

Bone mass and sex steroids in postmenarcheal adolescents and adult women with Type 1 diabetes mellitus[☆]

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Abstract

Objective: The aim of this study was to compare the bone mass in young adolescents and adult women with Type 1 diabetes mellitus (T1DM) and determine its relationship with sex steroid and sex hormone-binding globulin (SHBG) levels. **Design:** Cross-sectional study. **Patients:** We studied a group of adolescents and adult women with T1DM ($n=45$) and 50 healthy controls (C) matched by gynecological age and body mass index in a case-control study. Girls with menarche within the last 18–40 months ($n=17$ T1DM and 32 C) and adult women (age=30.4±1.4 years; $n=28$ T1DM and 18 C) were recruited. **Measurements:** Bone mass was evaluated with a GE Lunar Prodigy densitometer. Sex steroid levels were measured by radioimmunoassay. **Results:** Bone mass was lower in adolescents with T1DM than in control adolescents, but was similar in both groups of postmenarcheal girls after adjusting for age, lean, and fat mass. However, adult T1DM women exhibited lower adjusted and unadjusted ($P<.05$) Z-femoral neck (-0.2 ± 0.2 vs. 0.4 ± 0.2) and bone mineral content (BMC) (2306 ± 61 vs. 2645 ± 79 g) than adult controls. Adult controls and T1DM adults showed higher whole body BMC than adolescent controls and T1DM adolescents, respectively. Bone mass in T1DM did not correlate with estradiol, free estradiol, testosterone, SHBG, or HbA1c levels. **Conclusions:** The diminished bone mass observed in adult T1DM women does not appear to be related to sex steroid levels. In young adolescents with T1DM, the observed decrease in bone mass appears to be related to differences in body composition and age.

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1. Introduction

An increased risk of hip fracture has been described in women with Type 1 diabetes mellitus (T1DM) (Janghorbani, Van Dam, Willett, & Hu, 2007). In postmenopausal T1DM women, this risk increases 12 times, compared to women of the same age without diabetes (Nicodemus & Folsom, 2001).

Even middle-aged women with T1DM may exhibit an increased risk of developing a bone fracture (Strotmeyer, Cauley, Orchard, Steenkiste, & Dorman, 2006).

The relationship between hypoestrogenism and osteoporosis has been documented in nondiabetic women. In T1DM, hypoestrogenism, earlier menopause, and ovarian ageing (Soto et al., 2009), menstrual irregularities and pubertal delay have been described (Codner, 2008; Codner & Cassorla, 2009). These may be signs of estrogen deficiency (Codner et al., 2004; Gaete et al., 2009; Rohrer et al., 2007; Snell-Bergeon et al., 2008; Strotmeyer, Steenkiste, Foley, Berga, & Dorman, 2003). In addition, lower basal and stimulated estrogen levels have also been described in

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T1DM women (Codner et al., 2005; Salonia et al., 2006). In contrast, we observed higher testosterone levels in women with T1DM (Codner et al., 2006). These findings may represent a protective factor against osteoporosis.

Sex hormone-binding globulin (SHBG) levels have also been associated with a risk of osteoporotic fractures in nondiabetic, postmenopausal women. Higher levels of SHBG are associated with a higher frequency of osteoporotic fractures (Lee et al., 2008). This may be because high SHBG levels lead to lower unbound sex steroid levels. Patients with T1DM may exhibit increased SHBG levels due to lower insulin concentrations in the portal vein (Yki-Jarvinen, Makimattila, Utriainen, & Rutanen, 1995). This may represent another mechanism by which osteopenia is induced in T1DM women.

Studies conducted in the 1970s demonstrated osteopenia in children with T1DM and poor metabolic control (Rosenbloom et al., 1977; Santiago et al., 1977). Although several recent studies have demonstrated bone mass loss in adults with T1DM, whether such loss starts early in adolescence or later in life is controversial. A study by Liu et al. (2003) found that bone loss appears to start after the second decade of life. In contrast, Leger et al. (2006) demonstrated that diminished bone mass is already evident in girls younger than 20 years of age.

Bone mass increases progressively in healthy girls during the second decade of life (Chevalley, Bonjour, Ferrari, & Rizzoli, 2008; Lu, Cowell, Lloyd-Jones, Briody, & Howman-Giles, 1996). The age of menarche and the time that has elapsed since this event are important factors determining bone mass during adolescence (Chevalley et al., 2008; Lu et al., 1996). The aim of this study was to investigate whether low bone mass can be detected early during adolescence in girls with and without T1DM matched by gynecological age. In addition, we determined the bone mass of adult T1DM women of fertile age. Finally, we investigated the relationship of bone mass with sex steroids, SHBG and metabolic control.

