Two- and Four-Hour Tests Differ in Capture of C-Peptide Responses to a Mixed Meal in Type 1 Diabetes

Diabetes Care 2016;39:e76–e78 | DOI: 10.2337/dc15-2077

Karen D. Boyle,[1]
Lynette Keyes-Elstein,[1]
Mario R. Ehlers,[2] James McNamara,[3]
Mark R. Rigby,[4] Stephen E. Gitelman,[5]
Lia J. Weiner,[1] Kristen L. Much,[1] and
Kevan C. Herold[6]

Mixed-meal tolerance tests (MMTTs) are used in clinical trials to evaluate β-cell function in patients with new-onset type 1 diabetes (1,2). Some trials use a 4-h MMTT, whereas others use an abbreviated (2-h) protocol to reduce investigator and subject burden. In the T1DAL (Inducing Remission in Type 1 Diabetes With Alefacept) trial of patients with new-onset type 1 diabetes, the primary analysis using the 2-h test failed to reach statistical significance ($P = 0.065$), but a 4-h test did ($P = 0.019$) (3). We investigated the effect of abbreviating the test using data from 186 patients participating in three clinical trials conducted by the Immune Tolerance Network (3–5). Trials were approved by institutional review boards at the participating institutions. Written informed consent or assent was obtained.

Each patient contributed up to three 4-h MMTTs, conducted yearly, for a total of 506 paired 2- and 4-h observations. For this analysis, the 4-h assessment, which captures more of the complete hormonal response, was selected as the reference. The percent of the total 4-h C-peptide area under the curve (AUC) captured in the first 2 h ranged from 28% to 72%. Mean AUCs (mAUCs) were computed as 2- or 4-h AUCs divided by duration, 120 or 240 min, respectively. The correlation between the 2- and 4-h mAUCs was 0.98. Generally, the variability of the 2-h test was greater than that of the 4-h test (Fig. 1A). After adjusting for baseline, however, the variability was similar.

The standardized difference (Sdiff), the "distance" between the 2- and 4-h mAUCs measured in SD units, was used to evaluate associated factors. Both positive and negative differences exceeding 1 SD were observed for peak C-peptide values ≥0.6 pmol/mL (Fig. 1B). For peak values exceeding 1.6 pmol/mL, however, the 2-h mAUC generally overestimated the 4-h mAUC. Moreover, the 2-h mAUC generally overestimated the 4-h assessment when the time to peak was <120 min and vice versa for times >120 min (Fig. 1C).

As C-peptide levels may be associated with time, age, and treatment, we evaluated their impact on Sdiff. For low baseline Sdiffs, Sdiffs tended to increase over time and vice versa for high baseline values. For some individuals, however, Sdiffs were highly variable over time (Fig. 1D). Among untreated subjects, the distribution of Sdiffs among adults was shifted downward compared with pediatric subjects, who also had some extremely high values (Fig. 1E). At month 12, 56% of observations from the drug-treated subjects in the AbATE (Autoimmunity-Blocking Antibody for Tolerance in Recently Diagnosed Type 1 Diabetes) study had a positive difference between 2-h and 4-h mAUCs compared with 27% of observations for control subjects; the treatment effect was overestimated using the 2-h mAUC (Fig. 1F). In START (Study of Thymoglobulin to Arrest Newly Diagnosed Type 1 Diabetes), the treatment effect was underestimated with a 2-h test (data not shown).

Our findings may have important implications for designing studies. Because the impact of the abbreviated test is differential over time and by age and treatment groups, estimates and significance tests for 2- and 4-h assessments may be inconsistent. The variability of the C-peptide mAUCs may also affect sample size needs. Consideration of the methods used to measure C-peptide

