Journal of Diabetes and Its Complications 33 (2019) 390–397

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Lower serum osteocalcin concentrations in patients with type 2 diabetes and relationships with vascular risk factors among patients with coronary artery disease

L. Darwish [a,b,c,d], M.M. Nguyen [a,b,c,d], M. Saleem [a,d,e], K.A. Eakin [f], N. Herrmann [a,c,e,g], K.S. Sugamori [b], P.I. Oh [d], P. Yang [h], J. Mitchell [b], K.L. Lanctôt [a,b,c,d,e,g], W. Swardfager [a,b,c,d,*]

[a] Hurvitz Brain Sciences Program, Sunnybrook Research Institute, Toronto, Canada
[b] Department of Pharmacology & Toxicology, University of Toronto, Toronto, Canada
[c] Canadian Partnership for Stroke Recovery, Sunnybrook Research Institute, Toronto, Canada
[d] University Health Network Toronto Rehabilitation Institute, Toronto, Canada
[e] Neuropsychopharmacology Research Group, Sunnybrook Research Institute, Toronto, Ontario, Canada
[f] HBSc Program, Queen's University, Kingston, Ontario, Canada
[g] Department of Psychiatry, University of Toronto, Toronto, Canada
[h] Sunnybrook Academic Family Health Team, Sunnybrook Research Institute, Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Received 7 December 2018
Received in revised form 11 January 2019
Accepted 13 January 2019
Available online 17 January 2019

Keywords:
Osteocalcin
Coronary artery disease
Type 2 diabetes mellitus
Fitness
Metabolism
Endocrinology

ABSTRACT

Background: Lower serum concentrations of the osteoblast-derived protein, osteocalcin, have been associated with poorer glycemic control, insulin resistance and atherosclerosis, and with the development of type 2 diabetes (T2DM).

Methods: This study compares concentrations of two physiological forms of osteocalcin, carboxylated (cOCN) and uncarboxylated (unOCN), between participants with T2DM ($n = 20$) and age-, gender- and body mass index (BMI)-matched participants without T2DM ($n = 40$) among patients with coronary artery disease (CAD), and it explores relationships between osteocalcin concentrations and cardiovascular risk factors.

Results: Concentrations of unOCN (2.71 ± 1.86 vs. 4.70 ± 2.03 ng/mL; $t = -3.635$, $p = 0.001$) and cOCN (8.70 ± 2.27 vs. 10.77 ± 3.69 ng/mL; $t = -2.30$, $p = 0.025$) were lower in participants with T2DM. In participants without T2DM, concentrations of cOCN were associated with fitness (VO_{2Peak} rho $= 0.317$, $p = 0.047$) and lower body fat (rho $= -0.324$, $p = 0.041$). In participants with T2DM, lower unOCN was associated with HbA1c (rho $= -0.516$, $p = 0.020$). Higher body mass was associated with higher unOCN (rho $= 0.423$, $p = 0.009$) in participants without T2DM, but with lower concentrations of both unOCN (rho $= -0.590$, $p = 0.006$) and cOCN (rho $= -0.632$, $p = 0.003$) in participants with T2DM.

Conclusion: In patients with CAD, lower osteocalcin concentrations were related to type 2 diabetes, and to adverse fitness, metabolic and obesity profiles.

1. Introduction

Type 2 diabetes (T2DM) is a chronic metabolic disease and a major risk factor for cardiovascular morbidity and mortality. Studies have reported lower circulating concentrations of the osteoblast-derived protein osteocalcin in people with T2DM compared to those without T2DM.[1,2] In people with T2DM, lower circulating osteocalcin concentration have been associated with poorer glycemic control, and with measures of atherosclerosis.[3,4] It is therefore of interest to examine how osteocalcin concentrations differ among patients with cardiovascular disease between those with and without T2DM, and which metabolic and vascular factors are related to this difference.

Osteocalcin circulates physiologically in several carboxylation states, two of which are reliably quantifiable in humans.[5] Osteocalcin is synthesized by osteoblasts and is post-translationally γ-carboxylated on three Gla residues in a vitamin K-dependent manner to carboxylated osteocalcin (cOCN). It is thought that cOCN has effects on bone mineralization and bone turnover while attached to calcium in bone.[6,7] Clinically, lower circulating cOCN concentrations have been associated with insulin resistance, suggesting the need to evaluate these relationships in people both with and without T2DM.[8,9] In a meta-analysis of studies including

Declaration of interest: None.
Funding: The authors gratefully acknowledge financial support from the Canadian Institutes of Health Research (PJT-159711), NIDDK Diabetic Complications Consortium (DiaComp www.diacomp.org, grant number DK076169), Natural Sciences and Engineering Research Council of Canada (RGPIN-2017-0692), and the Centre for Collaborative Drug Research, University of Toronto.
* Corresponding author at: 1 King's College Circle, Toronto, ON M5S 1A8, Canada.
E-mail address: w.swardfager@utoronto.ca (W. Swardfager).

https://doi.org/10.1016/j.jdiacomp.2019.01.003
1056-8727/

measures for both serum osteocalcin and body mass index (BMI), circulating osteocalcin concentrations were significantly associated with BMI.[10] When studies were subgrouped by metabolic profile, the relationship between these two variables was driven by subgroups of participants with "metabolic syndrome", providing rationale for investigating groups of people with and without T2DM matched for BMI.[10]

In mice, decarboxylated osteocalcin is hormonally active, conferring widespread endocrine functions, including stimulation of β-cell proliferation, increasing insulin sensitivity in target tissues, and regulating fat mass.[6,11,12] In humans, these activities have yet to be substantiated; however, the fully uncarboxylated species (unOCN) has been associated with β-cell function among men with impaired glucose tolerance, prediabetes or T2DM.[9,13] Studies of older men with and without T2DM reported associations between unOCN and insulin sensitivity,[14] and with risk of T2DM.[15] The unOCN form in particular has also been related to cardiovascular disease in participants with and without T2DM.[16] In those with T2DM, unOCN, but not cOCN, was significantly correlated with measures of dyslipidemia.[17]

Differences in circulating osteocalcin concentrations, with carboxylation state-specific measurements, between well-matched groups of participants with and without T2DM, remain scarce. Some studies have provided analyses comparing osteocalcin species in participants with different measures of the metabolic syndrome,[17–19] but none have included well matched T2DM and non-T2DM groups. Moreover, it remains unknown to what extent these species are related to varying cardiovascular risk profiles in T2DM and non-T2DM populations. Here we compare both cOCN and unOCN between people with and without T2DM, all of whom have symptomatic coronary artery disease (CAD). Second, the study explores the relationships between cardiovascular risk factors and circulating osteocalcin concentrations within the T2DM and non-T2DM groups specifically.

