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FIRST TEST EFFECT IN INTRAVENOUS GLUCOSE TOLERANCE TESTING

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Abstract

Intravenous glucose tolerance testing (IVGTT) is a common test of β -cell function in which a glucose load is administered and insulin and/or C-peptide responses are monitored.

Aims—Since the first IVGTT may be more stressful and stress may alter β -cell secretion or hepatic insulin extraction, we asked whether there was a first test effect.

Methods—Insulin and C-peptide responses were compared from two sequential IVGTTs performed within 6 months during staging for the Diabetes Prevention Trial – Type 1 (DPT-1) in 368 people at high risk for type 1 diabetes. 1+3 minutes (min) insulin data were used because the first phase insulin response (and peak insulin concentration) occurs within this time frame. Areas under the curve (AUC) calculations represent early insulin or C-peptide responses from 0 through 10 min post-glucose challenge.

Results—More than half of all subjects were found to have first test values lower than the second. This was true for all measures of both insulin and C-peptide but the frequency was significantly different only for insulin measures corrected for basal and for insulin AUC ($p < 0.05$). However, for subjects ($n = 99$) whose 1+3min insulin response was $< 10^{\text{th}}$ percentile on the first test, there was a significant increase on the second test ($p < 0.05$). The C-peptide: insulin ratio did not change significantly between tests, indicating that differences are due to changes in β -cell secretion rather than hepatic insulin uptake.

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Conclusions—A statistically significant first test effect occurs during the IVGTT attributable to variations in insulin secretion rather than hepatic uptake.

INTRODUCTION

One of the most common methods of measuring β -cell function is the intravenous glucose tolerance test (IVGTT). A low peak insulin level, known as first phase insulin response (FPIR), is a strong risk factor for type 1 diabetes mellitus (T1DM), [1]. This test is often used for determining eligibility for clinical trials and so must be carefully performed and interpreted to ensure trial validity. It is possible, however, for several factors to influence the IVGTT.

Consistencies of the IVGTT have been investigated. While some have demonstrated that the intra-individual variation in FPIR to the IVGTT is high [2], others have shown that the IVGTT is reproducible [3, 4], particularly for insulin responses [5]. However, others have suggested that C-peptide be used as a marker of insulin secretion due to equimolar co-secretion of insulin and C-peptide. C-peptide measurement has several advantages including negligible hepatic extraction compared to considerable and variable insulin extraction, and constant within individual metabolic clearance rate, distribution and rate constants over varying metabolic states [6].

Stress responses have been associated with reduced glucose tolerance in several settings. In naïve cats, the stress of handling has been associated with both increased catecholamine levels and decreased glucose tolerance as assessed by the IVGTT [7]. Novel medical procedures, such as the first glucose tolerance test, may be similarly stressful in humans [8, 9]. Because the experience is less novel during a second test, the stress would be expected to be less.

A number of factors involved in the IVGTT results may be altered by a first test effect. Insulin extraction by the liver may vary, dependent on hormonal and other variables [10–12]. In addition, peripheral insulin clearance is not consistent and may vary with exercise [13], blood glucose levels [14] and other factors. These variables are likely to be responsible for at least some of the intraindividual variation in blood glucose and insulin levels described earlier. Finally, the insulin output of the β -cell in response to a challenge may be altered (potentiated or attenuated).

In this study, our main objective was to investigate whether insulin and/or C-peptide levels during an IVGTT might be altered by a possible first test effect. To address these issues, we explored the following specific aims: (1) determine whether there is a significant difference in insulin and/or C-peptide response between first and second IVGTTs and (2) if a significant difference is present, determine [a] the frequency and magnitude of this difference, [b] whether it is preferentially seen in certain groups, and [c] whether this difference is caused by a variation in β -cell secretion of insulin or hepatic uptake of insulin.

Our experimental approach involved analyzing data collected from candidates for entry into the Diabetes Prevention Trial – Type 1 (DPT-1), [15, 16]. As part of staging for this trial,

potential volunteers had at least two IVGTTs performed. The insulin and C-peptide responses to these two IVGTTs have been used to investigate these specific aims.

