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ORIGINAL ARTICLE

Observational Study

Serum levels of undercarboxylated osteocalcin are related to cardiovascular risk factors in patients with type 2 diabetes mellitus and healthy subjects

Sergio Sanchez-Enriquez, Isabel Thalia Ballesteros-Gonzalez, José Rafael Villafán-Bernal, Sara Pascoe-Gonzalez, Edgar Alfonso Rivera-Leon, Blanca Estela Bastidas-Ramirez, Jorge David Rivas-Carrillo, Juan Luis Alcala-Zermeno, Juan Armendariz-Borunda, Iris Monserrat Llamas-Covarrubias, Abraham Zepeda-Moreno

Sergio Sanchez-Enriquez, Isabel Thalia Ballesteros-Gonzalez, Edgar Alfonso Rivera-Leon, Juan Luis Alcala-Zermeno, Iris Monserrat Llamas-Covarrubias, Biochemistry Laboratory, Molecular Biology and Genomics Department, University Center of Health Sciences, University of Guadalajara, CP 44340 Guadalajara, Jalisco, Mexico

Sergio Sanchez-Enriquez, Edgar Alfonso Rivera-Leon, Blanca Estela Bastidas-Ramirez, Jorge David Rivas-Carrillo, Juan Luis Alcala-Zermeno, Abraham Zepeda-Moreno, Academic Group UDG-CA-533 Multidisciplinary Study of Chronic and Degenerative Diseases, Molecular Biology and Genomics Department, University Center of Health Sciences, University of Guadalajara, CP 44340 Guadalajara, Jalisco, Mexico

José Rafael Villafán-Bernal, Departamento de Jóvenes, Investigadores y Cátedras del Consejo Nacional de Ciencia y Tecnología (CONACYT), CP 03940 Benito Juárez, México

José Rafael Villafán-Bernal, Departamento de Cirugía, Centro Universitario de Ciencias de la Salud, Universidad Autónoma de Aguascalientes, CP 20131 Aguascalientes, Ags, México

Sara Pascoe-Gonzalez, Physiology Department, University Center of Health Sciences, University of Guadalajara, CP 44340 Guadalajara, Jalisco, Mexico

Blanca Estela Bastidas-Ramirez, Institute of Degenerative Chronic Diseases, Molecular Biology and Genomics Department, University Center of Health Sciences, University of Guadalajara, CP 44340 Guadalajara, Jalisco, Mexico

Juan Armendariz-Borunda, Head of Molecular Biology and Genomics Department, University Center of Health Sciences, University of Guadalajara, CP 44340 Guadalajara, Jalisco, Mexico

Abraham Zepeda-Moreno, Institute of Cancer Research in Child and Adolescent, University Center of Health Sciences, University of Guadalajara, CP 44340 Guadalajara, Jalisco, Mexico

Author contributions: Sanchez-Enriquez S and Zepeda-Moreno A designed the research; Ballesteros-Gonzalez IT, Villafán-Bernal JR and Pascoe-Gonzalez S performed the research; Rivera-Leon EA and Llamas-Covarrubias IM contributed new reagents/analytic tools; Bastidas-Ramirez BE and Rivas-Carrillo JD analyzed the data; Alcala-Zermeno JL and Armendariz-Borunda J wrote the paper.

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Correspondence to: Sergio Sanchez-Enriquez, MD, MSc, PhD, Biochemistry Laboratory, Molecular Biology and Genomics Department, University Center of Health Sciences, University of

Guadalajara, Sierra Mojada No. 950, Colonia Independencia, CP 44340 Guadalajara, Jalisco, Mexico. serlucis@hotmail.com
Telephone: +52-33-10585200-33644
Fax: +52-33-10585200-33644

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Abstract

AIM

To determine a potential relationship between serum undercarboxylated (ucOC) concentration and cardiovascular risk factors in type 2 diabetes (T2D) patients and healthy subjects (HS).

