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Original Article

Osteocalcin favoring glucose control is associated with decreased total hip bone mineral density-evidence from a cross-sectional study in T2DM male patients

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Abstract: Osteocalcin (OC), an osteoblast-derived protein, can regulate glucose metabolism. The release and activation of OC depend on bone resorption. It is possible that the process of OC favoring glucose control is associated with reduced bone mineral density (BMD). Our study aimed to test this speculation in males with type 2 diabetes mellitus (T2DM). A total of 196 adult males with T2DM were recruited, their hypoglycemic treatments were recorded. Serum Glycosylated hemoglobin A1c (HbA1c), OC, and BMDs at lumbar-spine1-4 (L1-4 BMD), total hip (TH BMD), and femoral neck (FN BMD) were measured. OC was inversely correlated to HbA1c (*r=-0.225, P=0.002*) and TH BMD (*r=-0.222, P=0.002*) in T2DM males. HbA1c had a positive correlation with TH BMD (*r=0.282, P=0.026*) in T2DM males with less than 10 years of diabetes, after controlling for age, BMI, insulin resistance, hypoglycemic treatment groups, and other potential confounders, but did not in those with more than 10 years of diabetes (*r=0.093, P=0.382*). In newly diagnosed T2DM patients without a medication history of hypoglycemic agents, HbA1c and TH BMD also had a positive correlation (*r=0.856, P=0.001*). However, the positive associations between HbA1c and TH BMD both disappeared after adjustments for OC in these T2DM patients. In conclusion, better glucose control is associated with decreased TH BMD in T2DM males with relative short diabetic duration, which is driven by the effect of OC. This supports the speculation, but additional human and animal studies are required to vigorously test this hypothesis.

Keywords: Bone mineral density, Glycosylated hemoglobin A1c, osteocalcin, type 2 diabetes mellitus

Introduction

Skeleton has long been considered as a target organ of T2DM, the detrimental effects of which on skeleton are multifactorial, altering bone metabolism, structure, and strength. It has been reported that T2DM patients have impaired bone quality compared to healthy controls [1, 2].

On the other hand, emerging evidence shows that bone is an endocrine organ involving inglucose metabolism, and osteocalcin (OC) is an essential mediator in this process [3]. OC is a protein initially synthesized by osteoblasts and is released from bone mineral matrix to the circulation during bone resorption. Serum total OC had long been used as a marker of bone turnover [4]. In 2007, Lee *et al.* [5] found that OC could also favor β cell proliferation, enhance insulin expression in β cells, and improve insulin sensitivity. An *in vivo* study demonstrated that long-term treatment of OC significantly improved glucose metabolism in gold thioglucose-induced hyperphagia and high-fat diet induced metabolic disease mice [6].

The skeleton-derived protein OC plays a role in glucose homeostasis regulation, but bone mass change in this process is less known. It has been suggested that skeleton regulated glucose metabolism via OC in a bone resorption-dependent manner, because under carboxylated OC (ucOC) was hormonally active in

mice, and the OC decarboxylation and activation was largely determined by bone resorption activity [7]. Furthermore, in an *in vivo* study, mutant mice harboring an increase in osteoclasts number had increased serum ucOC levels and improved glucose tolerance than WT animals, while osteoclasts ablation mice had decreased serum ucOC levels and reduced glucose tolerance, which also supported the notion that bone resorption participated in glucose metabolism [8].

Considering evidence form above research, it is reasonable to speculate that the process of OC favoring glucose control is associated with bone loss, that is, improved glucose control by OC is associated with decreased bone mineral density (BMD), and OC is considered to be a mediator in the relationship between glucose control and BMD. As T2DM has detrimental effects on skeleton, there isa possibility that the metabolic capacity of skeleton deteriorates with the process of diabetes and thus varies in subjects with different duration of diabetes. In this cross-sectional study, we tried to test the speculation by investigating the relationship between glucose control, indicated by Glycosylated hemoglobin A1c (HbA1c), and BMDs in T2DM males stratified by duration of diabetes. The role of OC in this relationship was also analyzed.

When exploring the relationship between HbA1c and BMDs, it was necessary to control for potential confounding factors. First of all, only adult males were included in this study for the consideration of that accelerated bone loss in postmenopausal women might confound the relationship between bone mass and glucose metabolism. Some other clinical factors affecting bone metabolism, including age, body mass index (BMI), diabetic duration, hyperinsulinemia, insulin resistance, hyperglycemia, hypercalciuria indicated by 24-hour urinary calcium (24 h urinary calcium), sex hormones including estradiol (E2) and testosterone (T), and microangiopathy indicated by 24 h proteinuria [9-12] were also adjusted in the analysis. Besides, factors that concurrently influenced bone metabolism and glucose metabolism, including serum parathyroid hormone (PTH), vitamin D indicated by serum 25-hydroxyvitamin D (25(OH)D), serum calcium (Ca), and uric acid (UA) [13-15] were also controlled in this study. Hypoglycemic agents significantly impact glucose metabolism in T2DM patients, and some hypoglycemic agents, including insulin, thiazolidinediones (TZDs), metformin, dipeptidyl peptidase-4 (DPP4) inhibitors, and glucagon-like peptide-1 (GLP-1) receptor agonists, have also been reported to affect skeleton [16, 17]. The influences of hypoglycemic treatments on glucose control and bone metabolism were also taken into account in the analysis.

