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Bone mineral density and its determinants in diabetes: the Fremantle Diabetes Study

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Abstract *Aims/hypothesis:* We assessed the effects of type 1 and type 2 diabetes on bone density and metabolism. *Materials and methods:* We analysed bone mineral density (BMD) measured at the hip, spine and forearm using dual energy X-ray absorptiometry in 34 patients with type 1 and 194 patients with type 2 diabetes. Patients were from the community-based Fremantle Diabetes Study, and findings for them were compared with those from normal age- and sex-matched control subjects from the local community. Biochemical and hormonal markers of bone metabolism were measured in a subset of 70 patients. *Results:* After adjusting for age and BMI, there was a lower BMD at total hip ($p<0.001$) and femoral neck ($p=0.012$) in type 1 men vs control subjects, but type 1 women and matched controls had similar BMD at each site. There was a higher BMD at total hip ($p=0.006$), femoral neck ($p=0.026$) and forearm ($p<0.001$) in type 2 women vs control subjects, but diabetes status was not associated with BMD in type 2 men after adjustment for age and BMI. Serum oestradiol, BMI, C-terminal telopeptide of collagen type 1 and male sex were consistently and independently associated with BMD at forearm, hip and femoral neck and explained 61, 55 and

50% of the total variance in BMD, respectively, at these sites. Spine BMD was independently associated with BMI and $\ln(\text{oestradiol})$. *Conclusions/interpretation:* Men with type 1 diabetes may be at increased risk of osteoporosis, while type 2 women appear to be protected even after adjusting for BMI. Low serum oestradiol concentrations may predispose to diabetes-associated osteoporosis regardless of sex.

Keywords Bone mineral density · Bone turnover markers · Diabetes mellitus · Dual energy X-ray absorptiometry · Sex hormones

Abbreviations 25OHD: 25-hydroxyvitamin D · ALP: alkaline phosphatase · BMD: bone mineral density · CTx: carboxyl-terminal type I collagen telopeptide · E2: oestradiol · FDS: Fremantle Diabetes Study · FSH: follicle-stimulating hormone · HRT: hormone replacement therapy · LH: luteinising hormone · PTH: parathyroid hormone · SHBG: sex hormone-binding globulin

Introduction

The relationship between diabetes and osteoporosis is complex. A history of diabetes, diabetes duration and chronic complications are associated with an increased risk of osteoporotic fracture [1, 2], while the fracture site may also be influenced by whether or not the patient has diabetes [3]. The association between low bone mineral density (BMD) and diabetes has most consistently been observed in type 1 patients [4, 5], with increased bone mineral loss attributed to early age at diagnosis, long duration, prolonged poor glycaemic control and high insulin doses [6–9]. In type 2 diabetes, the relationship is less clear, with reports of increased [10], decreased [11] or unchanged [12] bone mass and BMD, probably reflecting the heterogeneity of the disease and/or the different study methodologies used.

Several mechanisms may contribute to type 1 diabetes-associated osteopenia, including impaired vitamin D-para-

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thyroid hormone (PTH) regulation [9, 13] and altered bone mineral metabolism [14]. Increased bone resorption and decreased formation have been observed in young poorly controlled patients with type 1 diabetes of long duration [15, 16]. By contrast, low bone turnover has been reported in type 2 diabetes, particularly in insulin-treated patients [17]. Although sex steroids play a significant role in skeletal metabolism in the general population [18–20], there are few equivalent data from diabetic patients.

We therefore investigated the relationship between BMD and diabetes in well-characterised type 1 and type 2 patients from a representative community-based cohort who were matched with healthy non-diabetic controls from the local population. We also examined the role of bone turnover markers and sex steroids in modulating BMD in diabetes.

Subjects, materials and methods

Subjects

We studied 231 patients from the Fremantle Diabetes Study (FDS), a community-based prospective observational study conducted in a postcode-defined population of 120,097 surrounding Fremantle in Western Australia. The FDS protocol and present sub-study were approved by the Fremantle Hospital Human Rights Committee and informed consent was obtained from each patient before participation in both. Between 1993 and 1996, we identified 2,258 eligible patients and recruited 1,426 (63%) for annual assessments, including 127 with type 1 diabetes, 1,294 with type 2 and five with secondary diabetes. Details of identification and recruitment, sample characteristics, diabetes classification by clinical/laboratory criteria and particulars of non-recruited patients have been described elsewhere [21]. The present subset of FDS patients was a convenience sample of those without a self-reported history of osteoporosis who were not taking medication for the disease and who attended an annual assessment during 2000 and 2001. All the present patients were matched individually by age and sex with non-diabetic individuals recruited from the local healthy population whose BMD had been measured for determination of reference ranges [22].

