

# Undercarboxylated osteocalcin can predict insulin secretion ability in type 2 diabetes

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## Keywords

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## ABSTRACT

It has been reported that there is an intimate relationship between diabetes and bone metabolism including undercarboxylated osteocalcin (ucOC). In contrast, data on the relationship between ucOC and glucose metabolism are limited in type 2 diabetes. We recruited 50 Japanese patients with type 2 diabetes, and examined the association with ucOC on the insulin secretion, evaluated by both glucagon loading test and meal tolerance test. UcOC was shown to correlate positively with the change in C-peptide response in the glucagon loading test and C-peptide response after eating a meal ( $P = 0.025$ ,  $P = 0.047$ ). Therefore, ucOC reflects the reserve capacity of  $\beta$ -cell function, such as the bolus insulin secretion ability in patients with type 2 diabetes.

## INTRODUCTION

Undercarboxylated osteocalcin (ucOC) is a secreted protein produced by bone, especially by osteoblasts, and it enters the general circulation unlike osteocalcin, which remains in bone<sup>1</sup>. UcOC can regulate insulin secretion and insulin sensitivity in rodents<sup>2,3</sup>. In humans, it is reported that ucOC is associated with insulin secretion, insulin resistance and the risk of type 2 diabetes in the general population<sup>4–9</sup>, although the relationship between ucOC and glucose metabolism is still obscure. Furthermore, there are only a few previous reports suggesting the effect of ucOC on insulin secretion ability or future glycemic control, especially in patients with type 2 diabetes. Therefore, we attempted to examine the association of ucOC with insulin secretion, estimated by both the glucagon loading test (GLT) and meal tolerance test (MTT), and their prognosis in patients with type 2 diabetes.

## METHODS

A total of 50 Japanese patients with type 2 diabetes who gave written informed consent were recruited (41 men and 9 postmenopausal women). They all were admitted to Yokohama Rosai Hospital, Yokohama City, Kanagawa, Japan, for diabetes education. The mean age, duration of diabetes, body mass index (BMI) and glycated hemoglobin (HbA1c) of the participants were  $59.2 \pm 1.43$  years,  $7.8 \pm 0.7$  years,  $26.2 \pm 0.5$  kg/m<sup>2</sup>

and  $9.4 \pm 0.2\%$  ( $79 \pm 2$  mmol/mol), respectively (Table 1). Participants who were treated with insulin and anti-osteoporosis drugs, such as vitamin D, vitamin K, bisphosphonate and so on, were not included. Because vitamin K insufficiency increases ucOC level, participants who had anemia, hemorrhagic diathesis and hepatic disorder were also not included. A total of 17, 19, 15, 19, 4 and 12 patients were treated with dipeptidyl peptidase 4 inhibitor, sulfonylurea, glinide, metformin, thiazolidinedione and alpha-glucosidase inhibitor, respectively. The prognoses of diabetes after 6 months were shown in 41 patients. We could not follow up nine patients' data because they had moved to a private clinician after leaving our hospital. They were allocated to two groups, with lower and higher ucOC levels separated by the median ucOC level (2.16 ng/mL). The lower and higher ucOC groups contained 18 male and 3 female patients, and 15 male and 5 female patients, respectively.

## Study methods

We carried out GLT and MTT on the third and second morning of hospitalization. For this, 1 mg of glucagon (Glucagon G Novo 1 mg) was given intravenously, and the sequence of serum C-peptide response (CPR) levels were measured at 0 and 6 min<sup>10</sup>, 25 kcal/ideal bodyweight solid breakfast (60% carbohydrate, 25% fat, 15% protein) was given, and serum CPR levels were measured at 0 and 120 min. UcOC was determined on the third morning of hospitalization by electrochemiluminescence immunoassay (EIDIA, Tokyo, Japan)<sup>11</sup>. The

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**Table 1** | Clinical findings of the patients

