Arginine is preferred to glucagon for stimulation testing of β-cell function

R. Paul Robertson,1 Ralph H. Raymond,2 Douglas S. Lee,3 Roberto A. Calle,3 Atalanta Ghosh,4 Peter J. Savage,5 Sudha S. Shankar,6 Maria T. Vassileva,7 Gordon C. Weir,8 and David A. Fryburg,9
Beta Cell Project Team of the Foundation for the NIH Biomarkers Consortium
1Pacific Northwest Diabetes Research Institute, Seattle, Washington; 2 R-Squared Solutions, Skillman, New Jersey; 3 Pfizer, Incorporated, Groton, Connecticut; 4 Janssen Research and Development, Raritan, New Jersey; 5 National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland; 6 Eli Lilly and Company, Indianapolis, Indiana; 7 Foundation for the National Institutes of Health, Bethesda, Maryland; 8 Joslin Diabetes Center, Boston, Massachusetts; and 9 ROI BioPharma Consulting, East Lyme, Connecticut

Submitted 24 March 2014; accepted in final form 11 August 2014

Robertson RP, Raymond RH, Lee DS, Calle RA, Ghosh A, Savage PJ, Shankar SS, Vassileva MT, Weir GC, Fryburg DA: Beta Cell Project Team of the Foundation for the NIH Biomarkers Consortium. Arginine is preferred to glucagon for stimulation testing of β-cell function. Am J Physiol Endocrinol Metab 307: E720–E727, 2014. First published August 26, 2014; doi:10.1152/ajpendo.00149.2014.—A key aspect of research into the prevention and treatment of type 2 diabetes is the availability of reproducible clinical research methodology to assess β-cell function. One commonly used method employs nonglycemic secretagogues like arginine (arg) or glucagon (glgn). This study was designed to quantify the insulin response to arg and glgn and determine test repeatability and tolerability. Obese overnight-fasted subjects with normal glucose tolerance were studied on 4 separate days: twice using arg (5 g iv) and twice with glgn (1 mg iv). Pre- and postinfusion samples for plasma glucose, insulin, and C-peptide were acquired. Arg and glgn challenges were repeated in the last 10 min of a 60-min glucose (900 mg/min) infusion. Insulin and C-peptide secretory responses were estimated under baseline fasting glucose conditions (AIRarg and AIRglgn) and hyperglycemic (AIRargMAX AIRglgnMAX) states. Relative repeatability was estimated by intra-assay correlation coefficient (ICC). Twenty-three (12 men and 11 women) subjects were studied (age: 42.4 ± 8.3 yr; BMI: 31.4 ± 2.8 kg/m²). Geometric means (95% CI) for baseline-adjusted values AIRarg and AIRglgn were 84 (75–95) and 102 (90–115) μU/ml, respectively. After the glucose infusion, AIRargMAX and AIRglgnMAX were 395 (335–466) and 483 (355–568) μU/ml, respectively. ICC values were >0.90 for AIRarg and AIRglgnMAX. Glucagon ICCs were 0.83, 0.34, and 0.36, respectively, although the exclusion of one outlier increased the latter two values (to 0.84 and 0.86). Both glgn and arg induced mild adverse events that were transient. Glucagon, but not arginine, induced moderate adverse events due to nausea. Taken together, arginine is preferred to glucagon for assessment of β-cell function.

Arginine; glucagon; β-cell function; insulin secretion

There is considerable interest in the development of novel therapies that target the β-cell, especially agents or interventions that can potentially augment insulin secretion. Interrogation of treatment effects on β-cell function during the early and later phases of drug development require clinical methodologies that are both scientifically reliable and operationally feasible. Furthermore, there remains an unmet need for techniques that assess disease progression and β-cell preservation, in particular those that can be used feasibly both in a controlled experimental setting as well as in large population-based trials.