Clinical assessment

Data relating to diabetes, comorbidities and other relevant variables (including exercise levels and tobacco and alcohol consumption) from the FDS annual assessment prior to entry into the present sub-study (an average of 6 months beforehand) were available for each patient. At the present sub-study assessment, weight and height were remeasured. All patients completed a supplementary questionnaire which contained questions relating to (1) conditions predisposing to osteoporosis (family history, hepatic and renal disease, thyroid and parathyroid disease,

inflammatory conditions including rheumatoid arthritis, malabsorption, glucocorticoid use, menopausal status and use of hormone replacement therapy [HRT]); (2) details of past fractures; (3) prior use of any treatment for osteoporosis or any medication influencing bone metabolism; and (4) details of previous bone density measurements. Additional data on fractures were obtained from hospitalisations recorded in the Western Australian Data Linkage System [23].

Nephropathy was taken as a urinary albumin:creatinine ratio >3.0 mg/mmol on a first-morning urine sample. Peripheral sensory neuropathy was defined as a score of $>2/8$ on the clinical portion of the Michigan Neuropathy Screening Instrument [24]. A subject was considered as having retinopathy if any grade of retinopathy, including maculopathy, was detected by direct and/or indirect ophthalmoscopy in one or both eyes and/or on more detailed assessment by an ophthalmologist. Self-reported stroke and transient ischaemic attack were amalgamated with prior hospitalisations to define baseline cerebrovascular disease status [23]. Patients were classified as having CHD if there was a self-reported history of, or hospitalisation for, myocardial infarction, angina, coronary artery bypass grafting, angioplasty, and/or definite myocardial infarction on a Minnesota-coded electrocardiogram [25].

Bone densitometry

Each patient attended for measurement of BMD at hip, spine (L1–L4) and forearm using dual-energy X-ray absorptiometry (QDR-4500W; Hologic, Bedford, MA, USA) from which T-scores were generated for diabetic patients. For the control subjects for whom T-scores had not been generated, the formula (BMD-young normal mean)/SD was used. Osteoporosis was defined as a T-score at the hip of less than -2.5 [26] and osteopenia as a T-score of -1.0 SD to -2.5 SD. The CV of T-scores was 1.1% at the spine and 1.0–1.2% at the hip [27]. Valid BMD data for two or more sites were obtained for 229 patients (99.1% of the sample), of whom 34 had type 1 and 194 type 2 diabetes.

Measurement of biochemical and hormonal variables

Stored sera from 33 of the 34 type 1 patients were available for measurement of markers and determinants of bone turnover comprising serum calcium, phosphate, albumin, osteocalcin, carboxyl-terminal type I collagen telopeptide (CTx), alkaline phosphatase (ALP), 25-hydroxyvitamin D (25OHD), total testosterone, estradiol (E2), sex hormone-binding globulin (SHBG), luteinising hormone (LH), follicle-stimulating hormone (FSH) and plasma PTH. In addition, sera from a subgroup of 37 of the 194 type 2 diabetic patients, matched for sex and as closely as possible for serum creatinine with the type 1 patients, were also retained for the same range of assays.

Serum calcium, phosphate, albumin and ALP were measured spectrophotometrically (Cobas Integra 800;

Roche Diagnostics, Castle Hill, NSW, Australia). Between-day CV was 1.8% at 2.0 mmol/l calcium, 3.8% at 0.7 mmol/l phosphate and 7.8% at 234 U/l ALP. Plasma PTH, serum beta-CTx and osteocalcin were measured using electrochemiluminescent immunoassays (Elecys 2010 analyser; Roche Diagnostics) with between-day CVs of 4.7% at 3.6 pmol/l for PTH, 4.3% at 0.39 mg/ml CTx and 1.8% at 68 ng/ml osteocalcin. Total serum testosterone was measured by a coated-tube RIA and E2 by a double-antibody RIA (DPC-BioMediq, Doncaster, VIC, Australia). Between-day imprecision was 8.5% at 14 nmol/l testosterone, 11.9% at 44 pmol/l E2 and 10.0% at 121 pmol/l E2 with the lower limit of E2 detection at 10 pmol/l. SHBG, LH and FSH were measured by chemiluminescent immunoassay (Immulite 2000 analyser; DPC-BioMediq). Between-day imprecision was 5.5% at 16 U/l LH, 5.5% at 14.4 U/l FSH and 9.4% at 74 nmol/l SHBG. 25OHD was measured by RIA (Diasorin; Vital Diagnostics, Cleveland, QLD, Australia). Between-day imprecision was 8.4% at 43 nmol/l. Free testosterone was calculated for patients with E2 <150 pmol/l [28].