2. Material and methods

2.1. Subjects

We studied young 49 adolescent (17 T1DM) girls who had their menarche within the last 18–40 months, as well as 46 adult women (20–39 years of age, 28 T1DM). Both groups were matched by gynecological age and body mass index (BMI) with control subjects.

Patients with DM1 were included in this study if they fulfilled the following inclusion criteria: persistent severe insulinopenia, diagnosed with DM1 from the onset of the disease. Exclusion criteria were specific types of DM; Type 2 DM; honeymoon period defined as HbA1c and daily insulin dose lower than 7% and 0.5 U/kg per day, respectively (Couper & Donaghué, 2007; Lombardo et al., 2002);

abnormal thyroid function; elevated creatinine levels; presence of micro- or macrovascular complications, and presence of other concomitant chronic conditions such as genetic syndromes; celiac disease; and renal, liver or cardiac disease or undernourishment. Most of the patients had DM1 duration for at least three years. Only two patients had DM1 for 2 years. Insulin dose was not an exclusion/inclusion criterion. The patients did not have any history of bone disorder.

The control group included women with regular menstrual cycles, no history of hyperandrogenism, and normal blood glucose levels. Exclusion criteria for both groups of women included any condition that might affect bone metabolism. Such conditions included celiac disease, non-treated hypothyroidism/hyperthyroidism, amenorrhea of any etiology, pregnancy during the last six months, and the use of calcium supplements, biphosphonates, oral contraceptives, or other steroid drugs.

The study was approved by the Institutional Review Board of San Borja-Arriarán Hospital. Adult women signed informed consents. Parents and adolescents signed consent and assent forms, respectively.

2.2. Laboratory study

Body composition and bone mineral density (BMD) of the spine, femoral neck, and whole body, as well as whole body bone mineral content (BMC), were evaluated with a Lunar Prodigy densitometer coupled to software Encore 2005, version 9.30.044 (GE Lunar Corp, Madison WI, USA). The Z-score was calculated using the NHANES III and Del Río-Carrascosa references for adults and adolescents (del Rio et al., 1994).

An early morning blood sample was obtained in both groups of adult women during the follicular phase (Days 1–7). This blood was used for the measurement of estradiol, testosterone (T), and sex hormone binding globulin (SHBG), as previously described (Codner et al., 2005). The free estradiol value was calculated using Vermeulen's formula (Vermeulen, Verdonck, & Kaufman, 1999). HbA1c levels were measured using a commercially available automatic system (DCA 2000, Bayer Diagnostics, Tarrytown, NY, USA).

2.3. Statistical analysis

The Z-scores and bone mineral density were compared in T1D and control adolescents. The same comparison was made in adult women. Bone mineral content (BMC) was also compared between adults and adolescents.

Bone density parameters were analyzed using a Kolmogorov–Smirnov test for normally distributed data. The analysis of clinical and anthropometrical variables was performed using a Student's *t* test and an χ^2 -test. Unadjusted differences in bone mass were analyzed with a Student's *t* test. The variables were adjusted for age, total lean mass,

and total fat mass using a linear regression analysis. The adjusted and unadjusted analyses are presented.

In adult women with T1DM, correlations between HbA1c, diabetes duration, total estrogen, free estrogen, SHBG and testosterone levels were determined using Pearson's correlation analysis. A linear regression analysis was conducted for adult women with T1DM, to determine the effect of HbA1c, diabetes duration, and daily insulin dose on bone mineral density. An additional analysis assessed the effect of total estrogen, free estrogen, SHBG, and testosterone levels on bone mineral density. Results are expressed as mean±S.E.

3. Results

The clinical characteristics of patients and controls are shown in Table 1. By study design, gynecological age and BMI of both groups were similar. Menarche occurred later in T1DM, explaining the older age of the T1DM adolescent group. T1DM adolescents and adults had similar metabolic control; however, diabetes duration was longer in T1DM adults, as expected.

