001	
PTH (pg/mL)	49.16 ± 27.88 (16.00–90.00)	46.18 ± 22.07 (16.00–90.00)	29.03 ± 10.21 (17.00–47.00)
Significance	p < 0.033	p < 0.011 *p < 0.876	

IGF-1, insulin-like growth factor-1; 25(OH)D, 25 hydroxy cholecalciferol; PTH, parathormone.
Data are presented as the mean ± SD (range); p: vs. control; *p: significance vs. patients with sufficient 25(OH)D.

Discussion

Skeletal involvement in patients with T1DM has a complex pathogenesis and, despite much research on this problem, many questions remain unanswered. In the present study, serum level of calcium was significantly lower, while inorganic phosphorus was significantly higher in diabetic patients compared with controls. Lower serum calcium levels may be the result of the decrease in duodenal calcium absorption and an increase in its urinary excretion in diabetic patients (32). The high PO_4 serum level observed in this study could be explained by physiological feedback mechanism to reduced serum calcium in order to maintain the solubility product constant. The present study revealed a significant decline in the mean value of 25(OH)D in diabetic patients than controls. Moreover, 52.8% of diabetic patients had 25(OH)D deficiency (<50 nmol/L). This study reported that *Z*-score and bone status of arm and BMD, *Z*-score and bone status of ribs were significantly decreased in patients with insufficient compared to those with sufficient 25(OH)D. Similarly, Littorin and his colleagues (33) reported that 54% of patients with TIDM at diagnosis demonstrated levels of 25(OH)D below 80 nmol/L. The lower levels found at diagnosis compared with control subjects support the idea that vitamin D deficiency may be an important factor behind the development of type 1 diabetes, perhaps with an immunological background (34). Huynh et al. (35) reported the reduction in serum levels of 25(OH)D levels (<50 nmol/L) in 22% of children with T1DM. They also reported that lower measured 25(OH)D levels were associated with acidemia in children with new-onset T1DM and generally increase when the acidosis resolves. A number of studies have demonstrated impaired 1-alpha-hydroxylase activity and conversion of 25(OH)D to 1,25(OH)2D with induced chronic metabolic acidosis (36). It is also possible that the acidotic state results in a reduction of vitamin D-binding proteins and a corresponding reduction in measured 1,25(OH)2D levels in accordance with the Michaelis–Menten equilibrium (35). Svoren et al. (37) reported significant vitamin D deficiency was found in 76% of 128 children with type 1 diabetes [insufficiency, 61% (n = 78); deficiency, 15% (n = 19)]. The criteria used to define vitamin D sufficiency, insufficiency, and deficiency were 25(OH)D levels of ≥30 ng/mL (≥75 nmol/l), 21–29 ng/mL (52.5–72.5 nmol/L), and ≤20 ng/mL

Table 4. Dual energy X-ray absorption (DEXA) parameters of insulin dependent diabetes mellitus patients (=36)

Parameters	BMD (g/cm^2)	BMC (g)	CSA (cm^2)	*Z*-score	Abnormal bone status		
					Osteopenia	Osteoporosis	Total (n = 36)
Head	1.30 ± 0.20 (0.69–1.64)	201.19 ± 42.87 (107.90–287.50)	157.63 ± 32.84 (96.10–203.80)	−0.17 ± 0.53 (−0.67–0.90)	—	—	—
Leg	0.90 ± 0.12 (0.68–1.13)	328.76 ± 86.09 (121.65–460.25)	357.21 ± 65.16 (186.50–497.00)	−2.60 ± 1.87 (−5.82 to −0.18)	7 (19.4%)	21 (58.3%)	28 (77.8%)
Arm	0.68 ± 0.10 (0.50–0.91)	124.93 ± 161.61 (35.15–727.50)	115.85 ± 59.35 (53.65–294.41)	−1.77 ± 1.33 (−3.34 to 1.83)	10 (27.8%)	19 (52.8%)	29 (80.6%)
T-spine	0.64 ± 0.29 (0.23–1.31)	132.87 ± 137.05 (21.50–583.90)	191.17 ± 162.21 (83.60–872.60)	−2.74 ± 2.22 (−5.83 to 2.41)	5 (13.9%)	24 (66.7%)	29 (80.6%)
Pelvis	0.66 ± 0.21 (0.22–1.14)	165.09 ± 95.43 (19.30–382.50)	250.60 ± 105.83 (22.70–407.70)	−3.72 ± 2.40 (−7.78 to 2.21)	2 (5.6%)	29 (80.6%)	31 (86.1%)
Ribs	0.69 ± 0.12 (0.43–0.86)	82.64 ± 44.19 (25.45–200.45)	126.23 ± 67.37 (51.75–327.80)	−3.24 ± 1.70 (−6.02 to −0.70)	5 (13.9%)	27 (75.0%)	32 (88.9%)
L-spine	0.52 ± 0.20 (0.04–0.95)	60.98 ± 34.56 (13.70–13.30)	109.48 ± 38.60 (43.70–183.10)	−3.41 ± 1.58 (−6.31–0.43)	2 (5.6%)	30 (83.3%)	32 (88.9%)
Total body	0.72 ± 0.12 (0.51–0.98)	1101.45 ± 406.99 (320.90–1733.40)	1491.13 ± 390.15 (536.20–2099.80)	−3.12 ± 1.60 (−5.56 to 1.72)	5 (13.9%)	29 (80.6%)	34 (94.4%)