Statistical analysis

Statistical analyses were performed using SPSS for Windows (version 11; SPSS, Chicago, IL, USA) and SAS for Windows (version 8.02; SAS Institute, Cary, NC, USA). Data are presented as proportions, means±SD, geometric mean (SD range) or, in the case of variables not conforming to a normal or log-normal distribution, median [interquartile range]. Two unrelated groups were compared by Fisher's exact test, Student's *t*-test or Mann-Whitney *U* test. Two related normally distributed groups were

compared using the paired *t*-test. Since age and BMI are known risk factors for low BMD [29, 30], general linear modelling was used to compare BMD between type 1 and type 2 diabetes after adjusting for these variables. Likewise, after adjusting for age and BMI, the effect of diabetic status on BMD was examined using a Generalised Estimating Equations procedure that recognised matching. The Wald statistic for type 3 Generalised Estimating Equation analysis was reported. Multiple linear regression using forward conditional modelling with entry *p*<0.05 and removal *p*>0.10 was used to determine independent associates of BMD. A significance level of *p*<0.05 was used throughout.

Results

Subject characteristics

We studied 23 men and 11 women with type 1 diabetes, and 108 men and 86 women with type 2 diabetes. Details of these patients and the matched controls are shown in Tables 1 and 2. The type 1 males were younger, had lower BMI, higher HDL-cholesterol, and longer disease duration than the type 2 males, but glycaemic control, BP, serum creatinine and neuropathy were not significantly different by diabetes type. The 11 type 1 women were significantly younger, had lower BMI, systolic BP and serum triglycerides, but higher HbA_{1c}, and were less likely to have neuropathy than the 86 with type 2 diabetes, but disease duration and serum creatinine were not significantly different. A higher proportion of women with type 2 diabetes were postmenopausal with approximately 1 in 10 on HRT.

Table 1 Demographic, physical and biochemical variables in patients with type 1 and type 2 diabetes

	Females		<i>p</i> value	Males		<i>p</i> value
	Type 1	Type 2		Type 1	Type 2	
Number	11	86	–	23	108	–
Age (years)	43.5±9.7	65.5±9.6	<0.001	54.4±14.3	66.0±9.1	0.001
Diabetes duration (years)	9.7 [7.1–27.0]	9.1 [7.1–11.9]	0.74	18.8 [11.5–30.1]	8.7 [6.9–12.7]	<0.001
BMI (kg/m ²)	26.1±5.2	31.9±6.1	0.004	25.9±3.2	29.0±4.4	0.002
Fasting plasma glucose (mmol/l)	8.3 [4.8–16.0]	8.6 [7.4–10.9]	0.62	8.7 [7.1–12.5]	8.8 [7.4–10.3]	0.76
HbA _{1c} (%)	8.2 [7.9–8.9]	7.4 [6.5–8.4]	0.043	7.8 [6.7–8.7]	7.4 [6.5–8.2]	0.17
Systolic BP (mmHg)	111±14	137±19	<0.001	146±27	144±20	0.71
Diastolic BP (mmHg)	66±11	68±11	0.56	75±7	76±10	0.76
Total serum cholesterol (mmol/l)	5.4±0.9	5.2±1.0	0.50	5.0±1.4	5.1±1.2	0.77
HDL-cholesterol (mmol/l)	1.52±0.44	1.33±0.70	0.39	1.51±0.44	1.21±0.39	0.002
Serum triglycerides (mmol/l)	1.0 [0.6–1.8]	1.7 [1.0–2.8]	0.001	1.1 [0.5–2.2]	1.5 [0.9–2.6]	0.009
Serum creatinine (µmol/l)	83±13	75±24	0.28	122±67	94±33	0.06
Peripheral neuropathy (%)	18.2	57.7	0.022	47.6	52.4	0.81
Postmenopausal (%)	33.3	90.8	<0.001	–	–	–
Hormone replacement therapy (%)	0	10.5	0.59	–	–	–

Data are proportions, means±SD, geometric mean (SD range) or median [interquartile range]

Table 2 Bone mineral density and T-score comparisons for cases and age- and sex-matched controls by type of diabetes

