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Trabecular Bone Histomorphometry in Humans with Type 1 Diabetes Mellitus

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Abstract

Patients with Type 1 Diabetes Mellitus (DM) have markedly increased risk of fracture, but little is known about abnormalities in bone micro-architecture or remodeling properties that might give insight into the pathogenesis of skeletal fragility in these patients. We report here a case-control study comparing bone histomorphometric and micro-CT results from iliac biopsies in 18 otherwise healthy subjects with Type 1 Diabetes Mellitus with those from healthy age- and sex-matched non-diabetic control subjects. Five of the diabetics had histories of low-trauma fracture. Transilial bone biopsies were obtained after tetracycline labeling. The biopsy specimens were fixed, embedded, and scanned using a desktop μ CT at 16 micron resolution. They were then sectioned and quantitative histomorphometry was performed as previously described by Recker et al. 1988.[1] Two sections, >250 μ m apart, were read from the central part of each biopsy. Overall there were no significant differences between diabetics and controls in histomorphometric or micro-CT measurements. However, fracturing diabetics had structural and dynamic trends different from nonfracturing diabetics by both methods of analysis. In conclusion, Type 1 Diabetes Mellitus does not result in abnormalities in bone histomorphometric or micro-CT variables in the absence of manifest complications from the diabetes. However, diabetics suffering fractures may have defects in their skeletal microarchitecture that may underlie the presence of excess skeletal fragility.

Keywords

histomorphometry; micro-CT; diabetes; trabeculae; bone

1. Introduction

Diabetes Mellitus (DM) results in hyperglycemia because of an absolute (Type 1 DM) or relative (Type 2 DM) insulin insufficiency. This leads to many complications, both microvascular (affecting retina, nerves, kidneys and other tissues) and macrovascular

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(resulting in cardiovascular, peripheral and cerebrovascular disease). Recently there have been reports of diabetic complications affecting the skeleton resulting in increased fracture risk. Type 1 DM patients have as much as 6–7 times the relative risk for hip fracture compared to the non-diabetic population.[2–4] While lower bone mineral density is reported to be more prevalent in DM [5,6], and may account for some of this increase in fracture risk, it cannot explain all of it.[2] Bone quality features such as variation in bone remodeling rates and microarchitectural defects may explain more of the risk. Low rates of bone formation have been shown in rat and mice models of Type 1 DM,[7,8] along with reduced trabecular bone structure and strength in rat models of Type 1 DM [9] but it is unclear whether this is true in humans. Most estimates of bone remodeling rates in diabetic humans have been assessed with serum or urinary bone biomarkers, [10–13] but these studies have not provided consensus. Bone remodeling rates as assessed at the tissue level through histomorphometric analysis of fluorochrome labeled transiliac bone biopsies provides a more direct method. Histomorphometric data from eight diabetic subjects were published in 1995,[14] including 6 subjects with Type 2 DM and two male subjects with Type 1 DM. The authors concluded that bone remodeling was reduced in both types of DM. Bone microarchitecture defects can be quantified using micro-computed tomography (micro-CT). [15] Here we report complete histomorphometric and three-dimensional (3-D) micro-CT data on iliac biopsies from 18 subjects with Type 1 Diabetes Mellitus obtained at a time when diabetic complications were not clinically or biochemically apparent, and compare them to age and sex matched healthy controls.

2. Materials and Methods

2.1 Design

This was a cross-sectional, case-control study of bone histomorphometric and micro-CT variables from iliac biopsies in otherwise healthy subjects with Type 1 DM compared with the same data from healthy age- and sex- matched non-diabetic control subjects.

2.2 Subjects

We recruited a convenient sample of 23 Type 1 DM subjects (8 males, 15 females) and concurrently an equal number of age-matched (within 5 years) and sex-matched control subjects from 2008 through 2011. We used newspaper, TV, radio and website advertising specifically recruiting for healthy diabetics and healthy subjects. In compliance with the World Medical Association Declaration of Helsinki –Ethical Principles for Medical Research Involving Human Subjects, all subjects provided a signed written consent approved by the Creighton University Institutional Review Board.

