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# Differential pattern for regulating insulin secretion, insulin resistance, and lipid metabolism by osteocalcin in male and female T2DM patients

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

**B 1,2 Xuefei Rui\***  
**A 1 Bei Xu\***  
**C 1 Junlei Su**  
**D 1 Chunping Pan**  
**E 1 Chenyu Zhan**  
**F 1 Bin Su**  
**E 1 Hong Li**  
**C 1 Jiying Wang**  
**A 1,2,3 Hui Sheng**  
**B 1 Shen Qu**

1 Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China  
2 Department of Clinical Medicine, Nanjing Medical University, Nanjing, China  
3 Advanced Institute of Translational Medicine, Tongji University, Shanghai, China

\* Dr. Xuefei Rui and Dr. Bei Xu contribute equally to this work

**Corresponding Author:** Hui Sheng, e-mail: shenghui@tongji.edu.cn

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**Background:** Osteocalcin has been reported to be relevant to glucose and lipid metabolism, indicating it may stimulate insulin secretion and improve insulin resistance. Yet the difference between male and female patients is still not clear.

We aimed to investigate the difference in serum osteocalcin, and its association with glucose, lipid metabolism, pancreatic function, insulin sensitivity, and resistance in male and female middle-aged and elderly type 2 diabetic (T2DM) patients.


**Material/Methods:** 739 T2DM patients were included. After measurement of body mass index (BMI), the levels of fasting plasma glucose (FPG), insulin (FINS), C peptide (FC-P), 2-h post-OGTT plasma glucose (2h-PG), HbA1C, and osteocalcin were determined. Homeostasis model assessment of  $\beta$ -cell function (HOMA-%B), homeostasis model assessment of insulin sensitivity (HOMA-%S), and homeostasis model assessment of insulin resistance (HOMA-IR) were calculated.

**Results:** Females had higher osteocalcin concentration than males ( $P < 0.05$ ). In males, serum osteocalcin was negatively correlated with HbA1C, FPG, and 2-h PG ( $P < 0.05$ ), but positively with 2-h post-OGTT C peptide (2hC-P), 2-h post-OGTT serum insulin (2h-INS), and HOMA-%B ( $P < 0.05$ ). In females, serum osteocalcin was negatively correlated with HbA1C, FPG, triglyceride (TG), and HOMA-IR ( $P < 0.05$ ), but positively with 2-h C-P, 2-h INS, HOMA-%B, HOMA-%S, and high-density lipoprotein (HDL) ( $P < 0.05$ ). In all subjects, serum osteocalcin was inversely correlated with HbA1C, FPG, and 2-h PG ( $P < 0.05$ ), but positively with 2-h C-P, 2-h INS, HDL, and HOMA-%B ( $P < 0.05$ ).

**Conclusions:** Osteocalcin might improve glucose metabolism through enhancing insulin secretion in males, and through increasing insulin secretion and improving insulin resistance in females with T2DM. Osteocalcin probably also plays an important role in lipid metabolism.

**MeSH Keywords:** **Lipid Metabolism • Glucose Metabolism Disorders • Osteocalcin • Diabetes Mellitus**

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## Background

Osteocalcin (OC; also known as bone Gla protein, BGP) is a secreted 5-kDa protein that is the most prevalent noncollagenous protein of 49 amino acids in bone. It is synthesized exclusively in certain cells of the osteoblast lineage: mature osteoblasts and osteocytes. Post-translational modification of OC (gamma-carboxylation at 3 Gla residues) allows it to tightly bind to the calcium ions in hydroxyapatite (HA). OC is largely found in the bone, but a small amount of OC circulates in the blood, and the serum OC level has been considered a marker of bone formation and remodeling. It has been postulated that OC normally functions in the bone to maintain the normal calcification and inhibit mineralization, perhaps in order to prevent osteocytes from becoming completely embedded in mineral. OC has an important clinical role in osteoporosis diagnosis and treatment [1,2].