[1]Rho Federal Systems Division, Chapel Hill, NC
[2]Immune Tolerance Network, San Francisco, CA
[3]National Institute of Allergy and Infectious Diseases, Rockville, MD
[4]Janssen Research & Development, Pharmaceutical Companies of Johnson & Johnson, Spring House, PA
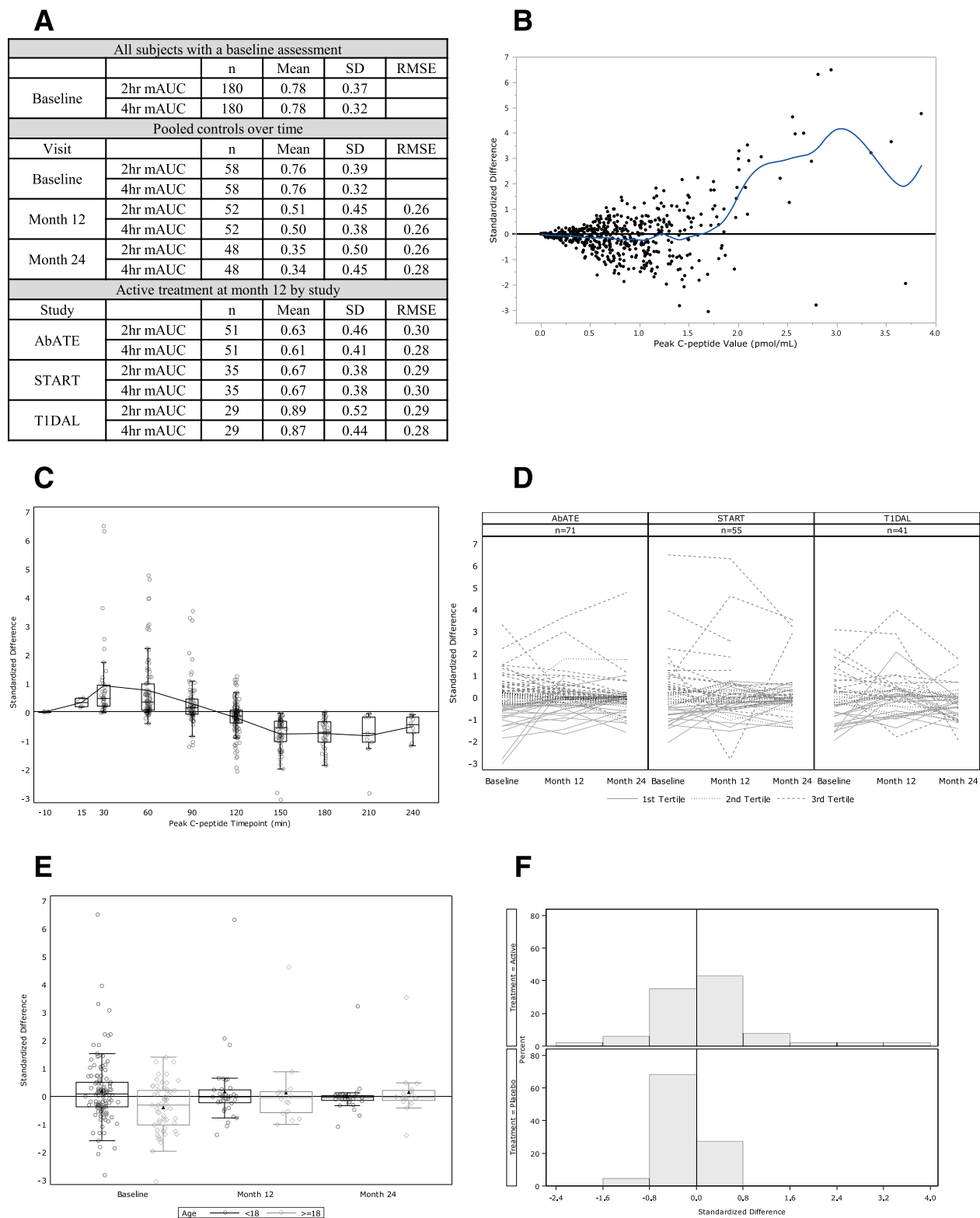[5]Department of Pediatrics, University of California, San Francisco, San Francisco, CA
[6]Department of Immunobiology and Internal Medicine, Yale University, New Haven, CT

Corresponding author: Karen D. Boyle, karen_boyle@rhoworld.com.