The diabetic subjects had a diagnosis of Type 1 DM clinically defined as onset before age 50, with an acute presentation or diabetic ketoacidosis, with normal body mass index (BMI) at the time of diagnosis. If history was equivocal, glutamic acid decarboxylase (GAD) antibodies > 1.45 U/mL were used to confirm the diagnosis. All diabetic subjects were on insulin treatment. Glycated hemoglobin (HbA1c) was used to determine glucose control before the biopsy. Good health of all subjects was determined by medical history, clinical examination and blood and chemical analysis including: complete metabolic panel, thyroid stimulating hormone, intact parathyroid hormone, phosphorus, 25-hydroxyvitamin D, and transglutaminase antibody titers. All the female subjects were premenopausal. None of the subjects were on medications that are known to interfere with bone metabolism including steroids, anticonvulsants, diuretics, bisphosphonates, metformin, and glitazones. The subjects were excluded if they had overtly progressive renal disease defined as a calculated glomerular filtration rate (GFR) less than 60 mL/min/1.73m², a history of cancer other than skin cancer, unstable angina, myocardial infarction, uncontrolled hypertension, untreated

hypothyroidism, hyperthyroidism, malabsorption, metabolic bone disease, active rheumatoid arthritis or collagen disease. Of the 23 subjects that were recruited, 1 subject's biopsy was not obtained because of obese body habitus, 2 subjects were found to have an excluding diagnosis (celiac disease and renal failure respectively) and 2 subjects did not have comparator control data available at the time of analysis, leaving 18 pairs to be reported.

2.3 Analytical Methods

2.3.1 Bone Mineral Density—Bone mineral density (BMD) and bone mineral content measurements were made by dual energy X-ray absorptiometry (DXA) with a Hologic 4500 instrument (QDR4500A model, Hologic Inc. Waltham, MA). The anterior/posterior spine, whole body and left hip were measured. The densitometer was operated by certified radiological technicians and the coefficient of variation for repeated measures of BMD was 1.0%

2.3.2 Laboratory Analytical Methods—Glycated hemoglobin (HgA1c) was measured in our clinical laboratory using Roche Integra 700 (Roche Applied Sciences, Indianapolis, IN) and Beckman Coulter UniCel 600i DxC analyzers (Beckman Coulter, Brea, CA). Serum 25- hydroxyvitamin D [25(OH)D] levels were analyzed in the Creighton University Osteoporosis Research laboratory by liquid phase radioimmunoassay, IDS kit (ImmunoDiagnostic Systems, Fountain Hills, AZ). Serum creatinine was measured in our clinical laboratory using Roche Integra 700 analyzers (Roche Applied Sciences, Indianapolis, IN) and Beckman Coulter UniCel 600i DxC (Beckman Coulter, Brea, CA). Insulin like growth factor 1 (IGF-1) was measured by ARUP Laboratories (Salt Lake City, UT) using quantitative chemiluminescence immunoassay.

2.3.3 Transilial Bone Biopsy—Each subject was given *in vivo* double tetracycline labeling as follows: oral tetracycline hydrochloride 250 mg four times daily for three days (label 1), followed by 14 days drug free interval, and then tetracycline hydrochloride 250 mg four times daily for three days (label 2). The biopsy was performed 5 to 14 days after the last label was administered. A transilial biopsy was performed at a standard site approximately 2 cm posterior and inferior to the anterior superior spine using a trephine of 7.5 mm inner diameter (Medical Innovations International, Inc., Rochester, Minnesota).[16]

2.3.4 Bone Specimen Preparation and Evaluation—The specimens were fixed, embedded, sectioned and evaluated as described previously.[1] Two sections were read from the central part of the biopsy, taken 250 μ m apart. All the microscope work was performed on an Olympus Microscope (BX 60, Hirschfeld Instruments St. Louis). The sections were read using an operator interactive, semi-automatic image analysis system (Bioquant, Nashville, TN) linked to a camera lucida Optronics DEI -750CE (Meyer Instruments, Inc. Houston, Texas). The camera was calibrated for each magnification with a stage micrometer. All raw data were entered from the Bioquant directly into an Excel file (Microsoft, Redmond, WA) where the calculations were performed. Measured and calculated variables in trabecular bone histomorphometry used here are described elsewhere. [17] Variables were named according to the nomenclature scheme approved by the American Society for Bone and Mineral Research.[17] Every width measurement was converted to a thickness expression by a correction for obliquity ($\pi/4$) and the corrected value used in every volume expression.[1] The term for mineralizing surface (MS/BS) used the doubly label surface plus half the singly label surface in calculations of dynamic histomorphometric variables.[18]