RESEARCH DESIGN AND METHODS

315 participants were first-degree (age range 3 to 45 years), and 53 were second degree relatives (age range 3 to 20 years) of patients with T1DM. Mean age was 16.5 ± 11.43 years.

Staging for the DPT-1 consisted of confirming islet cell autoantibody (ICA) positivity, measuring insulin autoantibody (IAA) status, assessing FPIR to intravenous glucose, assessing oral glucose tolerance test (OGTT), and determining presence or absence of HLA-DQA1*0102/DQB1*0602 (a protective haplotype that excluded subjects from participation), [17, 18]. 3,483 relatives screened during enrollment for the DPT-1 were ICA positive. A total of 2,523 (72.4% of ICA⁺ individuals) underwent staging for DPT-1. Subjects found to be positive for ICA underwent IVGTTs.

IVGTTs were performed by certified personnel in both inpatient and outpatient settings. A 25% glucose solution was infused as a square wave bolus over 3 minutes (min) ± 15 sec with a dose of 0.5g/kg body weight glucose (maximum dose 35g). Samples were collected 10 and 4 min before the infusion was initiated and 1, 3, 5, 7 and 10 min after the infusion was complete. C-peptide and insulin concentrations were measured by radioimmunoassay at each time point by the DPT-1 β -Cell Function Core Laboratory, as previously described [15, 16].

Insulin values measured during the IVGTT at one and three minutes (1+3min) were added together to determine the FPIR. The FPIR in siblings, offspring, and second-degree relatives was below threshold if it was below the 10th percentile for this group ($<100 \mu\text{U}$ per milliliter for subjects ≥ 8 years; $<60 \mu\text{U}$ per milliliter for subjects < 8 years of age). Those responding at $<10^{\text{th}}$ percentile of normal for 1+3min insulin or who were ICA and IAA positive underwent a second, confirmatory IVGTT. Those with a FPIR below the threshold on two occasions were considered to have a projected five-year risk of developing diabetes of $> 50\%$ and were deemed eligible for the parenteral insulin prevention trial. All assays were performed as previously described [15] and details of the DPT-1 recruitment process are outlined in [15, 16]. In no case did participants included in this study demonstrate: diabetes during an OGTT, other diseases, or ongoing use of medications that influence glucose tolerance. There were equal number of male and female participants enrolled (184 in each group). Only subjects who underwent two IVGTTs within 6 months were included in the analysis (n=368).

Statistical analysis

For each IVGTT, the 1+3min time points, 1+3min minus basal, area under the curve (AUC), and AUC minus basal were calculated both for insulin and C-peptide values. 1+3min data were used because the first phase insulin response (and peak insulin concentration) occurs within this time frame. Area under the curve calculations also represents the early insulin or C-peptide responses from 0 through 10 min post-glucose challenge. Basal levels for any

given test were taken to be the average of the two pre-glucose challenge values. Statistical significance was set at $p < 0.05$.

The differences between the first and second IVGTT were calculated for both insulin and C-peptide values. For each measurement, the percent of subjects with lower results on their first IVGTT was calculated, and tested for significance with the Sign test. Paired differences between the first and second IVGTTs were tested for significance using paired t-tests. This analysis was also performed on subsets with the first test above or below the 10th percentile.

Additionally, linear regression was used to model these differences by age and gender. The ratio of C-peptide values to the insulin values was calculated and compared by use of the paired t-test. All tests of significance were two tailed. Statistical analyses were performed using SAS software (SAS Institute, Cary, NC) and conducted by the Data Monitoring Unit (DMU) of the DPT-1 located at the University of South Florida, Tampa, FL.

Slight variations in sample sizes were due to incomplete data sets for some individuals and to the use of an abbreviated IVGTT protocol early in the study.

RESULTS

Difference between the first and second tests

The percentage of subjects whose first test result was lower than the second by any amount is shown in table 1. Over 50% of subjects demonstrated a first test response lower than the second test for all insulin and C-peptide measurements, but with significance being observed for insulin measurements only (1+3min minus basal $p = 0.027$, AUC $p = 0.049$ and AUC minus basal $p = 0.002$). Average results for each variable for test 1 and test 2 are shown in table 2. Significant differences were seen in the 1+3min minus basal ($p = 0.017$) and AUC minus basal (0.030) statistic for average C-peptide measures.