METHODS

A cross-sectional study was conducted on 140 subjects classified into two groups, 70 with T2D and 70 HS. Medical history and physical examination with anthropometric measurements were obtained from all subjects. Body fat percentage was determined by bioelectrical impendency analysis. Serum ucOC concentration was determined by enzyme immunoassay, while serum levels of insulin and hsCRP were obtained using high sensitivity enzyme-linked immunosorbent assay. Insulin resistance was determined using the homeostasis model assessment-IR. Lipid profile [triglycerides, total cholesterol (TC), high-density lipoproteins (HDL-c), low density lipoproteins (LDL-c), very low-density lipoproteins] was determined by spectrophotometry and standard formulas when applicable.

RESULTS

The T2D patient group showed significantly higher values of waist circumference, waist-to-hip ratio, systolic blood pressure (SBP), diastolic blood pressure (DBP), current smoking, and alcohol use when compared to the HS group ($P < 0.05$). We observed a significantly lower serum ucOC concentration in T2D than in HS (1.5 ± 1.4 *vs* 2.3 ± 1.8, $P < 0.05$). In the whole study population, ucOC concentration was inversely correlated with body mass index (BMI) ($r = -0.236$, $P < 0.05$), fasting plasma glucose ($r = -0.283$, $P < 0.01$) and HDL-c ($r = -0.255$, $P < 0.05$); and positively correlated with LDL-c/HDL-c ratio ($r = 0.306$, $P < 0.05$) and TC/HDL-c ratio ($r = 0.284$, $P < 0.05$). In the T2D group, serum ucOC concentration was inversely correlated with BMI ($r = -0.310$, $P < 0.05$) and body-fat percentage ($r = -0.311$, $P < 0.05$), and positively correlated with DBP ($r = 0.450$, $P < 0.01$). In HS group a positive correlation between serum levels of ucOC and SBP ($r = 0.277$, $P < 0.05$) was observed.

CONCLUSION

Serum ucOC is a potential marker for cardiovascular risk in Mexicans because it is related to adiposity parameters, blood pressure and lipid profile.

Key words: Bone; Osteocalcin; Glucose metabolism; Diabetes; Cardiovascular risk

Core tip: Lower levels of undercarboxylated osteocalcin (OC) are found in diabetic patients as this hormone is involved in various glucorregulatory mechanisms; however evidence regarding its role in cardiovascular disease development is still pending. Here we show the correlation between levels of undercarboxylated OC and markers of cardiovascular risk.

Sanchez-Enriquez S, Ballesteros-Gonzalez IT, Villafán-Bernal JR, Pascoe-Gonzalez S, Rivera-Leon EA, Bastidas-Ramirez BE, Rivas-Carrillo JD, Alcala-Zermeno JL, Armendariz-Borunda J, Llamas-Covarrubias IM, Zepeda-Moreno A. 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(1): 11-17 Available from: URL: http://www.wjgnet.com/1948-9358/full/v8/i1/11.htm DOI: http://dx.doi.org/10.4239/wjd.v8.i1.11

INTRODUCTION

Osteocalcin (OC) is a non-collagenous peptide composed of 49 aminoacids and produced by osteoblasts. Circulating OC has two variants, carboxylated (cOC) and undercarboxylated (ucOC)[1-3]. While cOC has high affinity for calcium ions contained in hydroxyapatite, is actively involved in osteogenesis[4] and enhances osteoclast maturation[5], ucOC functions as a regulatory hormone in glucose metabolism improving insulin sensitivity and secretion, β-cell mass and glucose tolerance[6,7]. Low serum OC levels and the index ucOC/cOC are associated with increased fasting plasma glucose (FPG) and IR in several cross-sectional and prospective studies[8-12] and with risk for developing type 2 diabetes (T2D)[10,13]. On the other hand, bone metabolism, T2D and cardiovascular diseases (CVD) interact with one another through complex mechanisms[14-21]. For example, bone-related proteins like OC, osteopontin (OPN), osteoprotegerin (OPG) and receptor-activated nuclear factor-k B ligand have been correlated with atherosclerotic arteries, suggesting a potential role of bone molecules in the pathophysiology of vascular disease[22,23]. Furthermore, a previous study showed that OC was inversely correlated with peripheral atherosclerosis markers (intima-media thickness and ankle-brachial pulse-wave velocity) in T2D patients[23]. This data suggests that OC might be involved in the development of CVD in T2D patients. However, direct evidence regarding ucOC concentrations and cardiovascular risk factors (CVRF) in humans is limited. The present study was designed to determine the correlation

