Undercarboxylated Osteocalcin: A Promising Target for Early Diagnosis of Cardiovascular and Glycemic Disorders in Patients with Metabolic Syndrome: A Pilot Study

Blanca Riquelme-Gallego 1,2, Laura García-Molina 1,2, Naomi Cano-Ibáñez 1,2,3, Francisco Andújar-Vera 4,5, Sheila González-Salvatierra 2,6, Cristina García-Fontana 2,7,8,*, Aurora Bueno-Cavanillas 1,2, Manuel Muñoz-Torres 2,6,7,8,*,† and Beatriz García-Fontana 2,7,8,*

1 Department of Preventive Medicine and Public Health, University of Granada, 18016 Granada, Spain; brique@ugr.es (B.R.-G.); lgarmol@ugr.es (L.G.-M.); ncaiba@ugr.es (N.C.-I.); bueno@ugr.es (A.B.-C.)
2 Instituto de Investigación Biomédica (ibs.GRANADA), 18014 Granada, Spain; sgsalvatierra@ugr.es
3 Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), 28029 Madrid, Spain
4 Department of Computer Science and Artificial Intelligence, University of Granada, 18071 Granada, Spain; franciscoluisandujar@gmail.com
5 Andalusian Research Institute in Data Science and Computational Intelligence (DaSci Institute), 18014 Granada, Spain
6 Department of Medicine, University of Granada, 18016 Granada, Spain
7 CIBER de Fragilidad y Envejecimiento Saludable (CIBERFES), Instituto de Salud Carlos III, 28029 Madrid, Spain
8 Endocrinology and Nutrition Unit, University Hospital Clínico San Cecilio, 18016 Granada, Spain
9 Correspondence: cgfontana@hotmail.com (C.G.-F.); mmt@mamuto.es (M.M.-T.); bgfontana@fibao.es (B.G.-F.); Tel.: +34-958023460 (C.G.-F.); +34-958-24-61-24 (M.M.-T.); +34-958023460 (B.G.-F.)
† These authors contributed equally to this work.

Abstract: Lifestyle changes are causing an exponential increase in the prevalence of obesity and metabolic syndrome (MetS) worldwide. The most frequent complications of these are the development of diabetes (T2D) and cardiovascular disease (CVD). Accurate tools are needed to classify the cardiovascular risk (CVR) in the MetS population. In recent years, numerous biomarkers of bone metabolism have been associated with CVR. The aim of this study was to determine the levels of undercarboxylated osteocalcin (ucOC) in a cohort of patients with MetS and to analyse its association with MetS parameters and CVR as well as with T2D prevalence. A longitudinal study was conducted in which a MetS population was followed for one year. Weight change, adherence to the Mediterranean diet (MedDiet), ucOC levels, MetS parameters and CVR were analysed and CVR was calculated using different scores. Our results showed a decrease of CVR associated with a better adherence to the MedDiet resulting in higher HDL-C and ucOC levels though the improvement of MetS risk factors. This bone protein appeared as a potential biomarker to classify CVR in the MetS population, especially for MetS patients without prevalent T2D. Furthermore, ucOC serum levels could be good predictors of T2D prevalence.

Keywords: metabolic syndrome; cardiovascular risk; diabetes; osteocalcin; Mediterranean diet

1. Introduction

In recent years, several studies have reported a strong association between osteoporosis (OP) and cardiovascular disease [1,2]. An association between lipid profile disturbances and lower bone mineral density (BMD) has been described [3], as well as a key role of the angiogenesis process on bone repair [4] and local vascularization changes in bone disorders such as OP, rheumatoid arthritis, bone cancer or metastasis [5]. These findings support the connection of both pathological processes [6].
The coexistence of both pathologies could be partially explained by the unbalance in bone formation and resorption processes, which could be involved in vascular complications too [7] and by the presence of common risk factors such as menopause, obesity, alcohol intake, smoking, sedentary lifestyle, aging, inflammation, hyperglycemia and unhealthy diets. Healthy diets, such as the Mediterranean diet (MedDiet), characterised by a high intake of plant-based foods contribute to the increase and maintenance of BMD [8]. On one hand, although those dietary components produce alkalinity, specifically potassium and magnesium [9], on the other hand, high intakes of fiber can enhance calcium absorption and inhibit bone resorption by osteoclasts while maintaining the bone-forming activity of osteoblasts [10]. A MedDiet is characterised by a high intake of Monounsaturated fats (MUFA), which are associated with the maintenance of BMD and a lower incidence of fractures [11]. However, a high intake of saturated fats increases osteoclast survival and reduces intestinal calcium absorption, increasing its excretion in the urine. They are also associated with increased expression of inflammatory genes (TNFa, IL-4, IL-17 and P53), leading to increased incidence of fracture [12]. Finally, the MedDiet targets adequate protein intake, which is necessary for the formation and maintenance of bone tissue, as well as stimulating the action of insulin-like growth factor 1, which in turn promotes bone growth and increases calcium absorption [13]. Classically, bone has been exclusively assigned a support and protector function; however, recent findings have shown the involvement of some bone proteins on homeostasis and energy metabolism regulation [14–16]. Osteocalcin (OC), a non-collagenous protein synthesized by osteoblast, has been associated with atherosclerotic disease parameters, such as pulse wave velocity (PWV) and intima-media thickness (IMT) in type 2 diabetes mellitus (T2D) patients [17] and in patients with prevalent atherosclerosis [18]. Nevertheless, its association with insulin secretion and insulin sensitivity has been the most remarkable disclosure [19]. The undercarboxylated fraction of OC is released into the bloodstream, acting directly on the pancreatic β cells and the adipocytes, improving glycemic homeostasis and increasing energy expenditure [20,21].

Metabolic syndrome (MetS) is a conjunction of central obesity and some cardiovascular risk (CVR) factors which lead to high socioeconomic costs globally every year [22–24]. MetS patients show 1.5 times more risk of all-cause mortality [25], 2 times more risk of cardiovascular mortality [26] and 5 times more risk of developing T2D than the healthy population [26,27]. However, large differences in cardiovascular risk among MetS patients have been described due to the great heterogeneity among its components [20]. On the other hand, MetS has been linked to the progression of other pathologies such as bone fragility and osteoporosis [28,29]. In this context, these patients set a good example of connection between atherosclerosis and bone demineralization processes. Despite the evidence that shows OC and undercarboxylated OC (ucOC) as energy metabolic regulators [30–32], and their association with individual CVR parameters in humans [33–35], the involvement of ucOC on global CVR in MetS patients remains unclear.

The aim of this study was to analyze the usefulness of the serum ucOC levels as an indicator of global cardiovascular and T2D risk in a cohort of MetS patients in order to characterize properly these patients with a high heterogeneity to establish early preventive and therapeutic strategies for patients at higher risk.

