Distribution of C-Peptide and Its Determinants in North American Children at Risk for Type 1 Diabetes

Ping Xu,[1] Xiaoning Qian,[1] Desmond A. Schatz,[2] David Cuthbertson,[1] Jeffrey P. Krischer,[1] and the DPT-1 Study Group

OBJECTIVE

To determine basal and stimulated C-peptide percentiles in North American children and adolescents at risk for type 1 diabetes (T1D) and to examine factors associated with this distribution in the Diabetes Prevention Trial–Type 1 (DPT-1).

RESEARCH DESIGN AND METHODS

We included 582 subjects aged 4–18 years at randomization in the DPT-1 trials. A 2-h oral glucose tolerance test (OGTT) was performed at baseline and every 6 months during the 5-year follow-up period. The percentile values of C-peptide after baseline OGTT were estimated according to age, BMI *Z* score (BMIZ), and/or sex categories. Conditional quantile regression was used to examine the relationship between C-peptide percentiles and various independent variables.

RESULTS

The basal and stimulated C-peptide levels increased significantly as age and BMIZ increased ($P < 0.05$). Both age and BMIZ had a stronger impact on the upper quartile of C-peptide distributions than the lower quartile. Sex was only significantly associated with stimulated C-peptide. Higher stimulated C-peptide levels were generally observed in girls compared with boys at the same age and BMIZ ($P < 0.05$). HLA type and number of positive antibodies and antibody titers (islet cell antibody [ICA], insulin autoantibody, GAD65A, and ICA512A) were not significantly associated with C-peptide distribution after adjustment for age, BMIZ, and sex.

CONCLUSIONS

Age-, sex-, and BMIZ-specific C-peptide percentiles can be estimated for North American children and adolescents at risk for T1D. They can be used as an assessment tool that could impact the recommendations in T1D prevention trials.

Type 1 diabetes (T1D) is a metabolic disease characterized by elevated blood glucose levels due to insufficient insulin production (1–3). It results from an autoimmune process that leads to destruction of pancreatic β-cells. C-peptide and insulin are released simultaneously from the pancreas with the cleavage of proinsulin. Measurement of C-peptide production under a standardized condition provides a sensitive assessment of β-cell function (4–8).

C-peptide levels have been used as a surrogate outcome for preserved β-cell function in intervention trials conducted in new-onset patients. Even earlier assessment (i.e., in at-risk subjects) of C-peptide and its precipitating or determinant

factors is critical. Most previous studies have reported only means and SDs assuming that measurement of C-peptide production follows a normal distribution (9,10). However, C-peptide profiles may follow a nonnormal distribution, which could substantially impact interpretation. When traditional statistical linear regression techniques are deployed such as ordinary least square and general linear model, a departure from normality can result in inaccurate estimates of C-peptide. Accurate risk characterization is critical in the design of prevention trials.

A number of environmental and genetic factors are known to be associated with T1D. Environmental factors, such as exposure to enteroviral infections and cow's milk, have been identified as potential triggers of T1D in epidemiological and immunological studies (11–13). Previous studies also established that factors, such as age, sex, BMI, relationship to proband, HLA type, and antibody titer levels, were related to progression of clinical T1D onset in children and adolescents at risk (14,15). These factors contribute to the risk for T1D independently or interactively at different prediabetes stages. The distribution of C-peptide may or may not depend on one or more of these factors that are linked to clinical disease onset. Taking into account the above observations and considering the importance of early detection of low C-peptide or preservation of C-peptide in subjects at risk for T1D (as the best strategy for the prevention of T1D), we aimed in this study to determine percentiles for basal and stimulated C-peptide in children and adolescents at risk for T1D and to examine factors associated with the distribution of C-peptide using the data from one of the largest T1D prevention trials.