Material and methods

Study participants

The data of T2DM patients who referred to the Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, School of Medicine, Tongji University from March 2013 to March 2016 were collected. All patients were diagnosed with T2DM according to Word Health Organization criteria. The T2DM patients recruited in this study were males who were older than 35 years and were considered to obtain peak bone mass. The Exclusion Criteria were as follows: (1) the presence of diseases affecting bone metabolism, including hyperparathyroidism, hypoparathyroidism, thyroid disorders, rheumatoid arthritis, Cushing disease, steroid-induced osteoporosis, and renal osteodystrophy; (2) a recent history of longer (>3 months) periods of immobilization; (3) a history of malignant diseases and hepatic impairment; (4) acute or chronic infections; (5) alcoholism, chronic gastrointestinal diseases, and chronic treatment over the last 6 months with adrenal or anabolic steroids, calcitonin, bisphosphonates, or PTH. Finally, a total of 196 males with T2DM were recruited. 185 males were established T2DM patients using hypoglycemic agents, and they were further divided into two subgroups according to the median of diabetic duration (10 years) in the analysis. 11 males were newly diagnosed T2DM patients without a medication history of hypoglycemic agents. The data of patients were analyzed anonymously after data collection. The study was approved by the ethical committee of the Shanghai Tenth People's Hospital.

Demographic measurements

Body weight (to 0.1 kg) was measured by a balance beam scale, and height (to 0.001 m) was measured by a wall-mounted stadiometer. BMI

(kg/m^2) was calculated by dividing the weight for the height square. Age, diabetic duration, admission date, and smoking status were recorded. T2DM patients using hypoglycemic agents had a stable hypoglycemic treatment under the guidance of experienced doctors, and the hypoglycemic agents they used within three months before referring to the hospital were also recorded. Hypoglycemic treatments were divided into groups in the analysis on the basis of hypoglycemic agents affecting bone metabolism.

Biochemical measurements

Biochemical measurements were performed on blood samples drawn from each patient at 8 a.m., after a 10-h overnight fast. HbA1c was determined by high performance liquid chromatography (HPLC) with the coefficient of variation (CV) of 1.11%; Fasting plasma glucose (FPG) was assayed by a routine laboratory biochemistry auto analyzer; Fasting insulin (FinS) (Biosource., Belgium) and fasting C-peptide (FCP) (Immunotech., Czech Republic) were measured by immunoradiometric assays; the intra- and inter assay coefficients of variations (CVs) for the former were 1.6-2.2% and 6.1-6.5%, respectively, and 2.3-3.0% and 3.5-5.1%, respectively, for the latter. Serum 25(OH)D was determined by double antibody radioimmunoassay (DiaSorin., Stillwater, MN, and Linco Research., St. Charles, MO), with inter assay CVs being 9.3%. Intact PTH (iPTH) was assayed by chemiluminescence (Siemens Healthcare Diagnostics., United Kingdom); its intra- and inter-assay CVs were 4.2-5.7% and 6.3-8.8%, respectively. Serumβ isomer of the C-terminal telopeptide of type I collagen (β-CTX) was detected by electrochemiluminescence (Roche Diagnostics., Switzerland); the intra- and inter-assay CVs were below 10.0%. Serum total OC was assayed by using an N-MID Osteocalcin ELISA kit (Roche diagnostic Ltd., Switzerland). Serum Ca and phosphate (P) concentrations, and biochemical measurements of liver and kidney function, including serum alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), creatinine (Scr), and UA, were assayed by a routine laboratory biochemistry autoanalyzer.

BMDs measurements

BMDs at lumbar-spine1-4 (L1-4 BMD), total hip (TH BMD), and femoral neck (FN BMD) were measured by dual-energy X-ray absorptiometry (DXA) (Hologic (QDR-4000), USA).

Updated homeostasis model assessment (HOMA2)

HOMA Calculator Software (University of Oxford) [18] was used to calculate an updated homeostasis model assessment of insulin resistance(HOMA2-IR) by variables of FPG and FCP.

Statistical analysis

The Kolmogorov-Smirnov test ($n \geq 50$) and Shapiro-Wilk test ($n < 50$) were applied to assess the normality of the continuous data. Skewness distribution data were expressed as median (interquartile range), and normal distribution data were expressed as mean ± SD. Variables were compared by Mann-Whitney U test or Kruskal-Wallis H test for skewness distribution continuous data, and independent-samples t test or one-way ANOVA for normal distribution continuous data. Pearson correlation and Spearman correlation were used to evaluate the correlation coefficients for normal- and skewness-distributed variables, respectively. Partial correlation analysis was used to assess the correlations with adjustments. Multivariate linear regression analysis using stepwise and enter methods were performed, respectively, to investigate the independent predictors of the dependent variable. A 2-tailed $P<0.05$ was considered significant. The statistical analysis was performed by using SPSS 22.0 software (SPSS, Inc).

