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# Undercarboxylated osteocalcin in patients with newly diagnosed type 2 diabetes after blood glucose regulation

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**Introduction** Until recently, endocrinologists have considered the bone to be a target for hormones such as sex steroids, parathyroid hormone, and calcitonin. Recent studies have suggested a new role for bone tissue by producing osteocalcin (OC), a protein hormone that affects insulin production and sensitivity, glucose utilization, and energy expenditure.<sup>1</sup>

OC is a bone-specific protein secreted by osteoblasts consisting of 46 to 50 residues; it undergoes posttranslational modification by vitamin K-dependent  $\gamma$ -carboxylation of 3 glutamic acid residues.<sup>2</sup> Undercarboxylated OC (ucOC) has less than 3 carboxylated residues and a lower affinity for the bone. Fully carboxylated and undercarboxylated OC forms are found in serum.

Studies on mice models, in which OC production was inactivated or increased, provided data on the role of circulating OC—particularly its undercarboxylated fraction—on energy expenditure and the regulation of insulin secretion.<sup>3</sup> In humans, blood OC levels were significantly lower in patients with diabetes than in nondiabetic controls, and the levels were inversely related to fat mass and blood glucose (BG).<sup>4</sup> In patients with poorly managed diabetes, increased OC levels were observed after only a month of treatment and glycemic control.<sup>5</sup> In patients with type 2 diabetes, OC levels increased after improving glycemic control.<sup>6</sup>

Most clinical studies investigating the possible metabolic effects of (or associations with) OC levels did not distinguish between ucOC and total OC (TOC). Experimental data suggest that ucOC is involved in metabolism.<sup>7</sup> Improved glycemic control appears to increase TOC but does not necessarily have the same effect on ucOC. Additional

studies are required to clarify the effects of improved glycemic control on this marker. The present study aimed to investigate the association and changes of serum ucOC levels and glycated hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) in patients with type 2 diabetes over 3 months of lifestyle improvement.

**Patients and methods** This study included 57 consecutive male and female patients with type 2 diabetes (according to the World Health Organization criteria), aged between 19 and 79 years. The exclusion criteria were as follows: malignant diseases, kidney or liver diseases, metabolic bone disorders, glucocorticoid treatment, hormonal contraception, hormone replacement therapy, and androgen treatment. Participants were outpatients or inpatients at the Department of Internal Medicine, Zagreb Clinical Hospital Centre. All patients were examined by the same specialist throughout the study. Clinical assessment included the measurement of height, weight, body mass index (BMI), as well as calculation of the homeostatic model assessment of insulin resistance (HOMA-IR) and insulin sensitivity (HOMA\_%S). The values were calculated from fasting BG (FBG) and fasting insulin using the HOMA calculator (<https://www.dtu.ox.ac.uk/homacalculator/>).

Blood was collected at baseline and at 3-month follow-up after overnight fasting. Six biochemical parameters were measured: ucOC (Takara Bio Inc., Kusatsu, Shiga, Japan), TOC, FBG, fasting insulin, HbA<sub>1c</sub>, and bone turnover marker (crosslaps telopeptide), measured using Roche instruments and reagents (Roche Diagnostics, Mannheim, Germany).

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**TABLE 1** Clinical parameters of the study group at baseline and at 3-month follow-up

| Parameter              | Baseline    | Follow-up   | P value |
|------------------------|-------------|-------------|---------|
| Weight, kg             | 88.0 (25.0) | 85.0 (23.0) | <0.001  |
| BMI, kg/m <sup>2</sup> | 29.2 (4.6)  | 28.7 (4.4)  | <0.001  |
| HbA <sub>1c</sub> , %  | 8.0 (2.7)   | 6.5 (1.3)   | <0.001  |
| FBG, mmol/l            | 9.0 (2.4)   | 7.0 (0.8)   | <0.001  |
| Insulin, uU/ml         | 18.0 (17.4) | 21.5 (15.5) | 0.69    |
| HOMA %S                | 39.7 (32.2) | 47.8 (48.3) | 0.38    |
| HOMA-IR                | 2.5 (2.2)   | 4.1 (2.1)   | 0.31    |
| TOC, ug/l              | 11.8 (4.4)  | 12.9 (6.2)  | 0.10    |
| UcOC, ng/ml            | 2.0 (1.8)   | 1.4 (1.9)   | 0.47    |
| UcOC/TOC ratio         | 0.11 (0.1)  | 0.13 (0.1)  | 0.58    |