The micro-CT measurements were performed on intact bone biopsies to capture differences in three-dimensional (3D) trabecular bone architecture and volumetric density. All biopsies

were scanned at 16 micron resolution. A typical measuring procedure consisted of a scout view, selection of the examination volume, automatic positioning, measurement and off-line reconstruction and evaluation. The intact bone biopsies were measured using a compact cone-beam type tomograph or desktop μ CT (μ CT40, Scanco Medical AG, Bassersdorf, Switzerland). A microfocus X-ray tube with a focal spot of 10 μ m and 55 kVp, 172 μ A (300 ms integration time) was used as a source, allowing excellent bone versus marrow contrast due to the pronounced photoelectric effect. A standard convolution-back projection procedure with a Shepp and Logan filter was used to reconstruct the CT images in 1024×1024 pixel matrices. An average of 300 (each 16 microns thick) image slices from the mid region of each biopsy were analyzed. During trabecular bone analyses, the contours (area of interest) were drawn approximately 0.5mm away from the edge of bone biopsy in order to avoid any debris and artifacts (Figure 1). A Digital AlphaStation 500/333 workstation (Digital Equipment Corporation, Maynard, MA, USA) was used to steer the stepping motors, to synchronize rotation, axial shift, and data recording, as well as for the image reconstruction. We measured/calculated bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular spacing (Tb.Sp), connectivity density (Conn.D), bone volumetric density (apparent [Ap.Den] and tissue [Tiss.Den]), and structure model index (SMI).

2.3.5 Statistical Analysis—Descriptive statistics were generated using the statistics package, PASW Statistics 18.0 (SPSS Inc., Chicago, Illinois). Nonparametric expressions were reported (Median and interquartile range [IQR]) as many of the variables were not normally distributed (Anderson-Darling test, using the statistics routines of SAS Version 9.2, SAS Institute Inc., Cary, NC, USA). Comparisons between cases and controls were done using Wilcoxon Signed Ranks test [PASW Statistics 18.0 (SPSS Inc., Chicago, Illinois)]. The diabetics who had sustained fractures were compared to the non-fracturing diabetics using a Mann Whitney U test [PASW Statistics 18.0 (SPSS Inc., Chicago, Illinois)]. Bivariate correlations were performed using Spearman's rho [PASW Statistics 18.0 (SPSS Inc., Chicago, Illinois)]. Statistical significance was set at $P < 0.05$.

3. Results

We analyzed data from 18 subjects (7 male, 11 female) with a diagnosis of Type 1 Diabetes Mellitus and 18 control subjects who fulfilled the enrollment criteria. See Table 1 for a description of the subjects' demographics. They had an age range of 20–47 years and the diabetic subjects had been diagnosed with diabetes for 3–36 years. Of the diabetic subjects, 5 had previously sustained a non-traumatic fracture or fractures, defined as less than or equal to a fall from a standing height (digit and facial fractures were not included). Descriptions of the fracturing subjects are outlined in Table 2. Table 3 describes pertinent laboratory data. The diabetic subjects had a wide range of glucose control with HgA1c values ranging from 5.5–10.1%, and no differences in either vitamin D status or creatinine when compared to the control subjects. The diabetics did have significantly lower levels of insulin like growth factor 1 (IGF-1). Table 4 describes the DXA results of diabetic and controls as well as the fracturing diabetics. There were no significant differences in DXA variables between cases and controls or between the fracturing diabetics and the nonfracturing diabetics.