2. Methods

2.1. Participants

This retrospective study included 60 participants who were enrolled in a year-long exercise-based cardiac rehabilitation program at the University Health Network Toronto Rehabilitation Institute Cardiac Rehab Program. Twenty participants with T2DM were matched 2:1 for age, gender and BMI category to 40 who did not have T2DM. All participants had CAD as indicated by referral for myocardial infarction, cardiac stent, coronary artery bypass graft, or angiographic evidence of ≥50% blockage in at least one major coronary artery.[20] Identification of participants with diagnosed T2DM (HbA1c > 6.4%, FBG > 7 mmol/L) was completed through clinical chart review. Those in the non-T2DM group did not have a clinical diagnosis of T2DM, use any anti-diabetic medications, or have an HbA1c over 6.4%. Any participants with acute medical illnesses, neurodegenerative or neuropsychiatric diagnoses, active cancer, bone disease or pregnancy were excluded. Participants were screened using the standardized Mini Mental State Exam to exclude those with cognitive impairment; those with scores of <24 were excluded.[21]

2.2. Demographics & clinical characteristics

Sociodemographic information and medical history, including metabolic disease, cardiac factors and medications were collected through participant interviews and chart review. The number of main coronary arteries blocked, and percentage of blockage within those arteries, was recorded from clinical angiograms as measures of CAD severity. Anthropometric data including weight, height, and BMI, were collected from medical records. Body fat percentage was measured by bioelectric impendence.[22] Insulin was measured using an enzyme-linked immunosorbent assay (ELISA; ab200011 Abcam, Toronto, ON, Canada) and glucose was measured using a standard glucometer (Bayer, Mississauga, ON, Canada). Homeostatic model of insulin resistance (HOMA-IR) was calculated using the formula: [fasting insulin (μU/L) × fasting glucose (nmol/L)] / 22.5.[23] HbA1c, cholesterol and triglycerides were assessed by standard lab testing at Sunnybrook Health Sciences Centre.

A cardiopulmonary fitness test was performed to determine peak oxygen uptake (VO_{2Peak}). The standardized symptom-limited graded exercise stress test was performed using a cycle ergometer (Ergoline 800 EL, Ergoline, Bitz, Germany). A calibrated metabolic cart (V_{max} Encore, SensorMedics, California) was used to collect breath-by-breath gas samples to determine VO_{2Peak}, which was standardized to body mass (mL/kg/min) to yield a highly reliable and reproducible measure of fitness.[24]

2.3. Osteocalcin measurements

This study measured fasting morning carboxylated osteocalcin (cOCN; glutamic acid residues at positions 17, 21 and 24 are carboxylated), and uncarboxylated osteocalcin (unOCN; none of the three glutamic acid residues carboxylated). Fasting blood was drawn (0900 h ± 30 mins) and collected blood samples were centrifuged at 4 °C, 1000 rpm for 10 min. Serum was separated and stored at −80 °C until assayed. Serum concentrations of unOCN (Glu-OC MK118 Takara Bio) and cOCN (Gla-OC MK111, Takara Bio, Kusatsu, Japan) were quantified by ELISA.

2.4. Statistical analyses

Differences between groups with and without T2DM were tested with independent samples *t*-tests. In order to explore potential confounders, participant characteristics were compared between those with T2DM and those without using an independent samples *t*-test for continuous measures or a chi-squared test for categorical measures, and relationships between participant characteristics and serum osteocalcin measures were assessed using non-parametric tests (Spearman's rho or Mann-Whitney *U* tests) because they are less sensitive to possible outlier effects in small sample sizes. Potential confounders thus identified were included in analyses of covariance (ANCOVA) to test the independent effect of T2DM on serum osteocalcin concentrations.

A sample size of 20 cases and 40 controls provides 95% power (1-β error probability) to detect an effect size (d) of at least 1.0 in the difference of two independent means at a two-tailed error probability of 0.05.

We explored relationships between osteocalcin types and clinical characteristics in subgroups with and without T2DM using non-parametric tests. Post-hoc models were run in subgroups not using an insulin preparation. We assessed interactions between T2DM and participant characteristics in predicting osteocalcin concentrations as interaction terms in ANCOVA models.

3. Results

3.1. Participant characteristics

Characteristics of the 20 participants with T2DM and of the 40 without T2DM are reported in Table 1. Participants with T2DM had significantly different metabolic, fitness and lipid profiles compared to participants without T2DM (Table 1).

3.2. Differences in serum osteocalcin concentrations between T2DM and non-T2DM groups

Concentrations of both unOCN (2.71 ± 1.86 vs. 4.70 ± 2.03 ng/mL; $t = -3.635$, $p = 0.001$) and cOCN (8.70 ± 2.27 vs. 10.77 ± 3.69 ng/mL; $t = -2.30$, $p = 0.025$) were lower in participants with T2DM compared to participants without T2DM (Fig. 1).

Differences in unOCN ($F = 9.573$, $p = 0.003$) but not cOCN ($F = 1.843$, $p = 0.181$) persisted in a model adjusted for HOMA-IR. In a

Table 1
Participant characteristics.