	All	HbA1c <8.0%
Sex (male/female)	41/9	15/3
Age (years)	59.2 ± 1.43	57.9 ± 2.78
Duration of diabetes (years)	7.8 ± 0.7	4.9 ± 1.1
BMI (kg/m <sup>2</sup> )	26.2 ± 0.5	26.8 ± 0.9
Serum creatinine (mg/dL)	0.75 ± 0.02	0.77 ± 0.02
HbA1c, NGSP (%)	9.4 ± 0.2	7.4 ± 0.1
HOMA-β (%)	35.8 ± 3.6	49.3 ± 7.7
HOMA-IR	2.04 ± 0.2	1.70 ± 0.2
C-peptide index	1.47 ± 0.07	1.66 ± 0.12
Glucagon loading test		
C-peptide 0 min (ng/mL)	1.89 ± 0.08	1.91 ± 0.14
C-peptide 6 min (ng/mL)	3.76 ± 0.16	4.02 ± 0.32
ΔC-peptide, 6–0 min (ng/mL)	1.87 ± 0.11	2.11 ± 0.22
Meal tolerance test		
C-peptide 0 min (ng/mL)	1.83 ± 0.07	1.98 ± 0.14
C-peptide 120 min (ng/mL)	5.39 ± 0.26	6.88 ± 0.56
ΔC-peptide, 120–0 min (ng/mL)	3.41 ± 0.24	4.62 ± 0.53
ucOC (ng/mL)	2.80 ± 0.22	3.41 ± 0.44

BMI, body mass index; HbA1c, glycated hemoglobin; HOMA-β, homeostatic model assessment of β-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; NGSP, National Glycohemoglobin Standardization Program; ucOC, undercarboxylated osteocalcin.

relationships between ucOC and the changes in CPR (ΔCPR) in the GLT and MTT were analyzed. We also analyzed the ratio of patients who achieved HbA1c <7.0% after 6 months of leaving our hospital in both the lower and higher ucOC groups.

### Statistical analysis

The obtained dataset are presented as mean ± standard error of the mean. Correlations between ucOC and each CPR were tested by using the Spearman's correlation coefficient. Multiple regression analysis was used. Fisher's exact test was also used to compare the difference of prognosis between lower and higher ucOC groups. Sex was changed into a categorical variable, and  $P < 0.05$  was considered to be significant.

### RESULTS

The mean ucOC level was  $2.80 \pm 0.22$  ng/mL; CPR to glucagon was CPR at 0 min,  $1.89 \pm 0.08$  ng/mL; CPR at 6 min,  $3.76 \pm 0.16$  ng/mL; ΔCPR (6–0 min),  $1.87 \pm 0.11$  ng/mL; and CPR to meal intake was CPR at 0 min,  $1.83 \pm 0.07$  ng/mL; C-peptide at 120 min,  $5.39 \pm 0.26$  ng/mL; and ΔCPR (120–0 min),  $3.41 \pm 0.24$  ng/mL (Table 1).

Correlations between ucOC and each factor are shown in Table 2. ucOC was correlated with ΔCPR in GLT and CPR after eating a meal ( $r = 0.32$ ,  $P = 0.025$ ;  $r = 0.29$ ,  $P = 0.047$ ; Fig. 1); however, ucOC was not correlated with the duration of diabetes, BMI and HbA1c ( $r = -0.24$ ,  $P = 0.096$ ;  $r = 0.038$ ,

$P = 0.79$ ;  $r = -0.17$ ,  $P = 0.25$ ). Furthermore, ucOC showed no significant correlation with CPR after glucagon loading, ΔCPR in MTT, and both CPR before glucagon loading and eating a meal ( $r = 0.23$ ,  $P = 0.10$ ;  $r = 0.11$ ,  $P = 0.42$ ;  $r = 0.11$ ,  $P = 0.91$ ;  $r = 0.10$ ,  $P = 0.51$ ). ucOC was also correlated with homeostatic model assessment of β-cell function ( $r = 0.36$ ,  $P = 0.011$ ).

Furthermore, multiple regression analysis was carried out between ΔCPR in GLT, and sex, duration of diabetes, BMI, serum creatinine, HbA1c and ucOC. Multiple regression analysis could not detect a significant independent association between ΔCPR in GLT and ucOC (Table 3).