The hormonal levels are shown in Table 2. Similar levels of gonadotrophins, estradiol, free estrogen index and 17-OH-progesterone was observed in both groups. Adults with T1DM showed higher androstenedione levels than adult C. Testosterone levels were almost significantly higher in T1DM than C adults ($P=.08$). Adolescents with T1DM had lower free androgen index associated to a higher levels of SHBG.

Bone mass (Z-score) and BMC results are shown in Fig. 1. The spine, femoral neck and total body Z-scores were lower in adolescents with T1DM. However, after adjusting for age and body composition, bone mass was similar in both groups of adolescents. Total BMC was similar in both groups of adolescents.

Adults with T1DM exhibited lower Z-spine, Z-femoral neck, Z-whole body and BMC scores than control adults.

This difference persisted for Z-femoral neck and BMC after adjusting for age, total lean mass, and total fat mass. Adults with T1DM showed lower femoral neck BMD than control ($P=.012$), but this difference was not significant after adjusting for age and body composition. Control and T1DM adult women showed higher whole body BMC and spine BMD than controls and T1DM adolescents, respectively. Control adults exhibited higher femoral neck BMD than control adolescents ($P=.07$). In contrast, adults and adolescents with T1DM had similar femoral neck BMD.

Bone mineral density in adult women with T1DM was evaluated by Z-spine, Z-whole body, and BMC. Bone mineral density showed no correlation with HbA1c, insulin dose, time of diabetes duration, estradiol, testosterone, SHBG levels, or free estradiol index. Z-femoral neck showed a positive correlation with the duration of T1DM ($r=0.3$, $P=.03$).

Regression analysis failed to identify HbA1c, insulin dose, or the duration of the disease as significant factors in the determination of bone mass (Z-whole body, Z-spine, Z-femoral neck, or BMC) in adults with T1DM.

4. Discussion

We studied 45 women with T1DM, as well as 50 controls. We demonstrated that bone mineral density is diminished in adult T1DM women during their fertile years, but not in young, postmenarcheal adolescents. These results are similar to those observed by Liu et al. (2003), who described diminished BMD at the femoral neck and lateral spine in adult women with type 1 diabetes who were older than 20 years of age. In this same study, the diminished BMD was not observed in adolescent girls with this condition. In contrast, Leger et al. (2006) observed lower median total bone mineral content and lean body mass in adolescent girls with T1DM, compared to controls.

Table 1

The clinical characteristics of T1DM adolescents, control adolescents, adult T1DM women, and adult control women

	Adolescents		Adults	
	T1DM	Control	T1DM	Control
<i>n</i>	17	32	28	18
Gynecological age (years)	2.6±0.1	2.4±0.1	17.0±1.3	18.8±6.4
Age (years)	15.3±0.2	14.2±0.2 **	29.4±1.3	32.0±1.5
BMI (kg/m ²)	22.2±0.6	23.3±0.8	24.6±0.6	25.3±0.6
Diabetes duration (years)	6.3±0.8	-	11.9±1.3	-
HbA1c (%)	8.3±0.2	-	8.2±0.4	-
Menarche (age)	12.5±0.2	11.8±0.2 **	12.4±1.6	13.1±1.1
Length of the menstrual cycle (days)	32.3±3.6	29.9±0.5	31.3±9.2	28.7±1.7
Pregnancies (<i>n</i>)	0	0	1.5±2.1	0.7±1.2
Live children (<i>n</i>)	0	0	0.9±0.8	0.6±0.9
Menstrual irregularities (%)	11.8	6.3	17.9	0*

Metabolic control in subjects with T1DM is also shown. Data is shown as mean±S.D.

* $P=.06$.

** $P<.01$.

Table 2

Hormone levels of T1DM adolescents, control adolescents, adult T1DM women, and adult control women

	Adolescents		Adults	
	T1DM	Control	T1DM	Control
<i>n</i>	17	32	28	18
Luteinizing Hormone	3.2±2.5	3.1±2.5	5.5±3.1	5.7±1.7
Follicle Stimulating Hormone	5.1±2.2	5.2±1.1	5.2±2.7	6.4±5.0
Estradiol (pg/ml)	68.3±34	51.4±17	69.3±34	74.9±27
Testosterone (ng/dl)	0.40±0.1	0.48±0.2	0.60±0.3	0.46±0.1
Free Androgens Index	2.6±1.4 **	4.6±2.4	5.9±4.9	4.6±3.8
Free Estrogens Index	0.42±0.2	0.40±0.16	0.65±0.5	0.74±0.4
SHBG (nmol/L)	61.8±21.8 **	41.1±12.7	49.4±23.8	49.2±25.6
17-OH-progesterone (ng/ml)	1.0±0.6	1.2±0.5	1.6±0.7	1.3±0.4
Androstenedione (ng/ml)	1.5±0.5	1.4±0.6	1.8±0.8 *	1.3±0.4

Data is shown as mean±S.D.