BMC, bone mineral content (g); BMD, bone mineral density (g/cm^2).
Data are presented as the mean ± SD (range) or number (%) as appropriate; cross-sectional area (cm^2).

(≤50 nmol/L), respectively (38). The reduced 25(OH)D serum level in T1DM can be attributed to decreased synthesis of vitamin D-binding protein by the liver (32). Many epidemiologic and experimental investigations appear to indicate that vitamin D deficiency may be involved in the pathogenesis of T1DM. Receptors for 1,25(OH)2D are expressed in antigen-presenting cells and T-cells as well as in pancreatic beta cells (20). Vitamin D deficiency was also implicated in the impairment of insulin synthesis and secretion (18), while vitamin D supplementation has been demonstrated to attenuate cytokine-mediated pancreatic beta-cell destruction (19). Vitamin D receptor genotype also appears to be important in determining an individual's susceptibility to developing T1DM (39). Moreover, the reported inverse correlation between sunlight exposure and T1DM incidence is consistent with the hypothesis that vitamin D status modulates disease susceptibility (40). In this study, there was no correlation between the concentrations of 25(OH)D and HbA1c. This indicates that the diabetic state purse is a reason for low 25(OH)D levels and is not secondary to any hyperglycaemic or insulin-resistant state.

The present work also revealed that serum PTH levels were significantly higher in T1DM patients than controls. The explanation of this apparently increased PTH levels might be because of the functioning feedback mechanisms to the decrease in serum levels of calcium and 25(OH)D. Malabanan et al. (41) found that serum PTH concentration tends to increase when 25(OH)D concentration was below 50 nmol/L (20 ng/mL). On the other hand, most studies demonstrated normal or even low PTH concentrations in diabetic patients (42). The difference between our results and others could be explained by high prevalence of 25(OH)D insufficiency among our T1DM patients. Other investigators reported high serum concentration of glucose reversibly suppresses PTH secretion from cultured bovine parathyroid cells *in vitro* (43). Vitamin D deficiency results in hyperparathyroidism (44), through which it may influence glucose metabolism. Patients with hyperparathyroidism have an increased prevalence of diabetes and insulin resistance, and parathyroidectomy improves their glucose intolerance (45). In the present study, BMD of ribs and L-spine and *Z*-score of arms and ribs was positively associated with PTH serum levels, despite elevated PTH blood levels observed among our diabetic patients could result in increased bone resorption and decreased BMD. This observation could be explained either by decreased sensitivity among diabetic to the effects of PTH on bone resorption (46) or by anabolic responses of osteoblasts to PTH. The anabolic responses of osteoblasts to PTH have been studied intensively, with reports of enhanced

Table 5. Dual energy X-ray absorption (DEXA) parameters of subgroups of insulin dependent diabetes mellitus patients according to 25 hydroxy cholecalciferol [25(OH)D] status

