



The Effects of Sex, Menopausal Status, and Glucose Tolerance on Osteocalcin Levels in Endocrinology Outpatients: A Case-Control Study

Endokrinoloji Poliklinik Hastalarında Cinsiyet, Menopoz Durumu ve Glukoz Toleransının Osteokalsin Seviyeleri Üzerindeki Etkileri: Bir Vaka-Kontrol Çalışması

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ABSTRACT

Aim: This study aimed to evaluate total and uncarboxylated osteocalcin levels concerning gender, menopausal status, and glucose tolerance in endocrinology outpatients.

Material and Methods: A total of 178 endocrinology outpatients with oral glucose tolerance test (OGTT) findings were included. Data on anthropometrics [body mass index (BMI), waist circumference (cm), body fat percentage (BFP), and fat mass], glycemic parameters [fasting blood glucose (FBG), insulin, C-peptide, HbA1c, and insulin resistance (HOMA-IR)], blood lipids, and serum osteocalcin (OCN) levels [total osteocalcin (tOCN) and uncarboxylated osteocalcin (uOCN)] were compared with sex, menopausal status, and glucose tolerance status.

Results: No significant difference was noted in the tOCN and uOCN levels concerning gender and menopausal status. tOCN was negatively correlated with BMI, waist circumference, BFP, and fat mass in patients with normal glucose tolerance ($p<0.05$) and premenopausal women ($p<0.05$). Besides, tOCN was negatively correlated with BFP and fat mass in patients with prediabetes ($p<0.05$) and positively correlated with fasting insulin levels and HOMA-IR in the prediabetes group ($p<0.05$).

Conclusion: Our findings revealed no significant difference in tOCN and uOCN levels regarding sex, menopausal status or glucose tolerance. The likelihood of a more limited role and complex regulation of OCN-glucose homeostasis link in humans should be considered.

Keywords: Osteocalcin, Diabetes mellitus, Type 2, Adiposity, Glucose tolerance test, Menopause

ÖZ

Amaç: Bu çalışmada, endokrinoloji poliklinik hastalarında cinsiyet, menopoz durumu ve glukoz toleransına göre total ve karboksile olmayan osteokalsin düzeylerinin değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Oral glukoz tolerans testi (OGTT) bulguları olan toplam 178 endokrinoloji poliklinik hastası dahil edildi. Antropometri verileri [vücut kütle indeksi (VKİ), bel çevresi (cm), vücut yağ yüzdesi (BFP) ve yağ kütlesi], glisemik parametreler [açlık kan şekeri (FBG), insülin], C-peptid, HbA1c ve insülin direnç direnci (HOMA-IR)], kan lipidleri ve serum osteokalsin (OCN) seviyeleri [toplam osteokalsin (tOCN) ve karboksile olmayan osteokalsin (uOCN)] cinsiyet, menopoz durumu ve glukoz tolerans durumu ile karşılaştırıldı.



Bulgular: Cinsiyet ve menopoz durumu ile ilgili olarak tOCN ve uOCN düzeylerinde anlamlı bir farklılık kaydedilmedi. Glukoz toleransı normal olan hastalarda ($p < 0.05$) ve premenopozal kadınlarda ($p < 0.05$) tOCN ile VKİ, bel çevresi, BFP ve yağ kütlesi arasında negatif korelasyon vardı. Ayrıca tOCN, prediyabetli hastalarda BFP ve yağ kütlesi ile negatif ($p < 0.05$), prediyabet grubunda açlık insülin düzeyleri ve HOMA-IR ile pozitif korelasyon gösterdi ($p < 0.05$).

Sonuç: Bulgularımız cinsiyet, menopoz durumu veya glukoz toleransı açısından tOCN ve uOCN düzeylerinde anlamlı bir farklılık göstermedi. İnsanlarda OCN-glukoz homeostaz bağlantısının daha sınırlı bir rolü ve karmaşık düzenlenmesi olasılığı dikkate alınmalıdır. OCN-glukoz homeostaz mekanizmasının kanıtlanabilmesi için daha geniş çaplı çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Osteokalsin, Diyabetes mellitus, Tip 2, Şişmanlık, Glukoz tolerans testi, Menopoz

INTRODUCTION

Bone remodeling is a biological process, highly dependent on the organism's energetic status, suggesting the existence of a coordinated endocrine regulation with hormones (1). Leptin, adiponectin, and glucagon-like peptides 1 and 2 are hormones of energy metabolism that participate in the endocrine control of bone mass. On the other hand, osteocalcin (OCN), an osteoblast-derived protein and biochemical marker for bone formation, carries out the endocrine role of bone as implicated in glucose and energy homeostasis (1,2).