2. Materials and Methods

A longitudinal study was conducted in 296 patients from primary care. All patients had obesity or overweight and were diagnosed with MetS according to the NCEP ATP III definition [36], meeting at least three of CVR criteria (high BP, impaired FPG, HDL-C and TG levels or increased WC). In order to ensure menopause status, men and women were aged older than 55 and 60 years, respectively, to make CVR comparable between sexes [37,38]. Outpatients were consecutively recruited from December 2014 to December 2016 in Granada (Spain) and followed up for one year. All of them were Caucasian and did not
present active cancer or previous history of cancer, morbid obesity (≥240 kg/m²), any prevalent cardiovascular disease or bone diseases and/or any health problem that could interfere with the study protocol. The study was approved by the ethics committee of Granada in accordance with the principles of the World Medical Association’s Declaration of Helsinki. All patients signed the informed consent before being included. Anthropometric data were determined by standard procedures and sociodemographic variables were collected by questionnaire. Patients reported smoking status, pharmacological treatment and T2D prevalence. Systolic and diastolic BP (SBP and DBP) were obtained using a standard mercury sphygmomanometer reporting the mean BP by the equation \( \frac{2 \times DBP + SBP}{3} \) [39]. Diet quality and physical activity were assessed at baseline and after 6 and 12 months of follow-up by the 14 items MedDiet adherence [40] and the Nurses’ Health Study for Spanish population [41] questionnaires, respectively. Blood samples were collected after an overnight fast and conventional analyses of lipid profile (total, HDL and LDL-Cholesterol and triglyceride (TG) level), fasting plasm glucose (FPG) and glycated hemoglobin (HbA1c) were performed. Serum ucOC levels were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) according to Takara Bio, Japan instructions at baseline, 6 and 12 months of follow up. Intra- and inter-assay variations precision testing were consistent with reported by the manufacturer (6%-10% and 5.21%-8.33%, respectively).

Although all MetS patients were treated as usual from primary care professionals, they were recommended to follow a healthy MedDiet to increase the consumption of olive oil, fruits, vegetables and nuts and decrease the consumption of meat, simple sugars and saturated fats and encouraged to practice physical activity according to the WHO recommendations (at least 150–300 min of moderate-intensity aerobic physical activity; or at least 75–150 min of vigorous-intensity aerobic physical activity; or an equivalent combination of moderate- and vigorous-intensity activity throughout the week) [42].

In order to classify CVR in MetS patients, a Z-score (CV-ZS) was calculated at baseline and during follow-up using the NCEP ATP III CVR criteria [36]. The mean-centering and standard deviation (SD) normalization were sex-specific for each variable by the \( \frac{x - \text{mean}}{\text{SD}} \) equation. Global CVR punctuation was the average of the Z-scores of mean BP,TG levels, WC, FPG and the inverse Z-score of HDL-C [43]. The CVR Framingham score was estimated by the Wilson P.W. et al. equation including age, T2D prevalence, smoking and sex-adjusted CVR variables (total cholesterol, HDL-C, LDL-C, systolic and diastolic BP) [44]. The cut-off point corresponding to the 25th percentile of baseline log ucOC levels was used to evaluate the cardiometabolic profile and CVR of MetS patients at baseline and at 6 and 12 months follow-up.

Statistical analysis was performed using SPSS version 22.0 software (SPSS, Inc., Chicago, IL, USA). The normality of the variables was checked by Kolmogorov-Smirnov test and a log transformation was performed for serum ucOC levels. Continuous variables with normal distribution were presented as mean ± SD, and categorical variables were expressed as percentages. Student’s t and ANOVA tests for continuous variables and \( \chi^2 \) test for categorical variables were used to determine statistical differences among the means of two or more groups. ANCOVA model was performed when an adjustment by covariates was required. The Pearson’s correlation coefficient was used to analyze the associations between continuous variables. The variables influencing CVR such as age, sex, smoking, sedentary activities and pharmacologic treatment for MetS (lipid-lowering, antidiabetic and antihypertensive drugs) were identified by multiple linear regression analysis. Multiple logistic regression model was applied to analyze ucOC as an estimator of T2D. A ROC curve was performed to estimate the usefulness of circulating ucOC levels as a marker of T2D risk. Values of the area under the curve (AUC) higher than 0.75 indicate a good predictive performance [45]. Statistical significance was set at \( p < 0.10 \) for multiple linear regression analysis and at \( p < 0.05 \) (two tailed) for the other analyses.
3. Results

Baseline characteristics of the study population according to sex are shown in Table 1. The proportion of women and men was 21.7% and 20.0%, respectively, of patients that had been previously diagnosed with T2D considering the American Diabetes Association criteria [46], with no differences by sex. More than 90% of the patients were hypertensive, 72.6% were obese and 94% of the females and 87.4% of the males showed high risk waist circumference (WC). The prevalence of dyslipidemia was similar for both sexes, however men showed significantly lower baseline HDL-C levels and women showed significantly higher LDL-C levels. Regarding lifestyle habits, mean MedDiet index was 8.6, without significant differences by gender, and men showed a higher prevalence of smoking and sedentary habits. In addition, males showed significantly lower ucOC serum levels and higher CVR measured by CV-ZS and Framingham score.

**Table 1. Baseline characteristics of the study population by sex.**

|                  | Males (N = 135) | Females (N = 161) | p    |
|------------------|-----------------|-------------------|------|
| T2D (%)          | 20.0%           | 21.7%             | 0.697|
| >7 h of sedentary activity (%) | 57.0%          | 36.0%             | <0.001|
| Smoking (%)      | 16.3%           | 8.1%              | 0.029|
| Hypertension (%) | 93.3%           | 90.7%             | 0.270|
| **Mean**         | **SD**          | **Mean**          | **SD**| **p**  |
| Age              | 62.1            | 5.1               | 65.8 | 4.1   | <0.001|
| BMI (kg/m²)      | 32.5            | 3.5               | 32.8 | 3.9   | 0.537|
| WC (cm)          | 112.7           | 9.5               | 104.3| 9.6   | <0.001|
| Systolic BP (mm Hg) | 142.4          | 16.4              | 134.9| 17.2  | <0.001|
| Dyastolic BP (mm Hg) | 88.4           | 10.2              | 83.8 | 9.6   | <0.001|
| FPG (mg/dL)      | 101.0           | 20.3              | 102.4| 26.8  | 0.611|
| Total cholesterol (mg/dL) | 194             | 32                | 208  | 37    | <0.001|
| HDL-C (mg/dL)    | 46              | 10                | 53   | 10    | <0.001|
| LDL-C (mg/dL)    | 119             | 29                | 127  | 35    | 0.034|
| Triglycerides (mg/dL) | 165            | 77                | 168  | 72    | 0.798|
| HbA1c (%)        | 5.9             | 0.8               | 6.0  | 0.8   | 0.242|
| CV-ZS            | 1.0             | 2.5               | -0.9 | 2.8   | <0.001|
| Framingham score (%) | 16.7          | 6.3               | 10.2 | 4.8   | <0.001|
| Log ucOC (ng/mL) | 1.4             | 0.8               | 1.6  | 0.8   | 0.043|
| MedDiet index    | 8.5             | 2.1               | 8.6  | 1.9   | 0.679|

T2D: type 2 diabetes; WC: waist circumference; BMI: body mass index; BP: blood pressure; FPG: fasting plasma glucose; CV-ZS: cardiovascular risk Z-score; ucOC: undercarboxylated osteocalcin; MedDiet: Mediterranean diet.