Results

The characteristics of T2DM males using hypoglycemic agents

T2DM males with shorter diabetic duration (<10 years) were younger (*P=0.014*) and had significantly higher levels of β-CTX (*P=0.021*), ALP (*P=0.042*), 24 h urinary calcium (*P=0.016*), FN BMD (*P=0.011*), and TH BMD (*P=0.013*) than those with longer diabetic duration (≥10 years). Additionally, serum OC levels were marginally significantly higher in those with shorter diabetic duration (*P=0.075*). The levels of sex hormone (T) (*P=0.019*) and 24 h proteinuria (*P=0.010*) were lower in T2DM males with shorter diabetic duration (**Table 1**).

Table 1. The clinical characteristics of T2DM males using hypoglycemic agents

	All (n=185)	Diabetic Duration <10 (n=79)	Diabetic Duration ≥10 (n=106)	*p* value
Age (yr)	62 (56-68)	60 (55-66)	64 (58-70)	0.014
BMI (kg/m^2)	24.49 ± 3.58	24.70 ± 3.45	24.34 ± 3.67	0.497
Smoking[a] n (%)	94 (50.8%)	39 (49.4%)	55 (51.9%)	0.735
HbA1c (%)	8.7 (7.6-10.2)	8.7 (7.5-10.2)	8.8 (7.7-10.2)	0.495
FPG (mmol/L)	8.4 (6.5-13.4)	8.2 (6.4-11.2)	8.8 (6.5-14.4)	0.462
FinS (pmol/L)	8.56 (5.11-18.01)	8.27 (5.46-15.35)	9.43 (4.59-19.85)	0.635
FCP (μg/L)	1.84 (1.23-2.47)	1.98 (1.29-2.64)	1.66 (1.17-2.46)	0.247
HOMA2-IR	1.69 (1.05-2.39)	1.73 (1.20-2.43)	1.65 (1.01-2.39)	0.481
25(OH)D (nmol/L)	48 ± 15	48 ± 13	48 ± 17	0.966
iPTH (ng/L)	31.9 (23.2-42.4)	30.5 (22.0-43.6)	32.3 (23.9-41.6)	0.686
Ca (mmol/L)	2.26 ± 0.11	2.27 ± 0.10	2.26 ± 0.11	0.387
P (mmol/L)	1.2± 0.17	1.21 ± 0.18	1.20 ± 0.17	0.620
OC (μg/L)	12.01 (9.65-15.06)	12.92 (10.13-15.39)	11.28 (9.30-14.90)	0.075
β-CTX (μg/L)	0.30 (0.21-0.47)	0.34 (0.24-0.48)	0.27 (0.19-0.44)	0.021
ALP (U/L)	68.5 (57.0-79.8)	72.0 (60.3-80.8)	65.0 (54.6-78.6)	0.042
AST (U/L)	15.8 (13.8-19.6)	17.0 (13.7-22.0)	15.8 (13.8-18.0)	0.163
ALT (U/L)	16.8 (11.9-22.6)	17.5 (11.9-27.1)	15.9 (11.8-20.9)	0.131
UA (umol/L)	324.65 ± 82.13	319.26 ± 80.49	328.68 ± 83.48	0.442
Scr (umol/L)	71.7 (63.3-83.2)	68.8 (62.6-80.0)	74.3 (64.2-85.1)	0.129
T (nmol/L)	15.01 (11.62-19.09)	14.06 (10.52-17.43)	16.04 (12.50-19.65)	0.019
E2 (pmol/L)	94.43 (71.32-117.30)	91.30 (71.24-112.20)	98.82 (71.20-123.25)	0.304
24 h proteinuria (g/24 h)	0.088 (0.039-0.176)	0.060 (0.031-0.160)	0.107 (0.049-0.213)	0.010
24 h urinary calcium (mmol/24 h)	4.51 (2.63-6.98)	4.98 (3.19-7.91)	3.84 (2.21-6.42)	0.016
L1-4 BMD (g/cm^2)	0.990 ± 0.165	1.014 ± 0.164	0.971 ± 0.165	0.082
TH BMD (g/cm^2)	0.913 ± 0.143	0.944 ± 0.135	0.890 ± 0.145	0.011
FN BMD (g/cm^2)	0.788 ± 0.143	0.818 ± 0.125	0.765 ± 0.152	0.013
Hypoglycemic treatments				0.065
Insulin (%)	72 (38.9%)	27 (34.2%)	45 (42.5%)	
Metformin (%)	33 (17.8%)	19 (24.1%)	14 (13.2%)	
TZDs (%)	5 (2.7%)	2 (2.5%)	3 (2.8%)	
Insulin + Metformin (%)	22 (11.9%)	7 (8.9%)	15 (14.2%)	
Insulin + TZDs (%)	5 (2.7%)	1 (1.3%)	4 (3.8%)	
Metformin + TZDs (%)	9 (4.9%)	2 (2.5%)	7 (6.6%)	
Insulin + Metformin + TZDs (%)	3 (1.6%)	0 (0%)	3 (2.8%)	
Others[b] n (%)	36 (19.5%)	21 (26.6%)	15 (14.2%)	