A

All subjects with a baseline assessment					
		n	Mean	SD	RMSE
Baseline	2hr mAUC	180	0.78	0.37	
	4hr mAUC	180	0.78	0.32	
Pooled controls over time					
Visit		n	Mean	SD	RMSE
Baseline	2hr mAUC	58	0.76	0.39	
	4hr mAUC	58	0.76	0.32	
Month 12	2hr mAUC	52	0.51	0.45	0.26
	4hr mAUC	52	0.50	0.38	0.26
Month 24	2hr mAUC	48	0.35	0.50	0.26
	4hr mAUC	48	0.34	0.45	0.28
Active treatment at month 12 by study					
Study		n	Mean	SD	RMSE
AbATE	2hr mAUC	51	0.63	0.46	0.30
	4hr mAUC	51	0.61	0.41	0.28
START	2hr mAUC	35	0.67	0.38	0.29
	4hr mAUC	35	0.67	0.38	0.30
T1DAL	2hr mAUC	29	0.89	0.52	0.29
	4hr mAUC	29	0.87	0.44	0.28

B

C

D

E

F

Figure 1—Analysis of 2- and 4-h C-peptide AUCs during MMTTs. The C-peptide AUC was computed for 2 and 4 h at baseline and at months 12 and 24 using the trapezoidal rule; mAUCs were computed by dividing AUCs by the duration of the test, 120 or 240 min, as indicated. Sdiff equals the 2-h mAUC minus the 4-h mAUC in SD units. Within each trial, the values below the lower limit of detection were either assigned a value of one-half of the lower limit of detection (START and T1DAL) or 0 (AbATE). Only available data were used in these analyses; missing MMTTs were not imputed. *A*: 2- to 4-h C-peptide mAUC estimates. Data from all control subjects over 24 months are shown and from drug-treated subjects at month 12. Root mean square error (RMSE) was derived from ANCOVA models controlling for baseline mAUC. Six subjects without baseline data were excluded. For active treatment groups at baseline and month 24, SDs for 2-h tests were greater than for 4-h tests (data not shown) except for T1DAL at month 24 where SDs were 0.51 and 0.52 for 2-h and 4-h tests, respectively. *B*: Sdiff by peak C-peptide value. The peak C-peptide value is the observed measurement with the highest value. The solid line shows the average trend. *C*: Sdiff by time at peak C-peptide point. The top and bottom of each box represent the

responses is important in clinical trial design.

References

1. Greenbaum CJ, Beam CA, Boulware D, et al.; Type 1 Diabetes TrialNet Study Group. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. Diabetes 2012;61:2066–2073
2. Steele C, Hagopian WA, Gitelman S, et al. Insulin secretion in type 1 diabetes. Diabetes 2004; 53:426–433
3. Rigby MR, DiMeglio LA, Rendell MS, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Diabetes Endocrinol 2013;1:284–294
4. Gitelman SE, Gottlieb PA, Rigby MR, et al.; START Study Team. Antithymocyte globulin treatment for patients with recent-onset type 1 diabetes: 12-month results of a randomised, placebo-controlled, phase 2 trial. Lancet Diabetes Endocrinol 2013;1:306–316
5. Herold KC, Gitelman SE, Ehlers MR, et al.; AbATE Study Team. Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. Diabetes 2013;62: 3766–3774

75th and 25th percentiles, respectively. The line through each box is the median. The whiskers mark the last points within 1.5 times the interquartile range. The solid line connects the averages for each visit across C-peptide time points. *D*: Sdiffs over time for individuals by study. Lines connect Sdiffs over time for individual subjects. Line styles reflect baseline Sdiffs in tertiles. Six subjects without baseline data were excluded. Solid light gray lines represent the lower tertile (values <−0.34). Hashed dark gray lines represent the upper tertile (values >0.22). Black dotted lines represent the middle tertile. *E*: Sdiffs for untreated pediatric and adult subjects by visit. Box plots for pediatric and adult subjects are black and gray, respectively. See *C* for a description of box plots. Black triangles represent mean values. *F*: Sdiff at month 12 in the AbATE study. The distribution of Sdiffs was shifted to the right for treated subjects (top) indicating that the 2-h mAUC overestimated the 4-h mAUC. The distribution of Sdiffs was shifted to the left for placebo subjects (bottom) indicating that the 2-h mAUC underestimated the 4-h mAUC. Thus these changes suggest that the C-peptide responses changed differently in the drug and placebo subjects over time and that the precision of the 2-h test differed for the two groups. The opposite effect was seen in the START study (data not shown).