3.1 Histomorphometry

Structural histomorphometry data are described in Table 5. There were no differences between diabetics and controls or in fracturing diabetics and nonfracturing diabetics in structural measurements including bone volume as a percent of total volume (BV/TV), wall thickness (W.Th), osteoid thickness (O.Th), and trabecular measures of thickness (Tb.Th), number (Tb.N), or separation (Tb.Sp). Surface data presented in Table 6 also showed no

differences between diabetics and controls or in fracturing diabetics and nonfracturing diabetics in osteoid surface (OS/BS), osteoblast surface (Ob.S/BS), or eroding surface (ES/BS). Dynamic data are presented in Table 7 and Table 8. Diabetic subjects, when compared to controls, had no significant differences in mineral apposition rate (MAR), mineralizing surface (MS/BS), osteoid maturation time (Omt), mineralizing osteoid (MS/OS), mineralization lag time (Mlt), bone formation rate (BFR/BS or BFR/BV), formation period (FP), remodeling period (Rm.P), or Activation frequency (Ac.F). There was a significant inverse correlation between Activation frequency and age (Spearman's rho, 2-tailed significance, $p=0.025$).

3.2 Micro-CT

There were no significant differences between paired diabetic and control micro-CT variables of BV/TV, Tb.Th, Tb.N, Tb.S, connective density [Conn.D], structure model index [SMI], apparent density [Ap.Den], or bone tissue density [Tiss.Den](See Table 9). The fracturing diabetics did not differ significantly from the nonfracturing diabetics in any of the micro-CT derived variables.

4. Discussion

We saw no significant differences in histomorphometric or micro-CT analysis of transiliac biopsies from otherwise healthy subjects with Type 1 Diabetes Mellitus compared to age and sex matched controls. This is the first case-controlled, fluorochrome-labeled histomorphometric and micro-CT study of Type 1 diabetic humans that the authors are aware of. The only other published human studies contained histomorphometric data on two male subjects (age 37 and 63) with Type 1 DM.[14] They had diabetes for ~ 3 decades and had some degree of retinopathy and peripheral neuropathy. Glucose control was not reported and an existing set of histomorphometric data from premenopausal women were used for comparison. Our current study differs from the 2 subjects previously reported [14] in several respects: 1) ours were healthy subjects with no reported diabetic complications and none identified on screening, 2) ours had the disease an average of 15 years, and 3) ours were compared to age and sex matched control subjects who were recruited and analyzed concurrently with the cases. Krakauer et al reported a low mean bone formation rate ($3.37 \pm 3.45 \mu\text{m}^3/\mu\text{m}^2/\text{yr}$) compared to a median bone formation rate of $12.18 \mu\text{m}^3/\mu\text{m}^2/\text{yr}$ in our study),[14] indicating low remodeling in their group of subjects with Type 1 and Type 2 DM. The results are strikingly different from what we observed. We found that the activation frequency (a derived variable indicating remodeling) in this diabetic cohort of subjects was no different than age matched controls. The activation frequency that we saw in both groups of subjects was comparable to results from similarly aged subjects in other populations. For example, Parisien et al. found a mean Ac.F of 0.47/yr in 34 young Caucasian females (mean age 32 years) [19]. Vedi et al also found similar Ac.F (mean 0.51/yr) in 18 young adults with a mean age of 30 years [20].

This study examined diabetic subjects before diabetic complications could confound the subjects' bone health. Epidemiological studies of fracture risk have shown an increase in fracture risk in Type 1 DM but have not always had documentation of diabetic complications.[21,22] Those studies that have documented complications have observed that diabetics with complications have a much higher risk of fracture, as high as 33 times higher in some studies.[4,23] While we saw no difference between our diabetic cases and controls, this could imply that deficits in bone remodeling rates and/or microstructure could be directly or indirectly related to complications of the disease. To determine this will require further research on subjects with diabetic complications. An alternative explanation is that diabetes causes intrinsic material property defects at a level below what is identifiable by standard histomorphometry techniques. Some examples of this include defects in the

elasticity, toughness and strength of bone tissue. Chemical content of the bone tissue including size and shape of hydroxyapatite crystals, concentration of minerals in the bone matrix and organic makeup of the collagen molecules could also be effected by hyperglycemia either directly or indirectly. Intrinsic material property defects in diabetes may be related to collagen deterioration and less likely a result of mineral defects as tissue density is not different in the fracturing subjects. Diabetes could also directly affect the cells responsible for bone formation and bone maintenance.[24] The osteocyte may play a role in the bone's response to hyperglycemia and osmotic changes.[25] Some of these outcomes could potentially be explored by analyzing the remaining bone biopsy specimens from this current study.

