

ORIGINAL ARTICLE

Serum C-peptide and osteocalcin levels in children with recently diagnosed diabetes

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Abstract

Background: We explored the association of C-peptide (marker of secreted insulin), proinsulin and proinsulin /C-peptide ratio (PI/C) (markers of beta-cell endoplasmic reticulum [ER] stress) with undercarboxylated (uOC) and carboxylated osteocalcin (cOC) and their ratio (uOC/cOC) in children with recently diagnosed type 1 (T1D) or type 2 diabetes (T2D), and the correlation of these variables with partial remission (PR) in children with T1D.

Methods: Demographic and clinical data of children with new-onset diabetes (n = 68; median age = 12.2 years; 33.8% non-Hispanic White, 45.6% Hispanic/Latino, 16.2% African American and 4.4% other) were collected at diagnosis and during the first (V1), second (V2) and third clinical visits at 9.0, 32.0 and 175.7 weeks, respectively. Serum proinsulin, C-peptide, uOC and cOC values were measured 7.0 weeks after diagnosis. PR was defined as insulin dose-adjusted HbA1c (IDAA1c) ≤ 9 .

Results: In children with new-onset T1D with DKA (33.3%) or T2D (29.4%), Spearman's correlation coefficient revealed a positive association between the C-peptide levels and both uOC and uOC/cOC ratio. In T1D (n = 48), both higher serum C-peptide levels and low PI:C ratio were associated with higher BMI percentile ($\beta = 0.02$, $P = .001$; $\beta = -0.01$, $P = .02$, respectively) and older age at diagnosis ($\beta = 0.13$, $P = .001$; $\beta = -0.12$, $P = .001$, respectively). Furthermore, in children with T1D, C-peptide levels at V1 correlated with IDAA1c ≤ 9 at V1 ($P = .04$).

Conclusion: C-peptide levels are associated with a higher uOC and uOC/cOC ratio in paediatric diabetes. In new-onset T1D children, older age and higher BMI were associated with lower beta-cell stress and higher preserved function, which was predictive of PR on follow-up.

KEYWORDS

C-peptide, diabetes, osteocalcin, paediatrics

Sabek and Redondo contributed equally to this article.

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1 | INTRODUCTION

Paediatric diabetes incidence has increased significantly in recent years. Worldwide, type 1 diabetes (T1D) increased 2.8% from 1990 to 1999,¹ and the current T1D prevalence in the United States is 0.3% by 18 years of age.² Type 2 diabetes (T2D) prevalence in young people parallels the increase in obesity rates, and similarly increases with age^{3,4}; however, T2D in youth has been proposed to represent a distinct disease state from both T1D and adult-onset T2D, including a faster decline in β (beta)-cell function, and accelerated development of microvascular complications.^{5,6} Insulin resistance (IR) and transient compensatory hyperinsulinaemia precede and predict T2D.⁷ In T1D, progressive loss of β -cell function and mass are the key features of the disease, but insulin resistance can also occur in both youth^{8,9} and adults,¹⁰ particularly related to longer disease duration and/or higher body fat mass.¹¹ Interestingly, one study found that while IR was identified in T1D youth, it did not share similar features with metabolic syndrome as found in T2D, and instead represented a unique phenotype.⁹ Insufficient metabolic control resulting in chronic glucose toxicity and impaired muscle metabolism could be the primary cause of insulin resistance in T1D.⁸ The physiological increase in body weight with age and development requires a matched increase in β -cell mass and circulating levels of insulin in order to maintain appropriate glucose metabolism.¹² Insulin deficiency is present in all diabetes subtypes, and while β -cell mass loss in T1D is 70%-100%,¹³ it ranges from 0% to 65% in adults with T2D.^{14,15} The link between β -cell mass loss versus dysfunction is diverse and still incompletely elucidated in the T2D context.^{14,15} More perplexing, perhaps, is the relatively large variability in assessed β -cell mass from adult cadaveric samples without diabetes,¹⁵ as well as in both lean and obese individuals.^{15,16} There is no way to conclusively quantify β -cell mass in the living patient. Moreover, the timing and extent of this loss in the paediatric population with new-onset diabetes is doubly unknown, particularly as other influences including diabetes subtype, age at diagnosis, BMI and weight management approaches, sexual maturation and hormonal changes, and psychosocial determinants complicate treatment decisions in this sensitive cohort,¹⁷ and which are even further confounded by dynamic insulin requirements during the "honeymoon phase" of diabetes progression, transient diabetes remission by β -cell "rest" following exogenous insulin commencement, and timing of initiation of advanced insulin delivery systems including pumps and CGM.^{18,19} Therefore, layers of complexity exist with regard to β -cell mass and function loss, not least being the variable amount of endogenous starting material before pathological circumstances further diminish β -cell reserve.

