SUPPLEMENTARY DATA

Supplementary Material

C-peptide values recorded as below "Lower Limit of Detection" (LLD) were assigned the value of one-half the LLD. C-peptide AUC was calculated using the Trapezoidal method. Areas were then divided by the time period of the test, 120 minutes. The resulting AUC is then interpretable as "mean C-peptide" during the 2 hour test and this interpretation motivated the use of 1/2LLD.

Associations between C-peptide measures with baseline covariates were assessed with Spearman Correlation for continuous covariates and with ANOVA for categorical covariates.

Undetectable C-peptide on was defined as all timed values on the MMTT below the LLD. Non-significant decrease in C-peptide was defined either as the individual's CV between values as less than or equal to the median CV (9.7%) or defined as less than 1 assay difference SD (0.2634) of the MMTT/GST study (10). The time course of the proportion of subjects with detectable C-peptide values and the proportion of subjects with peak C-peptide ≥2.0 pmol were estimated with the Kaplan Meier method.

The relationship of C-peptide across time with baseline covariates was evaluated with mixed linear models. The models included random effects for subject, time and the interaction of these two variables. In this way, slopes relating C-peptide and time were thus estimated for each individual subject. Slopes of C-peptide v. time were then estimated for subgroups defined by age quartiles using the mean slope of individuals in that group and the resulting estimates displayed in Fan Plots.

The joint influence of baseline covariates on the longitudinal change in C-peptide was assessed with multivariable models. Only covariates that were found significant ($p<0.05$) in univariable analysis were included in the multivariable models. The significance of variables was determined with Wald statistics.

Since our goal was to model the course of C-peptide across time, we elected to use all time points available in order to gain the widest range of time. We therefore did not include the initial or "baseline" value of C-peptide as a covariate in the model. However, our statistical models were nonetheless adjusted for baseline C-peptide in the following manner: Consider the univariable model $E(Y) = \beta_0 + \beta_1 X + \beta_2 T + \beta_3 XT$ with covariate "X" and time variable "T". At "baseline", T=0, so that baseline C-peptide is estimated by the first two terms of the model. This estimate is included in each of the univariable and multivariable models and provides, therefore, a statistical adjustment for the starting value of C-peptide in our analyses.

Piecewise linear regression (Muggeo, 2003) was used to test the hypothesis that the slope of C-peptide AUC was the same before and after one year post baseline. The selection of the one-year time point for comparison was based on inspection of the plot of means across time and, therefore, this analysis is data driven.

To compare slopes between the three C-peptide endpoints (AUC, Peak and Fasting) a multivariate random effects linear model was fit. No covariate adjustment was used because multivariable analyses found different sets of covariates to be associated with each endpoint. The slopes were statistically compared using the multivariate Wilks' Lambda test. Following an overall significant test ($p<.0001$), individual pair-wise comparisons of the slopes were accomplished with preplanned contrasts at the 5% level of significance.

Patterns of change from baseline were investigated using three definitions of "responder". In two of the definitions, data from the MMTT/GST study were used to (a) determine assay variability and (b) the median Coefficient of Variation (CV) of the C-peptide AUC endpoint. The MMTT/GST study was a repeated measures study with two determinations taken on each subject approximately 1 week apart. Variance components analysis was used to estimate within subject variability of C-Peptide that was required for one responder definition, namely: any change from baseline ≥ -1 assay standard deviation. The other definition defined a

responder as occurring whenever the change in AUC was an increase, no change, or if the CV at the time point (defined to be $\sqrt{2} * \left| (Y_t - Y_0) / (Y_t + Y_0) \right|$, where Y denotes AUC and t denotes time) was less than the median CV of the MMTT/GST study, namely 0.097. The third definition is based on Herold (ref) who defined a responder as a subject whose follow-up C-peptide value was no more than 7.5% below baseline. Since the previous measures do not capture the pattern of serial changes, that is changes in C-peptide from one measurement to the next, we then considered the concept of a "rally" in which the change in two consecutive C-peptide AUC's is such that the latter represents an increase of at least 1 assay standard deviation from the former. For each of these definitions of remission and rally, subjects were classified dichotomously as having none during the two year period or having at least one such event. These data were then analyzed with logistic regression.