Table 2 shows the evolution of MetS risk factors (anthropometric measures, lipid and glycemic profile), MedDiet index, serum ucOC levels and CVR scores at the 6 and 12 month follow-up. The general population showed a general improvement especially at the 6 month follow-up. BMI, WC, HDL-C and BP decreased significantly, which slightly reduced total CVR measured by CV-ZS and Framingham scores. Mean MedDiet index increased above the recommended (nine points) and lipid and glycemic profiles showed an improvement, but only serum TG showed significative differences during the follow-up period. Serum HDL-C and ucOC levels increased significantly at the 6-month point but decreased again at the 12-month point, showing a significant correlation during the follow-up (Figure 1).
Figure 1. Relationship between serum ucOC levels and HDL-C at baseline, at 6 and 12 month follow-up. * Spearman correlation test < 0.05.
| Table 2. Evolution of MetS risk factors, CVS and serum ucOC levels. |
|---------------------------------------------------------------|
| **Baseline (n = 246)** | **6 Months (n = 227)** | **12 Months (n = 214)** |
|------------------------|------------------------|------------------------|
| **Mean/ N**            | **Mean/ N**            | **Mean/ N**            |
| **SD/ %**              | **SD/ %**              | **SD/ %**              |
| **BMI (kg/m²)**        | 32.4 3.6              | 31.6 3.9              | 31.3 4.1 | <0.001 |
| **Waist (cm)**         | 108.1 10.4            | 104.1 10.4            | 103.6 10.9 | <0.001 |
| **Mean BP (mm Hg)**    | 103.2 11.0            | 102.6 10.6            | 100.3 11.7 | 0.023 |
| **Pulse (bpm)**        | 71.0 10.0             | 68.0 9.0              | 69.0 11.0 | 0.002 |
| **MedDiet index**      | 8.5 2.0               | 9.9 2.8               | 9.8 3.1  | <0.001 |
| **FPG (mg/dL)**        | 102 24                | 99 24                 | 99 24 | 0.105 |
| **HbA1c (%)**          | 6.0 0.1               | 6.0 0.1               | 6.0 0.1 | 0.454 |
| **Total cholesterol (mg/dL)** | 201 36            | 205 39                | 200 38 | 0.201 |
| **HDL-C (mg/dL)**      | 50 11                 | 52 12                 | 51 11 | <0.001 |
| **LDL-C (mg/dL)**      | 123 33                | 124 34                | 123 33 | 0.706 |
| **Triglycerides (mg/dL)** | 167 74             | 158 72                | 156 77 | 0.002 |
| **Log ucOC (ng/mL)**   | 0.6 0.3               | 0.8 0.3               | 0.7 0.3 | <0.001 |
| **CV-ZS**              | -0.2 0.2              | -0.2 0.2              | -0.2 0.2 | 0.956 |
| **Framingham index (%)** | 12.4 0.6              | 12.0 0.6              | 11.5 0.6 | 0.125 |
| **Sedentary**          | 135 46                | 111 37                | 124 42 | <0.001 |

BMI: body mass index; BP: blood pressure; bpm: beats per minute; FPG: fasting plasma glucose; MedDiet: Mediterranean diet; ucOC: undercarboxylated osteocalcin; CV-ZS: cardiovascular risk Z-score. ANOVA analysis of variance for multiple comparisons of means.

Table 3 shows the variables correlated with serum ucOC levels. Apart from HDL-C levels, ucOC showed a significant negative correlation with HbA1c levels and CVR at baseline measured according to CV-ZS and Framingham, as well as with sex.

| Table 3. Variables correlated with serum ucOC levels (log) at baseline, at 6 and 12 month follow-up. |
|---------------------------------------------------------------|
| **Baseline (n = 246)** | **6-Months (n = 227)** | **12-Months (n = 214)** |
|------------------------|------------------------|------------------------|
| **MetS Patients**      | **r**      | **p**      | **r**      | **p**      | **r**      | **p**      |
| **Age**                | 0.065       | 0.312      | 0.122      | 0.065      | 0.141      | 0.039      |
| **FPG (mg/dL)**        | -0.102      | 0.110      | -0.036     | 0.603      | -0.177     | 0.013      |
| **HDL-C (mg/dL)**      | 0.244       | <0.001     | 0.147      | 0.036      | 0.155      | 0.038      |
| **LDL-C (mg/dL)**      | 0.049       | 0.452      | 0.072      | 0.307      | 0.139      | 0.059      |
| **Triglycerides (mg/dL)** | -0.113       | 0.079      | -0.058     | 0.408      | -0.021     | 0.773      |
| **HbA1c (%)**          | -0.193      | 0.007      | -0.190     | 0.012      | -0.265     | 0.002      |
| **Systolic BP (mm Hg)** | 0.017       | 0.788      | 0.041      | 0.558      | 0.072      | 0.319      |
| **Dyastolic BP (mm Hg)** | 0.012      | 0.848      | 0.071      | 0.304      | 0.179      | 0.013      |
| **CV-ZS score**        | -0.175      | 0.007      | -0.054     | 0.457      | -0.008     | 0.919      |
| **Framingham score (%)** | -0.200       | 0.008      | -0.068     | 0.390      | -0.082     | 0.324      |

MetS: Metabolic syndrome; FPG: Fasting plasma glucose; BP: Blood pressure; CV-ZS: Cardiovascular Z-Score.

Serum ucOC levels increased from baseline 6.06 ng/mL to 9.44 ng/mL at 6 months and decreased to 6.39 ng/mL at the 12-months follow-up. We compared ucOC levels between patients with and without prevalent T2D at baseline, at the 6 and 12 month follow-up. Since ucOC levels were correlated with sex, we adjusted for sex and age (Table 4).
Table 4. Comparison of serum ucOC levels (log) in MetS patients with and free of prevalent T2D at baseline and at the 6- and 12-month follow-up, adjusting for sex and age.

| Log ucOC (ng/mL) | MetS Patients (n = 234) | T2D Patients (n = 62) | p   |
|-----------------|-------------------------|-----------------------|-----|
|                 | Mean | CI (95%)                  | Mean | CI (95%) |      |
| Baseline (n = 246) | 1.61 | 1.50 - 1.72 | 1.02 | 0.82 - 1.23 | <0.001 |
| 6 months (n = 227) | 1.99 | 1.88 - 2.10 | 1.57 | 1.34 - 1.80 | 0.002 |
| 12 months (n = 214) | 1.70 | 1.60 - 1.81 | 1.14 | 0.93 - 1.34 | <0.001 |

MetS: metabolic syndrome; T2D: type 2 diabetes; ucOC: undercarboxylated osteocalcin; CI: confidence interval.