We saw no differences in any of the DXA results between diabetics or controls. While we did not match the subjects for BMD at the time of recruitment, this lack of difference in BMD eliminated variation in histomorphometric variables that depend on bone mass. This lack of difference in BMD is in contrast to other studies that have reported lower BMD in Type 1 DM compared to controls. Strotmeyer et al found a 3–8% lower BMD in 67 Type 1 diabetic premenopausal diabetic females compared to a group of non-diabetic women.[5] Unlike our study, over half of their subjects reported microvascular complications which may have an influence on their bone mass.[5] Although the authors were unable to find an effect of reported complications, disease duration or glucose control on BMD, they did find correlations between blindness and neuropathy on femoral neck BMD after adjusting for age and diabetes duration. [5] Mastrandrea et al also found lower BMD in 26 Type 1 diabetic women (mean age 27 years) compared to a group of controls at the total hip, femoral neck and whole body, but not at the spine.[6] They found no correlation between BMD and disease duration or glucose control and the degree of diabetic complications were not reported in this cohort. [6] As with risk of fracture, diabetic complications may play an important role in determining BMD. There are a few studies that found normal BMD in Type 1 DM, but these were small studies (one in 35 men [26], one in 31 premenopausal women [27]) and may not have had the power to see a difference. Vestergaard conducted a meta-analysis of BMD and fractures in Type 1 and Type 2 DM and while he showed overall a decreased BMD in the Type 1 diabetic subjects, he was able to calculate an expected relative fracture risk. The expected risk was 1.42, quite different from the observed relative risk of 6.94.[2] This perhaps points out that the real risk factor for fracture in Type 1 DM is bone quality rather than quantity. Although we did not focus on recruiting diabetics with a history of fracture, we did recruit 5 subjects who had previously fractured. This may be a result of recruitment bias, but we examined these subjects and compared them to the nonfracturing diabetics to find any identifiable bone characteristics. Because of the small sample, there were no statistically significant differences in any variables and the following interpretation is preliminary. However, on examination of the variables there are some trends that may be clinically significant. (See Table 10) BV/TV was lower (~27%) in fracturing diabetics by both 2-D histo and 3-D microCT methods of measurement. Other structural measurements such as Tb.Th and Tb.N were lower and Tb.Sp was higher by histomorphometric measurements, although do not show much difference when measured by micro-CT. Apparent density by microCT was lower (26%) which is concordant with a lower BV/TV and structure model index (conversion of plate-like to rod-like structure in trabecular bone) was higher (2-fold) (Table -10), indicating a probable microarchitectural deterioration in the fracturing diabetic subject. No difference in tissue density (Tiss.Den-Table 9) suggests that material property may not have been influenced in this study and that only trabecular structure deteriorated as reflected in BV/TV and apparent density decline in the fracturing diabetic subjects. OS/BS, Ob.S/BS and MS/BS were also lower. As a result, the derived dynamic variables such as BFR/BS, BFR/BV and Ac.F were lower in the fracturing subjects indicating lower remodeling. Overall the fracturing subjects had less bone structure as indicated by lower BV/TV and higher SMI which may be a result of lower

remodeling seen in some of the subjects. A larger study of diabetic subjects with fractures will be needed to identify particular bone characteristics these fracturing subjects have.

We also observed significantly lower IGF-1 levels in the diabetic subjects. This has been observed in other diabetic cohorts and has been postulated to contribute to low bone mass in diabetics. [28] We saw no correlation between IGF-1 levels and any of the histomorphometric or DXA variables so it is difficult to attribute effects of low IGF-1 on bone in our study. Low IGF-1 may simply be a marker for nutrient intake [29] or result from the insulin deficiency of Type 1 DM which impairs hepatic IGF-1 synthesis.[30]

The strengths of this study were that the control subjects were age and sex matched and analyzed concurrently with the diabetic cases, and the bone biopsies were processed and analyzed by standardized technology in a well established lab.