The recent study by Lee et al. [3] showed the *Esp*, a gene expressed in osteoblasts, could encode a receptor-like protein tyrosine phosphatase termed OST-PTP. It was demonstrated that mice lacking *Esp* (*Esp*<sup>-/-</sup>) displayed an increase in  $\beta$ -cell proliferation, insulin secretion, and sensitivity, causing hyperinsulinemia and hypoglycemia. Mice lacking OC (*Ocn*<sup>-/-</sup>) showed a decrease in  $\beta$ -cell proliferation, insulin secretion and sensitivity, adiponectin expression and energy expenditure. Ferron et al. also confirmed that OC had a role in promoting  $\beta$ -cell proliferation, insulin secretion and sensitivity, adiponectin expression, and energy expenditure [4]. They inferred that there was an interaction between bone and pancreas, especially between osteoblasts and adipocytes.

Recent studies have indicated that the skeleton as an endocrine organ plays important roles in the bone and energy metabolism depending on the insulin receptor on the osteoblasts. The insulin signaling in osteoblasts is necessary for whole-body glucose homeostasis because it can increase the OC activity. Several studies have reported the association between DM and osteoporosis [5–19].

However, differential patterns for regulating insulin secretion, insulin resistance, and lipid metabolism by osteocalcin in male and female T2DM patients are still unknown. We compared the serum OC concentration between male and female DM patients, and examined the correlation of serum OC with glucose and lipid metabolism in males and females, respectively.

## Material and Methods

### Material

We recruited 1200 patients from the Shanghai Tenth People's Hospital during July 2011 to October 2012. The inclusion criteria

included: 1) patients diagnosed with type 2 diabetes mellitus (T2DM) and 2) patients with an age range from 50 to 90 years (all female patients were postmenopausal women). The exclusion criteria included: 1) patients with acute or severe chronic complications, 2) patients with past or current medications (sex steroids, corticosteroids, bisphosphonates, calcitonin, SERMs) known to affect bone metabolism, 3) patients with current cancer, laboratory evidence of kidney or liver diseases, hyperthyroid disease, parathyroid disease, or other diseases related to the endocrine system, 4) patients with acute or chronic infections, and 5) patients with current trauma or operations. A total of 739 patients who met these criteria were included in this study: 311 men with a mean age of  $66.76 \pm 10.24$  years (range: 50 to 90 years) and 428 women with a mean age of  $68.93 \pm 10.46$  years (range: 50 to 90 years). The study protocol was approved by the local institutional review board, and all participants provided written informed consent.

### Body size measurements

Height (accurate to 0.1 cm) was measured at baseline using a wall-mounted stadiometer and body weight (accurate to 100 g) was measured using an electronic calibrated scale. Body mass index (BMI) was calculated as  $\text{weight}/\text{height}^2$  ( $\text{kg}/\text{m}^2$ ).

### BMD measurements

Bone mineral density (BMD) was measured by using DEXA with intra- and inter-assay coefficient of variation (CV) below 1%. The BMD of L1-4, right hip, and femoral neck was measured. The average BMD was calculated as  $(\text{L1-4 BMD} + \text{right hip BMD} + \text{femoral neck BMD})/3$ .

### Laboratory measurements

Blood samples were collected after 12-h overnight fasting for the detection of biochemical measurements, and the 75-g oral glucose tolerance test (OGTT) was performed according to the standardized clinical procedures. Serum OC was measured by using radioimmunoassay (RIA) [20]. Hemoglobin A1C (HbA1C) was determined by high-performance liquid chromatography (HPLC) with the coefficient of variation (CV) of 1.11% [21]. The plasma glucose was measured with the glucose oxidase method [22,23]. The plasma insulin (Biosource, Nivelles, Belgium) and C-peptide (Immunotech, Czech Republic) were determined by immunoradiometric assays with the intra- and inter-assay CVs of 1.6–2.2% and 6.1–6.5% and 2.3–3.0% and 3.5–5.1%, respectively [24]. The total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were all measured by using the enzymic method [25,26]. The above parameters and the function of liver and kidney were tested with a routine laboratory biochemistry autoanalyzer.