The values are expressed in median (interquartile range) for skewness distribution data, and mean ± SD for normally distribution data. [a]Smoking; yes or no. [b]Others; hypoglycemic agents including sulfonylureas, glinides, and glycosidase inhibitor. BMI; body mass index. HbA1c; Glycosylated hemoglobin A1c. FPG; fasting plasma glucose. FinS; fasting insulin. FCP; fasting C-peptide. HOMA2-IR; updated homeostasis model assessment of insulin resistance. 25(OH)D; 25-hydroxyvitamin D. iPTH; intact parathyroid hormone. Ca; serum calcium. P; serum phosphate. OC; osteocalcin. β-CTX; cross-linked n-telopeptide of type collagen. ALP; alkaline phosphatase. AST; aspartate aminotransferase. ALT; alanine aminotransferase. UA; serum uric acid. Scr; serum creatinine. T; testosterone. E2; estradiol. 24 h proteinuria; 24-hour proteinuria. 24 h urinary calcium; 24-hour urinary calcium. L1-4 BMD; bone mineral density at lumbar-spine 1-4. TH BMD; bone mineral density at total hip. FN BMD; bone mineral density at femoral neck. TZDs; thiazolidinediones.

The hypoglycemic treatments of T2DM patients are also shown (**Table 1**). The most commonly used single-drug treatment was insulin, and insulin plus metformin was the most commonly

Table 2. The correlations of HbA1c and BMDs with demographic and biochemical variables in T2DM males usinghypoglycemic agents

	HbA1c		L1-4 BMD		TH BMD		FN BMD	
	r	*P* value	r	*p* value	r	*p* value	r	*p* value
Age	-0.064	0.386	0.021	0.777	-0.203	0.006	-0.255	<0.001
BMI	-0.070	0.345	0.332	<0.001	0.301	<0.001	-0.231	0.002
Diabetic Duration	0.004	0.957	-0.122	0.098	-0.231	0.002	-0.257	<0.001
FPG	0.421	<0.001	-0.042	0.575	0.050	0.503	0.070	0.345
FinS	0.042	0.570	0.110	0.138	0.087	0.241	0.007	0.923
HOMA2-IR	-0.006	0.934	0.009	0.898	0.087	0.237	0.160	0.029
25(OH)D	-0.095	0.198	0.035	0.641	0.090	0.223	0.091	0.219
iPTH	-0.195	0.008	0.007	0.922	0.017	0.814	0.003	0.973
Ca	-0.007	0.930	0.003	0.965	-0.042	0.566	-0.024	0.750
P	0.098	0.185	0.021	0.775	0.125	0.091	0.186	0.011
OC	-0.225	0.002	-0.253	0.001	-0.222	0.002	-0.196	0.007
β-CTX	-0.080	0.278	-0.234	0.001	-0.227	0.002	-0.197	0.007
UA	-0.142	0.054	0.146	0.047	0.198	0.007	0.235	0.001
Scr	-0.104	0.159	0.035	0.636	-0.054	0.468	-0.048	0.519
T	-0.012	0.871	-0.239	0.001	-0.244	0.001	-0.223	0.002
E2	0.027	0.714	0.139	0.060	0.090	0.225	0.065	0.377
24 h proteinuria	0.242	<0.001	-0.015	0.839	-0.007	0.929	-0.046	0.531
24 h urinary calcium	-0.059	0.424	-0.204	0.005	-0.137	0.063	-0.125	0.091

Table 3. The correlations between HbA1c and BMDs in T2DM males using hypoglycemic agents

Diabetic Duration <10	Model 1		Model 2		Model 3	
	r	*p* value	r	*p* value	r	*p* value
L1-4 BMD	0.047	0.679	0.094	0.461	0.009	0.942
TH BMD	0.166	0.143	0.282	0.026	0.217	0.088
FN BMD	0.070	0.541	0.177	0.161	0.118	0.355
Diabetic Duration ≥10	Model 4		Model 5		Model 6	
	r	*p* value	r	*p* value	r	*p* value
L1-4 BMD	0.188	0.054	0.074	0.484	0.067	0.528
TH BMD	0.148	0.129	0.093	0.382	0.079	0.459
FN BMD	0.157	0.109	0.071	0.501	0.051	0.631

Model 1 and Model 4 are crude. Model 2 and Model 5 are adjusted for age, BMI, smoking status, FPG, FinS, HOMA2-IR, 25(OH)D, iPTH, Ca, UA, T, E2, 24 h proteinuria, 24 h urinary calcium, and hypoglycemic treatments. Model 3 and Model 6 are adjusted for all variables in Model 2 and Model 5 plus OC.

used multi-drug therapy. The distribution of hypoglycemic treatments in two diabetic duration subgroups was not statistically different (*P=0.065*). Among subjects using different hypoglycemic treatments, HbA1c values were not significantly different (*P=0.111*). Although, there were some differences in the bone turnover indicators, including OC (*P=0.047*) and β-CTX (*P<0.001*) among hypoglycemic treatment groups, there were no differences in L1-4 BMD (*P=0.300*), TH BMD (*P=0.282*), or FN BMD (*P=0.071*) (Supplementary Figure 1).