	T2DM ($n = 20$)	No T2DM ($n = 40$)	Chi-square or t	p
Sociodemographic				
Gender (% female)	20%	20%	0.000	>0.999
Age (years)	63.0 ± 6.7	63.0 ± 6.1	0.001	0.999
Ethnicity %			6.170	0.187
Caucasian	74%	90%		
Arab/Middle-Eastern	0%	3%		
Asian	5%	5%		
South Asian	16%	3%		
Black/African American/Afro-Caribbean	5%	0%		
Vascular factors %				
MI	55%	48%	0.300	0.584
CABG	45%	28%	1.838	0.175
PCI	55%	58%	0.034	0.854
Valvular heart disease	5%	8%	0.134	0.714
Congestive heart failure	5%	3%	0.259	0.611
Hypertension	100%	95%	1.034	0.309
VO_{2Peak} (mL/kg/min)	17.2 ± 5.2	20.8 ± 5.3	**−2.492**	**0.016**
Smoking history			2.035	0.362
Never smoked	40%	43%		
Current smoker	5%	0%		
Quit smoking	55%	58%		
No. coronary arteries involved	2.35 ± 0.70	3.29 ± 7.42	0.519	0.606
Cumulative stenosis	169.84 ± 58.66	139.00 ± 54.07	−1.693	0.098
Metabolic function				
Fasting glucose (mmol/L)	7.38 ± 2.40	5.06 ± 0.56	**4.264**	**<0.001**
Fasting insulin (pmol/L)	79.51 ± 74.77	35.79 ± 31.73	**2.487**	**0.021**
HOMA-IR	5.15 ± 6.39	1.32 ± 1.13	**2.650**	**0.015**
HbA1c (%)	6.90 ± 0.90	5.61 ± 0.30	**6.112**	**<0.001**
LDL (mmol/L)	1.29 ± 0.72	1.59 ± 0.57	1.770	0.082
HDL (mmol/L)	1.17 ± 0.28	1.38 ± 0.39	**2.095**	**0.041**
Triglycerides (mmol/L)	1.45 ± 0.71	1.07 ± 0.49	**−2.418**	**0.019**
Obesity measures				
BMI (kg/m^2)	32.7 ± 6.7	30.8 ± 4.7	1.149	0.260
Body fat %	37.8 ± 16.7	33.2 ± 8.2	1.145	0.162
Body mass (kg)	96.0 ± 22.1	90.3 ± 15.9	1.138	0.260
Medications				
Statin	100%	100%	–	–
Anti-platelet	95%	95%	0.000	>0.999
Beta blocker	85%	85%	0.000	>0.999
Antihypertensive	90%	68%	3.600	0.058
Metformin	70%	0%	**36.522**	**<0.001**
Calcium channel blocker	35%	13%	**4.219**	**0.040**
Vasodilator	30%	33%	0.039	0.844
Insulin	30%	0%	**13.333**	**<0.001**
Diuretic	25%	20%	0.196	0.658

Entries in bold are significantly different at an uncorrected p-value < 0.05.

model adjusted for fasting blood insulin only unOCN (F = 10.858, p = 0.002) but not cOCN (F = 1.473, p = 0.230) differed between T2DM and non-T2DM groups. In a model adjusted for fasting blood glucose concentrations, unOCN differed between groups (F = 5.530, p = 0.022) but cOCN did not (F = 3.445, p = 0.069). In a model adjusted for HbA1c neither unOCN (F = 2.304, p = 0.135) nor cOCN (F = 3.092, p = 0.084) differed between groups.

Differences in unOCN and cOCN persisted when adjusting for levels of HDL cholesterol (unOCN, F = 13.435, p = 0.001; cOCN, F = 5.018, p = 0.029), but only the difference in unOCN persisted when adjusting for triglycerides (unOCN, F = 10.313, p = 0.002; cOCN, F = 2.952, p = 0.091).

In a post-hoc subgroup analysis of those not using an insulin preparation, unOCN (F = 6.954, p = 0.011) but not cOCN (F = 2.616, p = 0.112) differed between T2DM and non-T2DM groups. This result persisted in models adjusted for HOMA-IR (unOCN, F = 9.829, p = 0.003; and cOCN F = 0.992, p = 0.325), fasting blood insulin concentrations (unOCN, F = 8.574, p = 0.005; and cOCN F = 0.831, p = 0.367) and fasting blood glucose (unOCN, F = 5.822, p = 0.020; and cOCN F = 3.681, p = 0.061), but not in a model adjusted for HbA1c (unOCN, F = 2.163 p = 0.148; and cOCN F = 2.962, p = 0.091).

3.3. Associations between participant characteristics and osteocalcin concentrations in groups of participants with and without T2DM

In participants with T2DM, both unOCN and cOCN were negatively associated with BMI (Table 2 & Figs. 2A and 3A, respectively; unOCN, rho = −0.538, p = 0.014; cOCN, rho = −0.556, p = 0.011) and body mass (Table 2 & Figs. 2B and 3B; unOCN, rho = −0.590, p = 0.006; cOCN, rho = −0.632, p = 0.003). In participants with T2DM, unOCN was associated with metabolic parameters, specifically HbA1c (Table 2 & Fig. 2D; rho = −0.516, p = 0.020), but cOCN was not (Table 2 & Fig. 3D; rho = −0.389, p = 0.090; and rho = 0.108, p = 0.651, respectively).

In participants without T2DM, cOCN was negatively associated with body fat percentage (Table 2 & Fig. 3C; rho = −0.324, p = 0.041) and positively associated with VO_{2Peak} (Table 2 & Fig. 3E; rho = 0.317, p = 0.047). In participants without T2DM, unOCN was positively associated with body mass (Table 2 & Fig. 2B; rho = 0.423, p = 0.009).

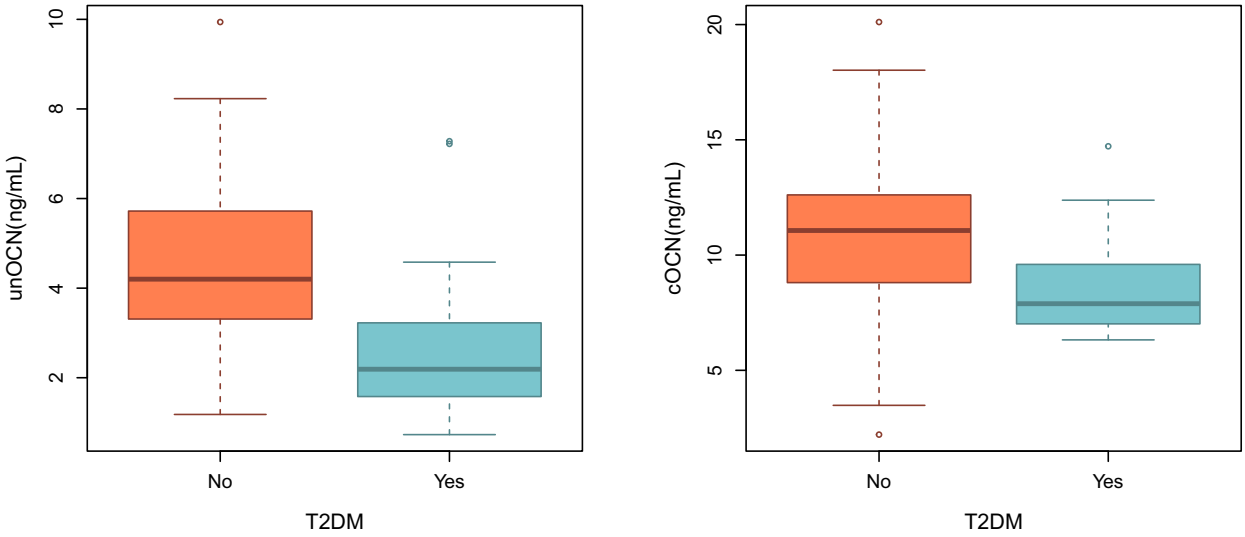

Fig. 1. Differences in (A) serum cOCN and (B) unOCN between participant groups with and without T2DM.