* *P*<.05.

** *P*<.01 T1DM vs. control.

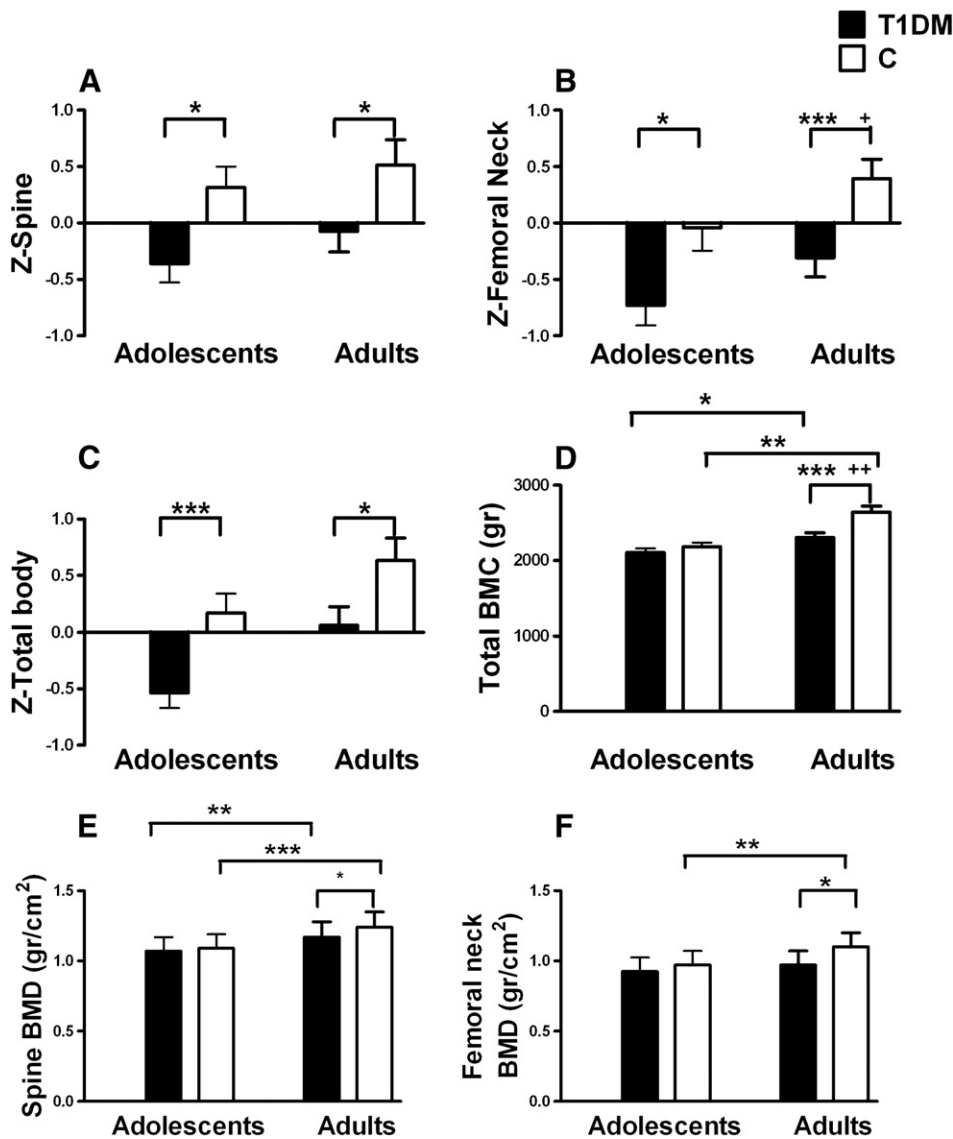


Fig. 1. BMD Z-score in adolescents and adults with and without T1DM. (A) Z-spine. (B) Z-hip. (C) Z-whole body. (D) BMC. (E) Spine BMD. (F) Femoral neck BMD. Black bars indicate patients with T1DM; white bars, control women. **P*<.05; ***P*<.01 ****P*<.001 for unadjusted Student's *t* test; +*P*<.05; ++*P*<.01 for adjusted analysis Student's *t* test.