					Abnormal bone status		
Parameters	BMD (g/cm^2)	BMC (g)	CSA (cm^2)	*Z*-score	Osteopenia	Osteoporosis	Total (n = 36)
Head							
Sufficient (n = 17)	1.27 ± 0.23	196.72 ± 40.41	158.65 ± 33.55	0.21 ± 0.55	—	—	—
Insufficient (n = 19)	1.32 ± 0.18	205.20 ± 45.67	156.73 ± 33.08	−0.13 ± 0.52			
Significance	$p < 0.561$	$p < 0.917$	$p < 0.665$	$p < 0.633$			$p < 1.00$
Leg							
Sufficient (n = 17)	0.92 ± 0.14	338.09 ± 96.40	360.04 ± 70.57	−2.77 ± 2.08	1 (5.9%)	11 (64.7%)	12 (70.6%)
Insufficient (n = 19)	0.89 ± 0.12	320.40 ± 77.44	354.67 ± 61.77	−2.46 ± 1.70	6 (31.6%)	10 (52.6%)	16 (84.2%)
Significance	$p < 0.418$	$p < 0.202$	$p < 0.396$	$p < 0.250$			$p < 0.134$
Arm							
Sufficient (n = 17)	0.70 ± 0.11	97.90 ± 77.51	114.01 ± 66.20	−1.38 ± 1.75	2 (11.8%)	9 (52.9%)	11 (64.71%)
Insufficient (n = 19)	0.66 ± 0.81	149.11 ± 210.10	117.50 ± 54.00	−2.11 ± 0.66	8 (42.1%)	10 (52.6%)	18 (94.74%)
Significance	$p < 0.163$	$p < 0.049$	$p < 0.571$	$p < 0.0001$			$p < 0.028$
T-spine							
Sufficient (n = 17)	0.66 ± 0.30	138.88 ± 148.64	186.43 ± 137.08	−2.52 ± 2.37	3 (17.6%)	11 (64.7%)	14 (82.35%)
Insufficient (n = 19)	0.62 ± 0.28	127.48 ± 129.68	195.41 ± 185.51	−2.94 ± 2.13	2 (10.5%)	13 (68.4%)	17 (89.47%)
Significance	$p < 0.995$	$p < 0.605$	$p < 0.768$	$p < 0.697$			$p < 0.891$
Pelvis							
Sufficient (n = 17)	0.69 ± 0.21	181.04 ± 101.26	261.59 ± 111.48	−3.45 ± 2.59	—	11 (78.6%)	11 (78.6%)
Insufficient (n = 19)	0.63 ± 0.21	150.81 ± 90.22	240.77 ± 102.55	−3.97 ± 2.59	2 (9.1%)	18 (81.8%)	20 (90.9%)
Significance	$p < 0.708$	$p < 0.671$	$p < 0.861$	$p < 0.742$			$p < 0.525$
Ribs							
Sufficient (n = 17)	0.67 ± 0.14	87.16 ± 43.18	122.54 ± 62.62	−2.83 ± 2.17	4 (23.5%)	9 (52.9%)	13 (76.47%)
Insufficient (n = 19)	0.60 ± 0.09	78.60 ± 45.86	129.52 ± 72.90	−3.37 ± 1.67	1 (5.3%)	18 (94.7%)	19 (100.0%)
Significance	$p < 0.007$	$p < 0.902$	$p < 0.679$	$p < 0.0001$			$p < 0.013$
L-spine							
Sufficient (n = 17)	0.55 ± 0.19	69.19 ± 37.30	121.06 ± 38.03	−3.46 ± 1.51	2 (11.8%)	14 (82.4%)	16 (94.12%)
Insufficient (n = 19)	0.50 ± 0.20	53.63 ± 31.06	99.11 ± 37.06	−3.37 ± 1.51	—	16 (84.2%)	16 (84.2%)
Significance	$p < 0.690$	$p < 0.185$	$p < 0.616$	$p < 0.809$			$p < 0.220$
Total body							
Sufficient (n = 17)	0.74 ± 0.12	1180.28 ± 391.30	1550.55 ± 320.32	−2.80 ± 1.65	3 (17.6%)	13 (76.5%)	16 (94.1%)
Insufficient (n = 19)	0.70 ± 0.12	1030.92 ± 418.16	1437.96 ± 445.48	−3.41 ± 1.59	2 (10.5%)	16 (84.2%)	18 (94.7%)
Significance	$p < 0.720$	$p < 0.534$	$p < 0.124$	$p < 0.849$			$p < 0.819$

BMC, bone mineral content (g); BMD, bone mineral density (g/cm^2).
Data are presented as the mean ± SD (range) or number (%) as appropriate; cross-sectional area (cm^2).

