Published in final edited form as:

Diabetes Metab Res Rev. 2011 September ; 27(6): 584–589. doi:10.1002/dmrr.1205.

Metabolic Tests to Determine Risk for Type 1 Diabetes in Clinical Trials

CJ Greenbaum¹, B Buckingham², HP Chase³, and J Krischer⁴

¹ Benaroya Research Institute, Seattle, WA

² Stanford University, Palo Alto, CA

³ Barbara Davis Center for Childhood Diabetes, Denver, CO

⁴ University of South Florida, Tampa, FL

Abstract

Aims—Evaluate the reproducibility and relationship of various metabolic tests conducted as part of the Diabetes Prevention Trial – type 1 diabetes (DPT-1)

Methods—Coefficients of variation, intraclass correlation coefficients, and Pearson correlations between the same metabolic tests performed at different times as well as the different tests were determined.

Results—Fasting samples on the same day had a CV of <10 for C-peptide, 11 for insulin and 2 for glucose. Testing on separate days approximately doubled the variance. Stimulated insulin values had less variance than fasting values and there was only a moderate correlation between fasting and stimulated values on each test. While highly correlated, C-peptide values from MMTT are significantly lower than that obtained during OGTT. Neither peak nor AUC C-peptide on OGTT was different between those with abnormal and normal glucose tolerance. Those with abnormal as compared with normal glucose tolerance had lower 30 minute C-peptide and a longer time to peak C-peptide.

Conclusions—A large, multi-center trial, with tests performed over a decade long period can provide robust data. C-peptide data from OGTT and MMTT differ, thus the same stimulation test should be used to evaluate changes in beta cell function over time. Worsening glucose tolerance is associated with lower C-peptide at 30 minutes and a delay in peak secretion on the OGTT. This DPT-1 data can be used in planning parameters for future studies including evaluation of new algorithms to determine risk of disease.

Keywords

Metabolic Tests; Type 1 diabetes; Prediction and Prevention

INTRODUCTION

The twin concepts that type 1 diabetes results from an immune mediated destruction of beta cells and that this process is far along prior to the clinical onset of disease is well established. Indeed, these concepts formed the basis for the notion that people could be identified prior to the onset of clinical disease, and that therapeutic strategies could be

Corresponding Author: Carla J. Greenbaum MD, Benaroya Research Institute, 1201 9th Ave, Seattle, WA 98101, cjgreen@benaroyaresearch.org, Phone: 206-515-5232, Fax: 206-515-5239.

Disclosure Statement: The authors have no conflicts to disclose

developed to prevent disease occurrence. Though therapies tested in recent large multi-center trials were unsuccessful in preventing or delaying diabetes, these studies verified algorithms to identify people at risk thus providing important information for planning of future intervention trials. For example, the Diabetes Prevention Trial – type 1 diabetes (DPT-1) showed that 41% of ICA+ relatives of people with type 1 diabetes with either low first phase insulin secretion as determined by IVGTT or impaired glucose tolerance on OGTT developed diabetes within 5 years(1). Similarly, 26 % of ICA+ relatives without the above metabolic abnormalities but with IAA+ developed diabetes within 5 years (2). These studies thus verified the notion that impaired insulin secretion occurs prior to the onset of clinical T1DM. However, the presence of the DPT-1 large data set also allows us to understand more details about the course of insulin secretion prior to diabetes diagnosis. The DPT-1 not only measured insulin secretion during IVGTT, enrolled subjects also underwent OGTT and MMTT testing at regular intervals with the hypothesis that there may be differences between these tests with respect to their use as risk indicators or surrogate markers for therapeutic effects or natural history. In this paper, we address the question as to the relative strengths and weakness of each of these metabolic tests in the context of a clinical trial to facilitate planning of future trials and to enhance our understanding of the T1DM disease process.

Important factors in selecting metabolic surrogate markers of the T1DM disease process include the reproducibility of the assay and tests in a large multi center clinical trial; the relationship of each type of test to the other; and what is the relationship of each test to glucose tolerance.