Studies of β-cell function have often used arginine and glucagon as stimulators of insulin and/or C-peptide secretion. Although the insulin secretory responses to arginine and glucagon are very similar, their mechanisms of action are different. Arginine is positively charged, which leads to depolarization of β-cells when it is taken up by cationic amino acid transporters (8). Depolarization opens voltage dependent calcium channels that allow entry of calcium into the cell, which then triggers insulin secretion. Glucagon stimulates insulin secretion by acting on both glucagon and glucagon-like peptide 1 receptors on β-cells (21). The important subsequent mechanism appears to be activation of adenyl cyclase to produce cyclic AMP, which can stimulate secretion through two pathways, the first being protein kinase A and the second being the CAMP-binding protein cAMP-guanine nucleotide exchange factor II (GEFII or Epac 2) interacting with Rim2, a target of the small G protein Rab3 that can mediate cAMP-dependent, PKA-independent exocytosis (16). Complicating interpretation of action at a cellular level is the well-recognized observation that systemically administered arginine also stimulates endogenous glucagon secretion (23). Thus, there may be direct as well as indirect effects of arginine on the β-cell.

In a clinical setting, these stimuli are given intravenously to elicit an acute insulin (AIR) or C-peptide response (ACR), which is the increment in insulin or C-peptide over their baseline values that occurs within 2–5 min after the intravenous injection (1, 4, 13, 14, 20, 27–32, 34, 37–39). These two stimuli have a major advantage over the use of intravenous glucose in that the acute insulin response to glucose disappears after fasting glucose levels exceed ~115 mg/dl (3). Glucose-induced insulin secretion may also undergo reversible failure that may be relatively independent of existing β-cell mass since a short period of treatment can lead to partial recovery of β-cell function, especially in prediabetes or early type 2 diabetes mellitus (11, 40).

Both arginine and glucagon are potentially valuable tools to determine AIRs and ACRs during studies of natural history of β-cell failure as well as during longitudinal studies of efficacy of drug therapy or other interventions for diabetes. However, with rare exceptions (32), the repeatability of AIRs and ACRs in response to arginine and glucagon has not been rigorously determined. A robust characterisation of the intrasubject variability for each of these two tests is a missing critical component to enable their use to assess the impact of an experimental therapy. Important additional issues are whether the use of
normal glucose tolerance as determined by oral glucose tolerance test.

### RESEARCH DESIGN AND METHODS

In this study, obese, otherwise healthy men and women in the age range of 30–65 yr and with BMI values of 27–40 kg/m² were recruited for the study, with the goal to achieve approximately equal recruitment (≥10 subjects) for each sex. The study protocol was approved by a central institutional review board (IRB; Integ Review, Austin, TX). All subjects reviewed and signed the IRB-approved informed consent document prior to participation in the study. All subjects were screened in a two-stage manner. At the first screening visit, medical history, physical examination, and overnight-fasted screening laboratory studies were performed. Subjects whose screening values of fasting glucose were <100 mg/dl were subjected to a 75-g oral glucose tolerance test and were included in the study only if their 2-h plasma glucose value was <140 mg/dl.

**Study design.** All subjects were admitted to the clinical research unit (ICON Development Solutions, San Antonio, TX) on two separate occasions, with admission occurring the night prior to the study procedures. During both admissions, subjects were given either arginine or glucagon stimulation tests in random order. During the second admission, which took place within 4 wk of the first, the order of arginine or glucagon was reversed from the first admission. All testing was conducted after an overnight (~12 h) fast. Each subject completed two arginine stimulation tests and two glucagon stimulation tests.

**Arginine stimulation test.** This protocol was taken from that published by Teuscher et al. (35). After baseline samples were drawn for glucose, insulin, and C-peptide at −10, −5, and 0 min, an intravenous injection of 5 g of arginine (given as 50% arginine HCl) was administered over 30 s, with time 0 set halfway through the arginine injection. Samples for plasma glucose, insulin, and C-peptide were collected from the contralateral arm at 2, 3, 4, 5, 7, and 10 min after the arginine injection. Immediately following the 10-min sample, glucose levels were elevated by a 60-min continuous infusion of glucose (as D20W) at a rate of 900 mg/min. Samples for glucose only were taken at 15, 30, and 45 min after the glucose infusion was begun. Then, at 50, 55, and 60 min, samples for glucose, insulin, and C-peptide were drawn. Immediately after the 60-min sample, 5 g of arginine was again administered intravenously over 30 s. Samples for glucose, insulin, and C-peptide were obtained at 62, 63, 64, 65, 67, and 70 min. Glucose was measured at 90 min to ensure that the subject’s value was returning to baseline. Following the return of circulating glucose to nearly normal levels, the intravenous lines were removed. Glucagon was also measured in the first of each of the arginine tests. Samples were collected in P800 tubes (Becton-Dickinson, Franklin Lakes, NJ). Glucagon stimulation test. The glucagon stimulation test was conducted as described for the arginine stimulation test, with the exception that 1 mg of glucagon (Eli Lilly, Indianapolis, IN) was given intravenously at times 0 and 60 instead of arginine.