between ucOC levels and CVRF in T2D and healthy subjects (HS).

MATERIALS AND METHODS

Study population

We performed a cross-sectional analysis of 140 subjects (70 T2D patients and 70 HS) aged 54.0 ± 5.0 and 51.8 ± 7.4, respectively attending the Program for Detection and Treatment of Congenital and Acquired Metabolic Diseases at a university center (Universidad de Guadalajara) in western Mexico. We obtained the medical history and physical examination from all subjects. Blood for biochemical determinations was drawn after an overnight fasting period. Subjects with hepatic, renal, parathyroid and thyroid dysfunction or taking medications known to influence bone or calcium metabolism, such as vitamin D, bisphosphonates, calcitonin, estrogen, tamoxifen or corticosteroids, were excluded.

Ethical considerations

This study was done according to the Ethical Principles for Medical Research Involving Human Subjects (Declaration of Helsinki) and was approved by the University of Guadalajara Ethics Committee. Informed consent was obtained from all participants before enrollment in the study.

Anthropometric and clinical measurements

Anthropometric measurements were performed in all subjects. Height was measured using a stadiometer with ± 0.001 m accuracy (Seca, Hamburg, Germany). Weight and body fat percentage (BFP) were determined using a fixed frequency (50 kHz) bioimpendance analyzer scale (TBF-300 Tanita, Tokyo, Japan) with an applied current of 0.8 mA. Body mass index (BMI) was calculated by Quetelet index (kg/m^2). Waist circumference (WC) was measured using a flexible measuring tape midway between the lowest rib and the superior iliac crest border in the mid axillary line. Hip circumference (HC) was measured around the buttocks at the greater trochanter level. Waist-to-hip ratio (WHR) was calculated by dividing WC/HC. After 10 min in the seated position, blood pressure was measured 3 times using an electronic aneroid sphygmomanometer (Omron, model HEM-7220 LA; Kyoto, Japan); the last 2 measures were averaged for analysis.

Biochemical analysis

Blood samples were collected after a 12-h overnight fast period. Samples were centrifuged at 4000 rpm for 10 min to obtain serum, which was stored at -70 ℃ for later processing. FPG was quantified by the glucose oxidase method (Biosystems, Barcelona, Spain). Serum triglycerides (TG), total cholesterol (TC), cholesterol bound to high-density lipoproteins (HDL-c) and cholesterol bound to low density lipoproteins (LDL-c) were determined by enzymatic colorimetric procedures (Biosystems, Barcelona, Spain). Cholesterol bound to very low-density lipoproteins (VLDLc) was calculated using Friedewald's equation (TG divided by 5)[24]. Fasting serum insulin (FINS) levels were measured by enzyme-linked immunosorbent assay (ELISA) (GenWay Biotech, Inc. San Diego CA, United States). Serum hsCRP concentration was quantified by high sensitivity ELISA (Abcam, CA, United States). Serum ucOC was determined by enzyme immunoassay (Takara Bio, Inc. Otsu, Japan).

Homeostasis model assessment-IR

The IR was estimated by homeostasis model assessment-IR (HOMA-IR) calculated according to the following formula: HOMA-IR = FINS (mU/L) × FPG (mmol/L)/22.5[25].

Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences v17.0 (SPSS, Inc., Chicago, Illinois). Every analysis was performed by a biomedical statistician. The Kolmogorov-Smirnov one-sample test was performed for assessing the sample cumulative distribution. Quantitative continuous variables were presented as mean ± SD. Normally distributed variables were analyzed using the two independent samples *t*-test. To analyze non-normally distributed data the Mann-Whitney *U* test was performed. Pearson's χ^2 test was used for categorical variable analysis (*i.e.*, gender, alcohol use, and current smoking). Pearson correlation coefficient was used to assess the strength of the association between ucOC concentration levels and CVRF. A P value < 0.05 was considered statistically significant.

RESULTS

Characteristics of subjects

Demographic and clinical characteristics of all subjects are shown in Table 1. Significant differences in WC, WHR, systolic blood pressure (SBP), diastolic blood pressure (DBP), current smoking, and alcohol use were observed between groups. Biochemical parameters according to study groups are shown in Table 2. TC, TG, HDL-c, LDL-c, VLDL-c, LDLc/HDL-c ratio, TC/HDL-c ratio, FPG, HOMA-IR, and ucOC were significantly different between groups (P < 0.05).

Correlation between serum levels of ucOC and CVRF

In the whole study population the serum ucOC concentration was inversely correlated with BMI, FPG and HDL-c, while it was positively correlated with both LDL-c/HDL-c ratio and TC/HDL-c ratio. In the T2D group and inverse correlation between serum levels of ucOC, body fat percentage, and BMI were observed. Conversely, serum levels of ucOC were positively correlated with DBP in this group. In the group of HS, a positive correlation between serum levels of ucOC and systolic pressure was observed (Table 3).

ucOC by DBP and SBP quartiles in T2D

Because of the strong correlation found between DBP and ucOC in T2D patients, participants were classified

Table 1 Demographic and clinical characteristics according to study group

Variable	T2D (n = 70) Mean ± SD	HS(n = 70) Mean ± SD	P value
Age (yr)	54.0 ± 5.0	51.8 ± 7.4	NS
Gender M/F	29/41	30/40	NS
Weight (kg)	75 ± 18.1	72.1 ± 14.8	NS
Height (cm)	159.6 ± 10.1	161.1 ± 7.5	NS
BMI (kg/m^2)	29.3 ± 6.5	27.6 ± 5.2	NS
WC (cm)	99.2 ± 18.1	90.7 ± 12.3	< 0.01
WHR	0.93 ± 0.14	0.87 ± 0.08	< 0.01
Fat percentage	34.5 ± 8.5	33.2 ± 8.7	NS
SBP (mmHg)	143.3 ± 21.5	113.3 ± 11	< 0.001
DBP (mmHg)	86 ± 12.3	74.9 ± 7.5	< 0.001
Current smoking n (%)	24 (38.1)	9 (27.3)	< 0.05
Alcohol use n (%)	29 (46)	2 (3.9)	< 0.001
Physical inactivity n (%)	32 (50.8)	31 (60.8)	NS

Statistical significances were determined using Student's t test (for data normally distributed) or Mann-Whitney test (for data not normally distributed) and χ^2 test (for qualitative variables). T2D: Patients with type 2 diabetes mellitus; HS: Healthy subjects; M/F: Male/female; BMI: Body mass index; WC: Waist circumference; WHR: Waist-to-hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; NS: Non statistically significant.