The independent association of ucOC serum levels (log) and CV-ZS was estimated through multiple linear regression model adjusting for age, sex, smoking, sedentary status, prevalent T2D and any MetS pharmacological treatment previously described. Baseline serum levels of ucOC appeared as an independent variable associated with CV-ZS for non-T2DM patients (Table 5). However, this association was not statistically significant at the 6- and 12-month follow-up analysis.

Table 5. Relationship between ucOC levels (log) and CVR.

| Sample                  | Time         | B     | CI  | p   |
|-------------------------|--------------|-------|-----|-----|
| MetS patients           | Baseline (n = 246) | -0.904 | -1.955 | 0.148 | 0.092 |
|                         | 6 months (n = 227) | -0.148 | -1.419 | 1.124 | 0.819 |
|                         | 12 months (n = 214) | -0.145 | -1.427 | 1.137 | 0.824 |
| MetS-No T2D patients    | Baseline (n = 246) | -1.317 | -2.417 | -0.217 | 0.019 |
|                         | 6 months (n = 227) | 0.014  | -1.164 | 1.376 | 0.869 |
|                         | 12 months (n = 214) | 1.014  | -0.305 | 2.333 | 0.131 |

MetS: metabolic syndrome; T2D: type 2 diabetes; CI: Confidence interval; T2D: Type 2 diabetes.

In order to analyze the influence of serum ucOC levels on CVR factors, they were compared according to the 25th percentile of the logarithm of baseline ucOC serum levels (0.92 ng/mL) previously reported [43] by ANCOVA analysis, adjusting for sex (Table 6). This cut-off point was applied at the 6- and 12-month follow-up. A cardiometabolic profile were significantly disturbed when ucOC levels were below 0.92 ng/mL, and thus CVR estimated by CV-ZS was higher for these patients during the follow up period.

Finally, the relationship between serum ucOC levels and T2D prevalence was analyzed by performing logistic regression including biological variables classically linked to T2D (age, sex, sedentary status, lipid profile and WC) in addition to ucOC serum levels as independent variables, showing a negative association (OR = 0.051; [0.011/0.226], p < 0.001).

An ROC curve was performed to analyze circulating ucOC ability to identify T2D prevalence. The model including ucOC serum levels best described T2D prevalence, since it showed the highest area under the curve (AUC = 0.894; p < 0.001). This trend remained at the 6- and 12-months follow-up (OR = 0.98; [0.96/0.99], p = 0.005 and (OR = 0.11; [0.01/0.70], p = 0.019 respectively) (Figure 2).
Figure 2. ROC curve to analyze the utility of ucOC serum levels (log) as an indicator of T2D prevalence in MetS patients at baseline and after the 6- and 12-month follow-up. ucOC: undercarboxylated osteocalcin. FPG: fasting plasma glucose; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ucOC: undercarboxylated osteocalcin.
Table 6. MetS and T2D factors according to the 25th percentile of baseline serum levels of ucOC (log) in the total sample of MetS patients.

| Log ucOC | Baseline  | 6 months | 12 months |
|----------|-----------|-----------|-----------|
|          | <0.92 ng/mL (n = 61) | ≥0.92 ng/mL (n = 185) | p | <0.92 ng/mL (n = 25) | ≥0.92 ng/mL (n = 202) | p | <0.92 ng/mL (n = 33) | ≥0.92 ng/mL (n = 181) | p |
|          | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| BMI (kg/m²) | 32.9 | 3.4 | 32.4 | 3.6 | 0.360 | 31.7 | 3.6 | 31.5 | 3.8 | 0.569 | 31.7 | 3.7 | 31.1 | 4.1 | 0.277 |
| WC (cm) | 107.7 | 9.6 | 107.2 | 10.2 | 0.312 | 105.6 | 9.8 | 103.6 | 10.2 | 0.464 | 106.1 | 10.8 | 102.7 | 10.7 | 0.143 |
| Mean BP (mm Hg) | 103.5 | 12.3 | 103.3 | 11.2 | 0.739 | 100.6 | 9.1 | 102.8 | 10.4 | 0.075 | 99.3 | 13.3 | 101.3 | 11.2 | 0.131 |
| HDL-C (mg/dL) | 45.1 | 7.7 | 50.4 | 10.3 | 0.002 | 47.2 | 8.9 | 53.5 | 12.2 | <0.001 | 47.9 | 8.8 | 52.4 | 10.9 | 0.030 |
| FPG (mg/dL) | 110.1 | 33.6 | 99.0 | 20.7 | 0.002 | 106.2 | 34.0 | 96.4 | 19.9 | 0.009 | 111.5 | 33.8 | 95.1 | 19.4 | <0.001 |
| Triglycerides(mg/dL) | 189.2 | 96.3 | 163.8 | 67.3 | 0.020 | 163.6 | 71.1 | 158.0 | 70.8 | 0.589 | 174.9 | 84.3 | 153.0 | 76.0 | 0.079 |
| HbA1c (%) | 6.3 | 1.0 | 5.8 | 0.7 | <0.001 | 6.3 | 0.8 | 5.8 | 0.6 | <0.001 | 6.4 | 0.9 | 5.8 | 0.6 | <0.001 |
| CV-ZS score | 1.0 | 3.4 | -0.3 | 2.4 | <0.001 | 0.6 | 2.9 | -0.3 | 2.5 | 0.035 | 0.7 | 3.0 | -0.5 | 2.5 | 0.007 |
| Framingham score (%) | 15.1 | 6.7 | 12.6 | 6.1 | 0.107 | 13.7 | 6.7 | 12.1 | 6.6 | 0.386 | 14.1 | 7.0 | 11.8 | 6.5 | 0.413 |

BMI: body mass index; WC: waist circumference; BP: blood pressure; FPG: fasting plasma glucose; CV-ZS: cardiovascular risk Z-score; ucOC: undercarboxylated osteocalcin.

4. Discussion

Our results showed a decrease of CVR associated with a better adherence to the MetDiet resulting in higher HDL-C and ucOC levels in MetS patients. Serum ucOC showed an association with HDL-C levels after one year of follow-up and seems to be a potential biomarker to classify CVR in the MetS population, especially for MetS patients without prevalent T2D. Finally, circulating ucOC appears to be a good predictor for the risk of developing T2D in MetS patients. Patients with ucOC levels below the 25th percentile showed six times more of possibility of suffering T2D and these results were confirmed at the 6- and 12-month follow-up. The reversion of MetS factors and the reduction of CVR through lifestyle habit improvement have been largely documented [38,39]. In this cohort of MetS patients an increase of mean MedDiet adherence score and a slight reduction of sedentary activities is observed. The consumption of olive oil and nuts is associated with the increase of HDL-C levels which correlates with ucOC levels during the follow-up, as previously reported in some studies [47–49].