RESEARCH DESIGN AND METHODS
Subjects
The Diabetes Prevention Trial–Type 1 (DPT-1) was a multicenter randomized, controlled clinical study in North America designed to determine whether it is possible to delay or prevent the clinical onset of T1D in individuals with autoimmunity. More than 100,000 nondiabetic relatives of subjects with T1D were screened to detect the presence of islet cell antibodies (ICAs). Individuals found to have ICAs were staged to determine

their risk of T1D based on genetic, immunologic, and metabolic characteristics. A total of 711 individuals were randomized into either a parenteral trial or an oral insulin trial according to their risk profiles. These randomized subjects were followed until T1D onset or up to 5 years. Previous analyses showed that the subjects failed to reach the primary end point of preventing diabetes. We analyzed 582 subjects aged 4–18 years at randomization. Among them, 224 (38.5%) subjects were 4–8 years old and 358 subjects (61.5%) were 9–18 years old. There were 350 boys (60.1%) and 232 (39.9%) girls.

Laboratory Measures
All assays including antibody assays were performed as previously described (16). For HLA-DQ typing, DNA was extracted from the buffy coats of peripheral blood leukocytes, and HLA-DQA1 and DQB1alleles were amplified by PCR with the use of sequence-specific probes. A high-risk HLA genotype was defined as having one of the following haplotype combinations: DQA1*0301-DQB1*0302, DQA1*0501-DQB1*0201, DQA1*04-DQB1*0201, or DQA1*0301-DQB*0201. No HLA DQB*0602s were included.

At baseline and every 6 months during the follow-up period, an oral glucose tolerance test was done after an overnight fast and blood samples were drawn at −10 and 0 min. An oral glucose load was then administered (1.75 g/kg, maximum 75 g). Blood samples were drawn at 30, 60, 90, and 120 min after glucose consumption. C-peptide levels were measured by radioimmunoassay in the DPT-1 β-cell function core laboratory (Seattle, WA). The basal C-peptide level in the current analysis was calculated as the mean of C-peptide levels at −10 and 0 min. The stimulated C-peptide was analyzed as the peak C-peptide level during a 2-h OGTT test. C-peptide was measured in nanograms per milliliter (1 ng/mL is equal to 0.333 nmol/L).

Statistical Methods
The descriptive statistics of both observed basal and stimulated C-peptide levels at baseline, such as their mean, minimum, median, maximum values, SDs, skewness, and kurtosis, were reported. A Kolmogorov-Smirnov goodness-of-fit test was used to test for the normality of its distribution (17).

A nonparametric approach, conditional quantile regression, was used to examine the relationship between baseline C-peptide percentiles and various independent variables. By extending the exclusive focus of the estimation of conditional mean functions in traditional regression models, the quantile regression approach allows examining of the entire distribution of the variable of interest (C-peptide here) rather than a single measure of the central tendency of its distribution. In addition, the quantile regression approach has its advantages over traditional regression models for its flexibility to allow the covariates to have different impacts at different percentiles of the distribution as well as its robustness with respect to departures from normality and skewed tails because it does not put any distributional assumption beforehand (18–20). The effect of potential covariates, including age categories (≥ 4 and ≤ 8 vs. ≥ 9 and ≤ 18 years), sex (female vs. male), BMI Z score (BMIZ) percentile categories (<85.0 vs. ≥ 85.0), relationship to proband (offspring, sibling, second degree), HLA type (high vs. low risk), number of positive antibodies, and antibody titer levels, were examined univariately first. Prepubertal subjects were defined as age <9 years (21–23). The significant covariates in the univariate model were then selected as the predictors in a multivariate model to account for the possible variations in determining the distribution of C-peptide. Lastly, the estimated percentile values of C-peptide were reconstructed and refined by the covariates that were both univariately and multivariately related to C-peptide distribution profile.

All tests of significance were two tailed. $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed with SAS (version 9.2; SAS Institute, Cary, NC).