Data from OCN knockout mice (*Ocn*^{-/-}) and mice with conditional deletion of the insulin receptors in osteoblasts (*Ob-IR*^{-/-}) revealed that both *Ob-IR*^{-/-} lines displayed impaired glucose tolerance, insulin sensitivity, and insulin secretion, accompanied by reduced serum uncarboxylated OCN (uOCN) concentrations (3). Also, data from *Esp*^{-/-} mice with loss of protein tyrosine phosphatase (OST-PTP) function revealed increased serum uOCN due to enhanced insulin signaling in osteoblasts facilitating decarboxylation and circulatory release of matrix embedded OCN (3,4). Thus, due to constitutive activation of insulin signaling via the loss of OST-PPT inhibitory action, *Esp*^{-/-} mice exhibited an anti-diabetic phenotype with severe hypoglycemia hyperinsulinemia, increased β -cell proliferation, and increased insulin sensitivity opposite to that of the *Ob-IR*^{-/-} mice (3,5).

Past studies in mice and cell cultures indicate that OCN participates in the regulation of glucose metabolism by acting as a bone messenger that affects both adipocytes and insulin-producing β cells, increasing insulin sensitivity and insulin secretion, respectively (6,7).

Therefore, a feed-forward regulatory loop between bone, pancreas, and adipose tissue has been suggested with insulin signaling in osteoblasts regulating the production of OCN and promoting its bio-availability by favoring decarboxylation (4,5) and the resulting increase in circulatory uOCN, enhancing insulin secretion and insulin sensitivity (4,8).

The role of OCN in glucose and energy metabolism was also shown in several cross-sectional and observational

studies in humans, indicating inverse relation of total OCN (tOCN) and/or uOCN with fasting blood glucose (FBG), fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR) index, and adiposity; however, a positive correlation of OCN with serum adiponectin and insulin secretion was observed in non-diabetic, pre-diabetic, and diabetic populations (8-10).

Consideration of OCN as the only biomarker of osteoblast activity that associates with oral glucose tolerance test (OGTT) in patients with type 2 diabetes mellitus (DM) (11) seems notable given the role of complex and yet unclarified interplay between increased insulin resistance and decreased insulin secretion by β cells in the pathogenesis of type 2 diabetes DM (2,9). This emphasizes the likely role of OCN as a risk factor and a therapeutic target in diabetes DM management (9,12). However, in contrast to animal studies, little direct evidence is available in humans. Hence, the actual presence, and more importantly, the physiological relevance of such a potential bi-directional link between OCN and glucose metabolism remains unclear (2,5,9,12,13).

Given the alteration in the normal biphasic process of bone remodeling and strong association of bone turnover biomarkers with certain parameters such as age, gender, menopausal status, and body composition (2,5,8), the present study was designed to evaluate tOCN and uOCN levels regarding gender, menopausal status, and glucose tolerance status in endocrinology outpatients who underwent OGTT.

MATERIAL and METHODS

Study Design

A descriptive cross-sectional study was designed. Study reporting was done per the STROBE guidelines (14). The study was approved by the Ethics Committee of Kocaeli University (date: December 27, 2010, IRB number: 2010/9-1/10). Written informed consent was obtained from the participants, and all procedures were done per the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration or comparable ethical standards.

Setting

The study was conducted at the endocrinology outpatient clinics of the Kocaeli University Hospital between November 2009 and May 2010. The endocrinology outpatients welcome daily around 65 patients.

Participants

A total of 4812 patients applied to the endocrinology outpatients during the study period. Of these, 288 underwent OGTT. Of the 99 patients diagnosed with prediabetes, seven refused to join, and three were excluded (two using medications, and one having hyperthyroidism). From the remaining cases, 24 were men, while 65 were women. The women were further categorized as premenopausal or postmenopausal (amenorrhea for at least 12 months). An equal number of healthy controls with normal glucose tolerance (NGT) were invited and included in the study (Figure 1). All participants were checked for the absence of hepatic or renal failure, malignancy, renal calculi, hyperparathyroidism (parathormone levels >65 pg/mL) and hyperthyroidism, or use of medications likely to influence bone turnover (including bisphosphonates, calcitonin, estrogen, testosterone, corticosteroids, calcium, and vitamin D).

Variables

The primary outcome variables of the study were serum tOCN (ng/mL) and uOCN (ng/mL) levels. Data on age

(years), anthropometrics [height (cm), weight (kg), body mass index (BMI; kg/m²), waist circumference (cm), body fat percentage (BFP; %), fat mass (kg), glycemic parameters [FBG (mg/dL), insulin (uU/ml), C-peptide (ng/mL), HbA1c (%), HOMA-IR], lipid parameters (total cholesterol (mg/dL), triglyceride (mg/dL), high density lipoprotein cholesterol (HDL-c; mg/dL), and low density lipoprotein cholesterol (LDL-c; mg/dL) levels were recorded in all groups. Normal glucose tolerance (NGT) was defined as FBG: <100 mg/dL and/or 2h-OGTT <140 mg/dL, while prediabetes was defined as FBG 100-125 mg/dL and/or 2h-OGTT 140-199 mg/dL.

Height was measured using a stadiometer, while body weight (kg), BMI (kg/m²), BFP (%), and fat mass were measured using the Tanita Body Composition Analyzer (Tanita BC-418 M 17, Japan). The waist circumference was measured in the midline between the iliac crest and the last rib edge using a 1 cm-wide metal measuring tape.