Data are presented as median (interquartile range).

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; HbA<sub>1c</sub>, glycated hemoglobin A<sub>1c</sub>; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA %S, homeostatic model assessment of sensitivity; TOC, total osteocalcin; ucOC, undercarboxylated osteocalcin

The calculated sample size for the assessment of a correlation between serum OC and HbA<sub>1c</sub> levels (using a coefficient of  $-0.3$ , based on the published data) for a power of 80% was 25 per group. An SD of HbA<sub>1c</sub> levels in the population was assumed at 2.5, based on the published data. Therefore, for a 1% difference in HbA<sub>1c</sub> levels, the sample size was 50, assuming at least half would reach a 1% decrease in HbA<sub>1c</sub> levels.

The study protocol followed current guidelines for proper conduct of the study and protection of participants. The identities of healthy controls and patients remained confidential. All subjects provided written informed consent to participate in the study.

**Statistical analysis** Numerical variables were tested for distribution normality using the Shapiro–Wilk test. As all variables showed nonnormal distribution, nonparametric tests were used for group comparisons (2-tailed Wilcoxon rank sum test for dependent samples) and correlations (2-tailed Spearman rank correlation analysis [ $r_s$ ]). The level of statistical significance was set at a  $P$  value of less than 0.05 (STATA/IC ver.14.2, StataCorp LLC, College Station, Texas, United States).

**Results** The study included 57 patients: 21 women (36.8%) and 36 men (63.2%). Hypertension was reported in 36 participants (63.2%); 47 patients completed the study.

Correlations of TOC and ucOC levels with BMI, insulin, and HOMA indexes were analyzed. We only identified a negative correlation between BMI and ucOC levels at baseline ( $r_s = -0.30$ ;  $P = 0.03$ ).

A change was observed at 3 months for body weight ( $P < 0.001$ ), BMI ( $P < 0.001$ ), HbA<sub>1c</sub> levels ( $P < 0.001$ ), FBG levels ( $P < 0.001$ ), but not for HOMA-IR ( $P = 0.31$ ). The mean ucOC level was lower at 3 months; however, the difference was

not significant. The difference in the ucOC/TOC ratio was also nonsignificant.

A change in HbA<sub>1c</sub> levels between baseline and 3 months did not correlate with a change in ucOC levels ( $r_s = -0.18$ ,  $P = 0.43$ ). A median drop in HbA<sub>1c</sub> levels at 3 months was 1.6%. The median ucOC level in participants with a drop in the HbA<sub>1c</sub> level exceeding 1.6% was 2.35 ng/ml, which was not higher ( $P = 0.22$ ) than in participants with a drop in HbA<sub>1c</sub> levels lower than 1.6% (1.21 ng/ml). A change in the FBG levels between baseline and 3 months also did not correlate with a change in ucOC levels ( $r_s = 0.22$ ,  $P = 0.32$ ). Finally, a change in BMI between baseline and 3 months did not correlate with a change in ucOC levels ( $r_s = 0.22$ ,  $P = 0.33$ ).