Our results point to ucOC as a potential biomarker capable of stratifying CVR in MetS patients. In this way, our results show a strong association between cardiovascular risk factors (CVRFs) and circulating ucOC and observing significative lower levels of ucOC in those MetS patients presenting higher CVR. Considering the close association of serum ucOC levels with CVRFs, we evaluated its role as a predictor of CVR in our study population. Our results showed that serum ucOC level is an independent estimator of CVR adjusting for lifestyle variables and pharmacologic treatment at baseline. This association was stronger when patients with prevalent T2D were excluded from the analysis since glucose levels are the main regulators on energy [50–53] and the presence of diabetes is the main risk factor for CVD rather than circulating ucOC. Agreeing with our results, other studies have previously reported the association between the individual CVR factors and total OC and ucOC [48,54,55]; however, this is the first study that shows an association with a total score of cardiovascular risk based on the MetS factors.

In order to validate the cut-off point established in our retrospective study as a threshold for stratifying CVR [43], the cardiometabolic profile of the study patients was analyzed during the follow-up period according to the cut-off point of 2.53 ng/mL ucOC. Our results showed an association between lower ucOC levels and worse cardiometabolic profile and, thus, with higher CVR in terms of CV-ZS score during the whole follow-up period. Similar results were reported in a cohort of elderly men, showing a positive correlation between higher ucOC/OC ratio and lower incidence of myocardial infarction [56].
The better results in terms of improvement of CVR associated with higher HDL-C and lower ucOC levels were found at the 6-month follow-up when the participants were more aware of the intervention program. However, the strength of ucOC as a predictor variable of CVR was weaker at this point since the proportion of patients with values of circulating ucOC lower than 2.53 ng/mL was very low due to the improvement of lifestyle habits. Nevertheless, a relaxation in lifestyle habits was observed after 12 months of follow-up, thus the number of patients with values of ucOC below 2.53 ng/mL was raised again and the predictive value of serum ucOC for CVR was nearly significant.

Based on the close association between ucOC and CVRFs, recent studies carried out in animal models have reported its effectiveness in vitro as a therapeutic treatment on atherosclerosis, showing an increase on capillary density and neovascularization and improving myocardial fibrosis [57,58].

Finally, the assessment of the usefulness of circulating ucOC for prediction of T2D risk in MetS patients positioned the ucOC as a powerful predictive strategy. Since MetS patients show an important heterogeneity, some of them may suffer higher glycemic disturbance, thus they will be at higher risk of T2D. Our results showed that patients with baseline levels below the cut-point of 2.53 ng/mL showed six times more of a risk of T2D. These results were confirmed at the 6- and 12-month follow-up, agreeing with previous findings [59]. These findings suggest that the measurement of serum ucOC levels could represent a promising screening tool in order to classify MetS individuals at high risk of developing T2D.

However, this study has some limitations. On the one hand, it is an observational study, so there is a possible residual confounding in the results due to a classification bias introduced by the self-reported data. On the other hand, because it is a population with CVR, it could lead to a social desirability bias, increasing the score on the lifestyle scales. In addition, total OC, N-MID OC, vitamin D and K, which may have an influence on serum ucOC values, were not determined. However, it should be noted that the main strength of this study is the novel assessment of CVR in patients with MetS using a global score of the accepted variables for the diagnosis of MetS, which allows a more accurate characterization of CVR in this heterogeneous population. Additionally, in contrast to other studies, confounding factors have been considered such as the presence of T2D and the pharmacological treatment for the management of MetS. This is therefore the first study to show the relationship between serum ucOC levels and the global CVR in this population. Furthermore, its considerable sample size and therefore its statistical power provides robustness to our results. Another strength is the longitudinal design, as the 12-month follow-up allows us to establish a causal relationship between weight loss due to the improvement of lifestyle and the decrease of CVR in MetS patients.

In conclusion, at the one year follow-up, it was shown that the improvement of lifestyle in a sample of MetS patients may have a double effect on their cardiometabolic health: on one hand through anthropometric changes and the improvement of MetS parameters, and on the other hand, by the increase of ucOC levels, which improves glucose homeostasis and insulin sensitivity by its action on beta pancreatic cells. Thus, ucOC is postulated as a good biomarker to classify T2D and cardiometabolic risk in such a heterogeneous population as MetS patients and to initiate early intervention in those at higher risk.

**Author Contributions:** Study design: A.B.-C., M.M.-T. and B.G.-F.; Study conduct: B.R.-G., L.G.-M., N.C.-I., B.G.-F., S.G.-S. and C.G.-F.; Data collection: B.R.-G., L.G.-M. and N.C.-I., Data analysis: B.G.-F., B.R.-G. and F.A.-V.; Data interpretation: B.R.-G., B.G.-F., A.B.-C., M.M.-T. and F.A.-V.; Drafting of the manuscript: B.R.-G., B.G.-F. and C.G.-F.; Reviewing the manuscript and approving final version of manuscript: All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Instituto de Salud Carlos III grants (PI18-00803, PI21/01069 and PI18-01235), co-funded by the European Regional Development Fund (FEDER) and by Junta de
Andalucía grant (PI-0268-2019). In addition, C.G.-F. and B.R.-G. are funded by postdoctoral fellowships from Instituto de Salud Carlos III and Junta de Andalucía (CD20/00022 and RH-0069-2021, respectively) and S.G.-S. is funded by a predoctoral fellowship from Instituto de Salud Carlos III (FI19/00118).

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Comité de ética de la investigación biomédica de la provincia de Granada (CEIM/CEI GRANADA) (N°0425-N-18) on July 27, 2018 for studies involving humans.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Acknowledgments:** The authors wish to thank all subjects who participated in the study as well as all study collaborators.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Reference**

1. Sambrook, P.N.; Chen, C.J.; March, L.; Cameron, I.D.; Cumming, R.G.; Lord, S.R.; Simpson, J.M.; Seibel, M.J. High Bone Turnover Is an Independent Predictor of Mortality in the Frail Elderly. *J. Bone Miner Res.* 2006, 21, 549–555. Available online: http://doi.wiley.com/10.1359/jbmr.060104 (accessed on 11 January 2021).

2. Van Der Klift, M.; Pols, H.A.P.; Geleijnse, J.M.; Van Der Kuip, D.A.M.; Hofman, A.; De Laet, C.E.D.H. Bone mineral density and mortality in elderly men and women: The Rotterdam Study. *Bone* 2002, 30, 643–648. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11934659 (accessed on 11 January 2021).

3. Ghorabi, S.; Shab-Bidar, S.; Sadeghi, O.; Nasiri, M.; Khatibi, S.R.; Djaferian, K. Lipid Profile and Risk of Bone Fracture: A Systematic Review and Meta-Analysis of Observational Studies. *Endocr. Res.* 2019, 44, 168–184. Available online: https://www.tandfonline.com/doi/full/10.1080/07435800.2019.1625057 (accessed on 20 April 2021).