**Table 1.** Baseline characteristics of all the patients.

Parameters	Male (n=311)	Female (n=428)	t	P
Age (yr)	66.76±10.24	68.93±10.46	-2.808	0.005
Height (cm)	169.97±5.84	158.56±5.33	27.151	<0.001
Weight (kg)	70.53±11.24	62.31±9.78	10.179	<0.001
BMI (kg/m <sup>2</sup> )	24.39±3.53	24.75±3.49	-1.326	0.185
Course of disease (yr)	10.04±7.45	12.45±7.87	-4.191	<0.001
L1-4 BMD (g/cm <sup>2</sup> )	0.97±0.17	0.88±0.15	4.788	<0.001
Right hip BMD (g/cm <sup>2</sup> )	0.85±0.15	0.80±0.15	2.957	0.003
Femoral neck BMD (g/cm <sup>2</sup> )	0.74±0.13	0.69±0.13	3.596	<0.001
Average BMD (g/cm <sup>2</sup> )	0.85±0.14	0.79±0.13	4.198	<0.001
BGP (ng/ml)	12.72±6.26	15.76±7.89	-5.847	<0.001
Ca (mmol/L)	2.21±0.13	2.24±0.13	-3.579	<0.001
P (mmol/L)	1.14±0.21	1.21±0.22	-4.328	<0.001
HbA1C (%)	9.00±0.24	9.02±2.39	-0.110	0.912
FPG (mmol/L)	8.32±3.12	8.38±3.34	-0.244	0.808
2-h PG (mmol/L)	16.49±4.73	16.74±4.98	-0.685	0.494
FC-P (ng/ml)	2.08±1.06	2.26±1.18	-2.099	0.036
2-h C-P (ng/ml)	4.86±2.80	5.39±2.77	-2.532	0.012
FINS (mU/L)	22.12±40.22	29.65±63.81	-1.961	0.050
2-h INS (mU/L)	43.57±58.98	58.02±88.78	-2.476	0.014
TC (mmol/L)	4.49±1.13	5.02±1.22	-5.985	<0.001
TG (mmol/L)	1.64±1.30	1.79±1.14	-1.647	0.100
HDL (mmol/L)	1.06±0.31	1.17±0.34	-4.475	<0.001
LDL (mmol/L)	2.67±0.90	3.04±1.02	-5.150	<0.001
HOMA-%B	66.06±47.56	71.04±51.13	-1.346	0.179
HOMA-%S	73.22±42.58	67.92±41.74	1.689	0.092
HOMA-IR	1.84±1.20	1.99±1.11	-1.764	0.078

Data are expressed as means ±S.D. BMI – body mass index; BMD – bone mineral density; BGP – bone Gla protein; FPG – fasting plasma glucose; 2hPG – 2-h post-OGTT plasma glucose; FC-P – fasting C peptide; 2-h C-P – 2-h post-OGTT C peptide; FINS – fasting serum insulin; 2hINS – 2-h post-OGTT serum insulin; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TC – total cholesterol; TG – triglyceride; HOMA-%B – homeostasis model assessment of  $\beta$  cell function; HOMA-%S – homeostasis model assessment of insulin sensitivity; HOMA-IR – homeostasis model assessment of insulin resistance.

### Homeostasis model assessment

Homeostasis model assessment of  $\beta$ -cell function (HOMA-%B), homeostasis model assessment of insulin sensitivity (HOMA-%S), and homeostasis model assessment of insulin resistance (HOMA-IR) were calculated by fasting plasma glucose (FPG) and fasting C peptide (FC-P). HOMA calculator

software (from the University of Oxford) was used to calculate the 3 parameters.