**Safety monitoring.** All study participants were assessed by the clinical research site staff for adverse events. The severity of each adverse event was assessed at onset by a nurse and/or physician. The following guidelines were used to assess severity: mild (awareness of

---

**Table 1. Demographics of participating subjects**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Height, m</th>
<th>Weight, kg</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means ± SD</td>
<td>42.4 ± 8.3</td>
<td>1.66 ± 0.1</td>
<td>86.5 ± 15.2</td>
</tr>
<tr>
<td>Range</td>
<td>30–57</td>
<td>1.49–1.83</td>
<td>65–125</td>
</tr>
</tbody>
</table>

Values are means ± SD; 23 obese men (n = 12) and women (n = 11) with normal glucose tolerance as determined by oral glucose tolerance test.

---

Fig. 1. Plasma glucose levels during arginine-induced insulin secretion and glucagon-induced insulin secretion studies. **Left:** the first intravenous pulse of arginine (5 g) was given at time 0; the continuous glucose infusion was started at 20 min, and at 80 min the second pulse of arginine was given. **Right:** glucagon (1 mg) was given instead of arginine. In both cases the target glucose level of ~300 mg/dl was reached in 60 min, although the values were slightly higher after the glucagon pulses.

---

**AJP-Endocrinol Metab** • doi:10.1152/ajpendo.00149.2014 • www.ajpendo.org
signs or symptoms but easily tolerated), moderate (discomfort enough to cause interference with usual activity), and severe (incapacitating with inability to work or do usual activity).

The relationship of an adverse event to a procedure undertaken in this protocol was classified using the World Health Organization criteria (5) as certain, probable, possible, unlikely, conditional/unclassified, not assessable/unclassifiable, or not related.

Assays. Samples from both the arginine stimulation tests and glucagon stimulation tests were collected in EDTA-containing tubes and assayed in the Immunochromal Core Laboratory, Mayo Clinic (Rochester, MN). Plasma glucose was measured enzymatically (Roche Cobas e311). Plasma insulin and C-peptide were measured by immunoassay (Cobas e411 Automated Immunoassay; Roche). Glucagon was measured by a commercial ELISA (Millipore).

Statistical methods. All derivation of parameters and statistical analyses were performed on a desktop with Windows 7 Ultimate (2009 Microsoft) using the R programming language version 2.15.1 (2012-06-22) with 64-bit processing capability. Baseline demographics and anthropometrics were summarized by mean, standard deviation (SD), and range (minimum to maximum). Frequency and severity of adverse events were tabulated by type of event.

The AIRs and ACRs at baseline glucose (AIRarg, ACRarg, AIRglgn, and ACRglgn) and during hyperglycemic levels (AIRargMAX, ACRargMAX, AIRglgnMAX, and ACRglgnMAX) were calculated as the difference between the baseline and the mean of the three highest insulin and C-peptide readings taken at 2, 3, 4, and 5 min after arginine or glucagon stimulation (35). The baseline insulin and C-peptide levels immediately prior to stimulation by arginine or glucagon were calculated as the mean of the −10-, −5-, and 0- or 50-, 55-, and 60-min time points for basal and hyperglycemic conditions, respectively, by subject and study visit. AIR and ACR values were corrected by subtracting baseline values from their respective AIR and ACR values. Insulin secretory reserve (ISRes) was calculated as the difference between ACRMAX and ACR values. The use of the term AIRMAX is not intended to imply that this is the absolute maximal insulin secretion that can be elicited. Clearly, the addition of other insulin secretagogues might augment insulin secretion further. Rather, the term is to reference the effect of arginine in the context of this methodology. The same consideration also applies to glucagon.