4. Beamer, B.; Hettrich, C.; Lane, J. Vascular Endothelial Growth Factor: An Essential Component of Angiogenesis and Fracture Healing. *HSS J.* 2010, 6, 85–94. Available online: http://link.springer.com/10.1007/s11420-009-9129-4 (accessed on 20 May 2021).

5. Carulli, C.; Innocenti, M.; Brandi, M.L. Bone Vascularization in Normal and Disease Conditions. *Front. Endocrinol.* 2013, 4. Available online: http://journal.frontiersin.org/article/10.3389/fendo.2013.00106/abstract (accessed on 2 May 2021).

6. Adami, S.; Braga, V.; Zamboni, M.; Gatti, D.; Rossini, M.; Bakri, J.; et al. Relationship between lipids and bone mass in 2 cohorts of healthy women and men. *Calcif. Tissue Int.* 2004, 74, 136–142. Available online: http://www.ncbi.nlm.nih.gov/pubmed/14668965 (accessed on 20 May 2021).

7. García-Martin, A.; Rozas-Moreno, P.; Reyes-García, R.; Morales-Santana, S.; García-Fontana, B.; García-Salcedo, J.A.; Muñoz-Torres, M. Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.* 2012, 97, 234–241.

8. Denova-Gutiérrez, E.; Méndez-Sánchez, L.; Muñoz-Aguirre, P.; Tucker, K.; Clark, P. Dietary Patterns, Bone Mineral Density, and Risk of Fractures: A Systematic Review and Meta-Analysis. *Nutrients* 2018, 10, 1922. Available online: http://www.mdpi.com/2027-6643/10/12/1922 (accessed on 3 June 2021).

9. Tian, L.; Yu, X. Fat, Sugar, and Bone Health: A Complex Relationship. *Nutrients* 2017, 9, 506. Available online: http://www.mdpi.com/2027-6643/9/5/506 (accessed on 5 June 2021).

10. Dreher, M. Whole Fruits and Fruit Fiber Emerging Health Effects. *Nutrients* 2018, 10, 1833. Available online: http://www.mdpi.com/2027-6643/10/12/1833 (accessed on 25 June 2021).

11. Trichopoulou, A.; Georgiou, E.; Bassiakos, Y.; Lipworth, L.; Lagiou, P.; Proukakis, C.; Trichopoulou, D. Energy Intake and Monounsaturated Fat in Relation to Bone Mineral Density among Women and Men in Greece. *Prev. Med.* 1997, 26, 395–400. Available online: https://linkinghub.elsevier.com/retrieve/pii/S0091743597901602 (accessed on 20 January 2021).

12. Mozaffari, H.; Djaferian, K.; Mofrad, M.D.; Shab-Bidar, S. Dietary fat, saturated fatty acid, and monounsaturated fatty acid intakes and risk of bone fracture: A systematic review and meta-analysis of observational studies. *Osteoporos Int.* 2018, 29, 1499–1506. Available online: http://link.springer.com/10.1007/s00198-018-4540-7 (accessed on 4 June 2021).

13. Ginty, F. Dietary protein and bone health. *Proc. Nutr. Soc.* 2003, 62, 867–876. Available online: https://www.cambridge.org/core/product/identifier/S0029665103001149/type/journal_article (accessed on 7 June 2021).

14. García-Martín, A.; Reyes-García, R.; Avila-Rubio, V.; Muñoz-Torres, M. Osteocalcin: A link between bone homeostasis and energy metabolism. *Endocrinol. Nutr.* 2013, 60, 260–263. Available online: http://www.ncbi.nlm.nih.gov/pubmed/23218238 (accessed on 17 June 2021).

15. Guedes, J.A.C.; Esteves, J.V.; Morais, M.R.; Zorn, T.M.; Furuya, D.T. Osteocalcin improves insulin resistance and inflammation in obese mice: Participation of white adipose tissue and bone. *Bone* 2018, 115, 68–82. Available online: http://www.ncbi.nlm.nih.gov/pubmed/29183784 (accessed on 20 July 2021).

16. Farhat, G.N.; Newman, A.B.; Sutton-Tyrrell, K.; Matthews, K.A.; Boudreau, R.; Schwartz, A.V.; Harris, T.; Tylavsky, F.; Visser, M.; Cauley, J.A.; Health ABC Study. The association of bone mineral density measures with incident cardiovascular disease in...
older adults. Osteoporos Int. 2007, 18, 999–1008. Available online: http://link.springer.com/10.1007/s00198-007-0338-8 (accessed on 20 March 2021).

17. Kanazawa, I.; Yamaguchi, T.; Yamamoto, M.; Yamauchi, M.; Kurioka, S.; Yano, S.; Sugimoto, T. Serum Osteocalcin Level Is Associated with Glucose Metabolism and Atherosclerotic Parameters in Type 2 Diabetes Mellitus. J. Clin. Endocrinol. Metab. 2009, 94, 45–49. Available online: https://academic.oup.com/jcem/article/94/1/45/2597641 (accessed on 20 May 2021).

18. Reyes-Garcia, R.; Rozas-Moreno, P.; Jimenez-Moleon, J.J.; Villoslada, M.J.L.; Garcia-Salcedo, J.A.; Santana-Morales, S.; Muñoz-Torres, M. Relationship between serum levels of osteocalcin and atherosclerotic disease in type 2 diabetes. Diabetes Metab. 2012, 38, 76–81.

19. Lin, X.; Zhang, X.; Guo, J.; Roberts, C.K.; McKenzie, S.; Wu, W.; Liu, S.; Song, Y. Effects of Exercise Training on Cardiorespiratory Fitness and Biomarkers of Cardiometabolic Health: A Systematic Review and Meta—Analysis of Randomized Controlled Trials. J. Am. Heart Assoc. 2015, 4. Available online: https://www.ahajournals.org/doi/10.1161/JAHA.115.002014 (accessed on 2 April 2021).

20. Movahed, A.; Larijani, B.; Nabipour, I.; Kalantarhormoz, M.; Asadipooya, K.; Vahdat, K.; Akbarzadeh, S.; Farrokhnia, M.; Assadi, M.; Amirinejad, R.; et al. Reduced serum osteocalcin concentrations are associated with type 2 diabetes mellitus and the metabolic syndrome components in postmenopausal women: The crosstalk between bone and energy metabolism. J. Bone Miner Metab. 2012, 30, 683–691.

21. 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. USA 2008, 105, 5266–5270. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18362359 (accessed on 22 April 2021).

22. Kanavos, P.; Van Den Aardweg, S.; Schurer, W. LSE Health, London School of Economics. Diabetes Expenditure, Burden of Disease and Management in 5 EU Countries. 2012. Available online: http://www.lse.ac.uk/lsehealthandsocialcare (accessed on 22 April 2021).