### Statistical analysis

All analyses were performed with Statistical Package for the Social Sciences version 13.0 (SPSS, Chicago, IL, USA). Data were

**Table 2.** Correlations of serum osteocalcin with anthropometric variables, glucose, and lipid metabolic indices.

Parameters	Male		Female		Total	
	r	P	r	P	r	P
Age	-0.139	0.014	-0.042	0.391	-0.061	0.100
BMI	-0.011	0.850	-0.114	0.021	-0.060	0.112
Course of disease	-0.099	0.082	-0.098	0.044	-0.066	0.072
HbA1C	-0.309	<0.001	-0.164	0.001	-0.222	<0.001
FPG	-0.262	<0.001	-0.162	0.001	-0.20	<0.001
2hPG	-0.274	<0.001	-0.087	0.074	-0.151	<0.001
FC-P	0.081	0.155	-0.057	0.243	0.015	0.687
2hC-P	0.201	<0.001	0.120	0.014	0.181	<0.001
FINS	-0.029	0.614	-0.080	0.098	-0.030	0.422
2-h INS	0.182	0.001	0.108	0.026	0.181	<0.001
TC	-0.078	0.174	0.013	0.796	0.032	0.395
TG	-0.033	0.567	-0.142	0.004	-0.054	0.143
HDL	-0.080	0.161	0.137	0.005	0.088	0.017
LDL	-0.060	0.292	0.036	0.466	0.038	0.308
HOMA-%B	0.287	<0.001	0.146	0.002	0.210	<0.001
HOMA-%S	-0.016	0.781	0.106	0.029	0.037	0.318
HOMA-IR	0.014	0.807	-0.107	0.027	-0.039	0.290

expressed as mean  $\pm$  standard deviation (S.D). Clinical characteristics were compared between 2 groups using the independent samples t test. Spearman correlation coefficients were calculated to assess the correlations of OC with anthropometric variables, glucose, lipid profile, insulin secretion, and insulin sensitivity and resistance. Multiple stepwise regression analysis was performed to determine the independent predictors of HOMA-%B, HOMA-%S, and HOMA-IR after adjustment for potential confounders. A value of two-tailed  $P < 0.05$  was considered statistically significant.

## Results

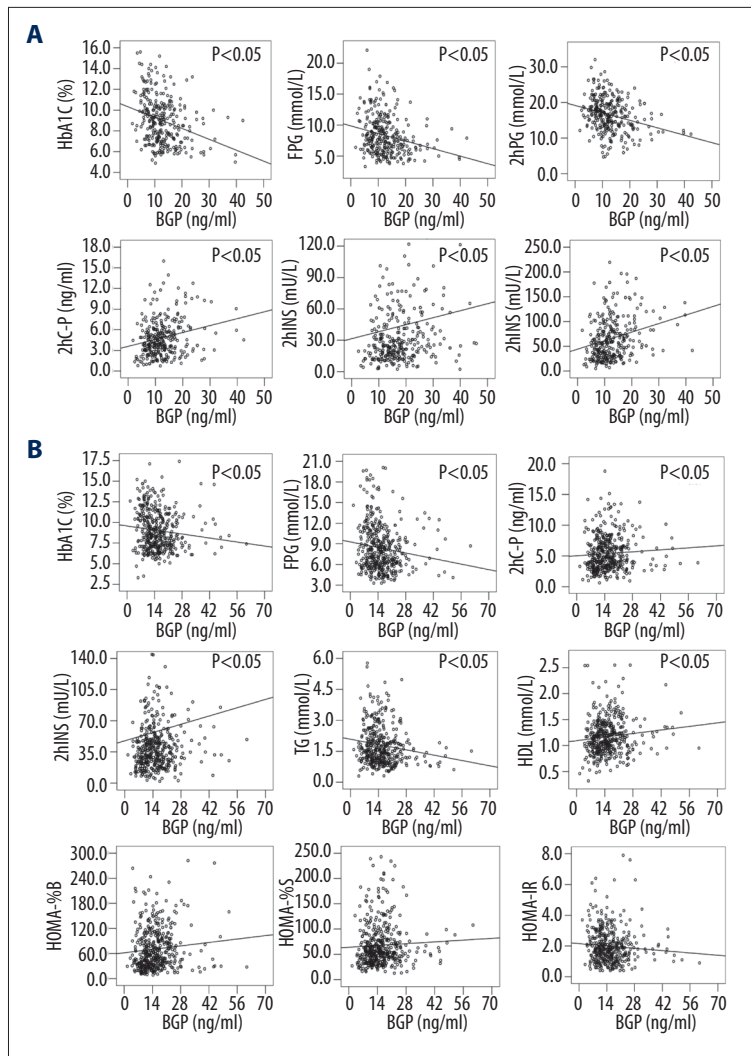
### Baseline characteristics of patients and measurements between males and females