METHODS

First-degree (ages 3–45 years) and second-degree (ages 3–20 years) relatives were screened for ICA. Subjects then underwent “staging” tests which involved confirming the presence of ICA, determining their HLA type, measuring IAA, and performing an IVGTT to determine first phase insulin release (FPIR). As previously described (1;2), FPIR was considered “above threshold” if the 1+3 minute insulin values from this test were >10th percentile for siblings, offspring, and second-degree relatives (>100 μ U/mL if age >8 years; >60 μ U/mL if age <8 years); and >1st percentile for parents (>60 μ U/mL). If the FPIR was found to be below threshold, a repeat IVGTT was done to confirm this result. If one test was below and the 2nd test above threshold, a third IVGTT was performed. Prior to enrollment in the intervention phase of the study, all subjects underwent OGTT. Subjects with an OGTT consistent with the diagnosis of diabetes were excluded from enrollment in the intervention studies. Thus relatives who were confirmed to be ICA+ and either confirmed to have below threshold FPIR or an abnormal (but not diabetic) OGTT were eligible for the high risk intervention protocol.

Subjects with a normal OGTT and above threshold FPIR along with a confirmed ICA and IAA were eligible for the intermediate risk intervention protocol.

Once in either intervention protocol, subjects underwent OGTT testing every six months since this was the primary outcome measure. Any time an OGTT was indicative of diabetes, a repeat OGTT was performed within 30 days. If this test confirmed diabetes, the subject was deemed to have reached endpoint and no longer followed in the study. If the test did not confirm diabetes, the subject resumed the every 6 month OGTT schedule. To mask investigators and subjects as to OGTT results, approximately 10% of OGTTs which were not indicative of diabetes were also repeated within 30 days.

In addition, individuals in the high risk intervention group underwent other metabolic testing annually, alternating MMTT with IVGTT testing. Individuals in the intermediate risk intervention underwent annual IVGTT assessments and MMTT at baseline, 36 months and study end.

As a quality assurance measure 5% of samples were assigned to be “split duplicates”. Thus, clinical sites received additional materials and labels to obtain duplicate blood samples during a test which was sent masked to the laboratory. During the DPT-1, this data was among the material regularly available to the laboratory monitoring and DSMB committees.

OGTT

As previously described (1;2), after overnight fasting, oral glucose (Sundex, Fisher) dose was consumed at 1.75 g/kg (maximum 75 g). Samples were obtained for glucose and C-peptide at -10, 0, 30, 60, 90, and 120 minutes.

Plasma glucose values were interpreted according to 1997 American Diabetes Association guidelines (3). Normal glucose tolerance was defined as fasting plasma glucose (FPG) <7.0 mmol/L (126 mg/dL) and 30, 60, 90 and 120-minute glucose <11.1 mmol/L (200 mg/dL). Abnormal glucose tolerance included all other categories including diabetes, impaired fasting glucose, impaired glucose tolerance, and indeterminate glucose tolerance (3).

IVGTT

As previously described (1;2), after overnight fasting, a 25% glucose solution was infused as a square wave bolus over 3 min. $\pm$ 15 sec with a dose of 0.5 g/kg body weight glucose (maximum dose 35 g) (4;5). Samples were for glucose, insulin, and C-peptide at -10, -4, 1,3,5,7, and 10 minutes. Insulin values at 1 and 3 minutes were added to determine FPIR. FPIR was above threshold if >10th percentile for siblings, offspring, and second-degree relatives (>100 μ U/mL if age >8 years; >60 μ U/mL if age <8 years); and >1st percentile for parents (>60 μ U/mL).

MMTT

As previously described(1;2), after overnight fasting, a liquid formula meal was consumed (Sustacal®/Boost®, Mead Johnson Nutritionals) (6 cal/kg body weight, maximum 360-kcal). Samples were obtained for glucose and C-peptide at -10, 0, 30, 60, 90, and 120 minutes.

All assays were performed as previously described (1), including ICA (indirect immunofluorescence), IAA (competitive fluid-phase radioassay), plasma glucose (glucose oxidase method), insulin (radioimmunoassay), C-peptide (radioimmunoassay)

STATISTICS

Intraclass correlations (ICC) were used as a measure of within subject correlation when a subject is tested/scored on the same test repeatedly. Pearson Correlations were used to evaluate the correlation between test variation (assuming independence).

