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Running Title: C-peptide Associations with BMI and Age

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Title: The Influence of Body Mass Index and Age on C-peptide at the Diagnosis of Type 1 Diabetes in Children Who Participated in the Diabetes Prevention Trial-Type 1

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The Type 1 Diabetes TrialNet and Diabetes Prevention Trial-Type 1 Study Groups (see Online-Only Supplement)

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ABSTRACT

Background

The extent of influence of body mass index (BMIZ) and age on C-peptide at the diagnosis of type 1 diabetes is unknown.

Objective

We studied the influence of BMIZ and age on C-peptide measures at and soon after the diagnosis of type 1 diabetes (T1D).

Subjects

Data from Diabetes Prevention Trial-Type 1 (DPT-1) participants <18.0 years at diagnosis was analyzed.

Methods

Analyses examined associations of C-peptide measures with BMIZ and age in 2 cohorts: oral glucose tolerance tests (OGTT) at diagnosis (n=99); mixed-meal tolerance tests (MMTT) <6 months after diagnosis (n=80). Multivariable linear regression was utilized.

Results

Fasting and area under the curve (AUC) C-peptide from OGTTs (n=99) at diagnosis and MMTTs (n=80) after diagnosis were positively associated with BMIZ and age ($p<0.001$ for all). Associations persisted when BMIZ and age were included as independent variables in regression models ($p<0.001$ for all). BMIZ and age explained 31%-47% of the variance of C-peptide measures. In an example, two individuals with identical AUC C-peptide values had an approximate 5-fold difference in values after adjustments for BMIZ and age. The association

between fasting glucose and C-peptide decreased markedly when fasting C-peptide values were adjusted ($r=0.30$, $p<0.01$ to $r=0.07$, n.s.).

Conclusions

C-peptide measures are strongly and independently related to BMIZ and age at and soon after the diagnosis of T1D. Adjustments for BMIZ and age cause substantial changes in C-peptide values, and impact the association between glycemia and C-peptide. Such adjustments can improve assessments of β -cell impairment at diagnosis.

Key Words

Type 1 diabetes, body mass index, age, C-peptide, children

INTRODUCTION

C-peptide indices are utilized to assess the loss of insulin secretion prior to and after the diagnosis of type 1 diabetes (T1D) in both clinical and research contexts. Clinically, these measures are used to differentiate T1D from type 2 diabetes and in guiding therapy. In addition, particularly in the United States, insurers have used C-peptide thresholds for determining whether insulin pumps and sensors should be covered. In the research setting, C-peptide measures are used to examine patterns of insulin loss in natural history studies (1), and as entry criteria and endpoints in clinical trials assessing interventions intended to prevent insulin loss (2-7).

In examining β -cell function for these purposes, it is essential to minimize other factors that could influence C-peptide levels apart from the pathology that is specific to T1D. Such factors include adiposity and age. Adiposity is known to be associated with insulin resistance (8,9). Although the direct influence of age on insulin resistance is less certain (10), age could influence insulin secretion by other mechanisms, such as possibly influencing β -cell mass (11). Thus, it is important to characterize the associations of C-peptide measures with indicators of adiposity such as BMI and age at the diagnosis of T1D to avoid a misinterpretation of β -cell function.

Since there is little information available regarding associations of C-peptide with BMI and age at the diagnosis of T1D, we have utilized Diabetes Prevention Trial-Type 1 (DPT-1) data to study the influence of BMI and age on C-peptide at the time of and soon after the diagnosis of T1D. In the DPT-1 study, a number of individuals were diagnosed by oral glucose tolerance testing which included C-peptide determinations. The findings presented below provide information that is relevant to assessments of β -cell function in both clinical and research settings.