RESULTS
Table 1 demonstrates the subjects' demographics and clinical and laboratory characteristics. The majority of subjects were white (92.71%), had high-risk HLA types (84.54%), and had three or four positive antibodies (84.88%) at baseline. Approximately 23% of subjects in this population were overweight or obese based on their BMIZ (≥ 85.0 percentile).

Table 1—Subjects' demographics and clinical characteristics (N = 582)

Age, years, mean (SD)	9.98 (3.82)
4–8, years, n (%)	224 (38.49)
9–18, years, n (%)	358 (61.51)
Sex, n (%)	
Female	232 (39.86)
Male	350 (60.14)
Race, n (%)	
White	529 (92.71)
Nonwhite	41 (5.23)
Unknown	12 (2.06)
Relationship to proband, n (%)	
Sibling	367 (63.06)
Offspring	156 (26.80)
Parent	1 (0.17)
Non–first degree relative	58 (9.97)
BMIZ percentile and age, mean (SD)	57.80 (29.38)
<85.0, n (%)	416 (71.48)
≥85.0, n (%)	132 (22.68)
Unknown	34 (5.84)
HLA risk, n (%)	
High risk	492 (84.54)
Low risk	88 (15.12)
Unknown	2 (0.34)
Number of positive antibodies, n (%)	
1	33 (5.67)
2	55 (9.45)
3	205 (35.22)
4	289 (49.66)
ICA titer, median (Q1–Q3)	160.00 (40.00–320.00)
IAA titer, median (Q1–Q3)	185.90 (69.20–450.30)
GAD65A titer, median (Q1–Q3)	0.260 (0.049–0.744)
ICA512A titer, median (Q1–Q3)	0.105 (0.013–0.714)

Q1, the first quantile; Q3, the third quantile.

The mean basal and stimulated C-peptide levels were 1.0 and 4.9 ng/mL, respectively. The median values were 0.6 ng/mL for basal and 3.5 ng/mL for stimulated C-peptide. The median values were less than the (arithmetic) mean values for both basal and stimulated C-peptides, which indicated that the distribution of C-peptide was right skewed. The kurtosis was higher for the basal C-peptide (4.6) than for the stimulated C-peptide (1.6). Higher value of kurtosis indicates a higher and sharper peak. These observations suggest that the C-peptide distributions do not conform to a normal distribution, and a formal Kolmogorov-Smirnov goodness-of-fit test for normality based on skewness and kurtosis rejected the hypothesis that the basal C-peptide or stimulated C-peptide is normally distributed ($P < 0.01$ for both basal and stimulated C-peptide).

Table 2 presents univariate quantile regression analyses. Relationship to proband, number of positive antibodies, ICA titer, GAD65A titer, and ICA512A titer were not associated with the C-peptide percentiles. Age, BMIZ percentile, HLA type, and insulin autoantibody (IAA) antibody titer were significantly related to the distribution of C-peptide, and these covariates were included in the multivariate models to account for all possible variations of C-peptide distribution. Sex was not associated with basal C-peptide but was significantly associated with stimulated C-peptide in univariate analyses. Therefore, sex was included in the multivariate model for the stimulated C-peptide only.

Table 3 reveals the results from the multivariate model. An age-related significant increase in C-peptide distribution was detected ($P < 0.001$). Sex was associated with stimulated C-peptide. Higher stimulated C-peptide percentiles were generally observed in girls compared with boys at the same age and BMIZ scores ($P < 0.05$). BMIZ scores were significantly associated with both basal and stimulated C-peptide distribution ($P < 0.001$), with no evidence of significant effect modification by either age or sex. As indicated in Fig. 1, both age and BMIZ had a stronger impact on the upper quartile of C-peptide distribution than the lower quartile. HLA type and IAA titer were no longer associated with the percentiles of basal or stimulated C-peptide after adjustment for age, sex, and BMIZ percentile. Further analysis demonstrated that age was significantly inversely related to IAA titer (data not shown). IAA titers were higher in the younger age-group ($P < 0.001$) compared with the older group, which indicates that IAA might be a confounding factor of C-peptide distribution rather than a determinant factor.