RESULTS

Reproducibility of individual measures

Coefficients of variation (CV) were determined for glucose, C-peptide, and insulin from both split duplicate samples and from two fasting samples on same subject 5–10 minutes apart during same test. Comparisons were also made between fasting samples for glucose

and C-peptide obtained up to 30 days apart (Table 1). There was no significant difference in CV of the samples for c-peptide, glucose, or insulin received from different clinical sites in the country or changes in split duplicate results over time. The intraclass correlation (r) was 0.600 (0.567 – 0.631 CI) for C-peptide samples done on tests 30 days apart and there was no effect of adjusting for fasting glucose or age on this relationship. The intraclass correlation coefficient for glucose was 0.107 (0.065–0.143CI) which did not change when adjusted for age. It was hypothesized that the differences in fasting glucose on different days may contribute to the increased CV of C-peptide measures performed on different days. However, testing the ratio of fasting C-peptide/fasting glucose did not appreciable impact the CV (mean 21.9, 20.1 STD).

Reproducibility of test outcomes

IVGTT—To determine the reproducibility of measures of stimulated insulin secretion, we compared the results from the last two IVGTT performed in subjects during the staging part of the study. There were 249 paired tests separated by at least 3 weeks, but performed within 26 weeks of each other (mean 114 days).

Insulin: First we explored the reproducibility of the IVGTT as assessed by categorical outcome. Of the 126 tests determined to be below threshold on the first visit, 112 or 89% were confirmed in that category on the second visit. Of those determined to be above threshold on the initial test, 82 tests (65%) were confirmed on the second visit (Kappa =0.23).

[Table 2] The intraclass correlation coefficient (r) on the 1+3 minute insulin values obtained from staging IVGTT tests was (r =0.656, 0.570 – 0.714 CI), and that calculated from AUC (r =0.690, 0.613 – 0.746 CI) and peak insulin (r =0.637, 0.554 – 0.703 CI) values. There was a significant decrease in the correlation between IVGTT tests when the fasting insulin was used as the only measure of comparison (r =0.471, 0.364 – 0.558 CI). Adjusting for the subject's age, fasting glucose, or fasting insulin did not make a significant improvement in reproducibility between tests.

C-peptide: [Table 2] The correlation coefficient of fasting C-peptide values obtained from two staging IVGTT tests was r =0.580 (0.493 – 0.658 CI). The correlation was improved with using either the peak or AUC C-peptide values (peak r =0.740 [0.678 – 0.791 CI]; AUC r = 0.721 [0.654 – 0.776 CI]). In all cases, adjusting for fasting C-peptide or glucose or subject's age did not significantly improve the reproducibility of the results.

Relationship between measures obtained during the same test

IVGTT—During the DPT-1, both insulin and C-peptide measurements were obtained as part of IVGTT. While both insulin and C-peptide are beta cell secretory products, there are significant differences in the metabolism of these hormones which are reflected in values measured in the peripheral blood. We thus wanted to understand the relationship of these measures within the same test.

The Pearson correlation coefficient was 0.67 when comparing 2110 samples in which fasting insulin and C-peptide was measured during the initial staging IVGTT. The relationship between the peak or AUC insulin and C-peptide values was better (0.85); however, adjusting for fasting C-peptide or glucose did not improve these correlations. Though all Pearson correlations were significant at the 0.001 level, the the correlation coefficients were quite low between the fasting insulin (0.14) or C-peptide values (0.11) and the fasting glucose value. Indeed, this was also true when examined between any measure of

glucose obtained during the IVGTT and stimulated insulin or C-peptide measurements (data not shown). All Pearson correlations were significant at the 0.001 level.

To better interpret this data, we also explored the relationship of fasting to stimulated values during the IVGTT for insulin and C-peptide separately. We found that fasting C-peptide was reasonably correlated with either AUC or peak C-peptide (0.68 for both), and that there was a strong correlation between peak and AUC C-peptide values (0.99). Similar data was observed for insulin in that fasting insulin was moderately correlated with peak (0.53) and AUC (0.58), while peak and AUC insulin were highly correlated (0.98). None of these correlations were improved by adjustments for fasting insulin, C-peptide, or glucose values. All Pearson correlations were significant at the 0.001 level.