The baseline characteristics of all the patients are shown in Table 1. A total of 739 T2DM patients (mean age  $68.01 \pm 10.41$  years [range: 50 to 90 years]; BMI:  $24.60 \pm 3.51$  kg/m<sup>2</sup>) were studied. Patients were divided into males ( $n=311$ ) and females ( $n=428$ ) (Table 1). The result showed a significant difference in the age, height, weight, course of disease, BMD, BGP, Ca, P, FC-P, 2hC-P, FINS, 2hINS, TC, HDL, and LDL between males and females ( $P < 0.05$ ), but there were no significant difference in BMI, HbA1C, FPG, 2-h PG, TG, HOMA-%B, HOMA-%S, and HOMA-IR between the 2 groups (Table 1).

### Correlations of serum OC with anthropometric variables, glucose, lipid profile, insulin secretion, and insulin sensitivity and resistance

The correlations of serum OC with glucose and lipid metabolic parameters are shown in Table 2. In males, the serum OC was negatively correlated with age ( $r=-0.187$ ,  $P < 0.001$ ), HbA1C ( $r=-0.277$ ,  $P < 0.001$ ), FPG ( $r=-0.234$ ,  $P < 0.001$ ), and 2-h PG ( $r=-0.255$ ,  $P < 0.001$ ), but positively with 2-h C-P ( $r=0.213$ ,  $P < 0.001$ ), 2-h INS ( $r=0.170$ ,  $P=0.002$ ), and HOMA-%B ( $r=0.274$ ,  $P < 0.001$ ). In females, serum OC was inversely correlated with BMI ( $r=-0.369$ ,  $P=0.029$ ), course of disease ( $r=-0.369$ ,  $P=0.029$ ), HbA1C ( $r=-0.369$ ,  $P=0.029$ ), FPG ( $r=-0.369$ ,  $P=0.029$ ), TG ( $r=-0.369$ ,  $P=0.029$ ), and HOMA-IR ( $r=-0.354$ ,  $P=0.037$ ), but positively with 2-h C-P ( $r=0.339$ ,  $P=0.047$ ), 2-h INS ( $r=0.339$ ,  $P=0.047$ ), HDL ( $r=0.339$ ,  $P=0.047$ ), HOMA-%B ( $r=0.339$ ,  $P=0.047$ ), and HOMA-%S ( $r=0.339$ ,  $P=0.047$ ). In all subjects, serum OC was negatively correlated with HbA1C ( $r=-0.165$ ,  $P=0.001$ ), FPG ( $r=-0.151$ ,  $P=0.002$ ), and 2-h PG ( $r=-0.107$ ,  $P=0.028$ ), but positively with 2-h C-P ( $r=0.115$ ,  $P=0.019$ ), 2-h INS ( $r=0.106$ ,  $P=0.030$ ), HDL ( $r=0.138$ ,  $P=0.005$ ), and HOMA-%B ( $r=0.139$ ,  $P=0.004$ ) (Figure 1).

To further determine which variables were independently associated with serum OC, multiple stepwise regression analysis was performed in men and women independently (Table 3). Results showed that course of disease, HbA1C, and 2-h PG



**Figure 1.** Correlations of serum osteocalcin with glucose and lipid metabolic indices. **(A)** significant correlations between BGP and glucose, lipid metabolic indices in male patients. **(B)** significant correlations between BGP and glucose and lipid metabolic indices in female patients.

**Table 3.** Multiple stepwise regression analysis showing variables independently associated with serum OC in males and females.