C-peptide level is considered the best surrogate marker of β -cell function.²⁰ Elevations in the fasting proinsulin-to-C-peptide ratio (PI:C) have been shown to precede the onset of T1D and are indicators of β -cell endoplasmic reticulum [ER] stress^{21,22} and persist after diagnosis. Furthermore, T1D patients in partial remission (PR), defined as insulin dose-adjusted HbA1c (IDAA1c) ≤ 9 ,²³ demonstrate higher proinsulin levels in the first 6 and 12 months following diagnosis relative to patients who did not remit, and thus, higher proinsulin

was positively associated with C-peptide, suggesting residual β -cell function and simultaneous β -cell distress.²⁴

The osteoblast-derived hormone osteocalcin (OC) has been implicated as a link between the skeleton and endocrine pancreas' role in glucose regulation, stemming from the observation that knock-out mice lacking the OCN gene have glucose intolerance, insulin resistance, impaired insulin secretion and abnormal accumulation of visceral fat.²⁵ Importantly, there is an established feed-forward regulation loop linking insulin, bone resorption and osteocalcin.^{26,27} Our group has previously demonstrated that OC is positively associated with insulin sensitivity and β -cell function in animal models in vivo, and in human islets, respectively,^{28,29} and others have shown that OC increased β -cell proliferation in mice through its receptor, GPRC6a.³⁰ During synthesis, OC undergoes a vitamin K-dependent carboxylation, which is incorporated into bone matrix,³¹ whereas the undercarboxylated form of OC (uOC) has been shown to be involved in energy metabolism and insulin action, and was proposed to be the biologically active form of the hormone.³² Higher uOC was correlated with improved peripheral and hepatic insulin sensitivity and β -cell function in nondiabetic, overweight adults.³² In a model of paediatric obesity and metabolic syndrome (MetS), decreased OC was shown to correlate with IR,³³ and in adult patients with T2D, and it was purported that uOC can predict insulin secretion ability.³⁴ We have previously shown an inverse relationship between uOC and haemoglobin A1c (A1c) in a paediatric population recently diagnosed with diabetes.³⁵ However, the potential correlation of retained β -cell function by C-peptide and proinsulin values, uOC, PR status in T1D, IR and diabetes subtype in this population is unknown.

The objectives of this analysis were to evaluate the relationships between C-peptide, proinsulin, uOC and cOC levels in children with recently diagnosed T1D and T2D. Better understanding of the factors associated with β -cell function early after the diagnosis of diabetes may improve current approaches for treatment paradigms.

2 | MATERIALS AND METHODS

2.1 | Patients and data collection

We prospectively evaluated children who presented with newly diagnosed diabetes at Texas Children's Hospital (Houston, Texas) between October 2010 and October 2011, and followed until October 2014, as previously described.³⁶⁻³⁸ Diabetes type (e.g. T1D and T2D) was classified based on clinical criteria.³⁹ Cases of secondary diabetes (e.g. monogenic, steroid-induced or cystic fibrosis-related diabetes) were excluded. The study was approved by the Institutional Review Board of Baylor College of Medicine/Texas Children's Hospital. Written, informed consent and assent as applicable were obtained from all participants. In this analysis, we included all children with available serum proinsulin, C-peptide, uOC and cOC data ($n = 68$).

Study data were collected at five time points, with dates presented as median interquartile range (IQR): at diagnosis; at 7.0 weeks after diagnosis (IQR: 4.3-8.9); at 9.0 weeks ($n = 68$) during the first