Supplementary Table 1 shows the estimated percentile values of basal C-peptide in children and adolescents with T1D ICA autoimmunity according to their age categories and BMIZ categories, respectively. Supplementary Tables 2 and 3 show the percentile values for boys and girls of all age and BMIZ groups combined. Percentile values were calculated for each group, even when the statistical tests indicated lack of differences between certain groups in lower percentiles. For all percentiles, stimulated C-peptide in lower-BMIZ girls was significantly different ($P < 0.03$) from that in high-BMIZ girls at both age periods. For boys, the same pattern was observed.

CONCLUSIONS

Previous findings from the DPT-1 study have shown that C-peptide is a good biomarker in predicting T1D onset in children at risk, with a level of prediction ability similar to that of glucose level (24,25). Longitudinal studies have shown that individuals at risk have a prolonged and gradual loss of C-peptide with the persistence of substantial β-cell function until at least 6 months before the onset of clinical disease (9,26,27). We believe that this is the first study to examine factors associated with C-peptide production in children and adolescents at risk for T1D. We found that ICA, IAA, GAD65A, and ICA512A antibody titers were not significantly associated with C-peptide distribution after adjustment for age, BMIZ, and sex in the samples from DPT-1. This

Table 2—Univariate quantile regression model for predicting C-peptide percentiles

	Basal C-peptide			Stimulated C-peptide		
	25th percentile	50th percentile	75th percentile	25th percentile	50th percentile	75th percentile
Sibling	0.25	0.11	0.54	0.18	1.00	0.46
Offspring	1.00	0.50	0.55	0.60	0.61	0.44
Second degree	0.08	0.40	0.12	1.00	0.15	0.84
Number of positive antibodies						
1 vs.4	0.06	0.10	0.11	1.00	0.27	0.85
2 vs. 4	0.49	1.00	0.57	0.32	0.51	0.19
3 vs. 4	0.33	0.09	1.00	1.00	0.65	0.30
IAA titer	0.13	0.05	0.29	0.16	<0.001	0.02
ICA titer	0.62	1.00	0.84	0.25	0.66	0.21
GAD65A titer	1.00	1.00	0.61	0.63	1.00	0.54
ICA512 titer	0.47	0.53	0.60	1.00	0.63	0.71
HLA (high vs. low risk)	0.05	0.05	0.09	0.54	0.02	0.31
Sex (female vs. male)	0.23	0.10	0.54	0.11	<0.001	0.01
BMIZ percentile (<85.0 vs. ≥85.0)	0.01	<0.001	<0.001	<0.001	<0.001	<0.001
Age (4–8 vs. 9–18 years)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data are P values.

is consistent with previous studies in children with newly diagnosed T1D (28–30). The relationships to proband or HLA type were also not found to be significant predictors of C-peptide. These data clearly demonstrate that the distributions of basal and stimulated C-peptide in a sample of children and adolescents with autoimmunity depend on BMIZ and age. Subjects with higher BMIZ have higher C-peptide at the considered percentiles than the subjects with lower BMIZ. Likewise, older children have higher C-peptide than children at a younger age. Interestingly, >30% (N = 68) of subjects who progressed to overt T1D at the end of study did not have an absolute decrease in stimulated C-peptide at the time of diagnosis from baseline. Approximately 75% of these subjects who were <9

years old at baseline were progressing through puberty, at which time there is a known increase in insulin production, and a total of 16% of subjects who had lower BMIZ percentile at baseline had increased BMIZ at the time of diagnosis. Thus, the decrease in C-peptide production at diagnosis may be considered not just a loss in absolute terms but also a failure to increase with age or BMIZ (31). A future longitudinal analysis to study the percentile changes before disease onset is warranted.