FBG, insulin and C-peptide was analyzed in blood samples collected at 08:00-08:30 following a fasting period of at least 10 hours, while blood glucose, insulin, and C-peptide levels were also determined using blood samples after 2 h hour 75 g OGTT. Glucose levels were determined with the glucose oxidase method. In contrast, plasma insulin and C-peptide levels were measured with the immunoassay method using the Advia Centaur XP (manufactured in Japan by

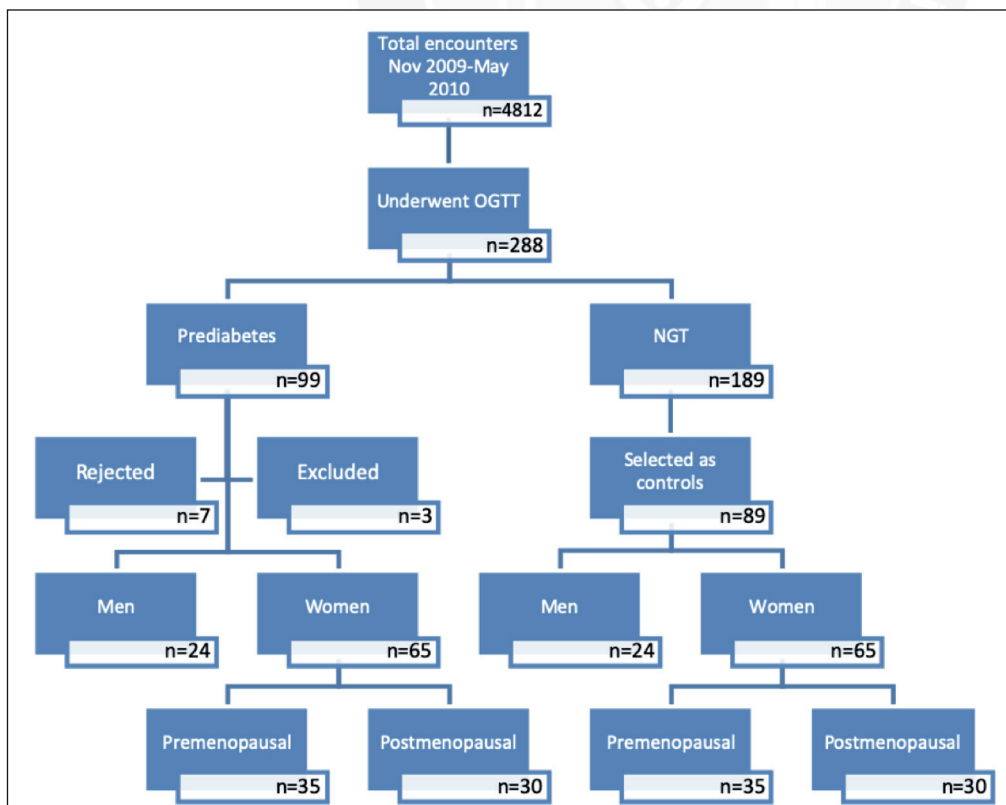


Figure 1: Patient flow chart
NGT: Normal glucose tolerance.

Kyowa Medex Co. Ltd for Siemens Healthcare Diagnostics, Camberly, UK) and Immulite 2000 XP (Siemens Healthcare Diagnostics, Tarrytown, USA) analyzers. HOMA-IR was calculated using the formula $\text{FBG (mg/dL)} \times \text{Fasting insulin (mU/L)} / 405$.

HbA1c levels were measured with the high-performance liquid chromatography (HPLC) method (Shimadzu, Japan), while cholesterol levels were determined spectrophotometrically using the C 16000 Architect (Abbott, Illinois, USA) analyzer. tOCN was measured with the ECLIA method using the Cobas analyzer (Roche Diagnostics GmbH, Mannheim, Germany). For uOCN measurements, blood samples were kept at $-80\text{ }^{\circ}\text{C}$ until analysis. All samples were analyzed simultaneously using the enzyme immunoassay method (Takara Biomedical Group, Otsu, Shiga, Japan)

Bias

All measurements were performed by the same researcher. Also, to eliminate selection bias, it was ensured that all participants were enrolled concomitantly during the study period.

Study Size

A total sample size of 46 participants (23 study+23 control) is required to compare two independent groups for a numerical variable using the Student's t-test with an effect size of 0.85, a two-way hypothesis, an alpha error of 5%, and a power of 80% (15).

Statistical Methods

Statistical analysis was made using the Number Cruncher Statistical System (NCSS) 2007 Statistical Software (Utah, USA). Distributions of the numerical variables were checked with the Shapiro-Wilk's test. tOCN, uOCN, age, anthropometric measurements, glycemic variables, and lipid parameters were compared between the groups as well as concerning the presence of prediabetes. The Chi-Square test or Fisher's exact test was used to compare categorical variables, while numeric variables were analyzed using the independent samples t-test or one-way ANOVA with post-hoc Tukey tests. Correlation analyses were performed with Pearson's correlation test. Data were expressed as mean \pm standard deviation (SD) or n (%), where appropriate. A p-value <0.05 was considered statistically significant.