clinical visit (V1) (IQR: 7.9-12.0); at 32.0 weeks (n = 58) during the second clinical visit (V2) (IQR: 28.0-32.0); and at 175.7 weeks (n = 36) at the third clinical visit (V3). Insulin autoantibodies were measured within 10 days of diagnosis to prevent potential seropositivity due to exposure to exogenous insulin. Other islet autoantibodies including GAD65, islet cell autoantigen 512 (ICA512/IA2) and zinc transporter 8 (ZnT8), as well as proinsulin, C-peptide and OC (undercarboxylated and carboxylated), were measured at the 7-week visit. Demographic information collected included date of birth, date of diagnosis, sex and race/ethnicity. Anthropometric information included weight and height measured at the first clinical visit to avoid the effect of dehydration on weight at the time of diagnosis. Biochemical data included glucose, A1c, pH, bicarbonate and beta-hydroxybutyrate measured at diagnosis. Insulin dose-adjusted A1c (IDAA1c) was calculated at V1, V2 and V3 by the equation $A1C \text{ (per cent)} + [4 \times \text{insulin dose (units per kilogram per 24 h)}]$.²³ Body mass index (BMI) was calculated in children older than 2 years based on height and weight and categorized using gender- and age-specific percentiles by Centers for Disease Control and Prevention criteria.⁴⁰ Obesity was defined as BMI \geq 95th gender-specific and age-specific BMI centile, and overweight as BMI centile \geq 85th to $<$ 95th. Diabetic ketoacidosis (DKA) was defined by venous blood pH $<$ 7.3 and bicarbonate $<$ 15 mEq/L.

2.2 | Laboratory methods

2.2.1 | Islet autoantibodies

GAD65, ICA512/IA2 and ZnT8 autoantibodies were measured by the radioligand binding assay as previously described^{41,42} as standardized in the International Combined Autoantibody Workshop.⁴³ The cut-off for GAD65 antibody (Ab) positivity was set at 45U/ml and established as the 98th percentile for healthy controls. Samples were considered ICA512/IA2 autoantibody (ICA512/IA2Ab) positive \geq 98th centile for healthy controls (30 RU/mL). For ZnT8 autoantibodies (ZnT8Ab), a cut-off was set at 15 RU/mL for ZnT8Arg and 26 RU/mL for ZnT8Trp based on the 98th centile observed in 50 healthy human control sera. Samples were considered ZnT8Ab positive if binding to either ZnT8Arg or ZnT8Trp was detected. The GAD65Ab assay showed 86% sensitivity and 93% specificity, and the ICA512/IA2Ab assay showed 66% sensitivity and 98% specificity, as defined by the Diabetes Autoantibody Standardization Program (DASP) workshop,⁴⁴ in which we participated. ZnT8 autoantibodies were not included in the workshop. Insulin autoantibodies were measured by the Quest Diagnostics Nichols Institute (San Juan Capistrano, CA, USA) by radioimmunoassay (RIA) with clinical sensitivity and specificity of 50% and 99%, respectively (positivity $>$ 0.4 U/mL).

2.2.2 | OC measurements

Serum cOC and uOC levels were measured by enzyme immunoassays (Human Gla-OC High Sensitive EIA Kit (MK111) and Glu-OC

EIA Kit (MK118), Takara Bio Inc). The range of the assays is 0.5-16 and 0.25-8 ng/mL, respectively, with assay sensitivity of 0.5 ng/mL for both kits.

2.2.3 | Proinsulin and C-peptide measurements

Random serum proinsulin was measured by ELISA (Human Total Proinsulin ELISA, ALPCO). Random serum C-peptide levels were obtained at diagnosis and measured by highly specific RIA (Human C-Peptide RIA Kit, Millipore Research Inc).

2.3 | Statistical analyses

The distribution of the continuous variables was evaluated for normality using the Shapiro-Wilk test. Demographic and clinical data were reported as frequencies and proportions for categorical variables and as median and interquartile range (IQR) for continuous variables (given continuous variables were not normally distributed). The Wilcoxon matched-paired signed-rank test was used to compare the difference in the BMI percentiles, and Stuart-Maxwell test was used for the difference in the categorical weight status between the two time points, at visit 1 versus at diagnosis. Spearman's correlation test was used to evaluate the correlation between continuous laboratory parameters. Spearman's correlation coefficients (ρ) and *P*-values were reported. Univariable and multivariable generalized linear model (GLM) analyses were used to explore the association between insulin hormone subsets (proinsulin, C-peptide and proinsulin/C-peptide ratio) at visit 1 and age, sex, race/ethnicity, BMI percentile, diabetes type and the presence of DKA at diagnosis. Mixed-effects logistic regression modelling in all patients was conducted to access the longitudinal association of laboratory parameters and the PR indication over time from visit 1 through visit 3 by IDAA1c \leq 9.0. All analyses were performed using Stata version 15.1 (StataCorp LLC). A *P*-value of $<$.05 was considered statistically significant.