	Female cases	Female controls	Unadjusted <i>p</i> value	Adjusted ^a <i>p</i> value	Male cases	Male controls	Unadjusted <i>p</i> value	Adjusted ^a <i>p</i> value
Type 1 diabetes								
Number	11	11	–	–	23	23	–	–
Age	43.5±9.7	43.1±8.8	0.93	–	54.4±14.3	54.7±14.7	0.94	–
BMI (kg/m ²)	26.1±5.2	24.9±5.0	0.53	–	25.9±3.2	27.3±2.4	0.10	–
Hip (g/cm ²)	0.885±0.123	0.949±0.128	0.19	0.06	0.947±0.178	1.087±0.178	0.002	<0.001
Hip T-score	-0.753±1.025	-0.218±1.063	0.24	0.06	-0.964±1.371	0.119±1.370	0.010	<0.001
Femoral neck (g/cm ²)	0.789±0.098	0.819±0.127	0.55	0.34	0.775±0.143	0.877±0.153	0.011	0.012
Femoral neck T-score	-1.064±0.982	-0.765±1.272	0.54	0.34	-1.857±1.303	-0.926±1.395	0.024	0.012
Spine (g/cm ²)	1.031±0.114	1.051±0.159	0.72	0.61	1.011±0.196	1.095±0.151	0.051	0.08
Spine T-score	-0.147±1.038	0.032±1.449	0.74	0.61	-0.726±1.783	0.035±1.373	0.11	0.08
Forearm (g/cm ²)	0.551±0.039	0.557±0.064	0.78	0.58	0.622±0.073	0.639±0.061	0.23	0.30
Forearm T-score	-0.259±0.756	-0.136±1.245	0.78	0.58	-1.059±1.359	-0.738±1.122	0.39	0.30
Type 2 diabetes								
Number	86	86	–	–	108	108	–	–
Age	65.5±9.6	64.8±11.1	0.66	–	66.0±9.1	66.3±9.6	0.80	–
BMI (kg/m ²)	31.9±6.1	25.1±3.5	<0.001	–	29.0±4.3	25.9±3.0	<0.001	–
Hip (g/cm ²)	0.993±0.173	0.848±0.118	<0.001	0.006	1.060±0.156	1.013±0.158	0.038	0.88
Hip T-score	0.148±1.440	-1.056±0.983	<0.001	0.006	-0.091±1.197	-0.456±1.218	0.029	0.88
Femoral neck (g/cm ²)	0.808±0.153	0.722±0.103	<0.001	0.026	0.851±0.128	0.802±0.129	0.010	0.34
Femoral neck T-score	-0.870±1.527	-1.726±1.035	<0.001	0.026	-1.167±1.168	-1.608±1.177	0.007	0.34
Spine (g/cm ²)	1.031±0.171	0.948±0.152	0.001	0.59	1.117±0.176	1.102±0.191	0.55	0.72
Spine T-score	-0.144±1.553	-0.897±1.379	0.001	0.59	0.239±1.600	0.101±1.737	0.54	0.72
Forearm (g/cm ²)	0.540±0.066	0.481±0.068	<0.001	<0.001	0.641±0.062	0.627±0.063	0.09	0.54
Forearm T-score	-0.462±1.291	-1.635±1.327	<0.001	<0.001	-0.702±1.143	-0.963±1.163	0.10	0.54

Data are means±SD

^aAdjusted for age and BMI using General Estimating Equation procedures that recognised matching

BMD in type 1 diabetes

BMD and T-score data are summarised in Table 2 and Fig. 1. Type 1 men had significantly lower BMD at hip and femoral neck compared with controls but total spine and forearm

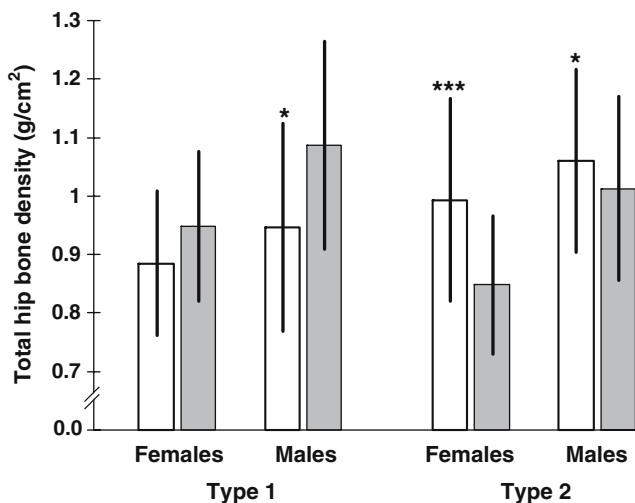


Fig. 1 Total hip bone mineral density (*unadjusted*) for cases with type 1 and type 2 diabetes (*open bars*) and age- and sex-matched controls (*filled bars*). **p*<0.05; ****p*<0.001

BMD were not significantly different. After adjusting for age and BMI, the lower BMD at femoral neck and hip in type 1 men vs controls persisted. There were no significant differences in BMD or T-score at any site in type 1 vs control women, although the observed differences were in the same direction as for the men.

Categories of bone density for the type 1 patients are shown in Table 3. A significantly higher proportion of type 1 diabetic men had osteopenia/osteoporosis at each site than controls. However, this was statistically significant only at the hip and femoral neck, and only at the latter site after adjustment for age and BMI. The distribution of BMD categories in women with type 1 diabetes did not differ from that in matched control subjects.