Table 2 Biochemical parameters according to study group

Variable	T2D (n = 70) Mean ± SD	HS (n = 70) Mean ± SD	P value
TC (mg/dL)	220.7 ± 84.7	181 ± 35.5	< 0.01
TG (mg/dL)[1]	140.9 (87.6-200.5)	108.0 (84.0-145.0)	< 0.05
HDLc (mg/dL)	43.1 ± 15.5	68.3 ± 18.0	< 0.001
LDLc (mg/dL)	129.8 ± 37.1	108.9 ± 33.1	< 0.05
VLDLc (mg/dL)	31.1 ± 18.6	25.8 ± 20.0	< 0.05
LDLc/HDLc	3 ± 1.7	2.2 ± 0.1	< 0.05
TC/HDLc	4.7 ± 1.9	3.4 ± 1.5	< 0.01
FPG (mg/dL)	161.9 ± 69.5	88.3 ± 9.0	< 0.001
FINS (mcUI/mL)	15.8 ± 7.0	13.8 ± 11.6	NS
HOMA-IR	6.8 ± 4.1	3 ± 2.6	< 0.001
hs-CRP (ng/L)	3 ± 3.1	2.1 ± 0.8	NS
ucOC (ng/mL)	1.5 ± 1.4	2.3 ± 1.8	< 0.05

[1]Values are expressed as median and interquartilar range. Statistical significances were determined using Student's t test (for data normally distributed) or Mann-Whitney test (for data not normally distributed). TC: Total cholesterol; TG: Triglycerides; HDLc: Cholesterol bound to high-density lipoproteins; LDLc: Cholesterol bound to low-density lipoproteins; VLDLc: Cholesterol bound to very low-density lipoproteins; LDLc/HDLc: LDLc/HDLc ratio; TC/HDLc: TC/HDLc ratio; FPG: Fasting plasma glucose; FINS: Fasting serum insulin; HOMA-IR: Homeostasis model assessment-insulin resistance; hs-CRP: High sensitivity C reactive protein; ucOC: Undercarboxylated osteocalcina; NS: Non statistically significant.

by DBP quartiles, and then ucOC serum levels were compared between quartiles. Also patients were classified according to ucOC quartiles and DBP was analyzed in each ucOC quartile. The ucOC serum levels were higher in T2D patients with DBP in Q4 than in Q1 (P < 0.05, Figure 1A). Additionally, DBP was higher in patients with ucOC in Q4 than those with ucOC in Q1 (P = 0.05, Figure 1B).

Table 3 Correlation between serum levels of undercaboxylated osteocalcin and cardiovascular risk factors

Variable	ucOC concentration (ng/mL) Whole population (n = 140)		T2D (n = 70)		HS (n = 70)	
	r	P value	r	P value	r	P value
BMI (kg/m^2)	-0.236	0.023	-0.310	0.046	-0.166	0.244
Body fat (%)	-0.201	0.054	-0.311	0.048	-0.126	0.379
SBP (mmHg)	-0.083	0.431	0.018	0.908	0.277	0.049
DBP (mmHg)	0.155	0.137	0.450	0.003	0.209	0.141
FPG (mg/dL)	-0.283	0.006	-0.286	0.070	-0.110	0.443
HDL-c (mg/dL)	-0.255	0.036	0.117	0.655	-0.096	0.503
LDL-c/HDL-c	0.306	0.015	-0.102	0.697	0.286	0.054
TC/HDL-c	0.284	0.019	-0.158	0.544	0.221	0.120

Correlations were determined using Pearson correlation coefficients. T2D: Patients with type 2 diabetes mellitus; HS: Healthy subjects; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; HDL-c: Cholesterol bound to high-density lipoprotein; LDL-c/HDL-c: Cholesterol bound to low density lipoprotein/HDL-c ratio; TC/HDL-c: Total cholesterol/HDL-c ratio; ucOC: Undercaboxylated osteocalcin.

DISCUSSION

In this study lower levels of ucOC were found in T2D when compared to HS. Although low levels of total OC and ucOC in T2D patients have been previously reported[11,26-28], there is little evidence describing the relationship between serum levels of ucOC and CVRF.

The association between ucOC concentration and T2D is consistent with *in vitro* and *in vivo* studies that link OC to energetic equilibrium[3,4]. According to previous reports, there is an inverse correlation between serum ucOC levels and; IR, BMI, BFP, FPG and HOMA-IR[7,22,23,29-31].