23. Lopez-Bastida, J.; Boronat, M.; Moreno, J.O.; Schurer, W. Costs, outcomes and challenges for diabetes care in Spain. Global Health 2013, 9, 17. Available online: http://www.ncbi.nlm.nih.gov/pubmed/23635075 (accessed on 23 January 2021).

24. Dunbar, S.B.; Khajvou, O.A.; Bakas, T.; Hunt, G.; Kirch, R.A.; Leib, A.R.; Morrison, S.; Poeher, D.C.; Roger, V.L.; Whitsel, L.P.; on behalf of the American Heart Association. Projected Costs of Informal Caregiving for Cardiovascular Disease: 2015 to 2035: A Policy Statement From The American Heart Association. Circulation 2018, 137, e558–e577. Available online: http://www.ncbi.nlm.nih.gov/pubmed/29632217 (accessed on 12 January 2021).

25. Berrington de Gonzalez, A.; Hartge, P.; Cerhan, J.R.; Flint, A.J.; Hannan, L.; Maclnnis, R.J.; Moore, S.C.; Tobias, G.S.; Anton-Culver, H.; Freeman, L.B.; et al. Body-Mass Index and Mortality among 1.46 Million White Adults. N. Engl. J. Med. 2010, 363, 2211–2219. Available online: http://www.nejm.org/doi/abs/10.1056/NEJMoia1000367 (accessed on 6 January 2021).

26. Mottillo, S.; Filion, K.B.; Genest, J.; Joseph, L.; Pilote, L.; Poirier, P.; Rinfret, S.; Schiffrin, E.L.; Eisenberg, M.J. The Metabolic Syndrome and Cardiovascular Risk. J. Am. Coll. Cardiol. 2010, 56, 1113–1132. Available online: https://linkinghub.elsevier.com/retrieve/pii/S0735109710026380 (accessed on 25 January 2021).

27. Ford, E.S.; Li, C.; Sattar, N. Metabolic syndrome and incident diabetes: Current state of the evidence. Diabetes Care 2008, 31, 1898–1904. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18591398 (accessed on 25 February 2021).

28. Kane, A.E.; Gregson, E.; Theou, O.; Rockwood, K.; Howlett, S.E. The association between frailty, the metabolic syndrome, and mortality over the lifespan. GerScience 2017, 39, 221–229. Available online: http://link.springer.com/10.1007/s11357-017-9967-9 (accessed on 25 May 2021).

29. von Muhlen, D.; Safii, S.; Jassal, S.K.; Svarthberg, J.; Barrett-Conner, E. Associations between the metabolic syndrome and bone health in older men and women: The Rancho Bernardo Study. Osteoporos Int. 2007, 18, 1337–1344. Available online: http://www.ncbi.nlm.nih.gov/pubmed/17492393 (accessed on 21 June 2021).

30. García-Martin, A.; Cortes-Berdonces, M.; Luque-Fernandez, I.; Rozas-Moreno, P.; Quesada-Charmecco, M.; Munoz-Torres, M. Osteocalcin as a marker of metabolic risk in healthy postmenopausal women. Menopause 2011, 18, 537–541.

31. Hwang, Y.-C.; Jeong, I-K.; Ahn, K.J.; Chung, H.Y. 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, 768–772.

32. Bullo, M.; Moreno-Navarrete, J.M.; Fernandez-Real, J.M.; Salas-Salvado, J. Total and undercarboxylated osteocalcin predict changes in insulin sensitivity and beta cell function in elderly men at high cardiovascular risk. Am. J. Clin. Nutr. 2012, 95, 249–255.

33. Prats-Puig, A.; Osiniri, I.; Soriano-Rodriguez, P.; Carreras-Badosa, G.; Buñuel-Álvarez, J.C.; Vila-Pablo, C.; de Zegher, F.; Ibáez, L.; Bassols, J.; López-Bermejo, A. Undercarboxylated osteocalcin relates to cardiovascular risk markers in offspring of families with metabolic syndrome. Atherosclerosis 2014, 233, 272–277. Available online: https://linkinghub.elsevier.com/retrieve/pii/S0021973814000815 (accessed on 11 February 2021).

34. Liu, J.-J.; Toy, W.C.; Wong, M.D.S.; Tan, C.S.H.; Tavintharan, S.; Wong, M.S.; Sum, C.F.; Lim, S.C. Elevated undercarboxylated and reduced carboxylated osteocalcin are associated with metabolic syndrome in middle age Asian females. Exp. Clin. Endocrinol. Diabetes 2013, 121, 329–333.

35. Zanatta, L.C.B.; Boguszewski, C.L.; Borba, V.Z.C.; Moreira, C.A. Association between undercarboxylated osteocalcin, bone mineral density, and metabolic parameters in postmenopausal women. Arch. Endocrinol. Metab. 2018, 62, 446–451. Available online: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2359-39972018000400446&lng=en&nrm=iso (accessed on 29 March 2021).
36. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001, 285, 2486–2497. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11368702 (accessed on 29 May 2021).

37. Mendelsohn, M.E.; Karas, R.H. The protective effects of estrogens on the cardiovascular system. N. Engl. J. Med. 1999, 340, 1801–1811. Available online: http://www.ncbi.nlm.nih.gov/pubmed/10362825 (accessed on 30 May 2021).

38. Pare, G.; Krust, A.; Karas, R.H.; Dupont, S.; Aronovitz, M.; Chambon, P.; Mendelsohn, M.E. Estrogen receptor-alpha mediates the protective effects of estrogen against vascular injury. Circ. Res. 2002, 90, 1087–1092. Available online: http://www.ncbi.nlm.nih.gov/pubmed/12039798 (accessed on 1 February 2021).

39. Benetos, A.; Rudnichi, A.; Safar, M.; Guize, L. Pulse Pressure and Cardiovascular Mortality in Normotensive and Hypertensive Subjects. Hypertension 1998, 32, 560–564. Available online: https://www.ahajournals.org/doi/10.1161/01.HYP.32.3.560 (accessed on 1 March 2021).

40. Schröder, H.; Fitió, M.; Estruch, R.; Martínez-González, M.A.; Corella, D.; Salas-Salvadó, J.; Lamuela-Raventós, R.; Ros, E.; Salaverría, I.; Fiol, M.; et al. A Short Screener Is Valid for Assessing Mediterranean Diet Adherence among Older Spanish Men and Women. J. Nutr. 2011, 141, 1140–1145. Available online: https://academic.oup.com/jn/article/141/6/1140/4689036 (accessed on 10 March 2021).

41. Martínez-González, M.A.; López-Fontana, C.; Varo, J.J.; Sánchez-Villegas, A.; Martínez, J.A. Validation of the Spanish version of the physical activity questionnaire used in the Nurses’ Health Study and the Health Professionals’ Follow-up Study. Public Health Nutr. 2005, 8, 920–927. Available online: https://www.cambridge.org/core/product/identifier/S1368980005001230/type/journal_article (accessed on 26 February 2021).