4. Discussion

In the present study, concentrations of both cOCN and unOCN were significantly lower in a group of CAD patients with T2DM compared to a well-matched group of CAD patients without T2DM. These results add to previous reports of lower total osteocalcin concentrations in T2DM compared to controls,[17,18,25] and to a previous report of lower cOCN and unOCN concentrations in T2DM in a population with high cardiovascular risk.[19]

Table 2
Associations between participant characteristics and osteocalcin concentrations in groups of participants with and without T2DM.

	T2DM ($n = 20$)				No T2DM ($n = 40$)			
	cOCN		unOCN		cOCN		unOCN	
	Rho or *U*	*p*	Rho or *U*	*p*	Rho or *U*	*p*	Rho or *U*	*p*
Sociodemographic								
Age (years)	0.113	0.635	0.112	0.638	−0.206	0.203	0.096	0.573
Gender (% female)	−0.054	0.820	−0.152	0.523	−0.222	0.169	−0.129	0.446
Vascular factors %								
MI	48.0	0.909	46.0	0.790	176.000	0.524	138.000	0.316
CABG	41.5	0.543	24.0	0.053	142.000	0.596	84.000	0.081
PCI	40.5	0.494	40.0	0.470	183.000	0.732	102.000	0.065
Valvular heart disease	4.0	0.340	9.0	0.931	20.000	0.068	28.000	0.638
Congestive heart failure	9.000	0.931	6.000	0.544	2.000	0.130	18.000	>0.999
Hypertension	–	–	–	–	**3.000**	**0.030**	31.000	0.788
VO_{2Peak}	−0.186	0.432	−0.163	0.492	**0.317**	**0.047**	−0.115	0.497
No. coronary arteries involved	0.067	0.799	0.469	0.058	0.135	0.454	−0.056	0.760
Cumulative stenosis	0.250	0.409	0.302	0.316	−0.043	0.814	0.333	0.067
Metabolic function								
Fasting glucose (mmol/L)	0.109	0.647	−0.348	0.132	−0.035	0.832	0.219	0.193
Fasting insulin (pmol/L)	−0.386	0.093	−0.338	0.145	−0.207	0.239	0.142	0.448
HOMA-IR	−0.389	0.090	**−0.444**	**0.050**	−0.266	0.129	0.208	0.262
HbA1C (%)	0.108	0.651	**−0.516**	**0.020**	−0.111	0.495	0.291	0.080
LDL (mmol/L)	0.355	0.125	0.032	0.895	−0.095	0.562	0.032	0.853
HDL (mmol/L)	0.239	0.310	0.114	0.631	−0.095	0.559	−0.091	0.590
Triglycerides (mmol/L)	−0.420	0.065	−0.358	0.121	−0.193	0.233	0.051	0.767
Obesity measures								
BMI (kg/m^2)	**−0.556**	**0.011**	**−0.538**	**0.014**	−0.249	0.121	0.312	0.060
Body fat %	−0.290	0.215	−0.360	0.119	**−0.324**	**0.041**	0.132	0.435
Body mass (kg)	**−0.632**	**0.003**	**−0.590**	**0.006**	−0.241	0.134	**0.423**	**0.009**
Medications								
Metformin	25.000	0.161	39.000	0.805	–	–	–	–
Statin	–	–	–	–	–	–	–	–
Anti-platelet	3.000	0.259	9.000	0.931	**0.000**	**0.018**	13.000	0.640
Beta blocker	25.000	0.958	21.000	0.634	75.000	0.306	88.000	0.837
Antihypertensive	12.000	0.450	15.000	0.705	125.000	0.145	156.000	1.000
Metformin	25.000	0.161	39.000	0.805	–	–	–	–
Calcium channel blocker	44.000	0.905	43.000	0.843	71.000	0.500	71.000	0.689
Vasodilator	41.000	0.934	38.000	0.741	151.000	0.479	134.000	0.484
Insulin	32.000	0.409	23.000	0.117	–	–	–	–
Diuretic	29.000	0.458	24.000	0.239	110.000	0.543	63.000	0.216

Entries in bold are significantly different at an uncorrected p-value < 0.05.