3 | RESULTS

Demographic, clinical and immunologic characteristics of children newly diagnosed with diabetes (n = 68; median [IQR] age = 12.2 years [9.8-13.8]; 33.8% non-Hispanic White, 45.6% Hispanic/Latino, 16.2% African American, 4.4% other; 70.6% T1D, 29.4% T2D) are presented in Table 1. Of patients with T1D (n = 48; age range: 2-18 years), 87.5% demonstrated autoantibody positivity (Table 1), and 16 (33.3%) had DKA at diagnosis, compared with none of the paediatric T2D patients (Table 1). The majority of T1D patients (68.1%) were of normal weight by BMI at diagnosis, whereas over 90% of the paediatric patients with T2D were overweight or obese by the same criteria (Table 1). Patients with T1D were treated with insulin, while patients with T2D were treated with lifestyle, metformin or insulin, according to standards of care for paediatric diabetes.¹⁷

TABLE 1 Characteristics of study participants

Characteristics	All (N = 68)	T1D (n = 48)	T2D (n = 20)	P-value
Age at diagnosis, median (IQR)	12.2 (9.8, 13.8)	11.8 (9.1, 13.4)	12.9 (11.5, 14.7)	.02
Male sex	33 (48.5)	27 (56.3)	6 (30.0)	.05
Race/Ethnicity				
Non-Hispanic White	23 (33.8)	21 (43.8)	2 (10.0)	.01
African American	11 (16.2)	5 (10.4)	6 (30.0)	
Hispanic/Latino	31 (45.6)	19 (39.6)	12 (60.0)	
Other	3 (4.4)	3 (6.3)	0 (0.0)	
DKA at diagnosis	16 (23.5)	16 (33.3)	0 (0.0)	.003
BMI percentile at diagnosis, median (IQR)	80.0 (45.0, 98.0)	60.0 (30.0, 85.0)	99.0 (96.5, 100.0)	<.001
Weight status category by BMI at diagnosis				
Underweight	3 (4.8)	3 (6.4)	0 (0.0)	<.001
Normal weight	33 (52.4)	32 (68.1)	1 (6.3)	
Overweight	6 (9.5)	4 (8.5)	2 (12.5)	
Obese	21 (33.3)	8 (17.0)	13 (81.3)	
BMI percentile at visit 1, median (IQR)	91.0 (75.3, 99.0)	85.5 (67.0, 92.1)	99.0 (97.0, 99.0)	<.001
Weight status category at visit 1				
Underweight				<.001
Normal weight	21 (31.3)	21 (44.7)	0 (0.0)	
Overweight	20 (29.9)	17 (36.2)	3 (15.0)	
Obese	26 (38.8)	9 (19.1)	17 (85.0)	
Glucose at diagnosis (mg/dL), median (IQR)	316.5 (223.0, 435.5)	323.0 (281.5, 476.0)	242.0 (126.0, 331.5)	.004
Carboxylated osteocalcin at visit 1, median (IQR)	30.0 (21.9, 40.3)	30.5 (22.3, 39.2)	29.5 (21.4, 43.0)	.74
Undercarboxylated osteocalcin at visit 1, median (IQR)	20.4 (13.8, 37.7)	20.4 (15.1, 25.8)	20.1 (8.0, 45.6)	.79
Undercarboxylated/carboxylated osteocalcin ratio at visit 1, median (IQR)	0.7 (0.4, 1.5)	0.7 (0.4, 1.4)	0.6 (0.3, 1.9)	.70
Proinsulin (pmol/L) at visit 1, median (IQR)	17.4 (7.5, 31.9)	12.5 (6.6, 21.7)	29.1 (19.4, 52.3)	<.001
C-peptide at diagnosis (ng/mL), median (IQR)	0.7 (0.4, 1.9)	0.5 (0.3, 0.7)	3.3 (1.8, 5.2)	<.001
C-peptide at visit 1 (ng/mL), median (IQR)	1.4 (0.6, 3.8)	1.0 (0.4, 2.5)	4.7 (2.8, 6.4)	<.001
Proinsulin/C-peptide ratio at visit 1, median (IQR)	33.1 (17.3, 66.7)	42.5 (20.9, 74.8)	19.3 (14.3, 28.5)	.00
Positive antibodies	42 (61.8)	42 (87.5)	0 (0.0)	<.001
IDAA1c at visit 1, median (IQR)	—	10.0 (9.2, 10.6)	—	—
IDAA1c at visit 1 ≤ 9	—	7 (19.4)	—	—
A1c at diagnosis, median (IQR)	11.8 (9.7, 12.9)	12.1 (11.0, 13.9)	9.8 (7.1, 12.0)	.00
A1c at diagnosis ≥ 7.5	50 (87.7)	38 (97.4)	12 (66.7)	.00
A1c at visit 1, median (IQR)	7.2 (6.5, 8.1)	7.4 (6.8, 8.2)	7.0 (6.1, 8.0)	.37
A1c at visit 1 ≥ 7.5	27 (41.5)	21 (45.7)	6 (31.6)	.30
A1c at visit 3, median (IQR)	—	8.4 (7.5, 10.3)	—	—
A1c at visit 3 ≥ 7.5	—	31 (77.5)	—	—
Severe hypoglycaemic episode at visit 3	—	4 (10.5)	—	—