Across subjects, insulin and C-peptide values for all parameters were reviewed by distribution plots and summary statistics to assess distribution assumptions and the identification of potential influential outliers. If determined to be log-normally distributed, then the natural log of the parameters was calculated prior to statistical analyses. Any outliers initially identified were further tested using the Grubbs test for a single outlier at the two-sided 0.05 level of significance. Sensitivity analyses were performed, including all data and excluding identified outliers. Subjects assessed had values for both study visits (matched pairs).

To determine whether between- and within-subject variance components differed by sex, AIR, AIRMAX, and ISRes for insulin values and ACR, ACRMAX, and CPSRes for C-peptide values were modeled using a mixed-effect ANOVA, treating sex difference as a fixed effect, subjects grouped by sex as a random effect, and visits as a repeated effect. To test whether between- and within-subject variances differed statistically by sex difference, log likelihood ratio tests were used to select among a full model assuming separate between- and within-subject variances by sex, a semireduced model assuming separate between-subject variances by sex with a common within-subject variance across sexes, and a fully reduced model assuming no evidence for sex differences with respect to between- and within-subject variances. All log likelihood tests were performed at the two-sided 0.05 significance level. As parameterized, the model automatically accounts for any sex differences in mean response with simultaneous estimation of between- and within-subject variance components.

Using the estimated variance components obtained separately for each stimulation test parameter, the intraclass correlation coefficient

Fig. 2. Plasma insulin levels during arginine-induced insulin secretion and glucagon-induced insulin secretion stimulation tests. Both arginine (left) and glucagon (right) elicited acute insulin responses (AIRarg and AIRglgn) before the glucose infusions that were potentiated by the glucose infusions (AIRargMAX and AIRglgnMAX).

AJP-Endocrinol Metab • doi:10.1152/ajpendo.00149.2014 • www.ajpendo.org
ICC was calculated as \( \frac{\sigma_{\text{between}}}{\sigma_{\text{between}} + \sigma_{\text{within}}} \). The ICC is a measure of the degree to which repeated measures within the same subject resemble each other (a measure of relative repeatability of a measure). Estimated values for ICC fall between 0 and 1, with higher values indicating greater repeatability of a measure (10).

Point estimates of the geometric mean response with 95% confidence bounds after exponentiation from the analysis on the natural log scale and geometric coefficients of variation corresponding to the between- and within-subject variance components were derived from the fitted model for the log-normally distributed results.

The correlation between parameters within a stimulation test and across tests was estimated with the Pearson’s correlation coefficient unless major departures from normality (Shapiro-Wilk statistic < 0.9) for either or both parameters were detected. In this case, the correlation was estimated by the Spearman’s rank correlation coefficient.

RESULTS

Demographics. This study enrolled 23 obese subjects: 12 men (52%) and 11 women (48%) with normal glucose tolerance. The mean (SD) age was 42 (8.3) yr, and the mean (SD) BMI was 31.4 (3.0). Complete summaries of baseline demographics are provided in Table 1.

Arginine- and glucagon-induced insulin and C-peptide secretion. Figure 1 summarizes the glucose time courses during the experiment. In response to the glucose infusion, plasma glucose concentrations rose from 93 mg/dl at baseline to 265 mg/dl after the glucose infusion. Figures 2 and 3 illustrate the arginine- and glucagon-stimulated insulin and C-peptide secretion under both the euglycemic and hyperglycemic states. As can be seen in the figures, both secretagogues elicited brisk and readily detectable responses. Figure 4 summarizes the glucagon response after arginine injection. Glucagon was not measured after the glucagon injection, as the resulting concentrations would have been far higher than the validation of the assay.