RESULTS

The results of 178 patients were analyzed. No significant difference was noted between study groups regarding tOCN and uOCN levels. Apart from significantly higher age and lower height in postmenopausal compared to premenopausal women, no significant difference was observed between the female groups regarding BMI, waist

circumference, body fat percentage BFP, and fat mass. Males were younger than postmenopausal females but older than premenopausal women; had significantly lower body fat percentage BFP and fat mass than premenopausal and postmenopausal women, and had higher body weights compared to the premenopausal group (Table 1).

On the other hand, premenopausal women had significantly lower mean C-peptide levels than males and significantly lower HbA1c levels than both males and postmenopausal females. However, no significant difference was noted between the study groups concerning serum levels of FBG, insulin, and HOMA-IR (Table 1).

Furthermore, premenopausal females had significantly lower total cholesterol levels than postmenopausal women and men and significantly lower LDL-c levels than postmenopausal females. Also, men had significantly higher triglycerides and lower HDL-c levels than premenopausal and postmenopausal women (Table 1).

Subgroup comparisons were made regarding the presence of prediabetes. Premenopausal patients with prediabetes were older than the NGT group. No significant difference was noted between patients with prediabetes and NGT in males and postmenopausal female groups per age (Table 2). Postmenopausal females with prediabetes had significantly higher mean BMI, waist circumference, body fat percentage BFP, and fat mass than the NGT group (Table 2). Also, patients with prediabetes had significantly higher mean FBG levels in males, premenopausal females, and postmenopausal females compared to the NGT group. On the other hand, higher HbA1c levels were observed in males, while higher C-peptide and HOMA-IR levels were observed in postmenopausal females (Table 2).

Considering the lipid parameters, the only significant change was in the postmenopausal female group with significantly higher triglyceride levels in patients with prediabetes than NGT. No significant difference was noted in tOCN and uOCN levels between the groups concerning glucose tolerance (Table 2).

There were significant correlations between some of the numerical variables. tOCN levels showed no correlation with age ($p>0.05$), but correlated negatively with BMI ($r=-0.205$, $p=0.008$), waist circumference ($r=-0.157$, $p=0.042$), BFP ($r=-0.235$, $p=0.002$), and fat mass ($r=-0.238$, $p=0.002$) in the overall study population. Subgroup analysis revealed that negative correlations of tOCN with BMI and waist circumference were evident only in the NGT group ($r=-0.275$, $p=0.011$ and $r=-0.245$, $p=0.025$, respectively) and premenopausal females ($r=-0.321$, $p=0.008$ and $r=-0.314$, $p=0.009$, respectively). Additionally, negative correlations of tOCN with BFP and fat mass were seen in the NGT ($r=-0.268$, $p=0.014$ and $r=-0.305$, $p=0.005$, respectively), prediabetes ($r=-0.253$, $p=0.02$ and $r=-0.22$, $p=0.044$, respectively), and

Table 1: Distributions of age, anthropometric measurements, glycemic indicators, lipid variables, and osteocalcin levels between the groups.

	Men (n=48)	Women (n=130)		p
		Premenopausal (n=70)	Postmenopausal (n=60)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Age	41.9±11.0*	32.2±9.4	54.5±5.7 ^{qqq}	<0.001
Anthropometrics				
Height (cm)	173.2±6.6*	160.8±6.5	157.0±6.1 ^{qqq}	<0.001
Weight (kg)	87.5±15.3 ^q	78.9±20.4	79.1±17.7	0.025
BMI (kg/m ²)	29.2±4.71	30.4±7.1	32.17±7.4	0.079
Waist circumference (cm)	101.4±11.9	95.6±14.9	99.9±15.7	0.075
Body fat percentage (%)	25.2±7.0*	37.9±7.9	40.5±6.9	<0.001
Fat mass (kg)	22.8±10.0*	31.2±13.7	33.0±12.6	<0.001
Glycemic parameters				
FBG (mg/dl)	99.1±11.9	101.1±12.27	102.3±10.5	0.379
Insulin (uU/ml)	8.9±8.1	7.8±5.1	6.6±5.3	0.181
C peptide (ng/ml)	2.5±0.8 ^{qq}	2.0±0.7	2.2±1.0	0.012
HbA1c (%)	5.4±0.6 ^q	5.1±0.5	5.5±0.4 ^{qqq}	<0.001
HOMA-IR	2.1±1.9	2.0±1.3	1.76±1.4	0.444
Lipid parameters				
Total cholesterol (mg/dL)	207.6±46.4 ^q	188.9±36.2	223.6±41.2 ^{qqq}	<0.001
Triglyceride (mg/dL)	184.6±100.1 ^{qqq,+}	121.6±55.5	145.0±73.1	<0.001
HDL-c (mg/dL)	39.7±7.1*	51.2±12.1	53.0±10.1	<0.001
LDL-c (mg/dL)	130.6±44.6	115.1±30.2	141.5±34.5 ^{qqq}	<0.001
tOCN (ng/mL)	14.0±7.7	11.4±4.5	12.3±5.7	0.059
uOCN (ng/mL)	3.9±1.1	3.9±1.3	3.9±1.3	0.989
Glucose tolerance, n (%)				
Normal	24 (50.0)	35 (50.0)	30 (50.0)	>0.05
Prediabetes	24 (50.0)	35 (50.0)	30 (50.0)	