METHODS

Subjects

Subjects who participated in the parenteral (12) and oral (13) insulin DPT-1 trials have previously been described in detail. All of the participants had islet cell autoantibodies and all were related to patients with T1D; neither the parenteral nor the oral insulin interventions showed efficacy. All participants included in the analysis were <18.0 years of age at diagnosis. Two analyses were performed from that cohort: a 2-hour oral glucose tolerance test (OGTT) analysis at the time of diagnosis, and a 2-hour mixed meal tolerance test (MMTT) analysis in the post-diagnostic period. For the OGTT analysis, all included were required to have BMI (kg/m²) measurements on the same date as the diagnostic OGTTs. Ninety-nine had BMI measurements on that date. Of those analyzed at diagnosis with OGTTs, 62 were in the parenteral insulin trial (intervention: 36; controls: 26) and 37 were in the oral insulin trial (intervention: 18; controls: 19). Criteria for inclusion in the MMTT analysis were the performance of MMTTs within 6 months after diagnosis and a BMI measurement within ± 6 months of the MMTT. Of the 80 included in the MMTT analysis 52 were in the parenteral insulin trial (intervention:30; controls 22) and 28 were in the oral insulin trial (intervention:11; controls: 17). The mean \pm SD interval from diagnosis to MMTTs was 0.24 \pm 0.10 years. Since each analysis had its own inclusion criteria, the participants could differ between the cohorts; overall, 53 individuals were included in both cohorts. DPT-1 was approved by institutional review boards at all participating sites, and written informed consents or assents as appropriate were obtained.

Procedures

As previously described (12,13), in both the parenteral and oral insulin DPT-1 trials, OGTTs were originally performed at 6-month (± 3 months) intervals for diagnostic surveillance. After an

oral glucose dose of 1.75 g per kilogram (maximum, 75 g of carbohydrate), fasting, 30, 60, 90 and 120 minute samples were obtained for glucose and C-peptide measurements. All of those included in the analysis were diagnosed by American Diabetes Association criteria. When an OGTT was within the diabetic range (fasting glucose values ≥ 126 mg/dl and/or 2-hr glucose values ≥ 200 mg/dl), a second OGTT was to be performed within 60 days unless clinically contraindicated. If the second OGTT was confirmatory, the age at the first diabetic OGTT was considered the age at diagnosis. The first diabetic OGTT (i.e., the diagnostic OGTT) was used for the OGTT analysis. For the MMTTs, glucose and C-peptide measurements were obtained before and after the consumption of a liquid formula meal (Sustacal/Boost, Mead Johnson Nutritionals; 6 kcal/kg body weight, maximum 360 kcal). Insulin dosing was held the morning of the OGTT until after test completion. Since children were analyzed, BMI Z-scores (BMIZ) were used for all analyses of BMI. The BMIZ values reflect BMI measures adjusted for age and sex based on Centers for Disease Control reference values (2000 growth charts). BMIZ is used as an indicator of the degree of adiposity.

Laboratory Measures

Plasma glucose levels were measured by the glucose oxidase method. C-peptide levels were measured by radioimmunoassay. Fasting C-peptide values in the undetectable range (< 0.2 ng/ml) were assigned a value of 0.1 ng/ml for the analyses. Three C-peptide measures were analyzed: fasting, area under the curve (AUC), and the 30-0 minute difference. The latter was included in the analysis, since it correlates with the first phase insulin response and declines during the latter stages of progression to T1D (14). The same C-peptide measures were used for the OGTT and MMTT analyses.

Data Analysis

Each of the analysis cohorts (OGTT and MMTT) were characterized using summary statistics, and two-sample t-tests and chi-square tests were used to compare factors and measures between groups of interest. Univariate and multivariable generalized linear regression models were used to examine associations of C-peptide variables with BMIZ and age. Models for each of the analysis cohorts were evaluated that adjusted for BMIZ alone, age alone, and for both. Pearson correlations were also utilized. Exploratory analyses supported the use of untransformed C-peptide measures; in addition, associations were similar when C-peptide values were log transformed.