	Independent variables	$\beta$	Sd. E	Standardized $\beta$	t	P
Male	Course of disease	-0.093	0.047	-0.111	-1.990	0.047
	HbA1C	-0.632	0.187	-0.226	-3.382	0.001
	2hPG	-0.188	0.088	-0.142	-2.132	0.034
Female	BMI	-0.239	0.112	-0.106	-2.125	0.034
	FPG	-0.297	0.118	-0.126	-2.524	0.012
Total	HbA1C	-0.376	0.142	-0.118	-2.645	0.008
	FPG	-0.211	0.103	-0.093	-2.055	0.040
	HDL	1.759	0.833	0.079	2.111	0.035

Age, BMI, course of disease, HbA1c, FPG, 2hPG, FC-P, 2hC-P, FINS and 2-h INS, TC, TG, LDL, and HDL served as independent variables.

were independently associated with OC in males. BMI and FPG were independently correlated with osteocalcin in females. In all subjects, HbA1C, FPG, and HDL were independently associated with OC.

#### Multivariate linear regression analysis: independent predictors of HOMA-%B

To further determine which variables were independently



**Table 4.** Multivariate linear regression analysis: independent predictors of HOMA-%B.

Independent variables		$\beta$	Sd.E	Standardized $\beta$	t	P
Male	HbA1C	-9.806	1.098	-0.462	-8.933	<0.001
	HbA1C	-11.843	0.893	-0.554	-13.263	<0.001
Total	Age	0.510	0.163	0.107	3.128	0.002
	Course of disease	-0.689	0.218	-0.108	-3.153	0.002
	HbA1C	-11.105	0.688	-0.520	-16.133	<0.001

Age, BMI, course of disease, HbA1c, BGP, TC, TG, LDL, and HDL served as independent variables.

**Table 5.** Multivariate linear regression analysis: independent predictors of HOMA-%S.

Independent variables		$\beta$	Sd.E	Standardized $\beta$	t	P
Male	BMI	-2.712	0.671	-0.224	-4.041	<0.001
	HbA1C	2.343	1.079	0.123	2.172	0.031
	TC	5.108	2.286	0.135	2.235	0.026
	TG	-8.868	1.896	-0.271	-4.677	<0.001
Female	Age	-0.711	0.196	-0.178	-3.619	<0.001
	BMI	-1.335	0.565	-0.112	-2.365	0.019
	Course of disease	0.733	0.259	0.138	2.834	0.005
	TG	-8.864	1.832	-0.242	-4.837	<0.001
	HDL	16.477	6.110	0.134	2.697	0.007
Total	LDL	6.001	1.998	0.147	3.004	0.003
	Age	-0.573	0.152	-0.142	-3.779	<0.001
	BMI	-2.067	0.432	-0.172	-4.788	<0.001
	Course of disease	0.550	0.202	0.102	2.727	0.007
	TC	4.481	1.493	0.128	3.001	0.003
Total	TG	-8.985	1.45	-0.258	-6.195	<0.001
	HDL	12.179	5.257	0.096	2.317	0.021

Age, BMI, course of disease, HbA1c, BGP, TC, TG, LDL, and HDL served as independent variables.

associated with HOMA-%B, multivariate linear regression analysis was performed (Table 4). Results showed that in males HbA1C ( $\beta=-0.462$ ,  $P<0.001$ ) was an independent predictor of HOMA-%B; in females HbA1C ( $\beta=-0.554$ ,  $P<0.001$ ) was an independent predictor of HOMA-%B; in all subjects age ( $\beta=0.107$ ,  $P=0.002$ ), course of disease ( $\beta=-0.108$ ,  $P=0.002$ ), and HbA1C ( $\beta=-0.520$ ,  $P<0.001$ ) were independent predictors of HOMA-%B.

**Multivariate linear regression analysis: independent predictors of HOMA-%S**

To further determine which variables were independently associated with HOMA-%S, multivariate linear regression analysis was performed (Table 5). Results showed that in males BMI ( $\beta=-0.224$ ,  $P<0.001$ ), HbA1C ( $\beta=0.123$ ,  $P=0.031$ ), TC ( $\beta=0.135$ ,  $P=0.026$ ), and TG ( $\beta=-0.271$ ,  $P<0.001$ ) were independent