**Discussion** During the 3-month follow-up, glucose regulation was improved in our study group by lifestyle change; however, no effect on ucOC levels was observed. Only ucOC levels were slightly lower at 3 months. However, this difference and the difference in the ucOC/TOC ratio were nonsignificant. Participants with a larger decrease in HbA<sub>1c</sub> levels tended to have a higher ucOC level at baseline, but without significance. Changes in ucOC levels between baseline and follow-up measurements did not correlate with the change in HbA<sub>1c</sub> or FBG levels, which is consistent with previous studies.<sup>8</sup> Kanazawa et al<sup>5</sup> measured TOC and ucOC levels in Japanese patients with type 2 diabetes before and after a month of improvement of glucose control. They found an increase in serum TOC levels, a nonsignificant increase in serum ucOC levels, and a decrease in the ucOC/OC ratio. However, participants ( $n = 50$ ) were on various anti-diabetic drugs. Another possible explanation for the differences in findings are the current limitations in the measurement of circulating ucOC levels and the lack of standardized techniques.<sup>8</sup>

An animal-model study by Lee et al<sup>1</sup> showed that OC gene knockout (OC<sup>-/-</sup> mice) could lead to elevated BG and insulin levels, as well as decreased insulin sensitivity, suggesting that OC could regulate glucose metabolism. After the discovery of the endocrine properties of ucOC in mice, it was necessary to evaluate the relationship between ucOC and glucose metabolism in humans. It is unclear whether ucOC has a similar function in humans, as the available evidence has only been partially confirmed. The use of anti-diabetic drugs might have been a confounding factor in previous human studies. The first direct evidence in humans showing that OC regulates energy metabolism might be provided by Confavreux et al.<sup>9</sup> In that study, 1 day after surgical resection of an OC-producing osteoid osteoma in 2 patients, ucOC levels decreased and serum glucose levels increased.

Previous studies have demonstrated that TOC levels correlate with FBG and HbA<sub>1c</sub> levels.<sup>4,5</sup> Few studies have measured both TOC and ucOC; therefore, the conclusions regarding the role of OC

carboxylation in glucose metabolism are far from definitive.

A Swedish study by Kindblom et al<sup>4</sup> showed that TOC levels were negatively correlated with FBG, fasting insulin, and HOMA-IR. Chinese research by Zhou et al<sup>10</sup> showed that TOC was not related to HOMA-IR in patients with type 2 diabetes.

A longitudinal study on elderly men, including those with diabetes, showed that an increase in ucOC levels was associated with improvements in the HOMA-IR, and that this association was limited to patients who were not treated with antidiabetic drugs.<sup>11</sup> Therefore, impaired glucose metabolism or drug interventions (or both) may make it more difficult to identify the correlation between ucOC levels and insulin resistance.

Razny et al<sup>12</sup> examined the relationship between insulin resistance and participants' ucOC and OC levels. They found that decreased ucOC levels may be an early sign of insulin resistance.

We did not observe a change in HOMA-IR, and it did not correlate with TOC or ucOC. Previous studies have demonstrated that TOC is negatively correlated with BMI,<sup>5,9</sup> while no correlation was reported for ucOC levels and BMI.<sup>9</sup> We observed a decrease in BMI between baseline and 3-month follow-up with only lifestyle change and no antidiabetic medication, but no correlation between the change in BMI and that in ucOC levels was seen.

This is the first prospective study designed to monitor the levels of 2 forms of OC (TOC and ucOC) in patients with type 2 diabetes before and after a 3-month improvement of glucose control without antidiabetic therapy in a European population. The strength of this study is that participants were newly diagnosed and treatment naive, and they were not prescribed any BG-lowering medications (before or during the study). However, the small number of participants is a critical limitation. Additionally, the inclusion of a wide range of BMIs (23–43 kg/m<sup>2</sup>), different age groups, and both sexes could account for the lack of changes in ucOC levels.

In conclusion, the improvement of glucose regulation in our patients with type 2 diabetes (achieved by lifestyle changes and without medication) was not associated with changes in the ucOC level, as might be expected by its role in energy metabolism. Further studies are needed to evaluate this relationship.

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