In our study, multiple associations between ucOC levels and CVRF in T2D patients were found. In T2D patients, ucOC concentration was inversely correlated with BMI and BFP and a positively correlated with DBP. In HS, a positive correlation between ucOc and SBP was also established. Additionally, those T2D patients with highest ucOC levels (Q4) had higher DBP than those with the lowest ucOC (Q1).

The inverse relationship between ucOC and BMI was previously reported by Chen *et al*[32] and Tan *et al*[33] in Chinese men, which might be explained by the role of OC in energy metabolism and by the observation that the OC knockout mice model has abnormal levels of visceral fat[6].

This might be the first report indicating a positive relationship between ucOC levels and DBP in T2D patients and a positive correlation of SBP with ucOC in HS. In non-diabetic young adults, Polgreen *et al*[34] found lower SBP in those having cOC and total OC in Q4 than in Q1, but did not report anything regarding ucOC and DBP. Although Chen, Tan and colleagues found a weak inverse correlation between DBP and total OC they did not did not determined ucOC levels[32,33] which

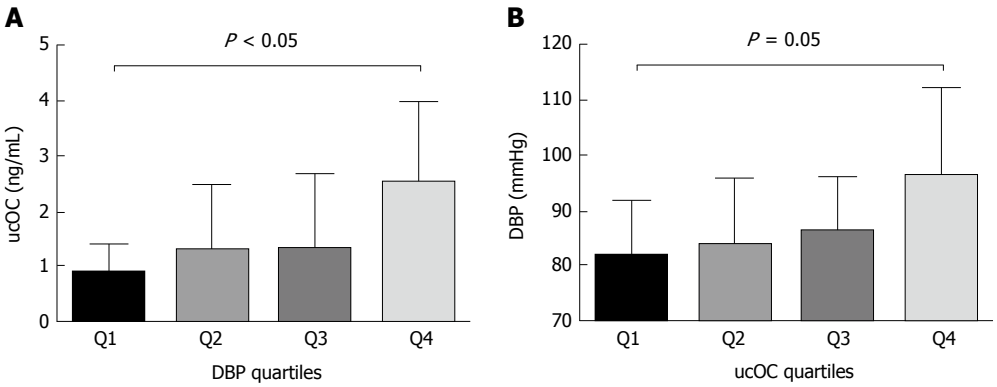

Figure 1 Correlation between undercaboxylated osteocalcin and diastolic blood pressure by quartiles. A: ucOC serum levels by DBP quartiles in type 2 diabetes; B: DBP (mmHg) by quartiles of serum ucOC. ucOC: Undercaboxylated osteocalcin; DBP: Diastolic blood pressure.

would've been desirable since various biological effects outside-the-bone have been endorsed to ucOC[6].

We also observed an inverse association between ucOC levels, BMI, FPG and HDLc, as well as a positive correlation with LDLc/HDLc and TC/HDLc ratios in the whole study population. These data suggest that serum levels of ucOC could be related to CVRF in both HS and T2D patients. These findings agree with the higher ucOC serum levels found by Okura *et al*[35] in hypertensive patients with carotid calcification than in those without carotid calcification, suggesting that ucOC might be a potential biomarker for carotid artery calcification. Our work also agrees with the results published by Kanazawa *et al*[23] who found that total serum OC concentration was negatively associated with atherosclerotic parameters (intima-media thickness and ankle-brachial pulse-wave velocity) independent of other CVRF in diabetic men. Other studies had reported that bone proteins such as matrix Gla protein, OPN and OPG are markers of vascular calcification and are expressed in arteries presenting atherosclerosis[22,23]. Moreover, T2D patients are particularly prone to develop CVD due to the role that diabetes plays in endothelial dysfunction, atherogenesis and vascular calcification[36-39].