BMI: Body mass index; **FBG:** Fasting blood glucose; **HOMA-IR:** Homeostatic Model Assessment of Insulin Resistance; **HDL-c:** High density lipoprotein cholesterol; **LDL-c:** Low density lipoprotein cholesterol; **tOCN:** Total osteocalcin; **uOCN:** Uncarboxylated osteocalcin
ANOVA and post-Hoc Tukey test

*p<0.001; compared to premenopausal and postmenopausal females

^qp<0.05, ^{qq}p<0.01 and ^{qqq}p<0.001; compared to premenopausal female

⁺p<0.05; compared to postmenopausal female

premenopausal females ($r=-0.295$, $p=0.015$ and $r=-0.27$, $p=0.026$, respectively) (Table 3).

Furthermore, uOCN negatively correlated with age in the overall study population ($r=-0.167$, $p=0.03$), in prediabetes ($r=-0.3$, $p=0.006$) and males ($r=-0.339$, $p=0.018$), but not in the NGT or female groups. uOCN had no correlation with any of the anthropometric parameters studied in the overall study population as well as in subgroups (Table 3). Lastly, amongst glycemic parameters, tOCN levels were positively correlated with fasting insulin levels and HOMA-IR in the overall study population ($r=0.191$, $p=0.013$ and $r=0.193$,

$p=0.012$, respectively) and in prediabetes ($r=0.246$, $p=0.024$ and $r=0.226$, $p=0.039$, respectively) but not in the NGT group, in males or females. A significant positive correlation between tOCN and FBG was found only in postmenopausal females ($r=0.281$, $p=0.044$) (Table 3).

DISCUSSION

Key Results

Our findings revealed no significant difference in tOCN and uOCN levels regarding gender, menopausal status, or glucose tolerance status. Significant negative correlations

Table 2: Comparison of the study variables between the groups regarding glucose tolerance.

	Men (n=48)			Women (n=130)					
				Premenopausal (n=70)			Postmenopausal (n=60)		
	NGT (n=24)	PreDM (n=24)	p	NGT (n=35)	PreDM (n=35)	p	NGT (n=30)	PreDM (n=30)	p
Age	40.0±8.6	43.7±12.8	0.250	28.9±8.3	35.4±9.4	0.003	53.31±5.24	55.7±6.0	0.129
Height (cm)	172.1±6.5	174.2±6.6	0.287	161.7±6.6	159.9±6.5	0.269	159.0±5.1	155.0±6.4	0.016
Weight (kg)	83.5±12.5	91.5±16.9	0.068	78.7±21.2	79.1±19.8	0.946	74.6±17.7	83.6±16.9	0.067
BMI (kg/m ²)	28.2±3.8	30.2±5.3	0.130	30.0±7.0	30.9±7.3	0.600	29.4±6.0	34.96±7.7	0.005
Waist circumference (cm)	98.3±10.5	104.6±12.5	0.064	94.7±15.5	96.5±14.4	0.628	94.0±13.6	105.7±15.7	0.006
Body fat percentage (%)	23.3±7.0	27.1±6.6	0.062	37.9±7.2	37.8±8.7	0.964	37.9±6.4	43.1±6.5	0.005
Fat mass (kg)	20.1±8.1	25.6±11.1	0.056	31.1±14.3	31.3±13.2	0.949	29.2±11.5	36.9±12.6	0.024
FBG (mg/dL)	89.8±6.0	108.3±8.5	<0.001	91.4±7.3	110.8±7.7	<0.001	94.4±6.2	110.3±7.5	<0.001
Insulin (uU/mL)	8.6±8.4	9.1±7.9	0.835	8.1±5.2	7.6±5.0	0.727	5.6±5.9	7.5±4.7	0.211
C peptide (ng/mL)	2.5±0.9	2.5±0.8	0.717	1.9±0.6	2.1±0.7	0.341	1.8±0.9	2.6±1.0	0.009
HbA1c (%)	5.2±0.5	5.6±0.5	0.004	5.1±0.5	5.2±0.4	0.295	5.5±0.4	5.6±0.3	0.541
HOMA-IR	1.9±1.8	2.4±2.0	0.325	1.8±1.2	2.1±1.4	0.396	1.4±1.4	2.2±1.3	0.037
Total cholesterol (mg/dL)	217.4±52.0	197.8±38.7	0.145	190.2±40.5	187.5±32.0	0.761	216.6±35.0	230.7±46.2	0.220
Triglyceride (mg/dL)	210.8±117.2	158.5±73.0	0.07	104.3±54.9	118.9±56.0	0.282	118.3±48.0	171.8±84.3	0.007
HDL-c (mg/dL)	40.4±6.9	39.1±7.4	0.535	53.3±12.6	49.1±11.2	0.151	55.7±9.3	50.3±10.3	0.055
LDL-c (mg/dL)	134.6±49.3	126.7±40.1	0.545	115.7±33.6	114.6±26.8	0.880	137.4±28.9	145.7±39.4	0.392
tOCN (ng/mL)	12.9±7.1	15.1±8.3	0.321	11.4±4.7	11.3±4.3	0.878	10.7±5.4	13.8±5.7	0.054
uOCN (ng/mL)	3.8±1.1	3.9±1.1	0.826	3.7±1.1	4.1±1.4	0.237	3.9±1.3	3.9±1.2	0.881