The limitations of this study were that we used a convenient sample of subjects. This will always be a limitation in any study using an invasive technique. Another limitation was that this group of diabetic subjects had reasonable glucose control (over half had HgA1c of <7%) [31] and may not be representative of poorly controlled diabetic patients. If bone remodeling and structure are dependent on glucose homeostasis, variations in glucose control could have an effect on bone which we were unable to observe in this study. We were also not able to assess the glucose control at the time of fracture in the subjects who had sustained fractures. Another limitation is that this was a cross sectional study and does not indicate what effect disease duration will have on the bones.

5. Conclusion

In summary, this cross-sectional case controlled study indicates that Type 1 Diabetes Mellitus does not have an effect on bone mineral density by DXA and bone histomorphometric characteristics and micro-CT variables before clinically apparent diabetic complications have manifested. The diabetic patient suffering from fracture may have unique microarchitectural bone defects and decreased bone remodeling. Further studies in patients with diabetic complications and those with fractures are warranted.

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Abbreviations

DM	Diabetes Mellitus
BMI	body mass index
GAD	glutamic acid decarboxylase
HgA1c	glycated hemoglobin
GFR	glomerular filtration rate
DXA	dual energy X-ray absorptiometry
BMD	Bone mineral density

25(OH)D	25- hydroxyvitamin D
IGF-1	Insulin like growth factor 1
BV/TV	bone volume fraction
Tb.Th	trabecular thickness
Tb.N	trabecular number
Tb.Sp	trabecular spacing
Conn.D	connectivity density
Ap.Den	bone volumetric apparent density
Tiss.Den	bone volumetric tissue density
SMI	structure model index
BV/TV	bone volume as a percent of total volume
W.Th	wall thickness
O.Th	osteoid thickness
Tb.Th	trabecular measures of thickness
Tb.N	trabecular number
Tb.Sp	trabecular separation
OS/BS	osteoid surface
Ob.S/BS	osteoblast surface
ES/BS	eroding surface
MAR	mineral apposition rate
MS/BS	mineralizing surface
Omt	osteoid maturation time
MS/OS	mineralizing osteoid
Mlt	mineralization lag time
BFR/BS or BFR/BV	bone formation rate
FP	formation period
Rm.P	remodeling period
Ac.F	Activation frequency

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Highlights

- We report a case-control study comparing bone histomorphometric and micro-CT results from iliac biopsies in Type 1 Diabetes Mellitus.
- There were no significant differences between diabetics and controls in histomorphometric or micro-CT measurements.
- However, fracturing diabetics had structural and dynamic trends different from nonfracturing diabetics by both methods of analysis.

Figure 1.
The 3D-image of bone biopsy showing the region of interest. An average of 300 micro-CT slices were analyzed for trabecular bone.

Table 1**Subjects Demographics^a**

	Diabetes	Control	P	Fracturing
N	18	18		5
Sex M,F	7,11	7,11		2,3
Age yrs	31.1 (25.4–38.0)	34.1(28.6–38.4)	NS ^b	37.1(22.2–45.9)
Dx yrs ^c	15(8–25)	na ^d		10(14–25)
Height cm	171(166–174)	172(164–175)	NS	170(160–174)
Weight kg	75.6(70.4–85.4)	68.3(59.2–85.3)	.043 ^e	74.9(69.9–89.0)
BMI	26.8(24.5–27.9)	23.9(21.5–24.9)	.02 ^e	27.1(25.7–30.4)

^aMedian (interquartile range)

^bNS = not statistically significant by Wilcoxon Signed Ranks Test

^cDx yrs = years since diagnosis of T1DM

^dna = not applicable

^eWilcoxon Signed Ranks Test

Table 2

Characteristics of Diabetic Subjects with Fracture

ID	Sex	Current Age	Age at Diabetes Diagnosis	Description of Fracture(s)	Age at last Fracture
1	M	21	7	R ankle, L ankle, elbow	18
2	F	22	12	R tibia	20
3	F	37	12	R tibia, fibula, ankle	36
4	M	45	35	L arm, knee, metatarsal	35
5	F	46	21	R proximal 5 th metatarsal	42