HbA1c has an independently positive association with TH BMD in T2DM males using hypoglycemic agents

The relationship of OC with glucose control and BMDs were investigated at first. Serum OC was inversely correlated to HbA1c (*r=-0.225, P=0.002*), as well as L1-4 BMD (*r=-0.253, P=0.001*), TH BMD (*r=-0.222, P=0.002*), and FN BMD (*r=-0.196, P=0.007*) (**Table 2**).

In next step, the relationship between HbA1c and BMD was explored in both correlation analysis and multivariate linear regression analysis, respectively. In correlation analysis, HbA1c was not significantly correlated to BMDs at three sites before adjustments in T2MD males with duration of diabetes less than 10 years (Model 1, **Table 3**) or in those with duration of diabetes more than 10 years (Model 4, **Table 3**). However, in partial correlation analysis, HbA1c displayed

Table 4. The independent association of HbA1c with TH BMD in T2DM malesusing hypoglycemic agents

Diabetic Duration <10	β	Standard Error	Standardized β	*p* value	Adjusted R^2
Model 1					0.203
HbA1c	0.015	0.006	0.249	0.019	
BMI	0.017	0.004	0.438	<0.001	
Age	-0.002	0.002	-0.144	0.188	
UA	<0.001	<0.001	-0.049	0.671	
T	-0.001	0.003	-0.036	0.738	
Model 2					
HbA1c	0.011	0.007	0.176	0.103	0.239
OC	-0.008	0.004	-0.241	0.038	
BMI	0.014	0.005	0.363	0.003	
Age	-0.003	0.002	-0.182	0.094	
UA	<0.001	<0.001	0.023	0.840	
T	<0.001	0.003	-0.018	0.865	
Diabetic Duration ≥10	β	Standard Error	Standardized β	*p* value	Adjusted R^2
Model 3					0.112
HbA1c	0.010	0.007	0.138	0.140	
BMI	0.008	0.004	0.198	0.046	
Age	-0.002	0.001	-0.119	0.195	
UA	<0.001	<0.001	0.212	0.026	
T	-0.004	0.002	-0.159	0.107	
Model 4					0.147
HbA1c	0.009	0.007	0.117	0.222	
OC	-0.003	0.003	-0.093	0.342	
BMI	0.007	0.004	0.175	0.087	
Age	-0.002	0.001	-0.124	0.177	
UA	<0.001	<0.001	0.209	0.029	
T	-0.004	0.002	-0.147	0.139	

Variables in Model 1 and Model 3 include HbA1c, BMI, age, UA, and T. Variables in Model 2 and Model 4 include all variables in Model 1 and Model 3 plus OC.

a positive correlation with TH BMD (*r=0.286, P=0.022*), after controlling for potential confounding factors, including age, BMI, smoking status, 25(OH)D, iPTH, Ca, FPG, FinS, HOMA2-IR, UA, 24 h proteinuria, 24 h urinary calcium, sex hormones, and hypoglycemic treatment groups in T2DM patients whose diabetic duration was less than 10 years (Model 2, **Table 3**). HbA1c did show any significant correlations with BMDs in those whose diabetic duration was more than 10 years (Model 5, **Table 3**).

As TH BMD, among BMDs at three sites, was the only one correlated to HbA1c after adjustments in partial correlation analysis, multivariate linear stepwise regression analysis was further performed to explore the independent predictors of the variances of HbA1c. BMDs at three sites and other factors showing associations with HbA1c, including FPG, iPTH, and 24 h proteinuria (**Table 2**) were used as independent variables in the analysis. At last, only TH BMD (β=4.221, P=0.015) and FPG (β= 0.194, P<0.001) entered the regression model and were responsible for the change in HbA1c (*adjusted R^2 for the model =0.173, P<0.001*) in T2DM patients who had less than 10 years of diabetes (data not shown). However, in T2DM patients with more than 10 years of diabetes, no BMDs entered the regression model (*adjusted R^2 for the model =0.148, P<0.001*) (data not shown).

When TH BMD was set as the dependent variable, in turn, in multivariate linear regression analysis using the enter method, it was

Table 5. The clinical characteristics of newly diagnosed T2DM patients

n	11
Age (yr)	43 (39-51)
BMI (kg/m^2)	25.86 ± 3.07
Smoking (%)	5 (45.5%)
HbA1c (%)	10.1 ± 1.0
FPG (mmol/L)	10.0 (7.9-11.8)
FinS (pmol/L)	10.79 ±5.30
FCP (μg/L)	2.01 ± 0.79
HOMA2-IR	1.75 (1.10-3.62)
25(OH)D (nmol/L)	42 (29-58)
iPTH (ng/L)	37.3 ± 11.5
Ca (mmol/L)	2.28 ± 0.11
P (mmol/L)	1.18 ± 0.20
OC (μg/L)	14.82 ± 3.57
β-CTX (μg/L)	0.43 ± 0.15
ALP (U/L)	70.1 (69.6-76.6)
AST (U/L)	17.1 ± 5.4
ALT (U/L)	23.8 ± 13.5
UA (umol/L)	317.71 ± 70.98
Scr (umol/L)	67.6 (61.9-71.5)
T (nmol/L)	12.47 ± 3.54
E2 (pmol/L)	92.39 ± 50.53
24 h proteinuria (g/24 h)	0.120 (0.088-0.224)
24 h urinary calcium (mmol/24 h)	5.74 ± 2.74
L1-4 BMD (g/cm^2)	0.961 ± 0.195
TH BMD (g/cm^2)	0.919 ± 0.155
FN BMD (g/cm^2)	0.816 ± 0.135

Values are expressed as median (interquartile range) for skewness distribution data, and mean ± SD for normally distribution data.