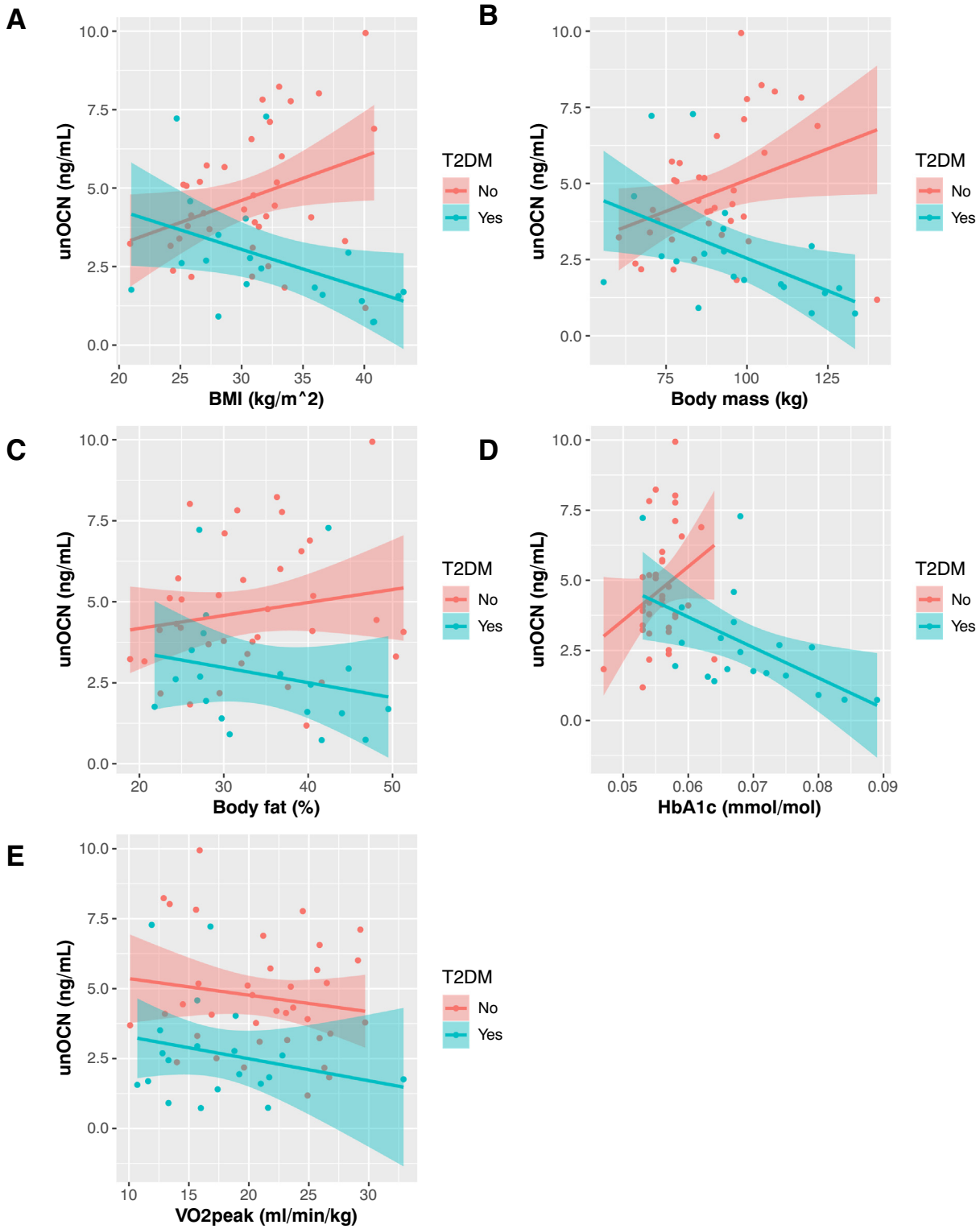

Fig. 2. Relationships between serum unOCN concentrations and clinical characteristics of those with and without T2DM. For obesity measures, there were significant interactions between BMI (A; F = 8.604, $p = 0.005$) and body mass (B; F = 9.775, $p = 0.003$), but not body fat (C; F = 1.684, $p = 0.200$), with T2DM in their relationships with unOCN concentrations, whereby those with greater obesity measures had lower unOCN in T2DM but higher unOCN in those without T2DM. For metabolic measures, there was a significant interaction between HbA1c and T2DM (D; F = 6.819, $p = 0.012$) in its relationship with unOCN concentrations. No significant relationships between VO_{2Peak} and unOCN were identified, nor an interaction with T2DM, in their relationships with unOCN concentrations (E; F = 0.033, $p = 0.856$).

Controlling for differences in metabolic parameters (fasting insulin, fasting glucose, and HOMA-IR) rendered the difference in cOCN between T2DM and non-T2DM groups non-significant. Although differences in unOCN persisted in these models, neither unOCN nor cOCN differed between participant groups when accounting for HbA1c. Glycated hemoglobin is an advanced glycated end product (AGE), a lipid or protein that is glycated in response to exposure to sugars, such as the hyperglycemic environment in T2DM. AGEs are associated with increased osteoclastogenesis and increased bone resorption[26,27] as well as decreased osteoblast differentiation[28] and they are proposed