predictors of HOMA-%S; in females age ( $\beta=-0.178$ ,  $P<0.001$ ), BMI ( $\beta=-0.112$ ,  $P=0.019$ ), course of disease ( $\beta=0.138$ ,  $P=0.005$ ), TG ( $\beta=-0.242$ ,  $P<0.001$ ), HDL ( $\beta=0.134$ ,  $P=0.007$ ), and LDL ( $\beta=0.147$ ,  $P=0.003$ ) were independent predictors of HOMA-%S; in all subjects age ( $\beta=-0.142$ ,  $P<0.001$ ), BMI ( $\beta=-0.172$ ,  $P<0.001$ ), course of disease ( $\beta=0.102$ ,  $P=0.007$ ), TC ( $\beta=0.128$ ,  $P=0.003$ ), TG ( $\beta=-0.258$ ,  $P<0.001$ ), and HDL ( $\beta=0.096$ ,  $P=0.021$ ) were independent predictors of HOMA-%S.

**Multivariate linear regression analysis: independent predictors of HOMA-IR**

To further determine which variables were independently associated with HOMA-IR, multivariate linear regression analysis was performed (Table 6). Results showed that in males age ( $\beta=0.144$ ,  $P=0.008$ ), BMI ( $\beta=0.114$ ,  $P=0.030$ ), course of

**Table 6.** Multivariate linear regression analysis: independent predictors of HOMA-IR.

Independent variables		$\beta$	Sd.E	Standardized $\beta$	t	P
Male	Age	0.017	0.006	0.144	2.651	0.008
	BMI	0.039	0.018	0.114	2.186	0.030
	Course of disease	-0.019	0.009	-0.118	-2.184	0.030
	TC	-0.199	0.059	-0.187	-3.393	0.001
	TG	0.453	0.051	0.491	8.883	<0.001
Female	Age	0.011	0.005	0.103	2.142	0.033
	TG	0.215	0.050	0.221	4.346	<0.001
	HDL	-0.567	0.165	-0.174	-3.446	0.001
	LDL	-0.145	0.054	-0.134	-2.670	0.008
Total	Age	0.013	0.004	0.122	3.484	0.001
	BMI	0.033	0.012	0.100	2.810	0.005
	TC	-0.141	0.040	-0.148	-3.497	<0.001
	TG	0.342	0.039	0.360	8.749	<0.001
	HDL	-0.293	0.142	-0.085	-2.068	0.039

Age, BMI, course of disease, HbA1c, BGP, TC, TG, LDL, and HDL served as independent variables.

disease ( $\beta=-0.118$ ,  $P=0.030$ ), TC ( $\beta=-0.187$ ,  $P=0.001$ ), and TG ( $\beta=0.491$ ,  $P<0.001$ ) were independent predictors of HOMA-IR; in females age ( $\beta=0.103$ ,  $P=0.033$ ), TG ( $\beta=0.221$ ,  $P<0.001$ ), HDL ( $\beta=-0.174$ ,  $P=0.001$ ), and LDL ( $\beta=-0.134$ ,  $P=0.008$ ) were independent predictors of HOMA-IR; in all subjects, age ( $\beta=0.122$ ,  $P=0.001$ ), BMI ( $\beta=0.100$ ,  $P=0.005$ ), TC ( $\beta=-0.148$ ,  $P<0.001$ ), TG ( $\beta=0.360$ ,  $P<0.001$ ), and HDL ( $\beta=-0.085$ ,  $P=0.039$ ) were independent predictors of HOMA-IR.

## Discussion

This cross-sectional study showed that serum OC level in females was higher than that in males, which is consistent with results of a previous report [25]. Because OC is mainly secreted from osteoblasts during bone turnover, this may suggest that the bone turnover rate in females could be higher than that in middle-age and elderly male T2DM patients. This is also supported by the lower BMD in the females than the males in the present study.

Several clinical trials have reported that serum OC level was significantly lower in T2DM patients than in healthy controls [25,28,29]. A recent study of Aoki et al. showed the serum OC level was significantly higher in pre-diabetic patients than in healthy controls [30]. These findings suggest that the OC level may change with the development of T2DM. In the present study, after adjustment for confounding factors, serum OC was found to be negatively correlated with HbA1C, FPG, and 2-h PG in males, and inversely with HbA1C and FPG in females. Many prospective studies and case-control studies have demonstrated

that serum OC is inversely correlated with HbA1C, FPG, and 2-h PG [20,21,28,31-35]. The results of the above reports are consistent with our findings and support the view that OC may help to regulate glucose metabolism.