To illustrate and further evaluate the influence of BMIZ and age on AUC C-peptide, coefficients for BMIZ and age for each of these fitted models were used to calculate an adjusted AUC C-peptide estimate from OGTTs at diagnosis and MMTTs after diagnosis. The influence of removing subject-specific BMIZ and age-related effects on AUC C-peptide was assessed by subtracting the subject-specific effects for BMIZ and age, according to the fitted models, from the actual observed AUC C-peptide value. We explored this delineation of effects added to the estimated AUC C-peptide that were specific to BMIZ and/or age for the individual difference from the average subject. In this context, those in the extremes for BMIZ or age in relation to the average cohort subject will have a greater adjustment to their AUC C-peptide than those whose BMIZ or age are near the overall average. An example of the formulaic approach for this functional adjustment is:

$$\tilde{y}_i = y_i - (\hat{y}_i - \widehat{y_{avg}})$$

$$\tilde{y}_i = y_i - (\widehat{\beta}_0 + \widehat{\beta}_1 BMIZ_i - (\widehat{\beta}_0 + \widehat{\beta}_1 \overline{BMIZ}))$$

$$\tilde{y}_i = y_i - (\widehat{\beta}_0 + \widehat{\beta}_1 BMIZ_i - \widehat{\beta}_0 - \widehat{\beta}_1 \overline{BMIZ})$$

$$\tilde{y}_i = y_i - (\widehat{\beta}_0 - \widehat{\beta}_0 + \widehat{\beta}_1 BMIZ_i - \widehat{\beta}_1 \overline{BMIZ})$$

$$\tilde{y}_i = y_i - (\widehat{\beta}_1 BMIZ_i - \widehat{\beta}_1 \overline{BMIZ})$$

where $\widehat{y}_i = \widehat{\beta}_0 + \widehat{\beta}_1 BMIZ_i$ is the predicted model-based estimate of AUC C-peptide for a specific subject and their corresponding BMIZ, $\widehat{y}_{avg} = \widehat{\beta}_0 + \widehat{\beta}_1 \overline{BMIZ}$ is the predicted model-based estimate of AUC C-peptide using the mean BMIZ from the overall analysis cohort, and y_i is the actual observed AUC C-peptide for the specific subject of interest. Thus, if our regression coefficient for BMIZ is 0.459, we would calculate the functional component of AUC C-peptide adjusted for BMIZ as $y_i - 0.459x(BMIZ_i - \overline{BMIZ})$. These adjustments were utilized to assess the influence of such a correction and the possible roles BMIZ and age have on AUC C-peptide at or soon after the diagnosis of T1D. The same methodology was used to assess the influence of BMIZ and age on the fasting C-peptide. Pearson correlations were used to assess the impact of associations between glucose and C-peptide variables.

For the figure, data was categorized according to tertiles; comparisons were made between the highest and lowest groups. OGTT and MMTT AUCs were calculated with the trapezoidal rule. The SAS 9.2 version was used for the analyses. All p-values are two-sided with a significance level set at 0.05.

RESULTS

There were 99 DPT-1 participants with OGTTs at diagnosis (mean±SD age at diagnosis: 11.3±3.3 years; mean±SD BMIZ at diagnosis: 0.42±1.13; 58% male) who were analyzed. Also, 80 DPT-1 participants were analyzed who had MMTTs within 6 months after diagnosis (age at MMTT: 11.8±3.4 years; BMIZ at MMTT: 0.52±1.08; 53% male). Characteristics of the participants are shown in Table 1, and values of C-peptide indices from the OGTTs and MMTTs are shown in Table 2. There were no differences between males and females for any of the C-peptide measures.

Regression coefficients for the associations of fasting C-peptide, 30-0 minute C-peptide difference, and AUC C-peptide from the OGTTs and MMTTs with BMIZ and age are shown in Table 3. There were substantial positive associations of the fasting and AUC C-peptide from the OGTTs with BMIZ and with age ($p<0.001$ for both). However, the 30-0 minute C-peptide difference was not significantly associated with BMIZ or with age.

The associations of the fasting and AUC C-peptide from the MMTTs with BMIZ and with age were also positive and significant ($p<0.001$ for all). Their magnitudes were similar to those for the OGTT associations. However, in contrast to the OGTTs, there was also a significant association of the 30-0 minute C-peptide difference with BMIZ ($p<0.05$). There were no significant associations of any of the C-peptide measures from the OGTTs or the MMTTs with gender.

Figure 1 shows C-peptide values for each OGTT time point according to BMIZ (A) and age (B) tertiles. The C-peptide values were significantly greater for the highest BMIZ tertile than for the lowest tertile at all OGTT time points ($p<0.01$ for all). The differences were even greater

between the highest and lowest age tertiles ($p < 0.001$ at all of the OGTT time points). For BMIZ, the values of the middle tertile were slightly higher than the values of the lowest tertile, whereas for age the values of the middle tertile were closer to the highest tertile.