Note: Bolded values represent statistical significance.

Values represent number and %, unless otherwise specified; BMI, body mass index; DKA, diabetic ketoacidosis; HbA1c, haemoglobin A1c; T1D, type 1 diabetes; T2D, type 2 diabetes; first clinical visit (V1) at 9.0 wk; second clinical visit (V2) at 32.0 wk; third clinical visit (V3) at 175.7 wk postdiagnosis. IDAA1c, insulin dose-adjusted A1c; "—" data not available; underweight, <5th percentile; healthy weight, 5th-84th percentile; overweight, 85-94th percentile; obese, ≥95th percentile. Significant differences were found between measurements at visit 1 versus at diagnosis for both BMI percentiles (Wilcoxon matched-paired signed-rank test $P < .001$) and categorical weight status (Stuart-Maxwell test $P = .001$).

TABLE 2 Correlations between carboxylated and undercarboxylated osteocalcin with proinsulin and C-peptide levels in children recently diagnosed with diabetes

	T1D patients without DKA		T1D patients with DKA		T2D patients	
	(n = 32)		(n = 16)		(n = 20)	
	Spearman's rho	P-value	Spearman's rho	P-value	Spearman's rho	P-value
Correlation with proinsulin at visit 1						
cOC	-0.02	.93	-0.04	.89	-0.15	.51
uOC	0.30	.10	0.23	.39	0.16	.49
uOC/cOC ratio	0.17	.37	0.37	.17	0.31	.18
Correlation with C-peptide at visit 1						
cOC	0.04	.84	-0.65	.65	-0.24	.34
uOC	0.09	.61	0.37	.16	0.26	.30
uOC/cOC ratio	0.00	.98	0.54	.03	0.39	.11
Correlation with proinsulin/C-peptide at visit 1						
cOC	0.07	.73	-0.12	.67	0.001	1.00
uOC	0.15	.43	-0.53	.03	-0.02	.93
uOC/cOC ratio	0.06	.75	-0.40	.13	-0.01	.96
Correlation with C-peptide at diagnosis						
cOC	-0.05	.80	-0.49	.06	-0.28	.24
uOC	0.20	.27	-0.09	.73	0.44	.06
uOC/cOC ratio	0.14	.44	0.18	.52	0.48	.04

Note: Spearman's correlation test was used to evaluate the correlation between continuous laboratory parameters. DKA (diabetic ketoacidosis) was measured at diagnosis. Bolded values represent statistical significance.

Interestingly, in children with T1D, only those who had DKA at diagnosis showed a significant, positive correlation between random C-peptide at V1 and undercarboxylated-to-carboxylated osteocalcin ratio (uOC/cOC), and a negative correlation between proinsulin/C-peptide ratio with uOC ($\rho = 0.54$, $P = .03$; and $\rho = -0.53$; $P = .03$, respectively). In children with T2D, uOC/cOC and uOC positively correlated with random C-peptide at diagnosis ($\rho = 0.48$, $P = .04$; and $\rho = 0.44$, $P = .06$, respectively) (Table 2), demonstrating the overlap in the presence of C-peptide and osteocalcin.