In addition, we considered that the capacity of insulin secretion was influenced by poor glycemic control at admission. Therefore, we identified 18 patients whose HbA1c was <8.0% and also analyzed this group. Their clinical findings are shown in Table 1. Correlations between ucOC and each factor are also shown in Table 2. We could find stronger correlations between ucOC and CPR after glucagon loading, ΔCPR in GLT, CPR after eating a meal and ΔCPR in MTT ( $r = 0.60$ ,  $P = 0.0088$ ;  $r = 0.67$ ,  $P = 0.0025$ ;  $r = 0.51$ ,  $P = 0.034$ ;  $r = 0.52$ ,  $P = 0.035$ ; Table 2).

Furthermore, the glycemic control of diabetes after treatment was compared between the groups with lower and higher ucOC levels. There were no significant differences in duration of diabetes, BMI and HbA1c in each group (9.5 vs 5.9 years,  $P = 0.079$ ; 26.2 vs 27.4 kg/m<sup>2</sup>,  $P = 0.41$ ; 9.7 vs 9.2%,  $P = 0.45$ ); however, the plasma fasting glucose level was significantly lower in the higher ucOC group than that in the lower ucOC group (160 vs 118 mg/dL,  $P = 0.0029$ ). The ratio of patients who achieved HbA1c <7.0% after 6 months in the higher ucOC group was significantly higher than that in the lower ucOC group (38.1 vs 75.0%,  $P = 0.028$ ). However, we could not find the association between the change of HbA1c after 6 months and ucOC level ( $r = 0.0098$ ,  $P = 0.95$ ), and there was no association between the change of HbA1c and ucOC level after adjusting for sex, duration of diabetes, BMI and baseline HbA1c (adjusted regression coefficient = 0.013,  $P = 0.91$ ).

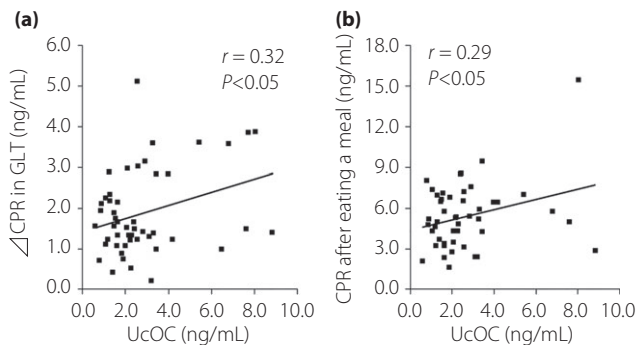
### DISCUSSION

The present report assessed the relationship of ucOC and insulin response with both GLT and MTT, and showed the association of ucOC and insulin secretion in a clinical situation with type 2 diabetic patients. In addition, the prognosis of diabetes was also assessed. Previous studies have shown the positive association between ucOC and homeostatic model assessment of β-cell function in the general population<sup>4,6</sup>. In addition, it was reported that ucOC was a significant predictor for circulating insulin level in the general population including patients with type 2 diabetes in part by using the multiple regression model<sup>9</sup>. In contrast, in patients with type 2 diabetes, ucOC was not correlated with fasting CPR<sup>12</sup>. There were only a few previous reports suggesting a correlation between ucOC and insulin secretion in the particular case of patients with type 2 diabetes.

**Table 2** | Correlations between undercarboxylated osteocalcin and each parameter

	All		HbA1c <8.0%	
	r	P	r	P
Sex (male/female)	0.20	0.16	0.30	0.22
Duration of diabetes (years)	-0.24	0.096	-0.33	0.20
BMI (kg/m <sup>2</sup> )	0.038	0.79	0.31	0.21
Serum creatinine (mg/dL)	-0.17	0.25	-0.0040	0.99
HbA1c, NGSP (%)	-0.13	0.39	-0.43	0.079
HOMA-β (%)	0.36	0.011*	0.75	<0.001*
HOMA-IR	0.27	0.13	0.60	0.013*
C-peptide index	0.15	0.29	0.53	0.025*
Glucagon loading test				
C-peptide 0 min (ng/mL)	0.11	0.91	0.30	0.23
C-peptide 6 min (ng/mL)	0.23	0.10	0.60	0.0088*
ΔC-peptide, 6-0 min (ng/mL)	0.32	0.025*	0.67	0.0025*
Meal tolerance test				
C-peptide 0 min (ng/mL)	0.10	0.51	0.27	0.29
C-peptide 120 min (ng/mL)	0.29	0.047*	0.51	0.034*
ΔC-peptide, 120-0 min (ng/mL)	0.11	0.42	0.52	0.035*

\*P < 0.05. BMI, body mass index; HbA1c, glycated hemoglobin; HOMA-β, homeostatic model assessment of β-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; NGSP, National Glycohemoglobin Standardization Program.