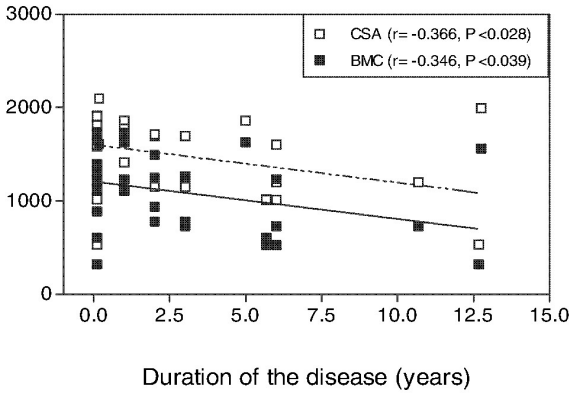

Fig. 1. Correlation between duration of disease (years) and bone mineral content (BMC, g), and cross-section area (CSA, cm^2) of total body.

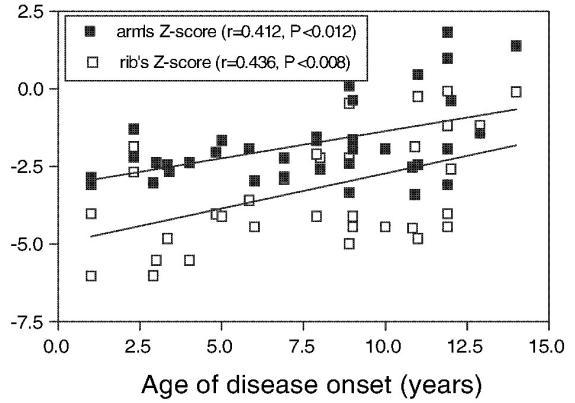

Fig. 2. Correlation between age of onset of the disease (years) and *Z*-score of arms and ribs.

recruitment, proliferation, and differentiation as well as reduced osteoblast apoptosis which were thought to be key regulatory components (21). Furthermore, PTH-induced increase in bone formation involved increased local production but not increased circulating levels of IGF-1 (47). Insulin plus PTH resulted in greater bone recovery in diabetic rats compared with insulin or PTH treatment alone (48). Therefore, it will be necessary to explore further the potential anabolic effect of PTH on bone cells, as its synergism with insulin may be an important feature of insulin's actions on bone.

Dysregulation of IGF-1 is well-recognized in children and adolescents with T1DM. In agreement with Emily et al. (49), serum level of IGF-1 was reduced in T1DM when compared with healthy controls in the present study. However, other investigators reported normal (50) or elevated (51) IGF-1 levels in T1DM. This difference can be contributed to different age, puberty stage, and metabolic control in different studies. IGF-1 functions as a key anabolic

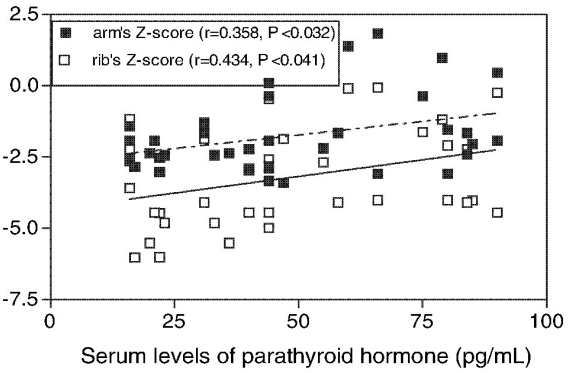

Fig. 3. Correlation between serum levels of parathyroid hormone (pg/mL) and *Z*-score of arms and ribs.

regulator of bone cell activity by decreasing collagen degradation and by increasing bone matrix deposition and osteoblastic cell recruitment (8). In addition, IGF-1 is a hormone that stimulates muscle protein synthesis and impairs protein degradation (52). The anabolic action of IGF-1 on muscle protein synthesis builds up the muscle mass and increases its strength (53), which is a well-known osteoprotective mechanism. The importance of circulating IGF-1 for bone metabolism is supported by many clinical studies, which have proved correlation between serum IGF-1 and bone density or bioptic parameters (osteoblastic activity, volume, and mineralization of the osteoid) (54). Circulating IGF-1 is produced primarily by the liver, and decreased levels in T1DM may result from reduced hepatic delivery of insulin (55). However, IGF-1 is also produced locally in muscle and bone tissues where it mediates important paracrine effects on bone metabolism (23).