Our results also demonstrate that boys have lower stimulated C-peptide compared with girls in all age-groups. However, basal C-peptide was not sex dependent. This may suggest that boys with autoimmunity are less likely to progress to overt disease than comparable girls and that the pathogenesis of

T1D among boys may be slower compared with girls. Overall β-cell function may need to be further reduced in boys than in girls to progress to T1D (32,33). In addition, girls may have less insulin sensitivity owing to higher BMIZ than boys at the same age.

The estimated values in Supplementary Tables 4–6 provide a powerful tool for the interpretation of C-peptide in children at different BMIZ categories and age-groups. Based on these values, careful attention to children with C-peptide values that fall on the 10th and 25th percentiles, according to their BMIZ classification and age, becomes important in the identification of subgroups of children progressing to clinical T1D. For example, at the 25th percentile of the basal distribution, the children between 9 and 18 years of age exceed

Table 3—Multivariate quantile regression model for predicting C-peptide percentiles (coefficient and its 95% CI)

	25th percentile			50th percentile			75th percentile		
	Estimate	95% CI		Estimate	95% CI		Estimate	95% CI	
Basal C-peptide									
HLA (low vs. high risk)	0.00	−0.15	0.15	0.10	−0.02	0.22	0.10	−0.13	0.33
IAA titer	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BMIZ percentile (<85.0 vs. ≥85.0)	−0.24	−0.33	−0.16	−0.31	−0.43	−0.19	−0.55	−0.72	−0.38
Age (4–8 vs. 9–18 years)	−0.34	−0.43	−0.25	−0.40	−0.48	−0.33	−0.60	−0.72	−0.48
Stimulated C-peptide									
Sex (female vs. male)	0.58	0.27	0.89	0.50	0.10	0.90	0.79	0.37	1.20
HLA (low vs. high risk)	0.26	−0.09	0.60	0.26	−0.29	0.81	0.27	−0.66	1.20
IAA titer	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BMIZ percentile (<85.0 vs. ≥85.0)	−1.00	−1.32	−0.68	−1.05	−1.54	−0.56	−1.85	−2.32	−1.39
Age (4–8 vs. 9–18 years)	−1.28	−1.52	−1.04	−1.50	−1.89	−1.10	−1.90	−2.29	−1.52
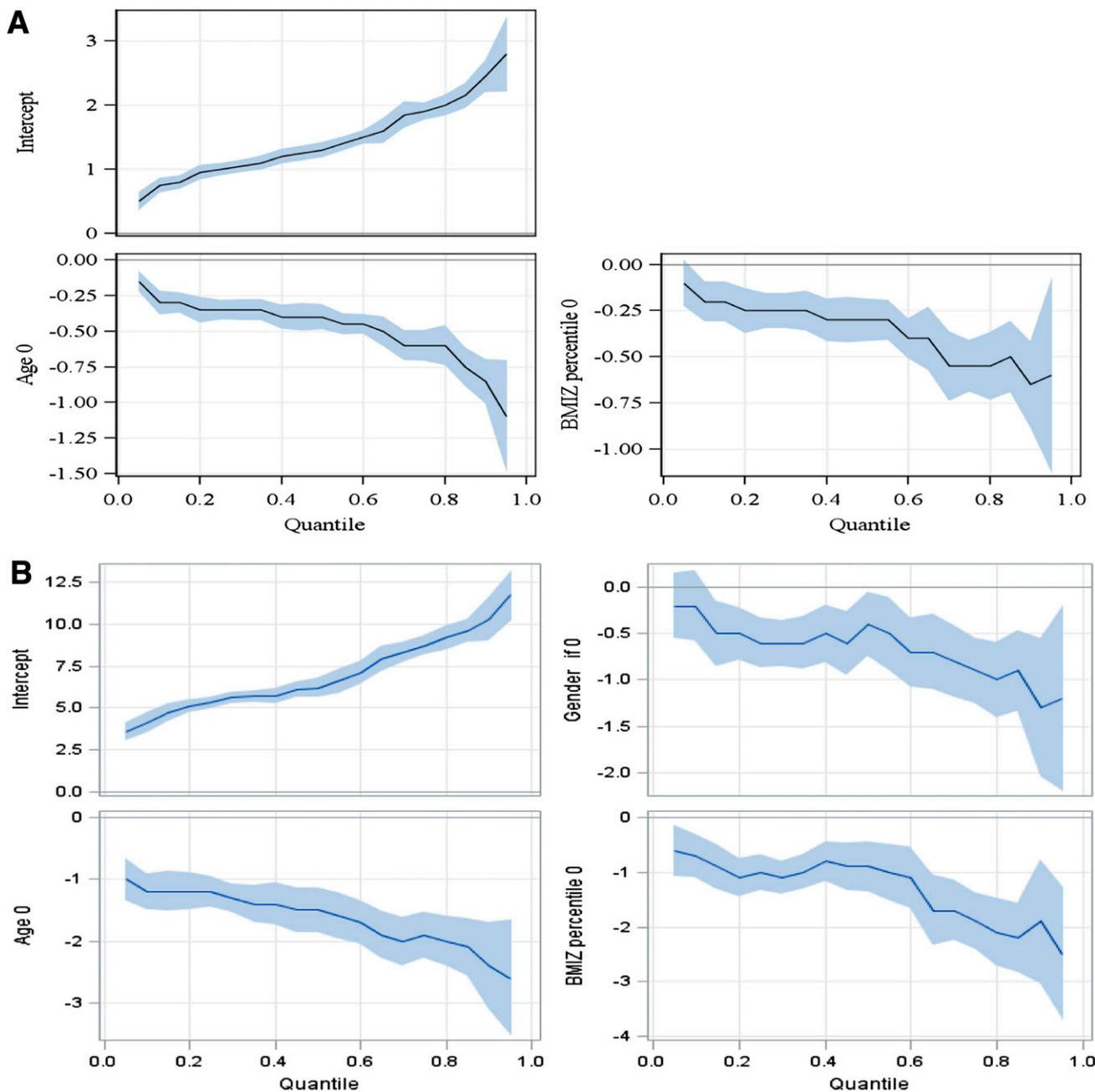
Figure 1—Effect of age, BMIZ, and/or sex on C-peptide by quantile. *A*: Effect on basal C-peptide (age 4–8 vs. 9–18 years, BMIZ <85.0 vs. ≥85.0 percentile) (estimated parameter by quantile for baseline fasting C-peptide with 95% CI). *B*: Effect on stimulated C-peptide (sex female vs. male; age 4–8 vs. 9–18 years; BMIZ <85.0 vs. ≥85.0 percentile) (estimated parameter by quantile for baseline peak C-peptide with 95% CI).