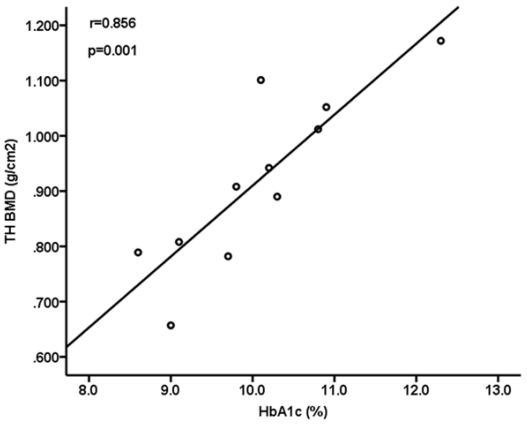

Figure 1. The correlation between HbA1c and TH BMD in newly diagnosed T2DM male patients.

observed that HbA1c was positively associated with TH BMD (β=0.015, P=0.019) independent of confounders in T2DM males whose diabetic duration was less than 10 years (Model 1, **Table 4**). In line with results above, HbA1c had no significant association with TH BMD (β=0.010, P=0.140) in those whose diabetic duration was more than 10 years (Model 3, **Table 4**).

HbA1c has a positive association with TH BMD in newly diagnosed T2DM male patients

The characteristics of newly diagnosed T2DM male patients without any usage of hypoglycemic agents are shown (**Table 5**). In multivariate linear stepwise regression analysis, it was also only TH BMD (β=5.700, P=0.001), among BMDs at three sites, that was responsible for the change in HbA1c (*adjusted R^2 for the model =0.702, P=0.001*) (data not shown). HbA1c and TH BMD displayed a significantly positive correlation (*r=0.856, P=0.001*) (**Figure 1**). Moreover, subjects in the lowest HbA1c tertile did possess lower TH BMD than those in the highest HbA1c tertile (*P<0.05*) (**Figure 2A**). OC also presented inverse associations with HbA1c (*P=0.043*) and TH BMD (*P=0.007*) in these patients (**Figure 2B**, **2C**).

OC mediates the positive association between HbA1c and TH BMD in T2DM males

In T2DM male patients with less than 10 years of diabetes, the significant correlation between HbA1c and TH BMD disappeared (*r=0.217, P=0.088*) after further adjustment of OC (Model 3, **Table 3**). In multivariate linear regression analysis using the enter method, when OC was added into the model, HbA1c was no longer associated with TH BMD (β=0.011, P=0.103) in these T2DM patients (Model 2, **Table 4**). In newly diagnosed T2DM patients, the positive association between HbA1c and TH BMD also disappeared after the adjustment of OC (*P=0.141*) (data not shown). These results suggested that the positive association between HbA1c and TH BMD was mediated by OC.

Discussion

The relationships between HbA1c and BMD have been reported to be inconsistent in T2DM

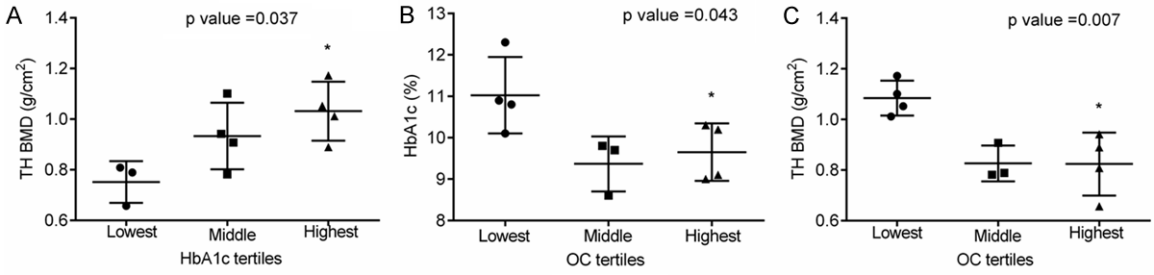

Figure 2. The associations between HbA1c, TH BMD, and OC in newly diagnosed T2DM male patients. A. TH BMD in tertiles of HbA1c. B. HbA1c in tertiles of OC. C. TH BMD in tertiles of OC. *p* value indicates comparison among OC tertiles or HbA1c tertiles. *; comparison with subjects in the lowest tertile, P<0.05.

patients from previous studies. There has been a reported inverse relationship, that is, poor glucose control was associated with decreased BMD [19], which agreed with the conception that T2DM or hyperglycemia was a detrimental factor to bone health. However, some studies including a meta-analysis reported a positive relation between HbA1c and BMD in T2DM patients [20-22], indicating that poor glucose control was associated with increased BMD, or conversely, better glucose control was associated with decreased BMD. Although the underlying reason for this was not fully elucidated, one of potential explanations for this was that the increased BMD in poorly controlled T2DM patients could be a result of hyperinsulinemia secondary to insulin resistance. Whereas, HbA1c maintained apositive association with TH BMD after adjustments for insulin levels, insulin resistance and other confounders in the present study, thus hyperinsulinemia or insulin resistance could not fully explain the increased BMD in poor controlled T2DM patients, or the positive relationship between HbA1c and BMD.