BMD in type 2 diabetes

BMD and T-score data for the type 2 patients and controls are summarised in Table 2 and Fig. 1. In contrast to the type 1 patients, both the men and women with type 2 diabetes had higher BMD and T-score at the hip (total and femoral neck) than the controls. In addition, in women with type 2 diabetes, spine and forearm BMD and T-score were higher than for control females. The differences in the women persisted at the femoral neck, total hip and forearm, but not

Table 3 Categories of bone density (normal/osteopenia/osteoporosis) for diabetic cases and age- and sex-matched controls

	Female cases (%)	Female controls (%)	<i>p</i> value ^a	Adjusted <i>p</i> value ^b	Male cases (%)	Male controls (%)	<i>p</i> value ^a	Adjusted <i>p</i> value ^b
Type 1								
Hip	54.5/45.5/0	54.5/45.5/0	1.00	0.96	47.8/43.5/8.7	78.3/21.7/0	0.014	0.048
Femoral neck	45.5/54.5/0	63.6/27.3/9.1	0.62	0.51	21.7/34.8/43.5	39.1/52.2/8.7	0.025	0.054
Spine	81.8/18.2/0	70.0/30.0/0	0.48	0.59	54.5/27.3/18.2	73.9/26.1/0	0.12	0.19
Forearm	81.8/18.2/0	72.7/27.3/0	0.56	0.63	52.2/34.8/13.0	60.9/34.8/4.3	0.36	0.57
Type 2								
Hip	76.7/20.9/2.3	44.2/48.8/7.0	<0.001	0.15	75.5/23.6/0.9	66.0/27.4/6.6	0.11	0.78
Femoral neck	54.7/31.4/14.0	18.6/55.8/25.6	<0.001	0.046	37.7/51.9/10.4	25.5/51.9/22.6	0.015	0.30
Spine	70.9/22.1/7.0	52.3/36.0/11.6	0.016	0.81	82.4/13.9/3.7	72.9/21.5/5.6	0.11	0.62
Forearm	69.0/22.6/8.3	32.1/47.6/20.2	<0.001	0.002	56.5/38.0/5.6	53.7/37.0/9.3	0.52	0.92

^aScore statistic for type 3 Generalised Estimating Equation analysis

^bAdjusted for age and BMI

at the spine, after adjusting for age and BMI. In the men, adjusting for age and BMI removed the effect of type 2 diabetes on BMD at the hip sites.

A higher proportion of type 2 diabetic men had normal BMD than controls but this was significant at the femoral neck only, and when adjusted for age and BMI was no longer apparent. Similarly for women, a significantly higher proportion of diabetic women had normal BMD at each site and a lower proportion with osteopenia and osteoporosis, compared with controls, and this persisted even after adjusting for age and BMI.

Demographic, physical and biochemical associates of BMD

In the men with type 1 diabetes, BMD was significantly lower at hip, spine and femoral neck ($p \leq 0.013$), but not at forearm ($p = 0.20$), compared with men with type 2 diabetes. After adjustment for age and BMI, these differences remained significant for the hip and femoral neck ($p \leq 0.033$), but not for the spine ($p = 0.24$). The women with type 1 diabetes had similar BMD to those with type 2 diabetes at the spine, forearm and femoral neck ($p \geq 0.46$),

Table 4 Univariate analyses of bone turnover markers and related hormones in 70 type 1 and type 2 diabetic men and women