Table 4 shows data from regression models with the C-peptide indices of the OGTTs and MMTTs as dependent variables, and BMIZ and age included together as independent variables for each model. Fasting and AUC C-peptide levels were significantly related to BMIZ and age in each model for both OGTTs and MMTTs. The R^2 (the proportion of the variance explained by BMIZ and age together in the models) for the associations of the fasting and AUC C-peptide with BMIZ and age varied from 0.31 to 0.47 for the OGTTs and MMTTs. Notable were the similarities of the multivariable coefficients in Table 4 to the univariate coefficients in Table 3. These findings indicate that the associations with BMIZ and age were largely independent of each other.

To demonstrate the extent of influence of BMIZ and age on C-peptide levels at diagnosis, we adjusted AUC C-peptide values to mean BMIZ and mean age values of the 99 children in the OGTT cohort and the 80 children in the MMTT cohort (Table 5). In the example shown, a 2.87 ng/ml/120 AUC C-peptide value of a 5 year-old child at the 10th BMIZ percentile of the OGTT cohort (BMIZ value=-1.13) would increase to an adjusted AUC C-peptide value of 4.91 ng/ml/120. Conversely, a 2.87 ng/ml/120 AUC C-peptide value in a 17 year-old adolescent at the 90th BMIZ percentile would decrease to an adjusted AUC C-peptide value of 0.97 ng/ml/120. Thus, although the actual AUC C-peptide values were the same for each child, the adjusted AUC C-peptide value (i.e., after removal of the influence of BMIZ and age) was approximately 5-fold higher in the younger and thinner child. A large divergence was similarly evident for the adjusted AUC C-peptide from the MMTTs.

We examined the extent to which BMIZ and age influenced the impact of C-peptide on glycemia at diagnosis. Whereas there was a significant association of fasting glucose values (log transformed) with unadjusted fasting C-peptide levels ($r=0.30$, $p<0.01$), there was little association when fasting C-peptide levels were adjusted for BMIZ and age ($r=-0.07$, n.s.). There were significant associations of AUC glucose with both unadjusted and adjusted AUC-C-peptide levels; however, the association tended to be stronger (negatively) with adjusted AUC C-peptide levels ($r=-0.37$, $p<0.001$ for adjusted; $r=-0.30$, $p<0.01$ for unadjusted).

DISCUSSION

The findings showed that at the diagnosis of T1D, an appreciable proportion of the variance of the fasting and AUC C-peptide from OGTTs was explained by BMIZ and age. Notably, the associations of the C-peptide indices with BMIZ and age were independent of each other in multivariable models. When the AUC C-peptide was adjusted for BMIZ and/or age, the C-peptide values differed markedly from the actual values.

There is an appreciable loss of C-peptide after diagnosis (15,16). Since MMTTs occurred on average about 3 months after the OGTTs, it appears that despite the loss of C-peptide after diagnosis, the magnitude of the association of C-peptide with BMIZ and age persists.

The independence of BMIZ and age in their associations with the C-peptide indices suggests that the basis for the associations differs between BMIZ and age. Evidence from prior studies are consistent with this finding. Whereas studies have consistently shown associations between insulin resistance and indicators of the degree of adiposity such as BMI in other populations (8,9), the relationship of insulin resistance with age is less certain (10). Since some β -cell characteristics are associated with age (11,17), it is possible that insulin secretion might be better sustained in older individuals who develop T1D. Age has been shown to be a mitigating factor for the risk of T1D (18) and for the loss of C-peptide levels soon after diagnosis (16).

The associations of C-peptide with BMIZ and age are clinically relevant, since C-peptide measurements are sometimes used to help differentiate the types of diabetes at diagnosis and to guide therapy. Also, low C-peptide values have been used as a criterion for providing insurance coverage of insulin pumps and glucose sensors. Thus, overweight and older children recently diagnosed with T1D could be less likely to obtain coverage than thinner and younger children.