OGTT—The relationship between fasting to stimulated C-peptide values during the initial staging OGTT was determined from 960 tests. The correlations were fasting and peak (0.65), fasting and AUC (0.71), peak and AUC (0.96). These correlations were not improved by adjustments for fasting glucose or C-peptide values. All Pearson correlations were significant at the 0.001 level.

Relationship between tests performed 30 days apart

The DPT-1 used three different beta cell stimulants (IV and oral glucose, oral “mixed meal”) to assess beta cell function after randomization. We wished to understand the relationship of beta cell function as assessed by each of these measures.

Comparisons between MMTT and IVGTT—Comparison of C-peptide values obtained up to 30 days apart on MMTT (n=406) and IVGTT (n=731) was done. There was only a moderate correlation between peak (0.53) or AUC (0.51) C-peptide values when the beta cell received two different stimulus (oral mixed meal vs IV glucose); however, this was only slightly different than the correlation of the fasting C-peptide values obtained for these test (0.62). All Pearson correlations were significant at the 0.001 level.

Comparisons between MMTT and OGTT—The peak and AUC C-peptide values obtained during MMTT and OGTT obtained up to 30 days apart were correlated (peak 0.69; AUC 0.76), while the relationship between fasting values was the same as seen above (0.66). All Pearson correlations were significant at the 0.001 level.

Notably, despite this correlation the OGTT mean C-peptide values (AUC 501.3 ng/ml $\pm$ 198.1; peak 5.36 $\pm$ 2.2 ng/ml) and glucose values (166.6 $\pm$ 41.6 mg/dl peak) were markedly greater than that seen with MMTT stimulation (C-peptide AUC 393.4 $\pm$ 171 ng/ml; C-peptide peak 4.49 $\pm$ 2.00 ng/ml; glucose peak 122.2 $\pm$ 23.7 mg/dl ($p < 0.001$ for each). [Figure 1]

Comparisons between OGTT and IVGTT—The relationship between fasting, peak, and AUC C-peptide values obtained during OGTT and IVGTT tests were determined for studies up to 30 days apart [Fasting 0.70; peak 0.57; AUC 0.62.]. All Pearson correlations were significant at the 0.001 level.

In the IVGTT, C-peptide as well as insulin measurements were made. We thus compared the relationship between the peak and AUC C-peptide obtained on the OGTT with peak and AUC insulin values obtained on the IVGTT. In both instances as well as comparing fasting values, while significant at the 0.001 level, the correlations were only moderate (fasting $r = 0.43$, peak $r = 0.39$, AUC $r = 0.46$) for tests separated by 30 days.

Relationship of secretion to glucose tolerance

During staging, the OGTT was categorized as normal (n=269) or abnormal (IFT, IGT, Diabetic, Indeterminate (n=683)). We compared the C-peptide values obtained during the initial staging IVGTT and these categories. Fasting C-peptide was 2.47ng/ml in AGT group and 3.51ng/ml in NGT group ($p < 0.001$). The peak C-peptide value from the IVGTT was 2.29ng/ml in AGT group, and 3.38ng/ml in those with NGT ($p < 0.0001$). This difference between groups was similar when expressed as C-peptide AUC (20.6 vs 30.4 ng/ml, $p < 0.0001$). There was no difference in fasting insulin values between the glucose tolerant and intolerant groups (13.2 vs 12.7 uU/ml, $p = 0.41$) even when adjusted for fasting glucose, while both the peak and AUC insulin values were markedly different between groups.