In children with T1D ($n = 48$), multivariable generalized linear model (GLM) analysis exploring the association between laboratory parameters (proinsulin, C-peptide and proinsulin/C-peptide ratio) and age, sex, race/ethnicity and BMI percentile at V1 revealed that older age and higher BMI were significantly correlated with higher levels of C-peptide ($\beta = 0.13$, $P = .001$; and $\beta = 0.02$, $P = .001$, respectively) (Table 3), implying higher retained β -cell function. Moreover, older age and higher BMI were significantly correlated with a lower proinsulin/C-peptide ratio ($\beta = -0.12$, $P = .001$; and $\beta = -0.01$, $P = .02$, respectively) (Table 3). However, there was no significant association of these factors in children with T2D ($n = 20$) (Table 3). There was a positive association between C-peptide and non-Hispanic White race/ethnicity in T1D and T2D patients (Table 3); this association was additionally shown by univariate GLM analysis in the same cohorts (Table S1). In T1D patients only, the univariate GLM analysis showed similar, significant associations between BMI at V1 with proinsulin ($P = <0.001$) and C-peptide ($P = <0.001$) (Table S1), as

well as significant associations of age at diagnosis ($P = .001$) and the proinsulin/C-peptide ratio ($P = .02$) (Table S1).

In children with T1D who had IDAA1c data available at V1 ($n = 48$), V2 ($n = 33$), and V3 ($n = 36$), the association of partial remission with proinsulin, C-peptide, proinsulin/C-peptide ratio, uOC, cOC and uOC/cOC levels measured at V1 was examined. Mixed-effects logistic regression modelling was performed to evaluate the association of IDAA1c ≤ 9 over time (from visit 1 through visit 3) as a dependent variable and individual laboratory parameters as independent variables (cOC, uOC, uOC/cOC ratio, proinsulin (pmol/L), C-peptide (ng/mL), proinsulin/C-peptide ratio). After adjusting for age and BMI percentile, the analyses showed a significant correlation of C-peptide at V1 with IDAA1c ≤ 9 (Table 4), while neither proinsulin nor proinsulin/C-peptide ratio was significantly correlated.

4 | DISCUSSION

The main finding of this study was that the uOC/cOC ratio was significantly correlated with C-peptide in children with new onset of diabetes in the early postdiagnosis time period, whether diagnosed with T1D and presenting with DKA at diagnosis, or T2D. We have previously shown that uOC and the uOC/cOC ratio are inversely correlated with A1c in children with recent-onset diabetes,³⁵ suggesting that higher uOC is associated with lower glucose levels and improved metabolic control. These new data further the evidence

TABLE 3 Multivariable generalized linear model (GLM) analysis exploring the association between insulin hormone subsets at visit 1 and age, sex, race/ethnicity, BMI percentile, diabetes type and DKA episodes

	Proinsulin		C-peptide		Proinsulin/C-peptide ratio	
	β coefficient (95% CI)	P-value	β coefficient (95% CI)	P-value	β coefficient (95% CI)	P-value
All Patients (n = 68)						
Age at diagnosis (years)	0.04 (-0.04, 0.13)	.33	0.10 (0.02, 0.17)	.02	-0.10 (-0.16, -0.05)	<.001
Male	0.18 (-0.36, 0.72)	.52	0.19 (-0.22, 0.59)	.37	0.20 (-0.21, 0.60)	.34
Non-Hispanic White	-0.09 (-0.61, 0.43)	.73	-0.25 (-0.67, 0.16)	.23	0.01 (-0.37, 0.39)	.96
BMI percentile at V1	0.03 (0.02, 0.05)	<.001	0.02 (0.01, 0.04)	<.001	-0.01 (-0.02, 0.00)	.02
T2D	0.26 (-0.38, 0.91)	.42	0.78 (0.27, 1.30)	.003	-0.34 (-0.81, 0.13)	.16
DKA	-0.14 (-0.78, 0.49)	.66	-0.43 (-0.93, 0.06)	.09	0.13 (-0.33, 0.59)	.58
T1D patients (n = 48)						
Age at diagnosis (years)	-0.12 (-0.19, -0.06)	<.001	0.13 (0.05, 0.22)	.001	-0.12 (-0.19, -0.06)	<.001
Male	0.42 (-0.12, 0.95)	.13	0.17 (-0.32, 0.66)	.49	0.42 (-0.12, 0.95)	.13
Non-Hispanic White	0.11 (-0.33, 0.54)	.64	-0.54 (-1.01, -0.07)	.03	0.11 (-0.33, 0.54)	.64
BMI percentile at V1	-0.01 (-0.02, 0.00)	.02	0.02 (0.01, 0.03)	.001	-0.01 (-0.02, 0.00)	.02
DKA	0.03 (-0.50, 0.56)	.92	-0.47 (-0.99, 0.05)	.07	0.03 (-0.50, 0.56)	.92
T2D patients (n = 20)						
Age at diagnosis (y)	-0.05 (-0.15, 0.06)	.37	-0.05 (-0.17, 0.06)	.37	-0.05 (-0.15, 0.06)	.37
Male	-0.13 (-0.64, 0.38)	.63	-0.40 (-1.00, 0.19)	.18	-0.13 (-0.64, 0.38)	.63
Non-Hispanic White	-0.49 (-1.26, 0.27)	.21	1.17 (0.28, 2.06)	.01	-0.49 (-1.26, 0.27)	.21
BMI percentile at V1	-0.01 (-0.08, 0.07)	.85	0.05 (-0.03, 0.13)	.19	-0.01 (-0.08, 0.07)	.85