However, the discovery of the endocrine role of bone may provide another possible explanation for the relationship between HbA1c and BMD. On account of the finding that skeleton regulated glucose metabolism through OC in a bone-resorption manner [7], it was speculated that the process of OC favoring glucose control could result in bone loss. In other words, serum OC improved glucose control and was associated with bone resorption or possible bone loss, therefore, improved glucose control was considered to be associated with decreased BMD, and OC could play a mediator role in the relationship between HbA1c and BMD. In this study, we have provided some evidence to support this speculation.

Firstly, OC was inversely associated with HbA1c, in line with findings in animals that OC beneficially affected glucose metabolism [5-7]. Although there was no direct evidence concerning the metabolic effects of OC in humans, changes in OC induced by PTH (1-84) and alendronate were associated with changes in metabolic indices in elderly women [13]. Moreover, OC had inverse associations with glucose profile, and insulin resistance index in general population and T2DM patients [23, 24]. These evidences support that OC is involved in the regulation of glucose metabolism in humans.

Secondly, bone resorption was also suggested to affect glucose metabolism in animals [8], but we failed to find a significant association between the bone resorption marker β-CTX and HbA1c in T2DM males. This is probably because that the assay of β-CTX underestimates bone resorption activity in diabetes [25, 26]. Even so, serum OC and β-CTX were significantly correlated (r=0.630, P<0.001) (data not shown), high levels of OC and β-CTX both were associated with decreased BMDs in this study, which was similar to the pathophysiological characteristics of high turnover osteoporosis. Taken together, these results indicated that OC not only improved glucose control, but also was associated with bone loss in T2DM males.

Thirdly and importantly, according to the speculation, improved glucose control was considered to be associated with decreased BMD, and their association was mediated by the effects of OC. In accordance with the speculation, the results of correlation analysis and multivariate linear regression analysis both

revealed that lower HbA1c was independently associated with decreased TH BMD after controlling for confounding factors in T2DM patients with diabetic duration less than 10 years, but not in those with longer diabetic duration. In newly diagnosed T2DM males without impacts from hypoglycemic agents, HbA1c also possessed a significantly positive relationship with TH BMD. The significant associations between HbA1c and TH BMD in T2DM males disappeared after further adjustments for OC, indicating the mediator role of OC in their association.

Xuan *et al.* [27] once observed a positive association between CTX and HbA1c in women with normal glucose tolerance (NGT), proposing a hypothesis that in NGT subjects, skeleton would combat against the subtle increase of HbA1c by enhancing bone resorption activity, but the skeleton becomes "exhausted" when diabetes develops because of decreased bone turnover makers in T2DM women. However, our results suggest that, in T2DM men with relative short diabetic duration, skeleton probably still possesses the ability to regulate glucose metabolism at the expense of bone loss, but the metabolic function of skeleton may "exhausted" after longer-term detrimental effects from T2DM.

It was worth noting that, among BMDs at three sites, only TH BMD was independently associated with glucose control. It has been demonstrated that hip possessed a higher cortical/cancellous ratio than lumbar spine, and T2DM patients had decreased cortical thickness in bone biopsy samples [28]. Moreover, T2DM subjects were observed to undergo accelerated hip bone loss in a prospective study [29]. These evidences imply a selective cortical bone loss in the hip of T2DM subjects; however, the question of whether the bone loss is attributed to the metabolic function of skeleton is unknown, as yet, and needs further investigation.

Admittedly, a positive association between HbA1c and TH BMD also indicates that poor glucose control is associated with higher TH BMD, but this does not imply that poorly controlled T2DM is associated with "better skeleton". Oei *et al.* [30] reported that T2DM patients with poor glucose control had higher fracture risks, even in the presence of higher BMD. Petit *et al.* [31] and Ishii *et al.* [32] found lower strength indices for compression and bending of bone in T2DM individuals. These fragility fractures, and impaired bone quality in T2DM patients could result from diabetes-related alterations in skeletal properties not captured by DXA, such as altered microarchitecture, accumulation of AGEs, and modified bone turnover [2]. Hence, adequate glucose control is still needed for bone health in T2DM patients.

Our study has several limitations: First of all, it was impossible to control for all confounders in clinical data analysis, but we considered as many as possible according to available evidence; We could not fully exclude the effects of hyperglycemia on bone metabolism in T2DM, but we controlled for glucose levels in the analysis; The impacts of hypoglycemic agents on glucose control and BMDs were completely excluded in newly diagnosed T2DM patients, and hypoglycemic treatment groups were also adjusted in T2DM males using hypoglycemic agents. Secondly, ucOC, the hormonally active form of OC in animal studies, was not measured, but serum total OC has also been indicated to have beneficial associations with glucose metabolism in humans [23, 24]. Thirdly, we assayed total sex hormones, rather than their bioactive forms in serum, which might slightly bias the results. Finally, this is a cross-sectional study with no causal effect to report and a small sample size, further larger prospective studies are needed.