	Females			Males		
	Type 1 (<i>n</i> =10)	Type 2 (<i>n</i> =14)	<i>p</i> value	Type 1 (<i>n</i> =23)	Type 2 (<i>n</i> =23)	<i>p</i> value
Age (years)	43.4±10.2	67.2±11.1	<0.001	54.4±14.3	64.3±9.4	0.008
BMI (kg/m ²)	25.4±4.9	30.7±5.5	0.024	25.9 ± 3.2	29.2±3.4	0.003
Serum albumin (g/l)	43.5 [42.0–44.5]	44.5 [41.3–47.0]	0.67	44.0 [42.0–46.0]	45.0 [44.0–47.0]	0.14
Serum calcium (mmol/l)	2.4 [2.0–2.5]	2.4 [1.3–2.5]	0.94	2.4 [2.2–2.5]	2.4 [2.1–2.6]	0.35
Serum phosphate (mmol/l)	1.2±0.2	1.2±0.2	0.99	1.2±0.2	1.1±0.1	0.31
Serum alkaline phosphatase (U/l)	77.0±39.4	80.8±36.1	0.82	69.7±30.5	61.2±29.2	0.39
Serum osteocalcin (ng/ml)	20.1 (14.4–28.2)	18.4 (12.7–26.6)	0.55	25.9 (13.3–50.6)	12.1 (6.9–21.4)	0.001
Serum carboxyl-terminal type I collagen telopeptide (ng/l)	0.15 (0.08–0.28)	0.17 (0.07–0.40)	0.71	0.25 (0.10–0.64)	0.21 (0.07–0.56)	0.51
Serum vitamin D (nmol/l)	61.7±15.9	71.1±18.9	0.23	64.9±17.4	72.3±27.7	0.33
Serum parathyroid hormone (pmol/l)	3.8 (2.3–6.4)	4.0 (2.4–6.7)	0.78	5.6 (2.7–11.9)	3.7 (2.2–6.1)	0.029
Serum sex hormone-binding globulin (nmol/l)	55.3±16.6	32.2±11.2	0.001	41.8±17.9	28.4±15.7	0.021
Total serum testosterone (nmol/l)	1.4 [1.2–1.5]	1.1 [0.7–1.5]	0.23	19.0 [13.6–24.7]	14.2 [10.8–16.7]	0.001
Serum free testosterone (nmol/l) ^a	0.02 ^b	0.02±0.02	0.49	0.38±0.09	0.33±0.11	0.16
Serum oestradiol (pmol/l)	212 (52–859)	18 (6–54)	<0.001	86 (49–151)	61 (23–163)	0.15
Serum luteinising hormone (U/l)	4.7 (1.4–15.8)	12.7 (5.0–31.9)	0.042	4.6 (2.3–9.1)	4.3 (2.0–9.4)	0.80
Serum follicle-stimulating hormone (U/l)	6.6 (1.7–26.6)	29.6 (9.2–95.4)	0.013	6.3 (2.5–15.6)	8.0 (3.4–18.9)	0.43

Values are means±SD, geometric mean (SD range) or median [interquartile range]

^aSerum free testosterone was calculated for patients with E2<150 pmol/l

^b*n*=2 with same value

Table 5 Independent associates of forearm, spine, hip and femoral neck BMD assessed by multiple linear regression analysis

	Females (<i>n</i> =24)			Males (<i>n</i> =46)			All patients (<i>n</i> =70)		
	<i>B</i> ^a	<i>p</i> value	Adjusted <i>R</i> ²	<i>B</i> ^a	<i>p</i> value	Adjusted <i>R</i> ²	<i>B</i>	<i>p</i> value	Adjusted <i>R</i> ²
Forearm									
BMI	–	–	–	0.008	0.003	0.168	0.005	0.001	0.056
ln(E2)	0.018	0.009	0.252	–	–	–	0.013	0.020	0.035
ln(CTx)	–	–	–	–	–	–	–0.021	0.010	0.061
Male sex	–	–	–	–	–	–	0.115	<0.001	0.458
All	–	–	0.252	–	–	0.168	–	–	0.610
Spine									
BMI	–	–	–	0.025	0.001	0.171	0.013	0.003	0.106
ln(E2)	0.036	0.013	0.222	0.068	0.039	0.067	0.052	0.002	0.081
All	–	–	0.222	–	–	0.238	–	–	0.19
Hip									
BMI	0.018	<0.001	0.447	0.024	<0.001	0.187	0.023	<0.001	0.288
ln(CTx)	–	–	–	–0.073	0.004	0.268	–0.061	0.002	0.118
ln(E2)	0.027	0.039	0.090	0.057	0.049	0.050	0.031	0.026	0.038
Male sex	–	–	–	–	–	–	0.147	<0.001	0.106
All	–	–	0.537	–	–	0.505	–	–	0.550
Femoral neck									
Age	–0.004	0.004	0.181	–	–	–	–	–	–
BMI	0.016	<0.001	0.271	0.016	0.004	0.148	0.017	<0.001	0.246
ln(CTx)	–0.062	0.029	0.113	–0.074	0.001	0.282	–0.057	0.001	0.100
ln(E2)	–	–	–	–	–	–	0.028	0.015	0.053
Male sex	–	–	–	–	–	–	0.088	0.003	0.096
All	–	–	0.565	–	–	0.430	–	–	0.495

^a*B* unstandardised regression coefficient

CTx Carboxyl-terminal type 1 collagen telopeptide; E2 oestradiol

but significantly lower BMD at the hip ($p<0.015$). After adjusting for age and BMI, BMD at both hip and femoral neck were significantly lower for the type 1 diabetic women ($p\leq 0.047$). However, when menopausal status and HRT were also included, only the difference in BMD at the hip remained significant ($p=0.023$).