The present study also showed that serum OC was positively correlated with 2-h C-P and 2-h INS in all individuals. In the study by Akiko et al., OC was found to stimulate Glucagon-like peptide-1 (GLP-1) secretion, which could stimulate insulin secretion in response to food intake [36], suggesting that OC may stimulate postprandial insulin secretion. A recent study of Bullo et al. revealed that serum OC was positively correlated with FINS [37]. These results suggest that OC may also stimulate fasting insulin secretion.

In this paper, serum OC was positively correlated with HOMA-%B in males, females, and all subjects. Some latest experiments indicated that serum OC was positively correlated with HOMA-%B [21,24,25,37]. These results were in accordance with our outcomes manifesting that OC may induce insulin secretion both in men and women.

This study shows that serum OC was positively correlated with HOMA-%S and negatively correlated with HOMA-IR in females, suggesting OC may help to improve insulin sensitivity and resistance in female patients. However, these associations were not found in males. These findings indicated that the correlation between OC and insulin sensitivity and resistance was obvious in females but not so in males. A Chinese study showed that serum OC was not associated with HOMA-IR in all participants [25]. A Korean study showed that serum

OC was inversely correlated with HOMA-IR in postmenopausal women [27]. However, some other studies in Western countries, including the USA and Sweden, showed that OC was negatively correlated with HOMA-IR in men [21,22,35,37] and all subjects [24,33,34,38,39], and positively correlated with HOMA-%S in all subjects [24]. It is speculated that differences in eating habits, physical features, and body composition (percentage of total body fat) may help to explain these diverse findings.

Our findings revealed that the serum OC was positively correlated with HDL in females and all participants, and negatively with TG in females. Recent clinical studies also uncovered a relationship between OC and TG and HDL in all individuals [20,25,34]. These recent results are consistent with our findings showing that OC may have relations with lipid metabolism. Some studies in Japan and Sweden demonstrated that OC was related to TG and HDL in men [22,35]; however, in our study no relationship between OC and lipid metabolic indices was found in males. These results are inconsistent with our findings, showing that it is probably due to differences in region, dietary and living habit, some medication use, and some other factors in males. These results suggest that bone may regulate lipid metabolism via OC through mechanisms that need further study.

Recent evidence in animal models suggests that OC, a hormone secreted during bone turnover, exerts regulatory effects on glucose and lipid metabolism. Consistent with this result, our results provide empirical evidence for the interactions of osteocalcin with markers of insulin secretion and sensitivity, and insulin resistance in humans. This evidence was confirmed by using 3 parallel statistical analyses based on multiple linear regression models. Results showed that serum OC was not

an independent factor influencing HOMA-%B, HOMA-%S, and HOMA-IR, and that HbA1C was an independent related factor influencing HOMA-%B in all subjects. In addition, BMI and several parameters of lipid metabolism were the independent predictors influencing HOMA-%S and HOMA-IR in all subjects. These results indicate that the effect of OC on the pancreas may not be so strong. That is to say, in addition to OC, there seems to be other factors affecting insulin secretion, sensitivity, and resistance. Our study also suggests a relationship between lipid metabolism and insulin sensitivity and resistance.

There are several limitations in this study. First, this was a cross-sectional study showing only association but not causality. Second, the influence of differences in gamma-carboxylation status of OC was not investigated. Third, different medications, especially treatment with insulin, might affect the results.

## Conclusions

In summary, we found higher serum OC levels in female than male T2DM patients. OC may have an important role in glucose metabolism, probably through enhancing insulin secretion in males and through both increasing insulin secretion and improving insulin resistance in females. OC maybe also play an important role in lipid metabolism in T2DM patients. The coupling mechanisms between bone metabolism and energy metabolism needs further study in the future.

## Conflicts of interest

None declared.

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