42. Physical activity. Available online: https://www.who.int/news-room/fact-sheets/detail/physical-activity (accessed on 18 May 2021).

43. Riquelme-Gallego, B.; García-Molina, L.; Cano-IBañez, N.; Sánchez-Delgado, G.; Andújar-Vera, F.; García-Fontana, C.; González-Salvaterra, S.; García-Recio, E.; Martínez-Ruiz, V.; Bueno-Cavanillas, A.; et al. Circulating Undercarboxylated Osteocalcin as Estimator of Cardiovascular and Type 2 Diabetes Risk in Metabolic Syndrome Patients. Sci. Rep. 2020, 10, 1840.

44. Wilson, P.W.F.; D’Agostino, R.B.; Levy, D.; Belanger, A.M.; Silbershatz, H.; Kannel, W.B. Prediction of Coronary Heart Disease Using Risk Factor Categories. Circulation 1998, 97, 1837–1847. Available online: https://www.ahajournals.org/doi/10.1161/01.CIR.97.18.1837 (accessed on 10 March 2021).

45. González, M. Bioestadística Amigable, 3rd ed.; Martínez-González, M.A., Sánchez-Villegas, A., Atucha, T., Estefania, A.; Faulin Fajardo, J., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; p. 596.

46. Alberti, K.G.; Zimmet, P.Z. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998, 15, 539–553. Available online: http://www.ncbi.nlm.nih.gov/pubmed/9686693 (accessed on 13 March 2021).

47. Pergola, G.; Triggiani, V.; Bartolomeo, N.; Nardecchia, A.; Giagulli, V.; Bruno, I.; Caccavo, D.; Silvestris, F. Independent Relationship of Osteocalcin Circulating Levels with Obesity, Type 2 Diabetes, Hypertension, and HDL Cholesterol. Endocr. Metab. Immune Disord. Targets 2016, 16, 270–275. Available online: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1871-5303&volume=16&issue=4&spage=270 (accessed on 20 March 2021).

48. Sanchez-Enriquez, S.; Ballesteros-Gonzalez, I.T.; Villafán-Bernal, J.R.; Pascoe-Gonzalez, S.; Rivera-Leon, E.A.; Bastidas-Ramirez, B.E.; Rivas-Carrillo, J.D.; Alcala-Zermeno, J.L.; Armendáriz-Borunda, J.; Llamas-Covarrubias, I.M.; 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. Available online: http://www.wjgnet.com/1948-9358/full/v8/i11/11.htm (accessed on 20 March 2021).

49. Alfadda, A.A.; Masoud, A.; Shaik, S.A.; 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, 1–6. Available online: https://www.hindawi.com/journals/ije/2013/197519/ (accessed on 11 February 2021).

50. Liu, D.-M.; Guo, X.-Z.; Tong, H.-J.; Tao, B.; Sun, L.-H.; Zhao, H.-Y.; Ning, G.; Liu, J.-M. Association between osteocalcin and glucose metabolism: A meta-analysis. Osteoporos Int. 2015, 26, 2823–2833. Available online: http://www.ncbi.nlm.nih.gov/pubmed/26089135 (accessed on 23 April 2019).

51. Kanazawa, I.; Yamaguchi, T.; Yamauchi, M.; Yamamoto, M.; Kurioka, S.; Yano, S.; Sugimoto, T. Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus. Osteoporos Int. 2011, 22, 187–194.

52. Kanazawa, I.; Yamaguchi, T.; Tada, Y.; Yamauchi, M.; Yano, S.; Sugimoto, T. Serum osteocalcin level is positively associated with insulin sensitivity and secretion in patients with type 2 diabetes. Bone 2011, 48, 720–725.

53. Iki, M.; Tamaki, J.; Fujita, Y.; Kouda, K.; Yura, A.; Kadowaki, E.; Sato, Y.; Moon, J.S.; Tomioka, K.; Okamoto, N.; et al. Serum undercarboxylated osteocalcin levels are inversely associated with glycemic status and insulin resistance in an elderly Japanese male population: Fujisawa-kyo Osteoporosis Risk in Men (FORMEN) Study. Osteoporos Int. 2012, 23, 761–770.

54. Tan, A.; Gao, Y.; Yang, X.; Zhang, H.; Qin, X.; Mo, L.; Peng, T.; Xia, N.; Mo, Z. Low serum osteocalcin level is a potential marker for metabolic syndrome: Results from a Chinese male population survey. Metabolism 2011, 60, 1186–1192.
55. Yeap, B.B.; Chubb, S.A.P.; Flicker, L.; McCaul, K.A.; Ebeling, P.R.; Beilby, J.P.; Norman, P.E. Reduced serum total osteocalcin is associated with metabolic syndrome in older men via waist circumference, hyperglycemia, and triglyceride levels. *Eur. J. Endocrinol.* 2010, 163, 265–272.

56. Yeap, B.B.; Alfonso, H.; Chubb, S.A.P.; Byrnes, E.; Beilby, J.P.; Ebeling, P.R.; Allan, C.A.; Schultz, C.; Hankey, G.J.; Golledge, J.; et al. Proportion of Undercarboxylated Osteocalcin and Serum P1NP Predict Incidence of Myocardial Infarction in Older Men. *J. Clin. Endocrinol. Metab.* 2015, 100, 3934–3942.

57. Qaradakhi, T.; Gadanec, L.K.; Tacey, A.B.; Hare, D.L.; Buxton, B.F.; Apostolopoulos, V.; Levinger, I.; Zulli, A. The Effect of Recombinant Undercarboxylated Osteocalcin on Endothelial Dysfunction. *Calcif Tissue Int.* 2019, 105, 546–556. Available online: http://www.ncbi.nlm.nih.gov/pubmed/31485687 (accessed on 14 November 2019).

58. Sadek, N.B.; Gamal, S.M.; Aboulhoda, B.E.; Rashed, L.A.; Shawky, H.M.; Gamal El-Din, M.M. The Potential Role of Undercarboxylated Osteocalcin Upregulation in Microvascular Insufficiency in a Rat Model of Diabetic Cardiomyopathy. *J. Cardiovasc. Pharmacol Ther.* 2020, 25, 86–97. Available online: http://journals.sagepub.com/doi/10.1177/1074248419876632 (accessed on 14 November 2019).

59. Villafán-Bernal, J.R.; Llamas-Covarrubias, M.A.; Muñoz-Valle, J.F.; Rivera-León, E.A.; González-Hita, M.E.; Bastidas-Ramírez, B.E.; Gurrola-Díaz, C.M.; Armendáriz-Borunda, J.S.; Sánchez-Enríquez, S. A Cut-Point Value of Uncarboxylated to Carboxylated Index Is Associated With Glycemic Status Markers in Type 2 Diabetes. *J. Investig. Med.* 2014, 62, 33–36. Available online: http://jim.bmj.com/lookup/doi/10.2310/JIM.0000000000000015 (accessed on 13 February 2021).