the basal C-peptide values of 1.00 ng/mL, identified as the cutoff point for increased risk of T1D among higher-BMIZ children. Similarly, at ages 9–18 years, the children with lower BMIZ achieved the 0.75 ng/mL cutoff point in the 25th percentile of the basal C-peptide distribution and 0.50 ng/mL cutoff point in the 10th percentile. Therefore, the cutoff point for C-peptide to classify subjects as having a loss of β-cell function may be different depending on their age, BMIZ, and/or sex.

Standard linear regression models, such as ordinary least square, are extensively used in statistical analyses. Despite their popularity, these conditional mean models have several limitations. When interest is in the percentiles of the conditional distribution rather than the mean, standard regression models may fail to provide the desired information because the assumption of normally distributed residuals with constant variance may not be justified. Standard regression

models are sensitive to outliers and can lead to unrealistic models if outliers are present in the data set. This is especially a problem if the sample size is moderately small and the error distribution is heavy tailed (17,18). Quantile regression overcomes these limitations of standard linear regression. It is robust in handling extreme value points and outliers for the outcome of interest. More importantly, it provides a more complete understanding of the impact of covariates on the

dependent variable across the whole distribution, especially toward the lower or upper tail of the distribution. The observed data in this sample show that the distribution of C-peptide is skewed to the right. The statistical inference based on the standard linear regression is inaccurate when the distributions are skewed and/or when the quantity of interest is the upper or lower tail of the distributions. This further promotes the quantile regression method for estimating percentiles of C-peptide and examining its relationship with the covariates.