Table 3Laboratory Data^a

	Diabetes	Control	P	Fracturing
N	18	18		5
HgA1c % ^b	6.8(6.3–8.3)	5.3(5.1–5.4)	<0.001 ^c	8.1(6.9–9.7)
25(OH)D nmol/L ^d	63(51–79)	68(56–86)	NS	62(52–73)
Creatinine mg/dl	0.8(0.7–0.8)	0.7(0.7–0.8)	NS	0.7(0.7–0.8)
IGF-1 ^e ng/ml	119(92–148)	158(127–240)	P=0.004 ^c	113(103–125)

^aMedian (interquartile range)^bHgA1c = Glycated hemoglobin^cWilcoxon Signed Ranks Test^d25(OH)D = 25-hydroxyvitamin D^eIGF-1 = Insulin-like growth factor 1

Table 4**DXA Results^a**

	Diabetes	Control	P	Fracturing
n	18	18		5
L1-L4 Spine BMD ^b g/cm ²	0.982(0.900 – 1.053)	1.079(0.955–1.137)	NS ^c	0.973(0.863–1.125)
L1-L4 Spine T-score	−0.7(−1.5–0.0)	0.3(−0.9–0.6)	NS	−0.7(−1.9–0.5)
Total Hip BMD g/cm ²	0.991(0.892 – 1.058)	0.970(0.885–1.093)	NS	0.862(0.841–1.045)
Total Hip T-score	0.0(−0.5 – 0.6)	0.1(−0.7–0.5)	NS	−0.8(−1.0–0.4)

^a Median (interquartile range)

^b BMD = bone mineral density

^c NS = not statistically significant by Wilcoxon Signed Ranks Test

Table 5**Structural Data^a**

Variable	Diabetes	Controls	P	Fracturing
n	18	18		5
BV/TV %	20.08(15.81–27.26)	23.80(18.54–26.66)	NS ^b	16.04(13.32–21.41)
W.Th μ m	31.2(27.1–32.2)	29.9(28.6–30.6)	NS	32.3(26.7–34.9)
O.Th μ m	6.75(6.08–7.78)	6.86(6.33–8.08)	NS	6.82(4.75–7.72)
Tb.Th μ m	124.6(102.4–149.9)	128.9(106.2–144.5)	NS	102.0(100.7–134.5)
Tb.N #/mm	1.61(1.40–1.79)	1.75(1.51–1.88)	NS	1.49(1.17–1.79)
Tb.Sp μ m	605(548–703)	560(521–654)	NS	660(554–841)

^aMedian (Interquartile Range)

^bNS = not statistically significant by Wilcoxon Signed Ranks Test

Table 6Surface Data^a

Variable	Diabetes	Controls	P	Fracturing
n	18	18		5
OS/BS %	9.9(3.8–11.8)	11.2(3.0–13.1)	NS ^b	4.1(1.9–10.8)
Ob.S/BS %	4.5(1.9–8.7)	4.2(1.6–9.3)	NS	1.2(0.9–7.9)
ES/BS %	1.0(0.8–1.4)	0.9(0.5–1.5)	NS	0.9(.8–1.6)

^aMedian (Interquartile Range)

^bNS = not statistically significant by Wilcoxon Signed Ranks Test

Table 7Dynamic Data^a

Variable	Diabetes	Controls	P	Fracturing
n	18	18		5
MAR $\mu\text{m}/\text{day}$	0.56(0.49–0.62)	0.54(0.49–0.58)	NS ^b	0.56(0.52–0.65)
MS/BS %	6.5(3.0–10.4)	6.6(3.3–11.4)	NS	2.7(1.7–11.4)
Omt d	12.5(10.5–13.9)	12.9(11.2–14.4)	NS	9.9(9.2–13.3)

^a Median (Interquartile Range)