Note: DKA (diabetic ketoacidosis) was measured at diagnosis. Bolded values represent statistical significance.

Abbreviations: CI, confidence interval; IDAA1c, insulin dose-adjusted A1c; V1, visit 1.

	Unadjusted		Adjusted*	
	OR (95% CI)	P-value	OR (95% CI)	P-value
cOC at visit 1	1.00 (0.94, 1.07)	.93	1.01 (0.94, 1.08)	.88
uOC at visit 1	0.99 (0.95, 1.03)	.63	0.98 (0.94, 1.03)	.47
uOC/cOC ratio at visit 1	1.02 (0.40, 2.63)	.96	1.08 (0.40, 2.93)	.88
Proinsulin at visit 1 (pmol/L)	0.98 (0.94, 1.03)	.49	1.00 (0.97, 1.04)	.90
C-peptide at visit 1 (ng/mL)	1.46 (0.85, 2.54)	.17	1.95 (1.03, 3.69)	.04
Proinsulin/C-peptide ratio at visit 1	1.00 (0.99, 1.01)	.91	0.98 (0.95, 1.01)	.16

Note: First clinical visit (visit 1) at 9.0 wk and the third clinical visit (visit 3) at 175.7 wk postdiagnosis.

Abbreviations: CI, confidence interval; cOC, carboxylated osteocalcin; IDAA1c, insulin dose-adjusted haemoglobin A1c; OR, odds ratio; uOC, undercarboxylated osteocalcin; uOC/cOC, undercarboxylated/carboxylated osteocalcin ratio.

*Adjusted in multivariate mixed-effects models for age and BMI percentile. Bolded values represent statistical significance.

TABLE 4 Mixed-effects logistic regression model for the association of laboratory parameters at visit 1 and IDAA1c ≤ 9 over time (from visit 1 through visit 3) in T1D patients (n = 36)

that uOC correlates with endogenous insulin presence as evidenced by C-peptide levels. These assertions have been supported by multiple other reports, showing an association between serum OC level and insulin sensitivity and secretion in patients without⁴⁵ and with T2D,^{34,46} as well as improvements in cardiovascular and glycaemic health.⁴⁷ Others have shown an inverse relationship of OC with hyperglycaemia in adults,⁴⁸ and of OC with BMI in overweight⁴⁹ and T1D⁵⁰ paediatric patients. Indeed, Takashi et al stated that uOC “reflects the reserve capacity of β -cell function”³⁴ and Prats-Puig et al showed that a higher uOC/cOC ratio in healthy children was associated with higher HOMA- β ($P < .01$),⁵¹ a metric of β -cell function. The data shown here, that the uOC/cOC ratio correlated with C-peptide values, further reinforce this link of sustained β -cell function. As such, osteocalcin could be used as an additional and contributing gauge of β -cell function within the diabetes context, as others have shown a positive association between uOC and C-peptide/glucose ratio in T1D patients aged 14–40 years as an indicator of endogenous insulin secretion.⁵² The aetiology of the diabetes subtypes presents unique opportunities to study the effect of osteocalcin in its undercarboxylated vs carboxylated forms and in models of loss of β -cell mass (primarily, T1D) vs loss of β -cell function (primarily, T2D).