It is important to note that log transformation of C-peptide AUC led to models in which time was no longer a statistically significant factor. These models were subsequently rejected since it is well established that C-peptide declines in time. Our data therefore suggests the relationship is not entirely linear or log-linear (in which decline is proportional in time).

"Spaghetti plots" suggested the presence of outliers. Using data from our previously published study of the reproducibility of MMTT, we calculated the standard deviation of the paired differences in AUC C-peptide and then, for each individual, compared their actual data to the line fit by the regression model for them. Those observations more than 1.96 SD's from the line were characterized as unexpected and individuals with at least 3 data points meeting this criteria were classified as outliers. Statistically, we would expect 0.223% of the subjects to be classified as outliers simply by chance. However, 13 subjects or 6.8% were classified as outliers in our study. As there were no characteristics among these 13 individuals that distinguished them from the rest of the subjects, this suggests that lack of fit by the linear regression model on the untransformed C-peptide might account for the high frequency of subjects classified as outliers. In fact, inspection of residual plots revealed that while the model estimates are indeed very close to the observed values, there is a tendency to underestimate the rate of decline by the model.

SUPPLEMENTARY DATA

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SUPPLEMENTARY DATA

Supplementary Table 1. Correlation between data from 2 and 4 hour MMTT

	Peak pmol/mL	AUC pmol/mL	AUC % Change from baseline
Baseline	0.992	0.952	-
12 months	0.998	0.986	0.960
24 months	0.997	0.990	0.981

Supplementary Table 2. Comparison of Slopes (reduction across time) for the C-Peptide Endpoints

C-Peptide Endpoint	**Mean Reduction/Month***	**SE**
AUC	-0.051509054	0.00211122
Maximum	-0.066874744	0.00298705
Fasting	-0.022568375	0.00169631

*slope of regression line.

Results presented are slopes from the regression of each endpoint on time, measured in months from start of study. Random effects regression models were used to accommodate correlations within subjects across time for each endpoint. The slopes were then statistically compared using the multivariate Wilks' Lambda test. Following an overall significant test ($p<.0001$), individual pair-wise comparisons of the slopes were accomplished with preplanned contrasts. Each contrast was statistically significant ($p<.0001$). Hence, the slopes are significantly different. Maximum C-peptide has the greatest reduction across time.