We also assessed whether secretion information obtained from the OGTT would also be different according to OGTT categories. Surprisingly this was not the case for peak C-peptide values in which the mean peak for those with AGT was 4.94 ng/ml and that for those with NGT was 5.19 ng/ml ($p = 0.15$) unless an adjustment was made for fasting C-peptide value. However, incorporating additional C-peptide values in the calculation for C-peptide AUC did demonstrate a difference between the glucose tolerance groups (C-peptide AUC for AGT=431.2 vs NGT 478.4 ng/ml; $p = 0.001$) Moreover, when a single time point was chosen to compare C-peptide values there were marked differences in glucose tolerance groups. Thus, those with AGT had less C-peptide at 30 and 60 minutes than those with NGT [Figure 2]. There was also a difference between groups at fasting and 120 minutes, but in this case, those with NGT had lower C-peptide values. While this data suggests that the 120 minute value in those with NGT is heading towards baseline, when the C-peptide/glucose ratio was calculated at 120 minutes there was no difference between glucose tolerance groups. The time to peak value was significantly different in the two groups. Those with AGT reached peak value at 99.1 ± 28 minutes, and those with NGT reached peak C-peptide value at 71.6 ± 29.8 minutes ($p < 0.001$).

Discussion

The DPT-1 performed multiple measures of beta cell function in the subjects identified at risk for type 1 diabetes. Since the pathogenesis of diabetes involves destruction of the beta cell, accurate and reliable assessment of beta cell function can serve as a surrogate marker of the disease process. The question is which tests are best to serve this purpose. Many factors would be involved in making this assessment, including standard determinations of reproducibility, the relationship between these measures and glucose tolerance, and practical issues of investigator and subjects' burden and cost. Thus one purpose of this analysis is to assist future investigators in choosing an outcome measure. In addition, by providing the first published data on the variability of these measures in the population, more accurate estimation can be made of the N required to use such measures as outcome markers.

Several key points can be made from the data presented. First, there was little difference in the variability between the QC samples tested in the laboratory for insulin, glucose, and C-peptide, and those fasting samples drawn separately at the local clinic. In addition, the variability was not different from site to site, or over the length of the study. To assess the reproducibility of the test itself and not variations due to progression of disease (i.e. pathophysiology), we looked at the variance when tests were performed up to 30 days apart finding that testing on separate days approximately doubled the variance. Importantly, we also found that the variances did not differ when adjusted for age or fasting C-peptide, insulin, or glucose value.

We next determined the reproducibility of the beta cell stimulated responses finding that stimulated as compared with fasting values are more consistent and thus better measures to

use as outcome markers over time. Of course, an alternative explanation is that the increased variance fasting insulin values reflects important changes in physiology over time that the stimulated values are insensitive in detecting. Not surprisingly, in both IVGTT and OGTT tests there were an excellent correlation between two measures of stimulated secretion, thus indicating that either the peak or AUC calculations could be used. However, there was only a moderate correlation between fasting insulin or C-peptide values and either of these stimulated measures – again indicating that these values represent different processes.

The key question therefore was the relationship of fasting versus stimulated secretion measures to glucose tolerance status. We found that fasting insulin did not differ between those with AGT and NGT while IVGTT stimulated values were markedly different between groups. This data supports the well known concept of loss of FPIR resulting in abnormal glucose tolerance.

We found no differences in peak C-peptide between those with normal and abnormal glucose tolerance status. Instead, the shape of the C-peptide curve in response to OGTT was different; those with NGT reaching peak value before those with AGT. Thus, the 30 minute C-peptide value was markedly different between the two glucose tolerance groups. Whether the metabolic data from an OGTT will be sufficient to determine risk of disease progression remains to be tested.

DPT-1 also had subjects undergo MMTT to assess the beta cell response to fats and proteins as well as glucose. We wished to understand whether this test provided additional information. Since a given individual's MMTT tests were separated by 1–3 years, we could not compare the reproducibility of the test per se without the confounding problem of changes in underlying physiology. The C-peptide and glucose values were markedly greater after OGTT as compared to MMTT stimulation. Although glucagon was not measured in DPT-1, the lower C-peptide and glucose values seen in the MMTT are likely due to the impact of glucagon secretion in response to the protein in the mixed meal. As there is great interest in understanding the how beta cell function changes during the transition period from at-risk status to clinical onset, these data confirm that longitudinal data will require using the same stimulation test over time, i.e. it would not be valid to compare the absolute C-peptide values obtained during OGTT in the pre-diabetes period with the C-peptide values obtained during an MMTT post-diagnosis.