Understanding variable factors of β -cell dysfunction and loss will help with prognosis and treatment decisions in all diabetes subtypes. In T1D, lack of residual β -cell function is associated with poor glucose control,³⁷ and is associated with diabetic ketoacidosis (DKA), which is life-threatening. In T2D, C-peptide levels also have clinical significance, as a study in T2D youth aged 16.1 ± 2.5 years with a median disease duration of 2.4 years showed that a decline in C-peptide levels was associated with deterioration of metabolic control and the consequent need for insulin therapy.⁵³

Of note, while uOC/cOC significantly correlated with C-peptide levels at diagnosis in children with T2D, children diagnosed with T1D and who presented with DKA at diagnosis showed a significant correlation with uOC/cOC at V1. These data suggest that because of the differences in β -cell function at *diagnosis* between type 1 and type 2, uOC and uOC/cOC ratio reflect differences in β -cell mass and islet functional status. That is, in newly diagnosed T2D children, uOC and uOC/cOC ratio are associated with glycaemic control and insulin sensitivity, whereas early after T1D diagnosis, uOC and uOC/cOC ratio are associated with residual β -cell functional mass. This differential temporal responses could be the result of β -cell “rest” following appropriate treatment initiation.⁵⁴ We did find a significant association of non-Hispanic White race/ethnicity with C-peptide in both diabetes subtypes. Additionally, in children with T1D, older age and higher BMI were significantly correlated with higher levels of C-peptide and thus lower proinsulin-to-C-peptide ratio. This finding is consistent with prior reports that C-peptide increases with age and BMI, possibly reflecting the increase in insulin secretion in response to puberty, ageing and obesity.^{12,55} We also noted that proinsulin was positively associated with BMI in participants with T1D, which was consistent with a higher C-peptide, suggesting that the lower proinsulin-to-C-peptide ratio implies no increase in low levels of β -cell ER stress, unlike evidence of elevations in proinsulin-to-C-peptide ratio

preceding the onset of T1D proposed by others.²² On the contrary, this evidence suggests that the β cell is able to process available endogenous proinsulin efficiently in the prediagnosis timeframe, supporting data from Brissova et al demonstrating that remnant β cells in T1D islets maintain regulated insulin secretion.⁵⁶ Furthermore, there was no significant finding of insulin resistance in the new-onset T1D subset by proinsulin or by correlation with uOC, which we speculate could occur only after longer disease duration, as Sims et al demonstrated that proinsulin secretion is a persistent feature of T1D.²¹ These conflicting data may further confound data interpretation and require further investigation. Moreover, as we found no significant correlation of C-peptide levels with either BMI or age in the T2D cohort, future studies with larger sample size are necessary to evaluate this relationship, as others have shown that chronic hyperglycaemia is inversely correlated with circulating OC levels in adult T2D patients.⁴⁸

In our study, children specifically with T1D who had IDAA1c ≤ 9 at V1 to V3 had significantly higher levels of C-peptide at V1, after adjustment for age and BMI. This observation is consistent with prior reports of IDAA1c as a marker of partial diabetes remission and indicates that endogenous insulin production is an important component of the partial remission metric. Neither proinsulin nor the proinsulin/C-peptide ratio was significantly correlated with IDAA1c ≤ 9 at V1–V3, suggesting that ER stress does not change in that discrete period of T1D disease progression. However, IDAA1c as a metric for partial diabetes remission does not account for carbohydrate intake, which is an inherent component of total daily insulin dosage. Instead, these data show support for monitoring endogenous C-peptide levels, and thus retained β -cell function, after diabetes diagnosis in children as part of a full clinical evaluation. The potential relationship between C-peptide, undercarboxylated osteocalcin and age at diagnosis with long-term outcomes and complication risk remains to be elucidated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to material presented in this study.

AUTHOR CONTRIBUTIONS

MJR and SNM provided the clinical data. MJR wrote/edited/reviewed the manuscript. DTN and EAG performed the statistical analysis and wrote/edited/reviewed the manuscript. CAB wrote/edited/reviewed the manuscript. DWF performed experiments and analysed the data. CSH provided the autoantibody measurements. OG contributed to experimental design. OMS conceived the idea, contributed to experimental design, analysed the data, wrote/edited/reviewed the manuscript and contributed to discussions. OMS

is the guarantor of this work. All authors have read and approved the final manuscript.

ETHICAL APPROVAL

The study was approved by the Institutional Review Board at Baylor College of Medicine/Texas Children's Hospital.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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