NGT: Normal glucose tolerance; **preDM:** Prediabetes; **BMI:** Body mass index; **FBG:** Fasting blood glucose; **HOMA-IR:** Homeostatic Model Assessment of Insulin Resistance; **HDL-c:** High density lipoprotein cholesterol; **LDL-c:** Low density lipoprotein cholesterol; **tOCN:** Total osteocalcin; **uOCN:** Uncarboxylated osteocalcin. Independent samples t-test

were noted between uOCN and age in males and women with prediabetes. tOCN correlated negatively with BMI, waist circumference, BFP, and fat mass in premenopausal women and in patients with NGT. In patients with prediabetes; a negative correlation was observed with tOCN, BFP and fat mass and a positive correlation was seen between fasting insulin and HOMA-IR levels.

Interpretation

Alongside decreased OCN levels in patients with prediabetes (16), inverse associations of tOCN and/or uOCN with FBG, HOMA-IR, and adiposity and positive correlation with insulin secretion were consistently reported both in diabetic and non-diabetics populations (8-10,16). However, no significant difference was noted in tOCN and uOCN levels between patients with prediabetes and NGT in our cohort, along with positive correlation of tOCN with fasting insulin and HOMA-IR levels and negative correlation with BFP and fat mass in patients with prediabetes.

Chronic exposure to hyperglycemia was associated with the inhibition of cell growth of an osteoblast-like cell line in humans and decreased OCN mRNA levels in mouse

osteoblasts (17,18). Hence, lower OCN levels in diabetes DM or prediabetes reported in cross-sectional studies are considered likely to be a consequence rather than a cause of hyperglycemia (2).

In fact, the presence of OST-PTP in osteoblasts has been associated with alteration in downstream insulin signaling that may sustain the insulin responsiveness of osteoblasts in situations leading to resistance at the receptor level in other tissues (12). This sustained release of OCN has been suggested to enable a homeostatic mechanism to compensate for the increased need for insulin in the presence of insulin resistance.

Accordingly, OCN was suggested to induce insulin secretion to overcome insulin resistance accompanying pregnancy and weight gain in mice studies (19). Similarly, circulating OCN was also reported to show a positive correlation with glycemic parameters in early-stage type 2 diabetes. At the same time, a gradual turn of OCN to a declining course has been suggested to occur by the progression of glucose intolerance toward overt diabetes DM (20). Hence, OCN may be related to an increase in insulin secretion in early-stage diabetic subjects with insulin resistance.

Table 3: Correlation of total and uncarboxylated osteocalcin levels with the study variables.

Study parameters		Total (n=178)		NGT (n=89)		Prediabetes (n=89)		Men (n=48)		Women (n=130)			
										Premenopausal (n=70)		Postmenopausal (n=60)	
		tOCN	uOCN	tOCN	uOCN	tOCN	uOCN	tOCN	uOCN	tOCN	uOCN	tOCN	uOCN
Age	r	-0.018	-0.167	-0.068	-0.05	-0.015	-0.3	-0.101	-0.339	-0.238	-0.231	0.152	-0.224
	p	0.822	0.03	0.536	0.653	0.893	0.006	0.495	0.018	0.051	0.058	0.283	0.111
Anthropometrics													
BMI (kg/m ²)	r	-0.205	-0.021	-0.275	-0.179	-0.21	0.075	-0.14	-0.152	-0.321	-0.042	-0.156	0.065
	p	0.008	0.785	0.011	0.103	0.056	0.499	0.344	0.301	0.008	0.736	0.268	0.645
Waist circumference (cm)	r	-0.157	-0.031	-0.245	-0.159	-0.144	0.05	-0.247	-0.192	-0.314	-0.024	-0.052	0.053
	p	0.042	0.690	0.025	0.148	0.192	0.653	0.091	0.192	0.009	0.849	0.713	0.709
Body fat percentage (%)	r	-0.235	-0.015	-0.268	-0.152	-0.253	0.088	-0.135	-0.141	-0.295	-0.061	-0.19	0.122
	p	0.002	0.848	0.014	0.167	0.02	0.427	0.361	0.340	0.015	0.622	0.178	0.390
Fat mass (kg)	r	-0.238	-0.011	-0.305	-0.133	-0.22	0.082	-0.125	-0.114	-0.27	-0.039	-0.169	0.087
	p	0.002	0.892	0.005	0.229	0.044	0.461	0.397	0.441	0.026	0.753	0.232	0.539
Glycemic parameters													
FBG (mg/dl)	r	0.048	0.025	-0.085	0.036	-0.08	-0.096	-0.02	-0.01	-0.013	0.083	0.281	-0.033
	p	0.538	0.746	0.443	0.747	0.469	0.387	0.893	0.946	0.913	0.501	0.044	0.814
Fasting insulin (uU/ml)	r	0.191	0.014	0.131	-0.133	0.246	0.159	0.247	-0.07	0.113	-0.017	0.126	0.163
	p	0.013	0.853	0.237	0.227	0.024	0.148	0.09	0.637	0.360	0.890	0.373	0.248
C peptide (ng/ml)	r	-0.039	0.013	-0.045	-0.059	-0.081	0.054	-0.021	-0.234	-0.13	0.069	-0.126	0.13
	p	0.616	0.864	0.686	0.595	0.465	0.622	0.885	0.109	0.290	0.575	0.375	0.356
HbA1c (%)	r	0.097	-0.004	0.079	-0.048	0.069	0.014	0.056	-0.034	0.01	-0.012	0.175	0.03
	p	0.211	0.956	0.477	0.663	0.532	0.897	0.707	0.820	0.933	0.925	0.215	0.830
HOMA-IR	r	0.193	0.034	0.115	-0.116	0.226	0.141	0.242	-0.055	0.12	0.014	0.156	0.161
	p	0.012	0.662	0.297	0.292	0.039	0.2	0.097	0.710	0.329	0.909	0.270	0.255