For all the type 2 patients, and after adjusting for age, sex and BMI using multiple linear regression, BMD at the hip and femoral neck were negatively associated with HbA_{1c} ($p\leq 0.003$) and ln(serum triglycerides) ($p\leq 0.009$). BMD at the spine was associated with diastolic BP ($p\leq 0.001$), while forearm BMD was not associated with metabolic control, BP or diabetes duration. BMD at the femoral neck was significantly associated with neuropathy ($p=0.022$) but not CHD, cerebrovascular disease, retinopathy or nephropathy. BMD at the other sites was not associated with these diabetic complications. There were insufficient numbers of type 1 patients to undertake similar analyses.

Biochemical markers of bone metabolism

Table 4 summarises biochemical data for the type 1 and a sample of the type 2 diabetic patients matched by sex and serum creatinine from whom sera were available ($n=70$). Men with type 1 diabetes had higher osteocalcin ($p=0.001$), PTH ($p=0.029$), SHBG ($p=0.021$) and total testosterone

($p=0.001$) than type 2 men, while in women with type 1 diabetes both SHBG ($p=0.001$) and E2 ($p<0.001$) were higher, and LH ($p=0.042$) and FSH ($p=0.013$) were lower, than in those with type 2 diabetes. No differences in albumin, ALP, calcium and CTx concentrations were found between type 1 and type 2 patients in the case of either men or women.

In multiple linear regression analysis of all 46 men with BMD as the dependent variable (Table 5), BMI was an independent predictor of BMD at each site. ln(E2) was significantly associated with BMD at spine and hip but explained less of the variance in BMD than BMI. ln(CTx) was associated with BMD at hip and femoral neck and accounted for a greater proportion of the variance in BMD than BMI. In the 24 women, ln(E2) was independently associated with forearm, spine and hip BMD. BMI was strongly associated with hip BMD and, together with ln(E2), accounted for 54% of the total variance in BMD at this site. BMI, ln(CTx) and age were independently associated with femoral neck BMD, accounting for 57% of the total variance.

When data from all 70 patients were pooled, BMI, ln(E2), ln(CTx) and male sex were consistently and independently associated with BMD at forearm, hip and femoral neck. The inclusion of these four variables explained 61, 55 and 50%, respectively, of the total variance in BMD at these sites, with BMI accounting

for no more than a half of this. Spine BMD was independently associated with BMI and $\ln(E2)$ only. Other variables considered for entry into the models included $\ln(PTH)$, insulin use, vitamin D, calcium and serum creatinine, but these were not independently associated with BMD. After adjusting for the variables in the models presented in Table 5, metabolic control, BP and vascular complications were not independently associated with BMD by site or sex, except for BMD at the spine in females. Total serum cholesterol added significantly to this model (regression coefficient, $B=0.11$, $p=0.005$, adjusted $R^2=0.45$). None of the 70 patients were taking HRT at the time of densitometry and few were smokers, regular alcohol consumers or (in the case of those with type 2 diabetes) insulin-treated, variables that did not prove significant in the various regression models assessed.

Fracture data

Analysis of fractures was limited by their small number in the Linked Database. Only two type 1 patients (5.9%) had been hospitalised for fracture between 1993 and 2003; one suffered two fractures (forearm and femur) while the second suffered a fractured metacarpal bone following a hypoglycaemic episode. Similarly, only 12 (6.3%) of the 189 type 2 patients with linked data were admitted with a fracture, seven before BMD was measured. For self-reported fracture, 55 patients had at least one before recruitment to the present sub-study. There was no correlation between fracture site and osteoporosis from self-reported or Linked Database fractures (data not shown).

Discussion

The present case-control analyses suggest that the patient's sex has an important effect on BMD in diabetes. Our men with type 1 diabetes had reduced BMD at hip and femoral neck compared with controls, which was independent of age and BMI. There was no such difference in the case of the women with type 1 diabetes in our cohort. Although age- and BMI-adjusted BMD in our type 2 males was similar to that of matched controls, the type 2 women had increased BMD at hip, femoral neck and forearm. In multivariate regression models involving a subset of patients, male sex remained a significant predictor of BMD together with CTx and serum E2. Up to two-thirds of the total variance in BMD at femoral neck, hip and forearm could be explained by these three variables and BMI.

Although we did not show any association between BMD and glycaemic control, disease duration, serum lipids, blood pressure or complications in our type 1 patients, diabetic status alone was an important predictor of lower BMD in the males. This finding is in contrast to two previous studies that showed reduced BMD at proximal femur in both men and women [12, 31]. Direct compar-

isons are, however, difficult, since, in one of these studies, the type 1 patients were significantly older than those in our community-based cohort and had been diabetic for >30 years, while in the other, type 1 patients were not matched with the control subjects [31]. Our data parallel the results of a longitudinal study that showed greater reductions in spine and total BMD in type 1 men than women over a two-year period [32]. However, the strongest associations between male sex and BMD in our sample were for sites other than the spine. An alternative explanation for the non-significant effects in type 1 women is a type II error, with the small sample size associated with $\leq 31\%$ power to detect a one-sided significant difference with the observed BMD values in this group.