SUPPLEMENTARY DATA

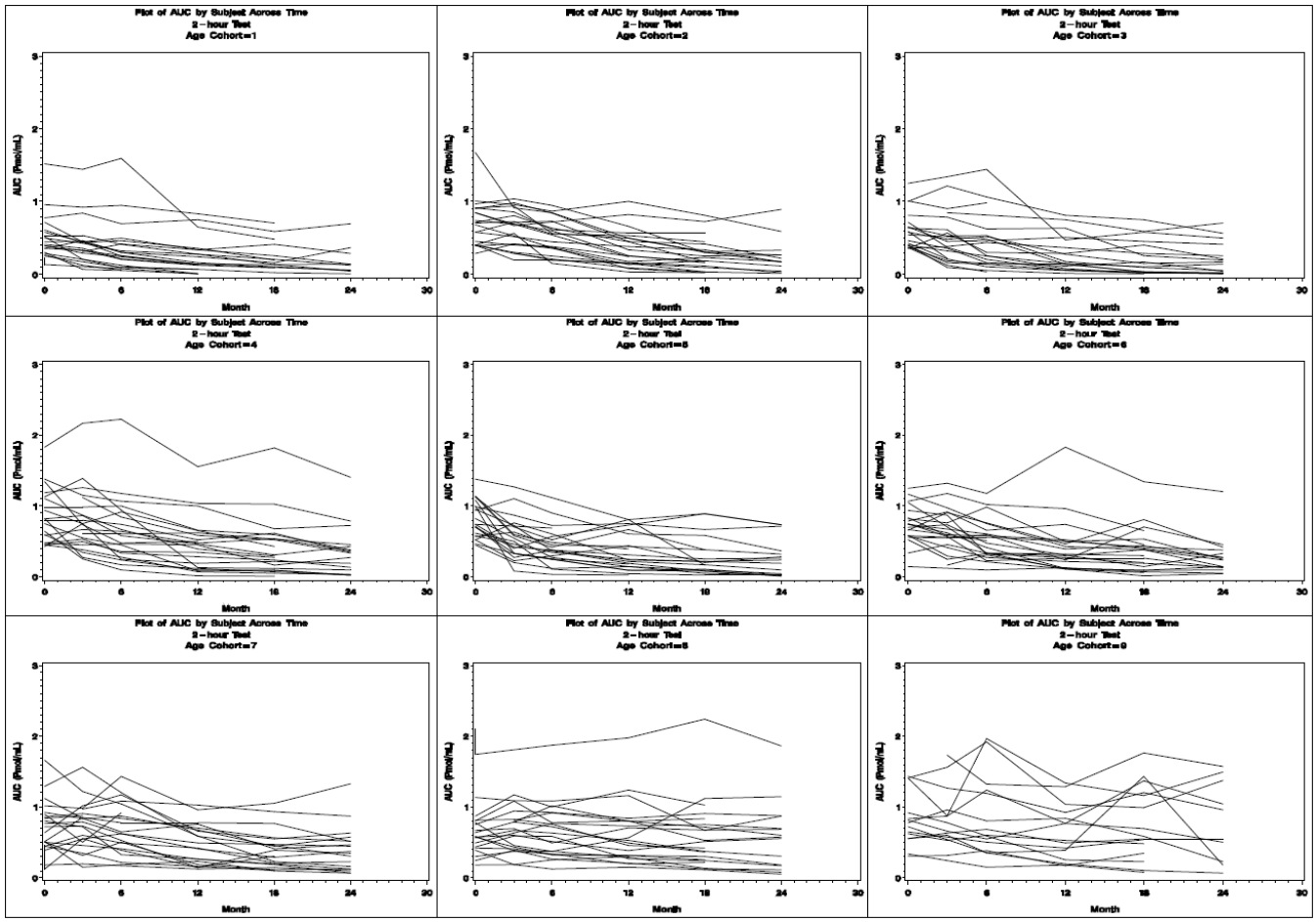
Supplementary Table 3. Variables Considered in Univariable Models

Age (years)
Age (categorical) 12 or under Between 12 and 17 Over 17
Race Asian Black/ African American White More than one race Other
Ethnicity Hispanic or Latino Not Hispanic or Latino
Gender Male Female
Duration of T1D (days)
Other autoimmune disease Yes No
BMI
zBMI
HLA DR3 or DR4; not DQB1*0602 Not DR3 or DR4; or with DQB1*0602
HbA1c
Insulin dose (units/kg)
mIAA (titer)
mIAA (% positive)
GAD65 (titer)
GAD65 (% positive)
ICA512 (titer)
ICA512 (% positive)
ICA (titer)
ICA (% positive)
CBC
Red blood cell count
Hemoglobin
Hematocrit
MCV
Platelet count
MCH
MCHC
White blood cell count
Pmnleuk
Lymphocytes
Monocytes
Eosinophiles
Basophils

SUPPLEMENTARY DATA

Supplementary Figure 1.

Individual trajectories in C-peptide AUC
Nine Age Cohorts of Approximately Equal Numbers



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Muggeo,VM: Estimating regression models with unknown break-points. *Stat Med* 22:3055-3071, 2003.