Healthy women achieve their peak bone mass during their late teens. Estrogen has an important role in the determination of peak bone mass (Armamento-Villareal, Villareal, Avioli, & Civitelli, 1992; Schoenau, 2006). The fact that T1DM women do not reach a peak in bone mass suggests that T1DM alters this process. The alteration may be related to abnormalities in ovarian function (Codner & Cassorla, 2009). Women with T1DM may exhibit a delay in menarche (Codner & Cassorla, 2009) and more menstrual cycle irregularities and use less contraceptive pills (Adcock et al., 1994; Gaete et al., 2009; Snell-Bergeon et al., 2008; Strotmeyer et al., 2003). All of these findings are associated with less estrogen exposure. We did not find a relationship between total or free estradiol index and bone mass. This may be due to the oscillating nature of estrogen secretion or to the fact that total estrogen exposure was not evaluated in this study. Another hypothesis is that despite normal estrogen levels, the bone is not responding normally to estrogen in T1DM women. This may be similar to estrogen's lack of beneficial effects on the cardiovascular system during fertile years in T1DM women.

Testosterone also has beneficial effects on bone mass. It acts directly through testosterone receptors expressed in bone tissue as well as through its aromatization to estradiol (Notelovitz, 2002). Women with hyperandrogenism, such as patients with polycystic ovary syndrome, have increased BMD compared to normal young women (Adami et al., 1998). Previously, we reported higher testosterone levels in T1DM patients (Codner et al., 2006). However, we did not find a correlation between testosterone and BMD in this study.

Recently, it has been reported that higher SHBG levels are associated with a higher risk of hip fracture (Lee et al., 2008). This increased risk occurs through a reduction in sex steroid hormones that are available to interact with receptors on bone. These receptors normally act by regulating the cellular response to sex steroids (Khosla, 2006). Theoretically, this could be another factor involved in the pathogenesis of osteopenia in T1DM. The lack of portal insulin delivery could lead to higher SHBG levels (Yki-Jarvinen et al., 1995) and lower free steroid levels. The fact that adolescents in our study had higher SHBG levels leads to the hypothesis that it may have a detrimental role during pubertal bone accrual (1994).

Insulinopenia or hyperglycemia may play a role in the pathogenesis of osteopenia in T1DM. We did not find an association between the degree of metabolic control, insulin dose, and bone mass. These results are similar to those reported by Liu et al. (Liu et al., 2003). Insulin has an anabolic role in bone (Thraikill, Lumpkin, Bunn, Kemp, & Fowlkes, 2005). In vivo and in vitro studies suggest that insulin promotes bone formation via pro-osteoblastic mechanisms. Insulin deficiency in animal models is associated with alterations in bone microarchitecture. These alterations may be prevented with insulin replacement. Hyperglycemia may be another mechanism of osteopenia

in T1DM (Paul, & Bailey, 1996). Chronic hyperglycemia generates higher concentrations of advanced glycosylation end-products in collagen. These products may reduce bone resistance (Huebschmann, Regensteiner, Vlassara, & Reusch, 2006; Paul, & Bailey, 1996).

Several other mechanisms have been associated with osteopenia in T1DM, including an increase in inflammatory markers (Schwartz, 2003). Other factors include increased osteoprotegerin-receptor activator of the nuclear factor kappa B ligand system (Galluzzi et al., 2005), decreased Insulin-like growth factor-I levels (Leger et al., 2006), genetic variants in the vitamin D receptor (Hauache et al., 1998), and collagen type 1 α -1 (Hampson et al., 1998).

One of the limitations of this study is the lack of bone turnover markers, however previous studies have not observed a relationship between BMD and these markers in patients with T1DM (Mastrandrea et al., 2008). Another limitation of this study is the number of studied subjects, which was relatively low.

In conclusion, our study suggests that the decreased BMD observed in adults with T1DM is apparently unrelated to sex steroid levels or metabolic control. In addition, we observed that during the years following menarche, girls with T1DM do not have decreased bone mass when measurements are adjusted by body composition and chronological age. However, during the ensuing years, adolescents with T1DM do not reach their peak bone mass. Our data suggests that the prevention of decreased bone mass should be pursued in all women with T1DM, independent of their metabolic control and endocrine characteristics.

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