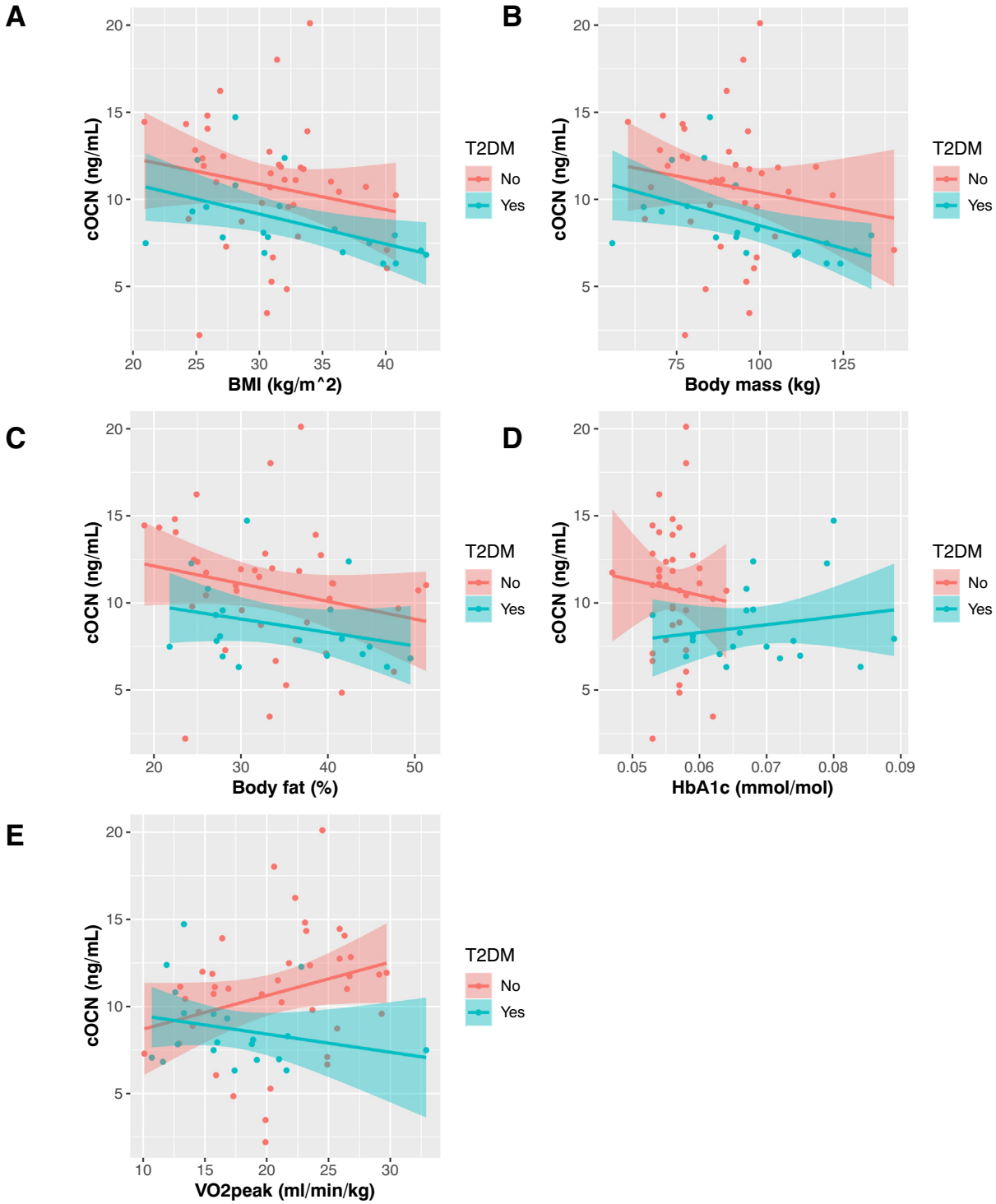

Fig. 3. Relationships between serum cOCN concentrations and clinical characteristics of those with and without T2DM. There were consistent trends for the obesity measures, BMI (A), body mass (B) and body fat (C) to be associated with lower cOCN concentrations, and these trends were consistent between groups with and without T2DM (no significant interactions). There was no significant interaction between HbA1c (D; $F = 0.470$, $p = 0.496$) and T2DM in their relationships with cOCN concentrations. No significant interaction between VO_{2Peak} and T2DM was identified in their relationships with cOCN (E; $F = 2.972$, $p = 0.090$), although VO_{2Peak} was associated with cOCN only in those without T2DM.

to mediate changes in vascularization of bone in T2DM.[29,30] Decreased osteoblast differentiation could contribute to decreased circulating concentrations of cOCN in T2DM. It is therefore possible that lower osteocalcin concentrations, particularly cOCN, are consequential of impaired bone homeostasis due to hyperglycemia in T2DM. In some populations, in adjusted models, serum osteocalcin is an independent predictor of obesity and metabolic function potentially implying a causal relationship.[31] Low osteocalcin could potentially cause metabolic deficits and, although not substantiated here, a pathogenic cycle might be hypothesized.

The finding that lower unOCN concentrations were associated with elevated HbA1c expand on previous reports of associations between lower total osteocalcin and HbA1c,[17,32,33] and confirm associations with lower unOCN specifically.[14,34] Elevated HbA1c is associated with cardiovascular

mortality both in people with and without diabetes, suggesting the possibility to examine unOCN too as a potential marker of cardiovascular outcomes in patients with CAD.[35]

In participants with T2DM, lower unOCN was also associated with poorer insulin sensitivity as measured by HOMA-IR. The result is broadly consistent with the hypothesis that insulin resistance would be associated with lower serum osteocalcin, because osteoblast insulin signaling increases cOCN production in mice, and therefore insulin resistance may impair cOCN production and unOCN release.[36] Although the results do not speak directly to a mechanism, the ability to maintain bone homeostasis in T2DM is clinically important, as people with T2DM are at increased fracture risk despite normal or elevated bone mineral density.[37]

Higher cOCN concentrations were associated with higher VO_{2Peak}, a reliable measure of cardiopulmonary fitness. An association between total osteocalcin and VO_{2Peak} has been previously reported among athletes,[38] but the relevant carboxylation state was not identified. Here, only cOCN was associated with VO_{2Peak}, suggesting a new perspective on the relationship between bone metabolism and fitness. In animals, restoring genetic deficiency in osteocalcin increased O_2 uptake[12] but no clear effects of osteocalcin on oxidative metabolism or respiratory function have been described in humans. In the present study, it is possible that those with a higher VO_{2Peak} were performing more exercise, and therefore they had higher bone turnover and elevated serum cOCN concentrations. Moreover, the association between VO_{2Peak} and cOCN was found only in people without T2DM. It is possible therefore, that impairment of osteoblast function by insulin resistance or accumulation AGEs in bone matrix might impair cOCN synthesis, even with exercise, in those with T2DM. While this study does not speak to potential mechanisms, given that lower VO_{2Peak} is an independent risk factor for poor cardiovascular outcomes and mortality among CAD patients, relationships between fitness and cOCN should be investigated further.

The observed relationships between lower cOCN and BMI, percentage body fat and body mass are also novel since previous studies have reported either total osteocalcin[39] or unOCN as the species related to these measures.[40,41] A relationship between BMI and serum osteocalcin has been reported in a meta-analysis, with the strongest correlation between these two variables being found among studies of those with "metabolic syndrome".[10] Our findings further clarify this by describing an interaction between T2DM and BMI in explaining unOCN concentrations. Higher body mass provides higher mechanical stress on the skeleton,[42] inducing an adaptive increase in bone remodelling,[43] which might explain an increase in unOCN with BMI in those without T2DM. However, in T2DM higher BMI was associated with lower concentrations of both cOCN and unOCN, which could indicate that compromised bone turnover, in combination with other factors such as hyperglycemia, AGEs, insulin resistance or insulin injection, may impair bone response to mechanical stress.

As a potential limitation, duration of diabetes was not controlled for; however, while osteocalcin can increase in some early stage T2DM cases,[44] differences between T2DM and non-T2DM groups persisted. Although the study was gender-matched, women were underrepresented in the present cohort as is common in cardiac rehabilitation populations,[45] and so results may not generalize to women. Longitudinal studies will be needed to determine relationships between osteocalcin and T2DM and CAD outcomes over time. The use of antidiabetic medications in the T2DM group are potential confounders; however, results were largely consistent in a subgroup not using insulin, and although metformin may increase osteocalcin levels due to its activation of pathways promoting osteoblast differentiation,[46] differences between groups persisted. As a further limitation, bone density and bone quality were not assessed; however, participants with bone disease were excluded. Finally, although cOCN and unOCN may be clinically meaningful markers, osteocalcin can be hormonally active in mice when decarboxylated at specific residues, and these partially decarboxylated species cannot yet be assessed readily in people. In the present study, small sample sizes precluded meaningful investigation of relationships between osteocalcin concentrations and participant characteristics shared by very few participants (e.g. without hypertension, using antiplatelet therapies). Additionally, lack of vitamin K measurements in our participants is a limitation as this may relate to the circulating proportion of unOCN, as shown in people given vitamin K supplements.[47]

Here, we describe carboxylation state specific associations between lower osteocalcin concentrations and adverse cardiovascular risk profiles in people with CAD. Lower cOCN was related to poorer fitness and obesity parameters and unOCN was related primarily to differences between those with and without T2DM, and to metabolic parameters and glycemic control within the T2DM group. Previously osteocalcin has been related to both T2DM and CAD. Here both unOCN and cOCN concentrations were significantly lower in CAD patients with T2DM compared to a well matched group of CAD patients without T2DM, although these differences were not independent of HbA1c. Further investigation of osteocalcin dysregulation in T2DM and its relationship with cardiovascular risk factors in people with and without T2DM are warranted, as are further studies to assess the clinical utility of serum osteocalcin concentrations as predictors of cardiovascular outcomes in patients with CAD.