Recent studies on animals suggest that OC may have beneficial effects on serum TG levels, but the clinical relevance of this remains elusive[29,31]. Although a significant correlation between serum OC concentration and TG was not found in our study, ucOC levels were inversely correlated with BMI and BFP in the T2D group and with BMI and HDL-c in whole population. This suggests a possible role of ucOC in lipid metabolism regulation. The discrepancy between our study and those conducted in animals could reside in the differences between human and animal lipid metabolism. Numerous studies regarding this issue suggest that TG are positively correlated with bone density while HDL-c is negatively correlated. These observations imply that a common mechanism of lipid and bone metabolism exists. However, more studies need to be performed in order to achieve proper understanding of the pathophysiological relationship between the OC levels and cardiovascular disease[40-49].

Virmani *et al*[41] have defined atherosclerosis as a chronic inflammatory process that can be accelerated by high blood pressure secondary to vasoactive peptides such as angiotensin and endothelin-1. Proinflammatory and prothrombotic risk markers play a very important role in the atheroma formation process, which contributes to the progression of vascular disease in T2D patients by activating inflammatory signaling and oxidative stress, both triggers of the endothelium injury process. Schurgers *et al*[39], found that matrix Gla protein was not carboxylated in atherosclerotic arteries, which could explain the positive correlation between uOC levels and SBP, DBP, LDL-c/HDL-c and TC/HDL-c, well known markers of cardiovascular disease[46,47].

To our knowledge, this is the first study proposing a link between ucOC serum levels and CVRF in a Mexican population. The main limitation of our study is that it cannot identify causal relationship due to its design. Additional studies are needed to determine specifically whether serum ucOC concentration could be considered an independent CVRF. Our findings will generate new hypotheses regarding the role of this protein, not only as a hormone in energy and bone metabolism and its well-studied role in regulation of glucose metabolism, insulin secretion and sensitivity; but also in endothelial dysfunction and as a marker of cardiovascular risk in patients with T2D.

Most reports that studied the relationship between OC and glucose metabolism include only total OC levels determination and data dealing with ucOC in T2D patients in Mexicans is scarce. Our study investigated serum ucOC levels in this population and thus provides information that helps further explore the role of OC in glucose metabolism and CVRF related to T2D.

Serum ucOC levels are related to CVRF in both T2D and HS. Specifically, ucOC is related to adiposity markers and blood pressure, as well as lipid profile. Thus, serum ucOC might be a cardiovascular risk marker in the Mexican population.

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COMMENTS

Background

Undercarboxylated (ucOC) is a non-collagenous peptide involved in various biological processes, including glucose metabolism. The relationship between low levels of ucOC and type 2 diabetes (T2D) is well established and some have proposed ucOC as a marker for metabolic risk. However, the role of ucOC and cardiovascular diseases (a common comorbidity in T2D) has not been well defined.

Research frontiers

Osteocalcin (OC) research now involves fields in energy metabolism, male fertility and brain development. Also a potential receptor to mediate its functions has been proposed, the GPRC6A receptor. However, in cardiovascular disease investigation, there is continuous interest in analyzing the role of OC in atherosclerosis indexes and vascular calcification.

Innovations and breakthroughs

The authors have described various correlations between ucOC levels and markers of cardiovascular risk such as blood pressure, high-density lipoproteins, body mass index and body fat percentage in a Mexican population. Other reports have analyzed the role of ucOC role in arterial calcification in hypertensive patients such as Okura *et al*, however no other group has studied its relationship with cardiovascular risk factors (CVRF) in T2D Mexican patients like in the present report.

Applications

The analysis postulates the possible emergence of ucOC as an independent CVRF in T2D patients.

Terminology

Osteocalcin: Non-collagenous protein that is found in the bone extracellular matrix and in the serum of circulating blood, it is produced by osteoblasts especially in the presence of vitamin D. This hormone exists in two forms: Carboxylated and undercarboxylated; Carboxylated osteocalcin: A form of osteocalcin in which its 3 residues of glutamic acid that reside at the 17, 21 and 24th positions are gamma carboxylated through a vitamin K depend process; Undercarboxylated osteocalcin: Osteocalcin that has less than 3 residues of carboxyglutamic acid.

Peer-review

This is a useful manuscript.

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