These findings also have implications with regard to clinical trials evaluating interventions for preserving β -cell function in recently diagnosed T1D patients. Such trials have used the AUC C-peptide from MMTTs as endpoints (2-7). Adjustments of the AUC C-peptide have been made for age in those trials, but not for the degree of BMI. Moreover, the findings are also relevant to the selection of subjects for trials, since C-peptide levels below a minimum threshold are used as an exclusion criterion. Without appropriate adjustments for BMI and age, younger and thinner children would more likely be excluded from clinical trials than overweight adolescents.

Since adjustments of C-peptide for BMIZ and age essentially exclude the portion of C-peptide that is attributable to those characteristics, we examined differences between unadjusted and adjusted C-peptide values with regard to their associations with glucose values. The analysis showed that the positive association between the fasting glucose and fasting C-peptide was especially impacted by the adjustment, indicating that the association was almost fully attributable to BMIZ and age. The adjustment of the AUC C-peptide resulted in a stronger inverse association with the AUC glucose, but the impact was smaller.

Despite the substantial impact of BMIZ and age on C-peptide values, adjustments might not always be warranted. For example, adjustments for age could obscure the reasonable possibility that β -cell compromise is less severe at the diagnosis of T1D in older individuals. Thus, for natural history studies of β -cell decline, age stratification might be more appropriate than age adjustment. As more is learned about the bases for the associations of C-peptide with BMIZ and age, decisions regarding the need for adjustment can be made with more certainty.

The adjustments demonstrating the extent to which BMIZ and age influenced C-peptide levels do not necessarily generalize to other populations, since the coefficients for the associations

could differ among populations. However, similar modeling procedures for adjustments could be utilized.

One of the limitations of the study was the lack of assessment of insulin resistance. We chose not to use indirect measures such as HOMA-IR, as they have not been validated in newly diagnosed T1D patients. In addition, since pubertal status was not ascertained at the time of diagnosis, we could not determine the extent of association between C-peptide indices and puberty. Insulin resistance has been shown to be associated with puberty (8). As a result of OGTT surveillance, it is likely that the diagnosis in DPT-1 occurred earlier than the typical diagnosis made clinically. Thus, the associations are not necessarily indicative of those at the time of a clinical diagnosis. Finally, since the analyses were cross-sectional, inferences could not be made on β -cell decline.

No prior studies have examined the impact of BMIZ and age on C-peptide indices from OGTTs at diagnosis, including their impact on associations between glucose and C-peptide. Recent studies (19,20) found that random C-peptide levels at diagnosis were higher in children with a greater degree of adiposity, and in older children. In a DPT-1 analysis, a correlation was observed between the AUC C-peptide from MMTTs and age in individuals at risk for T1D (21). A study of children and adult T1D patients within 3 months of their diagnosis found positive associations of the AUC C-peptide from MMTTs with BMIZ and age (16).

In conclusion, the findings indicate that BMIZ and age substantially and independently influence C-peptide levels at and soon after the diagnosis of T1D. These associations should be considered in both clinical and research settings. Although adjustments for BMIZ and age can add clarity to assessments of β -cell function, they should only be undertaken after determining their appropriateness for a particular objective.

AUTHOR CONTRIBUTIONS

Jay Sosenko analyzed data and wrote the manuscript. Susan Geyer provided statistical guidance and reviewed the manuscript. Jay Skyler conducted the study and reviewed the manuscript. Lisa Rafkin conducted the study and reviewed the manuscript. Heba Ismail reviewed the manuscript. Ingrid Libman reviewed the manuscript. Linda DiMeglio reviewed the manuscript. Carmella Evans-Molina reviewed the manuscript. Jerry Palmer conducted the study, reviewed the manuscript, and assisted in writing the manuscript. There was no conflict of interest for any of the authors.