Subgroup Analysis

To address whether these differences were seen preferentially in certain groups, correlations and comparisons were performed. No correlation was found between age ($p = 0.105$) or gender ($p = 0.979$) and IVGTT response.

Table 3 compares the insulin and C-peptide responses of subjects with a first test $< 10^{\text{th}}$ percentile on 1+3min insulin and table 4 compares those with a first test $< 10^{\text{th}}$ percentile. Approximately 25% of subjects had their first test $< 10^{\text{th}}$ percentile for insulin 1+3min (table 3). For all insulin (and C-peptide) measures, the differences between the first and second tests were significant for those who had a first test response $< 10^{\text{th}}$ percentile. The second test results were $< 10^{\text{th}}$ percentile in 37–41 percent of subjects (i.e. failed to confirm $< 10^{\text{th}}$ percentile). Whereas, in subjects whose first test was $< 10^{\text{th}}$ percentile, no difference was found for either C-peptide or insulin between first and second tests in the whole group, with approximately 91% of subjects confirming at $< 10^{\text{th}}$ percentile. The remaining participants (~9%) whose first test was $< 10^{\text{th}}$ percentile, failed to confirm on the second test, and these tests were significantly different for 1+3min, insulin AUC, and C-peptide AUC, at p-values of < 0.0001 , 0.0002, and 0.001 respectively (data not shown).

Figures 1 and 2 show the average insulin and C-peptide levels respectively during the IVGTT for all subjects and for those with a first test response below or above the 10th percentile. The second test response is consistently higher than the first for those with a first test < 10th percentile and similar between tests for those with a first test response ≥ 10th percentile as well as for the entire group. Figure 3 shows the 1+3min insulin percentiles in all subjects with a first test < 10th percentile. The second test was equal to or above the 10th percentile in 37/99 (37%) subjects.

β-cell secretion or hepatic uptake

Finally, to investigate whether the differences seen were due to variation in β-cell secretion or hepatic uptake, C-peptide to: insulin ratios were compared between IVGTTs. C-peptide: insulin 1+3min ratios were not significantly different between first and second IVGTTs (3.321 vs. 3.127, respectively; $p=0.2284$).

DISCUSSION

In our study, more than half of all subjects were found to have first test values lower than the second (Table 1). This was true for all measures of both insulin and C-peptide and the difference in frequency was significant for insulin measures corrected for basal and for insulin AUC, but not for C-peptide measures. In the whole group, C-peptide AUC minus basal and 1+3min minus basal were significantly lower in the first test compared with the second test. Thus, a measurable difference is appreciated. Though for the group as a whole, this difference is relatively small (Table 2), for some people it was quite large. For the 10% of subjects with the greatest change between tests, for example, the average change in the 1+3min value for insulin was 958.17 pmol/L and for C-peptide was 1481.00 pmol/L. Thus, these differences can cause a change in an individual's eligibility for entry into a study or treatment protocol. In subjects with a first test < 10th percentile, 37–41% failed to confirm on the second test. In subjects with a first test ≥ 10th percentile, over 90% confirmed on the second test.

When subjects are divided by their response to the first test, those whose initial response was less than the 10th percentile showed significant differences between tests for all insulin and C-peptide measures, while those whose response was ≥ 10th percentile did not show significant differences between tests for any insulin or C-peptide measure (p values range from 0.2156 to 0.8508). This occurred despite the larger sample size for the group of subjects who were not initially low responders ($n=269$) compared to the group of subjects who were low initial responders ($n=99$). Although not likely the case since the two IVGTTs were separated by a maximum of 6 months, the significant drop in some of the insulin and C-peptide measures in initial responders who failed to confirm on the second test could be explained by a progression towards development of diabetes.