**Figure 1** | Correlations between undercarboxylated osteocalcin (ucOC) and (a) the change in C-peptide response (ΔCPR) in the glucagon loading test (GLT), (b) CPR after eating a meal. UcOC was shown to correlate positively with ΔCPR in GLT and CPR after eating a meal ( $r = 0.32, P = 0.025; r = 0.29, P = 0.047$ ).

In the present study, ucOC was not correlated with CPR before glucagon loading or eating a meal, but was correlated positively with ΔCPR in GLT or CPR after eating a meal. In addition, ucOC was correlated with all ΔCPR in GLT, CPR after glucagon loading, ΔCPR in MTT and CPR after eating a meal, especially in the patients whose HbA1c levels were <8.0%. It is likely that the capacity of insulin secretion was influenced by poor glycemic control in the whole group analysis. These results showed that ucOC represents not basal, but bolus insulin secretion from β-cells in patients with type 2 diabetes. Therefore, we can dynamically evaluate the ability of insulin secretion by estimating ucOC. Mizokami *et al.*<sup>13</sup> reported that OC induced glucagon-like peptide-1 and thereby stimulated

**Table 3** | Multiple regression analysis between the change in C-peptide (6-0 min) in glucagon loading test and each parameter

	Adjusted regression coefficient	P
Sex (male/female)	0.22	0.078
Duration of diabetes (years)	-0.40	0.0017*
BMI (kg/m <sup>2</sup> )	0.51	<0.001*
Serum creatinine (mg/dL)	-0.049	0.69
HbA1c, NGSP (%)	0.058	0.63
UcOC (ng/mL)	0.13	0.28

BMI, body mass index; HbA1c, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program; UcOC, undercarboxylated osteocalcin. \*P < 0.05

insulin secretion in an animal model. In the present study, however, ucOC showed stronger positive correlation with GLT than MTT in patients whose HbA1c was <8.0%. Therefore, it is suggested that ucOC might directly stimulate β-cells without involving incretin, such as glucagon-like peptide-1 from the gut, and induces insulin secretion, especially in patients with the early phase of type 2 diabetes. In addition, Wei *et al.*<sup>14</sup> reported that a G protein coupled receptor called GPRC6A as an ucOC receptor is expressed in β-cells of the pancreatic islets. These data supported that ucOC directly stimulates β-cells.

We also reported an unique result in the present study: the relationship between ucOC and glycemic control after treatment. There is the possibility of relating ucOC with future glycemic control. It is considered that there is a stronger relationship between diabetes and bone metabolism through ucOC.

Diabetes is well known to make bones fragile, because mature osteoblastic cells become weakened by abnormal glucose metabolism<sup>15–18</sup>. Thus, it is speculated that some humoral factors derived from bones, including uOC, might stimulate  $\beta$ -cells for improving abnormal glucose metabolism. It is possible to consider that uOC plays a crucial role in protecting bone degradation in disturbance of glucose metabolism by normalizing glucose metabolism, which is achieved by uOC-induced insulin secretion.

In contrast, the present study had several limitations. First, sample sizes became so small because of limitations of carrying out both the glucagon loading test and meal tolerance test on the same patient just after admission. Second, we did not measure vitamin K levels and other bone turnover markers. Third, we did not exclude the influences of antidiabetic agents. Fourth, the present study was cross-sectional in design, and there was the absence of healthy controls. Thus, we require further studies in the near future.

In conclusion, uOC reflects the reserve capacity of  $\beta$ -cell function, such as the bolus insulin secretion ability in patients with type 2 diabetes. In addition, there is the possibility of relating uOC with future glycemic control. Therefore, the existence of strong relationship between diabetes and bone health could be confirmed in humans.

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#### DISCLOSURE

The authors declare no conflict of interest.

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