References

1. Rosato MT, Schneider SH, Shapses SA. Bone turnover and insulin-like growth factor I levels increase after improved glycemic control in noninsulin-dependent diabetes mellitus. Calcif Tissue Int 1998;63:107-11, https://doi.org/10.1007/s002239900498.
2. Achemlal L, Tellal S, Rkiouak F, et al. Bone metabolism in male patients with type 2 diabetes. Clin Rheumatol 2005;24:493-6, https://doi.org/10.1007/s10067-004-1070-9.
3. Kanazawa I, Yamaguchi T, Yamamoto M, et al. Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. J Clin Endocrinol Metab 2009;94:45-9, https://doi.org/10.1210/jc.2008-1455.
4. Khrimian L, Obri A, Ramos-Brossier M, et al. Gpr158 mediates osteocalcin's regulation of cognition. J Exp Med 2017;214:2859-73, https://doi.org/10.1084/jem.20171320.
5. Bonneau J, Ferland G, Karelis AD, et al. Association between osteocalcin gamma-carboxylation and insulin resistance in overweight and obese postmenopausal women. J Diabetes Complicat 2017;31:1027-34, https://doi.org/10.1016/j.jdiacomp.2017.01.023.
6. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;130:456-69, https://doi.org/10.1016/j.cell.2007.05.047.
7. Hauschka PV. Osteocalcin: the vitamin K-dependent Ca^{2+}-binding protein of bone matrix. Haemostasis 1986;16:258-72http://www.ncbi.nlm.nih.gov/pubmed/3530901.
8. Shea MK, Gundberg CM, Meigs JB, et al. γ-Carboxylation of osteocalcin and insulin resistance in older men and women. Am J Clin Nutr 2009;90:1230-5, https://doi.org/10.3945/ajcn.2009.28151.
9. Hwang YC, Jeong IK, Ahn KJ, Chung HY. The uncarboxylated form of osteocalcin is associated with improved glucose tolerance and enhanced beta-cell function in middle-aged male subjects. Diabetes Metab Res Rev 2009;25:768-72, https://doi.org/10.1002/dmrr.1045.
10. Kord-Varkaneh H, Djafarian K. Association between serum osteocalcin and body mass index: a systematic review and meta-analysis. Endocrine 2017;58(1):24-32, https://doi.org/10.1007/s12020-017-1384-4.
11. Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci U S A 2008;105:5266-70, https://doi.org/10.1073/pnas.0711119105.
12. Ferron M, McKee MD, Levine RL, Ducy P, Karsenty G. Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. Bone 2012;50:568-75, https://doi.org/10.1016/j.bone.2011.04.017.
13. Gower BA, Pollock NK, Casazza K, Clemens TL, Goree LL, Granger WM. Associations of total and undercarboxylated osteocalcin with peripheral and hepatic insulin sensitivity and beta-cell function in overweight adults. J Clin Endocrinol Metab 2013;98, https://doi.org/10.1210/jc.2013-1203.
14. Iki M, Tamaki J, Fujita Y, et al. Serum undercarboxylated osteocalcin levels are inversely associated with glycemic status and insulin resistance in an elderly Japanese male population: Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study. Osteoporos Int 2012;23:761-70, https://doi.org/10.1007/s00198-011-1600-7.
15. Yeap BB, Alfonso H, Paul Chubb SA, et al. Higher serum undercarboxylated osteocalcin and other bone turnover markers are associated with reduced diabetes risk and lower estradiol concentrations in older men. J Clin Endocrinol Metab 2015;100:63-71, https://doi.org/10.1210/jc.2014-3019.
16. Sanchez-Enriquez S, Ballesteros-Gonzalez IT, Villafán-Bernal JR, et al. Serum levels of undercarboxylated osteocalcin are related to cardiovascular risk factors in patients