In type 2 diabetes, both men and women had higher BMD than matched controls in the present study. However, in the type 2 diabetic men, the difference disappeared after adjusting for age and BMI. By contrast, diabetes was associated with higher BMD at three of the four sites measured in the women, independently of age and BMI. Our findings are in agreement with several previous studies that also demonstrated increased BMD at lumbar spine, femoral neck and forearm in type 2 diabetic patients [33], a relationship that was stronger for women [31, 34] even after adjusting for adiposity and lifestyle variables [34, 35]. The fact that diabetes status was a significant predictor of BMD in women independently of BMI may be partly explained by the anabolic effect that insulin has on bone tissue. Since type 2 diabetes is preceded by a period of insulin resistance, hyperinsulinaemia may confer a protective effect on BMD, either directly through elevated fasting insulin as demonstrated in diabetic and non-diabetic elderly men and women [36, 37] or indirectly through BMI [35]. Of potential relevance to the present findings is that there is a rise in serum insulin with advancing age in women from the 5th to the 8th decade and a concomitant decline in men, consistent with the greater BMD in our women with type 2 diabetes compared with matched controls.

We measured bone turnover markers and sex hormone levels in a subgroup of our patients to determine whether these variables help to explain the sex- and diabetes-specific differences in BMD. A number of recent studies outside the context of diabetes have reported the importance of E2 in maintaining BMD [18, 20, 38], and that low serum E2 rather than testosterone concentrations are a risk factor for osteoporosis in men [20] and women [18]. Our data provide evidence that this also applies in diabetes, with $\ln(E2)$ a strong independent predictor of BMD in women (forearm, spine and hip), men (spine and hip) and in all subjects combined (all sites). Diabetic men are reported to have higher serum E2 concentrations than control males [39], and these elevated levels can be further increased with improved glycaemic control [40]. In the total sample of 194 type 2 patients, HbA_{1c} was independently associated with BMD at hip and femoral neck and it may be that this relationship is due, in part, to higher serum E2 levels in the better-controlled patients. We did not find

that serum total testosterone, SHBG or free T predicted BMD at any site.

The bone resorption marker CTx was a significant independent predictor of BMD in women (femoral neck), men (hip and femoral neck) and in all subjects (forearm, hip and femoral neck), with a consistent inverse association between serum levels and BMD. We did not show any association between BMD and serum calcium, phosphate, ALP, osteocalcin and vitamin D in either type 1 or type 2 diabetes, a result that is consistent with those of other studies [31, 32]. These data suggest that diabetes-associated reductions in BMD relate primarily to increased bone resorption, and that bisphosphonate therapy could be valuable in the treatment of diabetic patients with, or at high risk of, accelerated skeletal calcium loss.

A valid assessment of the impact of BMD on fractures severe enough to justify hospitalisation was not possible since there were few such events in our patients. Although there were greater numbers of self-reported fractures, there was no association with BMD-defined osteoporosis. In larger-scale studies, type 1 diabetes increases the risk of fracture, especially in those with microvascular complications [2, 14, 41] and in females [42, 43]. In type 2 diabetes, both lower [44] and higher [1] rates of fracture have been reported, with disease duration and use of insulin increasing the risk [42]. A recent study has reported that type 2 diabetes was a risk factor for hip, proximal humerus and foot fracture despite the presence of high BMD [45]. This would suggest that the high BMD seen in our type 2 diabetic patients may not parallel bone quality or strength.

The present study had limitations. Our sample size was small, particularly in the case of type 1 patients. As a result, the present data should be interpreted as preliminary. Nevertheless, we had more comprehensive data on each patient than in most previous studies of osteoporosis in diabetes. Our data were cross-sectional and therefore it is not possible to impute causality based on our findings. Further large-scale prospective studies of diabetes-associated changes in BMD and its determinants would address these limitations.

The present results provide some evidence that men with type 1 diabetes are significantly disadvantaged with respect to hip BMD and are at greatest risk of developing osteoporosis. Indeed, >50% of our type 1 males had osteopenia or osteoporosis. By contrast, women with type 2 diabetes may have protection against fractures as a result of increased BMD at several sites. E2 and CTx appear to be strong predictors of BMD in diabetes. However, the balance of bone preservation and resorption is determined by additional variables that include male sex and BMI. We were not able to assess the relationship between BMD, bone fracture and diabetes status in our cohort, but continued acquisition of fracture data through the Linked Database may provide useful future outcome data from the present cohort.

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