In partial consistence with others (56, 57), we observed that both axial and peripheral bones were reduced in T1DM patients with highest reduction in total body (94.9%) followed by L-spine (88.9%), ribs (88.9%), pelvis (86.1%), arms (80.6%), T-spine (80.6%), and then legs (77.8%), meanwhile, no abnormal bone status was observed in the head bone status compared with a non-diabetic reference Egyptian population, implying a relatively rapid impact of T1DM on bone health. Gunczler et al. (58) reported significant deficits in L-spine BMD in >50% of children with T1DM ($n = 26$), mean age (12.1 ± 3.1 yr), and a mean duration of diabetes mellitus (DM) (4.3 ± 2.9 yr). Kemink et al. (42) found that, among middle-aged patients ($n = 35$) with T1DM (duration of DM, 8.5 ± 3.5 yr), 57% of females and 67% of males had osteopenia of femoral neck and/or L-spine and 14% of males met criteria for osteoporosis. In contrary, others had shown normal (3, 4) or even increased BMD in T1DM individuals (59). This discrepancy between reports may be because of several factors, including

differences in methods of BMD measurements, study design, and patient selection. In addition, in the present study, all the patients had been diagnosed before puberty and, at the time of the assessment, 5.6% had already achieved pubertal development. Thus, maximum peak bone mass was acquired during the course of the disease. This leads to high prevalence of affection of bone status in our T1DM patients. In this study, the axial bones were affected more than peripheral bones in T1DM patients. This can be explained by the fact that prepubertal children have accelerated growth in the peripheral skeleton, whereas during puberty, accelerated growth occurs in the trunk or axial skeleton (60). The pathophysiology of low BMD in T1DM is poorly understood. The lack of insulin had been linked to impaired gene expression with suppressed function and maturation and altered differentiation of the osteoblasts, coupled with inhibition of apoptosis (61). In this study, 61.10% of the participants had mean HbA1c values $\geq$8.2, indicating that the majority of our patients had chronic, mild to severe hyperglycemia during the previous 12 months. In agreement with others (3, 58, 62), no significant correlation between HbA1c level and any of measured bone status parameters was found in this study. Meanwhile, others (4) found a clearly negative association between BMD and HbA1c.

The association of bone status measured parameters in diabetic patients with variables such as age, BMI, and disease duration had been reported in different literatures. In consistence (63), low bone measured parameters were already apparent at the time of diagnosis in diabetic patients in the present study, indicating genotypic covariant predisposition may be more important than disease metabolic consequences on bone status. On the contrary, others reported that neither indexes of bone formation (64) nor BMD (3) were impaired in the recently diagnosed T1DM children. Also, in this study, the disease duration correlated negatively with BMC and CSA of total body. These results strengthen the need for deep systematic inquiry into bone metabolism in young patients with long duration diabetes. In this respect, Gunczler et al. (65) reported that disease duration had a negative impact on bone mass, although again, some data are contradictory and reported no relationship (3, 42, 58). Campos-Pastor et al. (57) reported that optimization of metabolic control could lead to a cessation of bone destruction after 7-yr duration of diabetes. In the present research, age of onset correlated positively with BMD and *Z*-score of arms and ribs suggesting that osteopenia in diabetic individuals might be attenuated with the progression of age. Puberty stage was correlated positively with BMD of ribs; BMC and CSA of L-spine and *Z*-score of arms. In the few studies in which pubertal stage of the patients was taken into consideration, BMD-*Z*-scores were found to have no relationship with pubertal stage (3, 59). It is well established that at the end of puberty, sex hormones determine epiphyseal growth plate fusion and decrease in biologic bone metabolism markers (66). Therefore, it could be speculate that the onset of puberty has a 'protective' role on bone, in opposition with potential bone loss, which is rather a complication of an early stage of the disease.

Limitation of the study

The major limitation of this study is the relatively small number of patients. Although all results have been tested statistically, the number of children with vitamin D deficiency was small. This imposes restriction on generalizing these results and calls for a larger study to confirm these preliminary findings. Despite this limitation, we believe that this study adds to the existing scarce literature on the effects of DM on bone loss in children and adolescents.

In conclusion, individuals with T1DM possess multiple risk factors for skeletal fragility mainly because of the insufficiency of 25(OH)D, IGF-1, and increase disease duration. However, because osteopenia-osteoporosis prevention is supported by nutritional, physical, and endocrine factors, it must be started early by encouraging intensive metabolic control in diabetic children. Physical activity, necessity for sunlight exposure and supplementing infants with calcium and vitamin D/fortification of food might be a safe and effective strategy for reducing the risk, and also in management, of T1DM. The cause of increased PTH in T1DM and its positive association with many of bone status measured parameters is evident by this study, but it needs further confirmation by a large number of cases as well as by population-based studies.