One possible explanation for this significant difference in response among the initial poor responders is regression towards the mean. In other words, those selected on the basis of low values on the first test would be expected to have higher values on the next test. Alternatively, stress associated with the first test could be another explanation for our findings. The first test may be more stressful since the subject has never had the procedure

before and does not know what to expect, and this stress might cause a low response to the first test [8, 9]. Familiarity with the procedure the second time might result in subjects being more relaxed. Unfortunately, we do not have data to document that the first test in our study was more stressful. A future direction would be to incorporate methods to measure stress during these procedures, such as standardized stress surveys, catecholamine or cortisol responses. Additionally, it would be interesting to explore whether insulin secretion in oral glucose or mixed meal tolerance testing has a similar first test effect.

Several models have been employed to investigate the effects of stress and stress hormones on β -cell and hepatic function. It is known that insulin influences the release of norepinephrine, thus indirectly affecting sympathetic nervous activity in a dose-dependent fashion [19]. Reciprocally, norepinephrine influences the release of insulin from the pancreas [19]. Epinephrine, another “stress hormone”, also suppresses insulin secretion, but is controlled independently. Both norepinephrine and epinephrine inhibit insulin secretion via the activation of α_2 -adrenergic receptors in the β -cell membrane [20]. In a study by Allen et al [9], the 1+3min insulin was found to be significantly higher on the second test in nine of 11 patients who underwent two IVGTTs, one to three weeks apart ($p < 0.05$). One 11-year-old boy in this study had a vasovagal episode prior to his first IVGTT and his 1+3min insulin was 2 μ U/ml. His epinephrine value at the time was 530 pg/ml, approximately 10 times the upper limit of normal (55 pg/ml). During a follow up IVGTT 3 weeks later; his 1+3min insulin was 78 μ U/ml. In patients with T1DM diabetes mellitus, acute psychological stress induced by mental arithmetic and public speaking and witnessed by significant increases in heart rate, blood pressure, plasma catecholamine and cortisol levels, has been shown to reduce basal insulin measurements [21]. Thus, the literature supports the idea that insulin secretion may be impaired during a stressful first test and could improve when the subject returns for a presumably less stressful second test.

Peripheral insulin levels can also be greatly affected by variations in hepatic insulin uptake. This is because insulin secreted from the pancreas immediately enters the portal venous system, traversing the liver before reaching the general circulation. This anatomic arrangement allows for the first pass hepatic extraction which averages 50% of the secreted insulin [14, 22, and 23]. Variation in this extraction is dependent on a number of variables including insulin concentration in the portal vasculature, obesity which decreases hepatic uptake [12], hormonal control [22, 24] and increased arginine level [12].

In order to determine whether the data presented here reflect changes in insulin and c-peptide secretion or changes in hepatic insulin uptake, comparing the peripheral C-peptide and the peripheral insulin responses is helpful. C-peptide is used because it is secreted with insulin in equimolar amounts, but its hepatic extraction is negligible and its clearance is constant within individuals, even through varied metabolic states [6]. Because a first test effect was observed with C-peptide levels, and because C-peptide levels are not affected by hepatic uptake, it can be concluded that there is at least some change in β -cell secretion contributing to the first test effect seen here.

To determine whether there was a first test effect on hepatic insulin uptake, it is useful to compare the ratios of C-peptide to insulin observed in the first and second tests. As

mentioned above, a change in this ratio between tests would imply a change in hepatic extraction while an unchanged ratio would imply that any difference was the result of a change in β -cell secretion. No significant change occurred in this ratio between the first and second tests strongly suggesting that the first test effect is primarily due to differences in insulin secretion. This finding is supported by the research of Ishida, et al who found that the stress of anesthesia and surgery does not significantly change the basal hepatic extraction of insulin in the dog [12].

Regardless of the cause of the difference in insulin responses to the first versus the second IVGTT, it is important to note that there appears to be a difference frequently large enough to impact an individuals' eligibility for entry into T1DM prevention clinical trials and therefore, affect study outcomes. This is especially important since an impaired insulin response to the IVGTT is a risk factor for future T1DM development. Thus, it is important for these trials to be designed in a way that will minimize any bias a first test effect might introduce. In particular, the decreased insulin response many people appear to have to an initial IVGTT is likely to inappropriately increase the number of people eligible for trials that require low β -cell function for entry if confirmation of the low response is not obtained and misleadingly show a strong therapeutic/preventative response for an agent that could be later approved for diabetes prevention in high risk populations.