All insulin and C-peptide parameters were determined to be log-normally distributed. One female subject had an extreme outlier value for one visit of the glucagon stimulation test \( (P < 0.001) \). The log likelihood ratio test comparing ANOVA models indicated no statistical difference \( (P \geq 0.05) \) when the full model, the semireduced model, and the fully reduced model were compared, indicating no evidence for sex differences with respect to between- and within-subject variances, with the exception of glucagon stimulation values \( \text{AIRglnMAX, ISRes, and ACRglnMAX,} \) which were heavily influenced by the single outlier. This outlier caused a significant increase in variance estimates and, as such, a decrease in calculated ICCs. A sensitivity analysis assessing these three parameters, excluding the outlier subject, showed no statistical significance, indicating common between- and within-subject variances with respect to sex difference. Variance and ICC estimates for these three values are presented with and without the outlier subject included in the analyses. All variance component results and ICC values presented were derived on the basis of the fully reduced ANOVA model.

Table 2 summarizes the insulin secretory responses to arginine and glucagon. In the euglycemic fasted state, arginine and glucagon increased insulin secretion to approximately nine- to 10-fold over the baseline value; these responses were augmented in the hyperglycemic state. Likewise, in the baseline glucose state, both arginine and glucagon stimulated significant increases in C-peptide secretion. These are summarized in Table 3. The choice of either insulin or C-peptide to quantify insulin secretion did not

![Fig. 3. Plasma C-peptide levels during arginine-induced C-peptide secretion and glucagon-induced C-peptide secretion stimulation tests. Both arginine (left) and glucagon (right) elicited acute C-peptide responses (ACRarg and ACRgln) before the glucose infusions that were potentiated by the glucose infusions (ACRargMAX and ACRglnMAX).](image)
matter; the correlations between AIRarg and ACRarg, as well as AIRargMAX and ACRargMAX, are >0.92 and >0.95, respectively.

Within-subject variability (σwithin) generally accounted for a small portion of the total variability (σbetween + σwithin), and as such, repeatability (ICC) of measures was estimated to be high. Using arginine, ICC values were 0.91, 0.91, and 0.90 for AIRarg, AIRargMAX, and arginine ISRes, respectively. Using glucagon, ICC values were 0.83, 0.34 (0.87, excluding outlier subject, \(P < 0.001\)), and 0.36 (0.86, excluding outlier subject, \(P < 0.001\)) for AIRglgn, AIRglgnMAX, and ISRes, respectively. Complete results of the insulin analyses results are presented in Table 2.

Comparable results were observed for C-peptide values with estimated ICCs of 0.85, 0.86, and 0.83 for ACRarg, ACRargMAX, and CPSRes, respectively. Likewise, glucagon stimulation test ICC values were 0.83, 0.33 (0.84, excluding outlier subject, \(P < 0.001\)), and 0.72 for ACRglgn, ACRglgnMAX, and CPSRes, respectively. Complete results of the C-peptide analyses results are presented in Table 3. All within-subject stimulation tests and across-subjects test correlations for insulin and C-peptide values were high and statistically significant. Results of the correlation analyses are presented in Table 4.

Adverse events. Table 5 summarizes the frequency of significant adverse events reported during the study. A greater number of subjects who received arginine experienced mild flushing and perioral paresthesias compared with the subjects who received glucagon, whereas a greater number of subjects receiving glucagon experienced mild nausea and dizziness (Table 5). No subjects receiving arginine experienced any moderate symptoms, whereas three subjects receiving glucagon experienced moderate nausea without vomiting.

Fig. 4. Plasma glucagon concentrations during arginine-induced insulin secretion. Arginine-stimulated glucagon responses both in the baseline normoglycemic state and during hyperglycemia. These responses were lower during hyperglycemia, which is consistent with the known ability of hyperglycemia to dampen glucagon secretion.