^a data calculated from double label plus half the single label

^b NS = not statistically significant by Wilcoxon Signed Ranks Test

Table 8Derived Dynamic Data^a

Variable	Diabetes	Controls	P	Fracturing
n	18	18		5
MS/OS %	72.3(61.5–100)	73.3(53.8–100)	NS ^b	96.1(63.9–100)
Mlt d	14.8(12.2–24.6)	18.1(11.4–26.0)	NS	13.6(9.2–14.8)
BFR/BS $\mu\text{m}^3/\mu\text{m}^2/\text{yr}$	12.18(4.94–22.63)	14.11(5.49–24.80)	NS	4.79(3.78–24.10)
BFR/BV %/yr	22.9(9.2–32.5)	21.1(11.4–34.1)	NS	9.4(7.5–35.9)
FP yrs	0.19(0.13–0.28)	0.19(0.13–0.36)	NS	0.15(0.12–0.24)
Rm.P yrs	0.22(0.16–0.31)	0.21(0.14–0.38)	NS	0.18(0.16–0.30)
Ac.F #/yr	0.42(0.18–0.69)	0.49(0.18–0.82)	NS	0.19(0.11–0.79)

^aMedian (Interquartile Range)^bNS = not statistically significant by Wilcoxon Signed Ranks Test

Table 9**MicroCT Data^a**

	Diabetes	Control	P	Fracturing
n	18	18		5
BV/TV%	22.1(17.4–30.0)	23.3(20.0–29.1)	NS ^b	17.8(13.5–23.3)
Tb.Th mm	0.16(0.13–0.20)	0.15(0.14–0.19)	NS	0.14(0.12–0.17)
Tb.N #/mm	1.44(1.35–1.57)	1.48(1.39–1.53)	NS	1.45(1.12–1.66)
Tb.S mm	0.63(0.60–0.68)	0.62(0.60–0.66)	NS	0.63(0.55–0.84)
Connectivity Density 1/mm ³	6.50(4.65–7.54)	5.94(4.82–7.40)	NS	6.44(3.56–9.42)
Structure model index	0.575(0.311–1.322)	0.452(0.204–0.810)	NS	1.194(0.567–1.51)
Apparent density mg/cm ^{3c}	230(183–286)	238(207–290)	NS	184(151–236)
Bone tissue density mg/cm ^{3c}	817(796–836)	810(788–821)	NS	820(791–843)

^aMedian (Interquartile Range)

^bNS= not significant by Wilcoxon Signed Ranks Test

^cVolumetric density is reported in mg/cm³ of calcium hydroxyapatite (HA)

Table 10**Fracturing Diabetics Variables^a**

	Fracturing Diabetics^b	Diabetics without Fracture
n	5	13
<i>Histomorphometric Variables</i>		
BV/TV %	16.04(13.32–21.41)	21.95(18.18–28.08)
Tb.Th μm	102.0(100.7–134.5)	125.2(112.4–162.2)
Tb.N #/mm	1.49(1.17–1.79)	1.74(1.54–1.79)
Tb.Sp μm	660(554–841)	558(544–638)
OS/BS %	4.1(1.9–10.8)	11.1(6.5–11.8)
Ob.S/BS %	1.2(0.9–7.9)	4.5(3.6–9.5)
MS/BS %	2.7(1.7–11.4)	6.9(4.1–10.1)
BFR/BS $\mu\text{m}^3/\mu\text{m}^2/\text{yr}$	4.79(3.78–24.10)	12.88(8.59–21.28)
BFR/BV %/yr	9.4(7.5–35.9)	26.2(11.6–32.4)
Ac.F #/yr	0.19(0.11–0.79)	0.46(0.30–0.71)
<i>Micro-CT Variables</i>		
BV/TV%	17.8(13.5–23.3)	24.6(19.2–30.8)
Structure model index	1.194(0.567–1.51)	0.435(0.236–1.256)
Apparent density mg/cm^3 ^c	184(151–236)	247(201–299)

^aMedian (Interquartile Range)^bNo significant differences were found using Mann Whitney U test^cVolumetric density is reported in mg/cm^3 of calcium hydroxyapatite (HA)