In our study, as shown in table 1, approximately one third of those subjects with a low first phase insulin secretion on their initial IVGTT failed to confirm on their second test. Therefore, it is important to confirm a low first test with a second IVGTT, even if this may appear to be more costly on the outset, but should eventually prove to be more cost effective, as it allows separation of those whose low response to the first IVGTT may reflects a first test effect rather than markedly impaired insulin secretion due to the T1DM disease process. In addition to avoidance of prevention study outcome result bias, confirming a low response to the initial IVGTT saves some (one third) individuals from being unnecessarily subjected to the possible harmful side effects of prevention medications, and the stress of being considered at risk for diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Average insulin levels $\pm 2SE$ with respect to time for all subjects (A), for those with first test less than 10th percentile (B) and for subjects with first test greater than 10th percentile (C)

Figure 2.

Average C-peptide levels $\pm 2SE$ with respect to time for all subjects (A), for those with first test less than 10th percentile (B) and for subjects with first test greater than 10th percentile (C)

Figure 3.
1+3min insulin percentiles in all subjects with a first test < 10th percentile (n= 99)

Table 1

Percentage of subjects with first test less than the second test

		% With test 1< test 2	N	P
Insulin	1+3	53.50%	368	0.1152
	1+3-basal	55.20%	368	0.0274
	AUC	55.10%	356	0.0491
	AUC-basal	58.10%	356	0.0020
C-peptide	1+3	52.40%	359	0.1639
	1+3-basal	52.40%	359	0.2429
	AUC	53.30%	351	0.2402
	AUC-basal	51.90%	351	0.5219

Table 2

Average results for each measure of insulin secretion for the first and second IVGTTs

		Test 1 Avg pmol/L	SD	Test 2 Avg pmol/L	SD	P	N
Insulin	1+3	833.73	772.50	867.63	744.45	0.2846	368
	1+3-basal	740.48	741.58	775.72	714.14	0.2574	368
	AUC	3446.50	3124.00	3524.40	2808.60	0.4615	356
	AUC-basal	2509.50	2797.20	2608.90	2486.30	0.3230	356
C-peptide	1+3	2268.80	1496.60	2348.80	1430.90	0.0744	359
	1+3-basal	1848.50	1323.60	1943.20	1261.40	0.0177	359
	AUC	10910.00	6739.00	11119.00	6416.90	0.2987	351
	AUC-basal	6696.50	5190.30	7048.80	4838.80	0.0309	351

Table 3

Comparison of first and second tests for subjects whose first test was less than the 10th percentile

		1 st test < 10 th percentile			
		Test 1 Avg pmol/L	Test 2 Avg pmol/L	P	% with second test < 10 th percentile
Insulin	1+3	260.95	387.30	<0.0001	37.37%
	1+3-basal	201.65	320.99	<0.0001	37.37%
	AUC	1166.60	1622.90	<0.0001	38.54%
	AUC-basal	567.82	959.76	<0.0001	38.54%
C-peptide	1+3	1056.00	1216.20	0.0124	39.36%
	1+3-basal	776.44	914.83	0.0088	39.36%
	AUC	5246.70	6038.40	0.0076	40.66%
	AUC-basal	2435.00	2979.40	0.0099	40.66%

Table 4

Comparison of first and second tests for subjects whose first test was greater than the 10th percentile

		1 st test 10 th percentile			
		Test 1 Avg pmol/L	Test 2 Avg pmol/L	P	% with second test 10 th percentile
Insulin	1+3	1044.50	1044.40	0.9975	91.08%
	1+3-basal	938.78	943.07	0.9177	91.08%
	AUC	4288.40	4226.50	0.6600	90.77%
	AUC-basal	3226.40	3217.80	0.9488	90.77%
C-peptide	1+3	2699.10	2750.50	0.3608	91.32%
	1+3-basal	2228.70	2308.00	0.1190	91.32%
	AUC	12893.00	12897.00	0.9859	91.15%
	AUC-basal	8188.00	8473.10	0.1701	91.15%