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Table 1. Characteristics of the OGTT and MMTT cohorts

	OGTT Cohort (n=99)	MMTT Cohort (n=80)
Age at Baseline (years)	9.0±3.1	9.3±3.3
Age at Diagnosis (years)	11.3±3.3	11.6±3.4
Age at MMTTs (years)	-----	11.8±3.4
Height (cm)	149.0±18.2	150.0±18.9
HeightZ	0.55±0.99	0.33±0.84
Weight (kg)	46.0±19.2	47.6±19.1
WeightZ	0.61±1.04	0.55±0.99
BMI (kg/m²)	19.8±4.5	20.2±4.5
BMIZ	0.42±1.13	0.52±1.08
HbA1c (%)	6.1±0.8	-----
Gender (% Male)	57.6	52.5

mean±SD values shown except for Gender

Table 2. Mean±SD C-peptide values of OGTTs at diagnosis and MMTTs within 0.5 years after diagnosis

	OGTT (n=99)	MMTT (n=80)
Fasting C-peptide (ng/ml)	1.34±0.89	1.02±0.63
30-0 Minute C-peptide Difference (ng/ml)	1.31±1.01	1.45±1.19
AUC C-peptide (ng/ml)/120	2.87±1.54	2.39±1.29

Table 3. Regression coefficients and R² (in parentheses) for univariate associations of C-peptide indices with BMIZ and age for OGTTs at diagnosis and for MMTTs within 0.5 years after diagnosis

OGTT (n=99)	BMIZ	Age (Years)
Fasting C-peptide (ng/ml)	0.41±0.07 (0.27)++	0.13±0.02 (0.23)++
30-0 Minute C-peptide Difference (ng/ml)	0.07±0.09 (0.01)	0.05±0.03 (0.03)
AUC C-peptide (ng/ml)/120 min	0.46±0.13 (0.11)++	0.23±0.04 (0.24)++
MMTT (n=80)		
Fasting C-peptide (ng/ml)	0.25±0.06 (0.19)++	0.07±0.02 (0.15)++
30-0 Minute C-peptide Difference (ng/ml)	0.31±0.12 (0.08)+	0.04±0.04 (0.02)
AUC C-peptide (ng/ml)/120 min	0.44±0.13 (0.13)++	0.15±0.04 (0.16)++

+p<0.05; ++p<0.001

Table 4. Regression coefficients from models that included C-peptide indices as dependent variables with BMIZ and age as independent variables for OGTTs at diagnosis and for MMTTs within 0.5 years after diagnosis

	OGTT (n=99)			MMTT (n=80)		
	BMIZ	Age (Years)	R ²	BMIZ	Age (Years)	R ²
Fasting C-peptide (ng/ml)	0.38±0.06++	0.12±0.02++	0.47	0.27±0.05++	0.07±0.02++	0.36
30-0 Minute C-peptide Difference (ng/ml)	0.06±0.09	0.05±0.03	0.03	0.31±0.12+	0.05±0.04	0.10
AUC C-peptide (ng/ml)/120 min	0.42±0.11++	0.22±0.04++	0.33	0.46±0.11++	0.16±0.04++	0.31

+p<0.05; ++p<0.001

Table 5. Examples of AUC C-peptide values adjusted for BMIZ, age, or both from regression coefficients for 2 individuals at opposite ends of BMIZ and age distributions with same AUC C-peptide at diagnosis

	AUC C-peptide=2.87 from OGTT at Diagnosis			AUC C-peptide=2.39 from MMTT after Diagnosis		
	Adjusted for BMIZ	Adjusted for Age	Adjusted for Both	Adjusted for BMIZ	Adjusted for Age	Adjusted for Both
Age=5 Years; BMIZ=10 th %tile	3.58	4.32	4.91	2.98	3.43	4.10
Age=17 Years; BMIZ=90 th %tile	2.17	1.56	0.97	1.76	1.60	0.90

Figure 1

Shown are mean AUC C-peptide levels during OGTTs according to BMIZ and age tertiles.

Those in the highest tertiles of BMIZ and age had markedly higher AUC C-peptide levels than those in the lowest tertiles. For BMIZ, the values of the middle tertile were slightly higher than the values of the lowest tertile, whereas for age the values of the middle tertile were closer to the highest tertile. The ages of those in the middle BMIZ tertile (10.6 ± 3.2 years) tended to be lower than the ages of those in the lowest 11.4 ± 3.6 and highest 11.8 ± 3.1 tertiles. After an adjustment for age, the AUC C-peptide values of the middle BMIZ tertile moved further from the lowest tertile (from 13% of the difference between the highest and lowest tertiles to 28% of the distance).