References

1. Ambler G, Fairchild J, Craig M, Cameron F. Contemporary Australian outcomes in childhood and adolescent type 1 diabetes: 10 years post the Diabetes Control and Complications Trial. J Paediatr Child Health 2006: 42: 403–410.
2. Holmberg A, Johnell O, Nilsson P, Nilsson J, Berglund G, Akesson K. Risk factors for fragility fracture in middle age. A prospective population-based study of 33,000 men and women. Osteoporos Int 2006: 17: 1065–1077.
3. De Schepper J, Smitz J, Rosseneu S, Bollen P, Louis O. Lumbar spine bone mineral density in diabetic children with recent onset. Horm Res 1998: 50: 193–196.
4. Brandao F, Vicente E, Daltro C, Sacramento M, Moreira A, Adan L. Bone metabolism is linked to disease duration and metabolic control in type 1 diabetes mellitus. Diabetes Res Clin Pract 2007: 78: 334–339.

5. Leger J, Marinovic D, Alberti C et al. Lower bone mineral content in children with type 1diabetes mellitus is linked to female sex, low insulin- like growth factor type 1 levels, and high insulin requirement. J Clin Endocrinol 2006: 91: 3947–3953.
6. Saggese G, Baroncelli GI, Bertelloni S. Puberty and bone development. Best Pract Res Clin Endocrinol Metab 2002: 16: 53–64.
7. Bloch CA, Clemons P, Sperling MA. Puberty decreases insulin sensitivity. J Pediatr 1987: 110: 481–487.
8. Yakar S, Rosen C, Beamer W et al. Circulating levels of IGF-1 directly regulates bone growth and density. J Clin Invest 2002: 110: 771–781.
9. Holick M. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers and cardiovascular disease. Am J Clin Nutr 2004: 80: 1678S–1688S.
10. Cashman K. Calcium and vitamin D. Novartis Found Symp 2007: 282: 123–138.
11. Zold E, Szodoray P, Gaal J et al. Vitamin D deficiency in undifferentiated connective tissue disease. Arthritis Res Ther 2008: 10: R123 (doi:10.1186/ar2533).
12. Maalouf G, Gannagé-Yared MH, Ezzedine J et al. Middle East and North Africa consensus on osteoporosis. J Musculoskelet Neuronal Interact 2007: 7: 131–143.
13. Pettifor JM. Nutritional rickets: deficiency of vitamin D, calcium, or both? Am J Clin Nutr 2004: 80: 1725S–1729S.
14. Thacher TD, Fischer PR, Strand MA, Pettifor JM. Nutritional rickets around the world: causes and future directions. Ann Trop Paediatr 2006: 26: 1–16.
15. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001: 22: 477–501.
16. Rewers M, Norris J, Dabelea D. Epidemiology of type 1 diabetes mellitus. Adv Exp Med Biol 2004: 552: 219–246.
17. Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. Diabetologia 2005: 48: 1247–1257.
18. Zeitz U, Weber K, Soegiarto D, Wolf E, Balling R, Erben R. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. FASEB J 2003: 17: 509–511.
19. Gysemans C, Cardozo A, Callewaert H et al. 1,25-Dihydroxyvitamin D3 modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in non-obese diabetic mice. Endocrinology 2005: 146: 1956–1964.
20. Adams J, Liu P, Chun R, Modlin R, Hewison M. Vitamin D in defense of the human immune response. Ann N Y Acad Sci 2007: 1117: 94–105.