In conclusion, the low CVs for glucose, C-peptide, and insulin values obtained from split duplicate samples, or two fasting samples obtained during the same test provide reassurance regarding the accuracy of such data for multi-center clinical trials conducted over a long period of time and account for the assay, blood draw, and handling variability. The variance found on tests conducted within 30 days is about double the values obtained the same day. When tests are repeated over a longer period of time (i.e. results from IVGTTs up to 26 weeks apart), the tests are less reproducible, reflecting the impact of disease progression. In this regard it is of interest to note that there was more variance in the fasting rather than stimulated levels. Further, there were only moderate correlations between fasting and stimulated values of either insulin or C-peptide. The data also indicate that, while highly correlated, the C-peptide values from an OGTT are not the same as those from the MMTT in this population. Finally, worsening glucose tolerance is associated with lower C-peptide at 30 minutes and a delay in peak secretion on the OGTT. This DPT-1 data can be used in planning parameters for future studies including evaluation of new algorithms to determine risk of disease.

Acknowledgments

Grant Support: DPT-1 was supported through cooperative agreements by the Division of Diabetes, Endocrinology and Metabolic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institutes of Health (NIH); the National Institute of Allergy and Infectious Disease (NIAID), NIH; the National Institute of Child Health and Human Development (NICHD), NIH; the National Center for Research Resources (NCRR), NIH; the American Diabetes Association (ADA); the Juvenile Diabetes Research Foundation (JDRF); and various corporate sponsors.

DPT-1 was supported through cooperative agreements by the Division of Diabetes, Endocrinology and Metabolic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institutes of Health (NIH); the National Institute of Allergy and Infectious Disease (NIAID), NIH; the National Institute of Child Health and Human Development (NICHD), NIH; the National Center for Research Resources (NCRR), NIH; the American Diabetes Association (ADA); the Juvenile Diabetes Research Foundation (JDRF); and various corporate sponsors.

The authors thank David Cuthbertson for statistical support and the DPT-1 Study Group Members and participants

Reference List

1. Diabetes Prevention Trial Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med.* 2002; 346:1685–1691. [PubMed: 12037147]
2. Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1. *Diabetes Care.* 2005; 28:1068–1076. [PubMed: 15855569]
3. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care.* 1997; 20:1183–1197. [PubMed: 9203460]
4. Bingley PJ, Colman P, Eisenbarth GS, Jackson RA, McCulloch DK, Riley WJ, Gale EA. Standardization of IVGTT to predict IDDM. *Diabetes Care.* 1992; 15:1313–1316. [PubMed: 1425095]
5. Chase HP, Cuthbertson DD, Dolan LM, Kaufman F, Krischer JP, Schatz DA, White NH, Wilson DM, Wolfsdorf J. First-phase insulin release during the intravenous glucose tolerance test as a risk factor for type 1 diabetes. *J Pediatr.* 2001; 138:244–249. [PubMed: 11174623]

Figure 1.
Mean C-peptide $\pm$ SE (ng/ml) during MMTT and OGTT performed within 30 days of each other

Figure 2. Mean C-peptide $\pm$ SE (ng/ml) during OGTT with normal (n=269) and abnormal glucose tolerance (n=683)

Coefficient of variation for glucose, C-peptide, and insulin values obtained on the same samples (split duplicate samples), on two separate fasting samples obtained the same day (two baseline samples), and on fasting samples obtained from tests performed within 30 days of each other.

TABLE 1

	SPLIT DUPLICATE SAMPLES			TWO BASELINE SAMPLES			Fasting samples 30 days apart		
	Glucose	C-peptide	Insulin	Glucose	C-peptide	Insulin	Glucose	C-peptide	
N	2061	2544	831	5714	7620	1981	1836	1816	
CV±Std %	1±5	10±10	11.3±13	2.12±4.2	9.3±12.2	11.6±12.9	5.9±9.0	22.2±20.6	

Intraclass correlation (R) and coefficient of variation (CV) on insulin and C-peptide values obtained during 249 paired IVGTT tests performed within 3 to 26 weeks of each other

TABLE 2

	Insulin			C-peptide			
	Fasting	1+3 minute	AUC	Peak	Fasting	AUC	Peak
R	.471	.656	.69	.637	.579	.72	.74
CV	24.98	23.27	22.13	21.91	27.6	18.9	18.9