The results of this study are based on a representative sample of children and adolescents with pre-T1D autoimmunity in North America. Our findings thus demonstrate the importance of age, BMIZ, and/or sex groups in C-peptide production in this population. Age-, sex-, and BMIZ-specific C-peptide percentiles are estimated for North American children and adolescents at risk for T1D and can be used as an assessment tool that could impact the recommendations in T1D prevention trials. Ideally, the use of age-, sex- and BMIZ-specific reference percentiles should use values specific to a normal population. Therefore, the estimated values at different percentiles describe an at-risk population and do not establish norms for healthy children. The majority of subjects in our sample (92%) were Caucasian, as is the case with T1D in North America. Thus, we also have limited data to assess the distribution of C-peptide production in minority groups. It remains unknown whether there is a difference in C-peptide production among different race or ethnicity groups.

Our study also suggests the possibility of developing age-, sex-, and BMIZ-specific cutoff values for identification of subjects with increased risk in prevention trials that target preserving β-cell function. It is important to establish that such specific cutoffs require demonstration of differential predictive ability and not simply demonstration of marginal distributions of the biomarker.

References

1. Rewers M, Norris J, Dabelea D. Epidemiology of diabetes. In *Immunology of Type 1 Diabetes*. 2nd ed. Eisenbarth GS, Ed. New York, Springer, 2004, p. 221–233
2. Palmer JP, Asplin CM, Clemons P, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 1983;222: 1337–1339
3. Eisenbarth GS, Moriyama H, Robles DT, et al. Insulin autoimmunity: prediction/precipitation/prevention type 1A diabetes. Autoimmun Rev 2002;1:139–145
4. Polonsky KS, Rubenstein AH. C-peptide as a measure of the secretion and hepatic extraction of insulin. Pitfalls and limitations. Diabetes 1984;33:486–494
5. Shapiro ET, Tillil H, Rubenstein AH, Polonsky KS. Peripheral insulin parallels changes in insulin secretion more closely than C-peptide after bolus intravenous glucose administration. J Clin Endocrinol Metab 1988;67:1094–1099
6. Eaton RP, Allen RC, Schade DS, Erickson KM, Standefer J. Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. J Clin Endocrinol Metab 1980;51:520–528
7. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41:368–377
8. Palmer JP, Fleming GA, Greenbaum CJ, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve β-cell function: report of an ADA workshop, 21-22 October 2001. Diabetes 2004;53:250–264
9. Sosenko JM, Palmer JP, Rafkin LE, et al.; Diabetes Prevention Trial-Type 1 Study Group. Trends of earlier and later responses of C-peptide to oral glucose challenges with progression to type 1 diabetes in diabetes prevention trial-type 1 participants. Diabetes Care 2010;33:620–625
10. Schatz D, Cuthbertson D, Atkinson M, et al. Preservation of C-peptide secretion in subjects at high risk of developing type 1 diabetes mellitus–a new surrogate measure of nonprogression? Pediatr Diabetes 2004;5:72–79
11. Verge CF, Howard NJ, Irwig L, Simpson JM, Mackerras D, Silink M. Environmental factors in childhood IDDM. A population-based, case-control study. Diabetes Care 1994;17:1381–1389
12. Akerblom HK, Vaarala O, Hyöty H, Ilonen J, Knip M. Environmental factors in the etiology of type 1 diabetes. Am J Med Genet 2002;115:18–29