In conclusion, OC is inversely associated with HbA1c and BMDs, a positive association between HbA1c and TH BMD is suggested to be driven by the effect of OC in T2DM males with relative short diabetic duration. These results supported the speculation that OC favoring glucose control is associated with decreased TH BMD in T2DM patients. Nevertheless, this hypothesis must be vigorously tested in prospective human and animal studies.

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Disclosure of conflict of interest

None.

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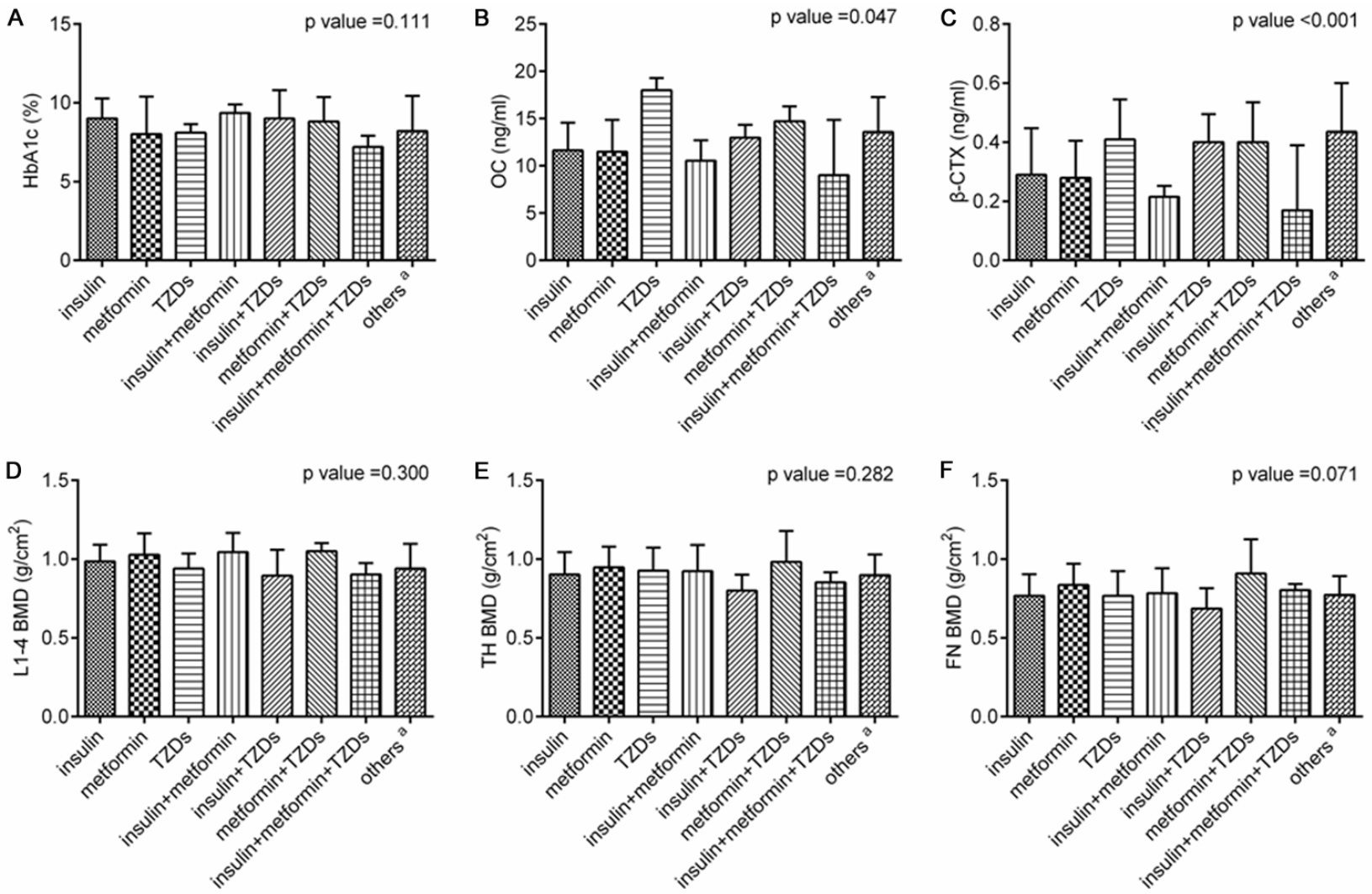

Supplementary Figure 1. HbA1c, bone turnover makers, and BMDs in hypoglycemic treatment groups. A. HbA1c concentrations in hypoglycemic treatment groups. B. Serum OC concentrations in hypoglycemic treatment groups. C. Serum β-CTX concentrations in hypoglycemic treatment groups. D. L1-4 BMD in hypoglycemic treatment groups. E. TH BMD in hypoglycemic treatment groups. F. FN BMD in hypoglycemic treatment groups. [a]others; hypoglycemic agents including sulfonylureas, glinides, and glycosidase inhibitor. *p* value indicates comparison among hypoglycemic treatment groups.