BMI: Body mass index; **FBG:** Fasting blood glucose; **HOMA-IR:** Homeostatic Model Assessment of Insulin Resistance; **tOCN:** Total osteocalcin; **uOCN:** Uncarboxylated osteocalcin; **NGT:** Normal glucose tolerance; **r:** Correlation coefficient.

Also, based on the demonstration of increased OCN levels and positive correlation of OCN with insulin secretion parameters in pregnant women with gestational diabetes mellitus (GDM) than those with NGT, OCN has been suggested to enhance insulin secretion in pronounced insulin-resistant states such as late pregnancy. At the same time, a potential effect of hyperinsulinemia on OCN secretion has also been emphasized (21). The same authors also reported that the disposition index, which refers to the ability to increase insulin secretion to cope with increased insulin resistance, was positively correlated with OCN in subjects with GDM. Accordingly, increased OCN levels in GDM indicate an early adaptation mechanism for impaired glucose tolerance, enabling coping with an increased insulin demand and dissipating with the onset of overt type 2 diabetes DM. Although our patients with prediabetes and NGT had similar levels of tOCN and uOCN, our findings seem to indicate that the regulation of glucose homeostasis

by OCN may be confounded in humans depending on the course of diabetes DM and the accompanying insulin resistance.

On the other hand, it was reported that baseline circulating OCN levels do not predict future diabetes DM development (2,22). Accordingly, based on the lack of an association between plasma OCN levels and the future development of diabetes DM compared to most cross-sectional data, it was suggested that OCN may not play a significant role in glucose homeostasis in humans (2,23). This also seems to be in accordance with the lack of any evidence related to an increased risk for incident diabetes DM in patients treated with drugs known to reduce circulating levels of OCN, such as bisphosphonates and raloxifene and strontium ranelate (24).

Nonetheless given the reported association of baseline OCN levels with future development of diabetes DM in other studies (25), consideration of the potential impact of several

factors related to bone metabolism on OCN levels has also been emphasized including age, gender, menopausal status, physical activity, smoking, renal function, and the use of some medications (2,5,8,20).

In our study, along with no change in anthropometrics among females concerning menopausal status, premenopausal females had a more favorable glycemic and lipid profile than postmenopausal females and males. In contrast, the presence of prediabetes was associated with significantly higher BMI, waist circumference, BFP, and fat mass compared to NGT only in postmenopausal females. However, no change in tOCN and uOCN was evident regarding menopausal status, and except a positive correlation between tOCN and FBG levels, no association was shown between tOCN and uOCN and anthropometrics or glycemic parameters in postmenopausal women. Our findings are in line with the lack of correlation between tOCN and FPG, fasting insulin, and insulin resistance parameters reported in a study among non-diabetic postmenopausal women from Turkey (26). In addition, Şahin et al. found a statistically significant difference in waist circumference and BMI in prediabetes patients compared to the control group (27). In contrast, higher levels of OCN in postmenopausal women compared to males and premenopausal women, (28) lower levels of OCN in postmenopausal women with type 2 diabetes DM compared to prediabetes and NGT (29), as well as an inverse relationship between OCN and glucose, HbA1c, HOMA-IR, and abdominal obesity parameters in postmenopausal women were reported in past studies (29,30).