with type 2 diabetes mellitus and healthy subjects. World J Diabetes 2017;8:11, https://doi.org/10.4239/wjd.v8.i1.11.
17. Alfadda AA, Masood A, Shaik SA, Dekhil H, Goran M. Association between osteocalcin, metabolic syndrome, and cardiovascular risk factors: role of total and undercarboxylated osteocalcin in patients with type 2 diabetes. Int J Endocrinol 2013;2013, https://doi.org/10.1155/2013/197519.
18. Razny U, Fedak D, Kiec-Wilk B, et al. Carboxylated and undercarboxylated osteocalcin in metabolic complications of human obesity and prediabetes. Diabetes Metab Res Rev 2017;33, https://doi.org/10.1002/dmrr.2862.
19. Díaz-López A, Bulló M, Juanola-Falgarona M, et al. Reduced serum concentrations of carboxylated and undercarboxylated osteocalcin are associated with risk of developing T2D in a high cardiovascular risk population: a nested case-control study. J Clin Endocrinol Metab 2013;98:4524-31, https://doi.org/10.1210/jc.2013-2472.
20. Swardfager W, Herrmann N, Marzolini S, et al. Verbal memory performance and completion of cardiac rehabilitation in patients with coronary artery disease. Psychosom Med 2011;73:580-7, https://doi.org/10.1097/PSY.0b013e318227fff9.
21. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12: 189-98, https://doi.org/10.1016/0022-3956(75)90026-6.
22. Forhan M, Zagorski BM, Marzonlini S, Oh P, Alter DA. Predicting exercise adherence for patients with obesity and diabetes referred to a cardiac rehabilitation and secondary prevention program. Can J Diabetes 2013;37:189-94, https://doi.org/10.1016/j.jcjd.2013.03.370.
23. Matthews DR, Hosker JR, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and fl-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985:412-9.
24. Milani RV, Lavie CJ, Mehra MR, Ventura HO. Understanding the basics of cardiopulmonary exercise testing. Mayo Clin Proc 2006;81:1603-11, https://doi.org/10.4065/81.12.1603.
25. Starup-Linde J, Eriksen SA, Lykkeboe S, Handberg A, Vestergaard P. Biochemical markers of bone turnover in diabetes patients—a meta-analysis, and a methodological study on the effects of glucose on bone markers. Osteoporos Int 2014;25: 1697-708, https://doi.org/10.1007/s00198-014-2676-7.
26. Ding KH, Wang ZZ, Hamrick MW, et al. Disordered osteoclast formation in RAGE-deficient mouse establishes an essential role for RAGE in diabetes related bone loss. Biochem Biophys Res Commun 2006;340:1091-7, https://doi.org/10.1016/j.bbrc.2005.12.107.
27. Catalfamo DL, Britten TM, Storch DI, Calderon NL, Sorenson HL, Wallet SM. Hyperglycemia induced and intrinsic alterations in type 2 diabetes-derived osteoclast function. Oral Dis 2013;19:303-12, https://doi.org/10.1111/odi.12002.
28. Sanguineti R, Storace D, Monacelli F, Federici A, Odetti P. Pentosidine effects on human osteoblasts in vitro. Annals of the New York Academy of Sciences, vol. 1126. ; 2008. p. 166-72, https://doi.org/10.1196/annals.1433.044.
29. Wang Z, Li L, Du R, et al. CML/RAGE signal induces calci cation cascade in diabetes. Diabetol Metab Syndr 2016;8:83.
30. Koike S, Yano S, Tanaka S, Sheikh AM, Nagai A, Sugimoto T. Advanced glycation end-products induce apoptosis of vascular smooth muscle cells: a mechanism for vascular calcification. Int J Mol Sci 2016;17, https://doi.org/10.3390/ijms17091567.
31. Hu WW, Ke YH, He JW, et al. Serum osteocalcin levels are inversely associated with plasma glucose and body mass index in healthy Chinese women. Acta Pharmacol Sin 2014;35(12):1521-6, https://doi.org/10.1038/aps.2014.92.
32. Bao YQ, Zhou M, Zhou J, et al. Relationship between serum osteocalcin and glycaemic variability in type 2 diabetes. Clin Exp Pharmacol Physiol 2011;38:50-4, https://doi.org/10.1111/j.1440-1681.2010.05463.x.
33. Maddaloni E, D'Onofrio L, Lauria A, et al. Osteocalcin levels are inversely associated with Hba1c and BMI in adult subjects with long-standing type 1 diabetes. J Endocrinol Investig 2014;37:661-6, https://doi.org/10.1007/s40618-014-0092-7.
34. Okuno S, Ishimura E, Tsuboniwa N, et al. Significant inverse relationship between serum undercarboxylated osteocalcin and glycemic control in maintenance hemodialysis patients. Osteoporos Int 2013;24:605-12, https://doi.org/10.1007/s00198-012-2003-0.
35. Cavero-Redondo I, Peleteiro B, Álvarez-Bueno C, Rodriguez-Artalejo F, Martínez-Vizcaíno V. Glycated haemoglobin A1c as a risk factor of cardiovascular outcomes and all-cause mortality in diabetic and non-diabetic populations: a systematic review and meta-analysis. BMJ Open 2017;7, https://doi.org/10.1136/bmjopen-2017-015949.
36. Wei J, Ferron M, Clarke CJ, et al. Bone-specific insulin resistance disrupts whole-body glucose homeostasis via decreased osteocalcin activation. J Clin Invest 2014;124: 1-13, https://doi.org/10.1172/JCI72323.
37. Marin C, Luyten FP, Van der Schueren B, Kerckhofs G, Vandamme K. The impact of type 2 diabetes on bone fracture healing. Front Endocrinol (Lausanne) 2018;9, https://doi.org/10.3389/fendo.2018.00006.
38. Koundourakis NE, Androulakis N, Dermitzaki E, Venihaki M, ANM. Effect of a 6-week supervised detraining period on bone metabolism markers and their association with ergometrics and components of the hypothalamic–pituitary–gonadal (HPG) axis in professional male soccer players. J Bone Miner Metab 2018:1-8.
39. Zhou M, Ma X, Li H, et al. Serum osteocalcin concentrations in relation to glucose and lipid metabolism in Chinese individuals. Eur J Endocrinol 2009;161:723-9, https://doi.org/10.1530/EJE-09-0585.
40. Tan A, Gao Y, Yang X, et al. Low serum osteocalcin level is a potential marker for metabolic syndrome: results from a Chinese male population survey. Metabolism 2011;60:1186-92, https://doi.org/10.1016/j.metabol.2011.01.002.
41. Kanazawa I, Yamaguchi T, Yamamoto M, et al. Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus. Osteoporos Int 2011;22:45-9, https://doi.org/10.1007/s00198-010-1322-2.
42. Iwaniec UT, Turner RT. Influence of body weight on bone mass, architecture and turnover. J Endocrinol 2016;230:R115-30, https://doi.org/10.1530/JOE-16-0089.
43. Robling AG, Turner CH. Mechanical signaling for bone modeling and remodeling. Crit Rev Eukaryot Gene Expr 2009;19:319-38, https://doi.org/10.1016/j.biotechadv.2011.08.021.Secreted.
44. Aoki A, Muneyuki T, Yoshida M, et al. Circulating osteocalcin is increased in early-stage diabetes. Diabetes Res Clin Pract 2011;92:181-6, https://doi.org/10.1016/j.diabres.2011.01.009.
45. Swardfager W, Herrmann N, Marzolini S, et al. Cardiopulmonary fitness is associated with cognitive performance in patients with coronary artery disease. J Am Geriatr Soc 2010;58:1519-25, https://doi.org/10.1111/j.1532-5415.2010.02966.x.
46. Jang WG, Kim EJ, Bae IH, et al. Metformin induces osteoblast differentiation via orphan nuclear receptor SHP-mediated transactivation of Runx2. Bone 2011;48: 885-93, https://doi.org/10.1016/j.bone.2010.12.003.
47. Binkley NC, Krueger DC, Engelke JA, Foley AL, Suttie JW. Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. Am J Clin Nutr 2000;72:1523-8, https://doi.org/10.1093/ajcn/72.6.1523.