13. van der Werf N, Kroese FGM, Rozing J, Hillebrands JL. Viral infections as potential triggers of type 1 diabetes. Diabetes Metab Res Rev 2007;23:169–183
14. Diabetes Prevention Trial–Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 2002;346:1685–1691
15. Sosenko JM, Krischer JP, Palmer JP, et al.; Diabetes Prevention Trial-Type 1 Study Group. A risk score for type 1 diabetes derived from autoantibody-positive participants in the diabetes prevention trial-type 1. Diabetes Care 2008; 31:528–533
16. Smirnov NV. Tables for estimating the goodness of fit of empirical distributions. Ann Math Stat 1948;19:279
17. Koenker R, Hallock K. Quantile regression: an introduction. J Econ Perspect 2001;15:143–156
18. Hao L, Naiman DQ. *Quantile Regression*. Thousand Oaks, CA, Sage Publications, 2007
19. SAS Institute Inc. The QUANTREG Documentation. In *SAS/STAT 9.2 User's Guide*. Cary, NC, SAS Institute Inc., 2008
20. Krarup T, Regeur L, Faber OK, Binder C. Insulin secretory reserve in insulin dependent patients at time of diagnosis and the first 180 days of insulin treatment. Acta Endocrinol (Copenh) 1980;95:359–363
21. Lee PA, Guo SS, Kulin HE. Age of puberty: data from the United States of America. APMIS 2001;109:81–88
22. Herman-Giddens ME, Wang L, Koch G. Secondary sexual characteristics in boys: estimates from the national health and nutrition examination survey III, 1988-1994. Arch Pediatr Adolesc Med 2001;155:1022–1028
23. Anderson SE, Dallal GE, Must A. Relative weight and race influence average age at menarche: results from two nationally representative surveys of US girls studied 25 years apart. Pediatrics 2003;111:844–850
24. Xu P, Beam CA, Cuthbertson D, Sosenko JM, Skyler JS, Krischer JP; DPT-1 Study Group. Prognostic accuracy of immunologic and metabolic markers for type 1 diabetes in a high-risk population: receiver operating characteristic analysis. Diabetes Care 2012;35:1975–1980
25. Xu P, Wu Y, Zhu Y, et al.; Diabetes Prevention Trial-Type 1 (DPT-1) Study Group. Prognostic performance of metabolic indexes in predicting onset of type 1 diabetes. Diabetes Care 2010;33:2508–2513
26. Sosenko JM, Palmer JP, Greenbaum CJ, et al. Patterns of metabolic progression to type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care 2006;29:643–649
27. Sosenko JM, Palmer JP, Rafkin-Mervis L, et al. Glucose and C-peptide changes in the perionset period of type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care 2008;31: 2188–2192
28. Jaeger C, Allendörfer J, Hatziagelaki E, et al. Persistent GAD 65 antibodies in longstanding IDDM are not associated with residual beta-cell function, neuropathy or HLA-DR status. Horm Metab Res 1997;29:510–515
29. Wang L, Lovejoy NF, Faustman DL. Persistence of prolonged C-peptide production in type 1 diabetes as measured with an ultrasensitive C-peptide assay. Diabetes Care 2012;35: 465–470

30. Nicole AS, Emily BT, Kevan CH. Natural history of β-cell function in type 1 diabetes. Diabetes 2005;54(Suppl. 2):S32–S39

31. Thunander M, Törn C, Petersson C, Ossiansson B, Fornander J, Landin-Olsson M. Levels of C-peptide, body mass index and age, and their usefulness in classification of diabetes in relation to autoimmunity, in adults with newly diagnosed diabetes in Kronoberg, Sweden. Eur J Endocrinol 2012;166: 1021–1029

32. Krischer JP, Cuthbertson DD, Greenbaum C; Diabetes Prevention Trial-Type 1 Study Group. Male sex increases the risk of autoimmunity but not type 1 diabetes. Diabetes Care 2004;27: 1985–1990

33. Weets I, Truyen I, Verschraegen I, et al.; Belgian Diabetes Registry. Sex- and season-dependent differences in C-peptide levels at diagnosis of immune-mediated type 1 diabetes. Diabetologia 2006;49:1158–1162