Lack of any correlation between OCN and glycemic parameters in our patients with NGT seems consistent with the suggestion of the effects of OCN in glucose metabolism to be minimal in people with NGT but augmented when the glucose metabolism is impaired (9). Notably, only a weak association was reported to exist between OCN and glucose metabolism, regardless of the fraction of OCN measured (23) and despite lower OCN levels in prediabetes than NGT (2). Likewise, some authors reported no significant difference in tOCN and uOCN between subjects with NGT, impaired glucose regulation, and type 2 diabetes DM (9) along with no correlation between tOCN or uOCN and glucose levels in subjects with NGT (23).

Nonetheless, given the inverse association of tOCN levels with BMI, BFP, and fat mass in patients with NGT as well as in the premenopausal females who had a more favorable glycemic profile, our findings support the inverse association of OCN with adiposity (31) and the likelihood of a glucose-independent bone-adipocyte interaction before the deterioration of glycemic control and glucose tolerance.

Along with OCN's direct action in inducing adiponectin expression, the regulation of bone mass via adiponectin was shown to occur without affecting glucose metabolism in

mice fed with a normal diet (32). In contrast, a role for adiponectin in insulin sensitivity was noted in mice fed with a high-fat diet (33). Also, change in visceral fat was shown to be the best predictor of the change in OCN after controlling for age, BMI, and change in insulin sensitivity, while a protective effect of OCN on obesity and insulin resistance has been suggested to occur, at least in part, owing to its ability to increase energy expenditure in brown adipose tissue and the skeletal muscle (1). Additionally, different concentrations of OCN were required to regulate β -cell and adipocyte gene expression and associated metabolic outcomes in wild-type mice (7). In the review written by Ottani et al in 2020, they described the regulation of lipid metabolism by low-dose uncarboxylated osteocalcin. They stated that low-dose uncarboxylated osteocalcin increases glucose and lipid metabolism by promoted the expression of adiponectin. High-dose uncarboxylated osteocalcin increases the number of adipocytes by triggering necroptosis in adipocytes(34) Thus, a threshold effect of OCN has been suggested with no further decrease in blood biomarkers of metabolic phenotype beyond the threshold also in humans (35). Hence, our findings support the unclear link of OCN-glucose homeostasis in humans (2), and emphasize a likelihood of a more limited role for OCN in human glucose metabolism (26) as well as a more complex endocrine function for OCN in humans.

In our research, tOCN and uOCN levels showed no difference concerning sex, while tOCN levels were inversely correlated with age only in men. This seems consistent with a past study indicating no correlation between age and OCN in women and similar OCN levels between men and women with type 2 diabetes DM (20). Also, an increase in age was reported to be associated with a decrease in OCN concentrations along with an increasing trend in OCN levels in postmenopausal women and men over the age of 70 (36).

Our findings revealed no correlation between tOCN and uOCN levels and anthropometric as well as glycemic parameters among males. Given that males had better anthropometric indices than females, lack of any correlation between OCN and glycemic and anthropometrics among males, alongside with a negative association of OCN with age in males seems to emphasize a need for future studies addressing the associations of OCN not only with body fat mass but also with lean body mass to clarify the interaction of lean tissue in the intricate relationships between fat, bone, and energy metabolism (37). Given the significant negative correlation of tOCN with age not only in males but also in all patients with prediabetes, our findings also support the role of elucidating the lifelong interaction between body composition and bone metabolism in better management of potentially adverse metabolic and skeletal outcomes, especially during the aging process. In addition Tanoglu et al showed that the verbal memory could be improved by diet and metformin therapy in newly diagnosed type 2 diabetic patients (38).

Limitations

Certain limitations of this study should be considered. First, our study was powered with a relatively high effect size. Studies with higher sample sizes could reveal other significant associations. Lack of data on other hormones involved in bone and energy metabolism, such as vitamin D, parathyroid hormone, and adiponectin, as well as the potential contribution of negative hormonal regulators of OCN such as leptin and glucocorticoids, and also the absence of data on the insulinogenic index and visceral and subcutaneous adipose compartments may be considered as other limitations of the current study. Nonetheless, the exclusion of patients with diseases and medications likely to influence bone turnover and the association of both tOCN and uOCN in our research provided reliable estimates for otherwise confounding variables.

CONCLUSION

In conclusion, our findings revealed no significant difference in tOCN and uOCN levels concerning sex, menopausal status, or glucose tolerance status. uOCN was not correlated with any anthropometric and glycemic parameter, while negatively correlated with age in males and in patients with prediabetes. tOCN was correlated negatively with all anthropometric indices in patients with NGT and premenopausal females, while correlated negatively with BFP and fat mass and positively with fasting insulin and HOMA-IR levels in patients with prediabetes. Our findings emphasize the likelihood of a more limited role and more complex regulation of OCN-glucose homeostasis link in humans as well as the potential modification of the crosstalk between bone and energy metabolism, depending on the course of diabetes DM and presence of insulin resistance. More extensive research is needed to elucidate the relevance of the potential link between bone and energy metabolism in humans.

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Author Contributions

Authors' contributions are equal.

Conflicts of Interest

The authors have no conflict of interest in this study.

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Ethical Approval

The study was approved by the Ethics Committee of Kocaeli University (date: December 27, 2010, IRB number: 2010/9-1/10).

Review Process

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