21. Poole K, Reeve J. Parathyroid hormone – a bone anabolic and catabolic agent. Curr Opin Pharmacol 2005: 5: 612–617.
22. Whitfield J, Morley P, Willick G. Parathyroid hormone, its fragments and their analogs for the treatment of osteoporosis. Treat Endocrinol 2002: 1: 175–190.
23. Garay-Sevilla M, Nava L, Malacara J, Wróbel K, Pérez U. Advanced glycosylation end products, insulin-like growth factor-1 and IGF-binding protein-3 in patients with type 2 diabetes mellitus. Diabetes Metab Res Rev 2000: 16: 106–113.
24. Pereira R, Canalis E. Parathyroid hormone increases mac25/insulin-like growth factor-binding protein-related protein-1 expression in cultured osteoblasts. Endocrinology 1999: 140: 1998–2003.
25. Farley J, Dimai H, Stilt-Coffing B, Farley P, Pham T, Mohan S. Calcitonin increases the concentration of insulin-like growth factors in serum-free cultures of human osteoblast-line cells. Calcif Tissue Int 2000: 67: 247–254.
26. Pastor MMC, Lopez-Ibarra PJ, Escobar-Jimenez F, Pardo SMD, Garcia-Cervigon A. Intensive insulin therapy and bone mineral density in type 1 diabetes mellitus: a prospective study. Osteoporos Int 2000: 11: 455–459.
27. Taylor S, Whincup P, Hindmarsh P, Lampe F, Odoki K, Cook D. Performance of a new pubertal self-assessment questionnaire: a preliminary study. Paediatr Perinat Epidemiol 2001: 15: 88–94.
28. Heap J, Murray M, Miller S, Jalili T, Moyer-Mileur L. Alterations in bone characteristics associated with glycemic control in adolescents with type 1 diabetes mellitus. J Pediatr 2004: 144: 56–62.
29. Iwanicka Z, Lewandowicz-Uszyńska A, Głab E, Kotschy B. Relationship between nitrogen oxide and the degree of metabolic control of diabetes mellitus type 1 in children and adolescents. Wiad Lek 2006: 59: 27–31.
30. Schlosser K, Schmitt CP, Bartholomaeus JE et al. Parathyroidectomy for renal hyperparathyroidism in children and adolescents. World J Surg 2008: 32: 801–806.
31. Eren E, Yilmaz N. Biochemical markers of bone turnover and bone mineral density in patients with b-thalassemia major. Int J Clin Pract 2005: 59: 46–51.
32. Carnevale V, Romagnoli E, D'Erasmo E. Skeletal involvement in patients with diabetes mellitus. Diabetes Metab Res Rev 2004: 20: 196–204.
33. Littorin B, Blom P, Scholin A et al. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). Diabetologia 2006: 49: 2847–2852.
34. Zemunik T, Skrabic V, Boraska V et al. FokI polymorphism, vitamin D receptor, and interleukin-1 receptor haplotypes are associated with type 1 diabetes in the Dalmatian population. J Mol Diagn 2005: 7: 600–604.
35. Huynh T, Greer R, Nyunt O et al. The association between ketoacidosis and 25(OH)-vitamin D3 levels at presentation in children with type 1 diabetes mellitus. Pediatr Diabetes 2009: 10: 38–43.
36. Kawashima H, Kraut JA, Kurokawa K. Metabolic acidosis suppresses 25-hydroxyvitamin in D3-1alpha-hydroxylase in the rat kidney. Distinct site and mechanism of action. J Clin Invest 1982: 70: 135–140.
37. Svoren BM, Volkening LK, Wood JR, Laffel LMB. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. J Pediatr 2009: 154: 132–134.

38. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005: 16: 713–716.
39. Nejentsev S, Cooper J, Godfrey L et al. Analysis of the vitamin D receptor gene sequence variants in type 1 diabetes. Diabetes 2004: 53: 2709–2712.
40. Pozzilli P, Manfrini S, Crinò A et al. Low levels of 25- hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 in patients with newly diagnosed type 1 diabetes. Horm Metab Res 2005: 37: 680–683.
41. Malabanan A, Veronikis I, Holick M. Redefining vitamin D insufficiency. Lancet 1998: 351: 805–806.
42. Kemink S, Hermus A, Swinkels L, Lutterman J, Smals A. Osteopenia in insulin-dependent diabetes mellitus: prevalence and aspects of pathophysiology. J Endocrinol Invest 2000: 23: 295–303.
43. Sugimoto T, Ritter C, Morrissey J, Hayes C, Slatopolsky E. Effects of high concentrations of glucose on PTH secretion in parathyroid cells. Kidney Int 1990: 37: 1522–1527.
44. Zitterman A. Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr 2003: 89: 552–572.
45. Taylor WH, Khaleeli AA. Coincident diabetes mellitus and primary hyperparathyroidism. Diabetes Metab Res Rev 2001: 17: 175–180.
46. Kleerekoper M, Nelson DA, Peterson EL et al. Reference data for bone mass, calciotropic hormone, and biochemical markers of bone remodelling in older (55–75) postmenopausal white and black women. J Bone Miner Res 1994: 9: 1267–1276.
47. Miyakoshi N, Kasukawa Y, Linkhart T, Baylink D, Mohan S. Evidence that anabolic effects of PTH on bone require IGF-I in growing mice. Endocrinology 2001: 142: 4349–4356.
48. Suzuki A, Kotake M, Ono Y et al. Hypovitaminosis D in type 2 diabetes mellitus: association with microvascular complications and type of treatment. Endocr J 2006: 53: 503–510.
49. Emily Y, Wactwski-Wende J, Richard P, Dmochowski J, Hovey K, Quattrin T. Dose low bone mineral density start in post-teenage years in women with type 1 diabetes? Diabetes Care 2003: 26: 2365–2369.
50. Tamborlane W, Hintz R, Bergman M, Genel M, Felig P, Sherwin R. Insulin-infusion-pump treatment of diabetes: influence of improved metabolic control on plasma somatomedin levels. N Engl J Med 1981: 305: 303–307.
51. Cohen M, Jastik A, Rye D. Somatomedin in insulin dependent diabetes mellitus. J Clin Endocrinol Metab 1977: 45: 236–239.
52. Frost R, Lang C, Gelato M. Transient exposure of human myoblasts to tumor necrosis factor-α inhibits serum and insulin-like growth factor-I stimulated protein synthesis. Endocrinology 1997: 138: 4153–4159.
53. Kanda F, Okuda S, Matsushita T, Takatani K, Kimura K, Chihara K. Steroid myopathy: pathogenesis and effects of growth hormone and insulin-like growth factor-I administration. Horm Res 2001: 56: 24–28.
54. Reed B, Zerwekh H, Sakhaee K, Breslau N, Gottschalk F, Pak C. Serum IGF 1 is low and correlated with osteoblastic surface in idiopathic osteoporosis. J Bone Miner Res 1995: 10: 1218–1224.
55. Frystyk J, Ritzel R, Maubach J et al. Comparison of pancreas-transplanted type 1 diabetic patients with portal venous versus systemic-venous graft drainage: impact on glucose regulatory hormones and the growth hormone/insulin-like growth factor-I axis. J Clin Endocrinol Metab 2008: 93: 1758–1766.
56. Rix M, Andreassen H, Eskildsen P. Impact of peripheral neuropathy on bone density in patients with type 1 diabetes. Diabetes Care 1999: 22: 827–831.
57. Campos-Pastor M, Lopez-Ibarra P, Escobar-Jimenez F, Serrano-Pardo M, Garcia-Cervigon G. Intensive insulin therapy and bone mineral density in type 1 diabetes mellitus: A prospective study. Osteoporos Int 2000: 11: 455–459.
58. Gunczler P, Lanes R, Paz-Martinez V et al. Decreased lumbar spine bone mass and low bone turnover in children and adolescents with insulin dependent diabetes mellitus followed longitudinally. J Pediatr Endocrinol Metab 1998: 11: 413–419.
59. Pascual J, Argente J, Lopez M et al. Bone mineral density in children and adolescents with diabetes mellitus type 1 of recent onset. Calcif Tissue Int 1998: 62: 31–35.
60. Bass S, Delmas P, Pearce G, Hendrich E, Tabensky A, Seeman E. The differing tempo of growth in bone size, mass, and density in girls is region-specific. J Clin Invest 1999: 104: 795–804.
61. Botolin S, McCabe L. Chronic hyperglycemia modulates osteoblast gene expression through osmotic and non-osmotic pathways. J Cell Biochem 2006: 99: 411–424.
62. Alexopoulou O, Jamart J, Devogelaer J, Brichard S, de Nayer P, Buysschaert M. Bone density and markers of bone remodeling in type 1 male diabetic patients. Diabetes Metab 2006: 32: 453–458.
63. Lopez-Ibarra P, Pastor M, Escobar-Jimenez F et al. Bone mineral density at time of clinical diagnosis in adult-onset type 1 diabetes mellitus. Endocr Prac 2001: 7: 346–351.
64. Bonfanti R, Mora S, Prinster C et al. Bone modeling indexes at onset and during the first year of follow-up in insulin dependent diabetic children. Calcif Tissue Int 1997: 60: 397–400.
65. Gunczler P, Lanes P, Paoli M, Martins R, Villaroel O, Weisinger J. Decreased bone mineral density and bone formation markers shortly after diagnosis of clinical type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2001: 14: 525–528.
66. Saggese G, Bertelloni S, Baroncelli G, Federico G, Calisti L, Fusaro C. Bone demineralization and impaired mineral metabolism in insulin-dependent diabetes mellitus: a possible role of magnesium deficiency. Helv Paediatr Acta 1989: 43: 405–414.