Table 2. Summary statistical analyses and ICC of insulin parameters

<table>
<thead>
<tr>
<th></th>
<th>Arginine Stimulation Test</th>
<th>Glucagon Stimulation Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AIRarg</td>
<td>AIRargMAX</td>
</tr>
<tr>
<td>Insulin, (\mu U/ml)*</td>
<td>84 (75, 95)</td>
<td>395 (335, 466)</td>
</tr>
<tr>
<td>ICC**</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>CV between subjects, %</td>
<td>38.9</td>
<td>57.5</td>
</tr>
<tr>
<td>CV within subjects, %</td>
<td>11.6</td>
<td>16.6</td>
</tr>
</tbody>
</table>

*AIR, acute insulin response; arg, arginine; ISR, insulin secretory reserve; glgn, glucagon; ICC, intraclass correlation coefficient; CV, coefficient of variation. **Because responses are log-normally distributed, all results are summarized by geometric means (95% confidence interval). ***Larger CV values (and lower ICC) driven by 1 subject for AIRglgnMAX and SecRSRV ICC. Values in brackets reflect removal of that subject.
DISCUSSION

This study was performed to characterize and compare the safety, tolerability, and performance of arginine and glucagon in repeated measures to assess β-cell function. Both fasted and glucose-potentiated states were used to assess acute insulin and C-peptide responses to arginine and glucagon. The primary outcome was the repeatability or, conversely, the variance in these parameters assessed in a test-retest paradigm, which would estimate the variability that could be encountered with repeat testing within and between subjects in a therapeutic trial. Overall, the results with arginine generally showed higher repeatability. The side effects with arginine were expected and caused mild discomfort. However, glucagon was associated with moderate levels of nausea in several subjects.

It is noteworthy that one test in one subject significantly impacted the estimated within-subject repeatability for glucagon. That extreme outlier was for visit 2 only; for visit 1, the subject’s value was well within the range of observations for the present study, which reports 92 tests with arginine and a greater number of within-subject replications to limit outlier results. To demonstrate this, we would need more repeat testing within and between subjects in a therapeutic trial. Nevertheless, it is not the one outlier result that really dissuades against the use of glucagon; it is the adverse event profile. Our intention is to have one of the secretagogues used in multicenter, longitudinal studies. With the observation of vasovagal responses with more severe nausea, it became apparent that the use of glucagon in the populations that we intend to study (patients with diabetes and prediabetes) poses a real risk to the participants. When studying many subjects, the likelihood that a subject might experience a significant decrease in blood pressure (due to the nausea), hypoperfuse a part of the coronary or cerebral circulations, and have an adverse event should be a factor in determining the future use of the test. We recognize that the chance is fairly low, yet since arginine did not yield this outcome in this limited population, we made the decision to proceed with only arginine and not glucagon. We recognize that the event should be a factor in determining the future use of the test.

One major benefit of these studies is that they provide needed information about the repeatability of these tests over time. Sarlund et al. (29) tested 10 nondiabetic and 20 elderly subjects with type 2 diabetes by injecting glucagon (1 mg) intravenously. Blood samples were drawn at baseline and 6 min after the glucagon injection. These tests were performed four times in each subject within 1 mo. No statistically significant differences were observed in baseline, stimulated, or post-stimulated C-peptide levels within the control or diabetic groups. sarlund et al. (29) found no significant differences when comparing the two groups. They concluded that there were no significant differences between the two groups. Given that there are similar responses that correlate well with one another (within subjects), it is highly likely that the arginine test would yield a similar conclusion to the glucagon test.

One major benefit of these studies is that they provide needed information about the repeatability of these tests over time. Sarlund et al. (29) tested 10 nondiabetic and 20 elderly subjects with type 2 diabetes by injecting glucagon (1 mg) intravenously. Blood samples were drawn at baseline and 6 min after the glucagon injection. These tests were performed four times in each subject within 1 mo. No statistically significant differences were observed in baseline, stimulated, or post-stimulated C-peptide levels within the control or diabetic groups. The results from the present study, after removal of the outlier test, are consistent with the work of Sarlund et al. (29) with regard to glucagon.

Table 3. Summary statistical analyses and ICC of C-peptide parameters

<table>
<thead>
<tr>
<th>Parameter Assessment</th>
<th>Correlation Coefficient (P &lt; 0.001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIRarg vs. AIRargMAX</td>
<td>0.90b</td>
</tr>
<tr>
<td>AIRglgn vs. AIRglgnMAX</td>
<td>0.76b</td>
</tr>
<tr>
<td>AIRarg vs. AIRglgn</td>
<td>0.57</td>
</tr>
<tr>
<td>AIRargMAX vs. AIRglgnMAX</td>
<td>0.84b</td>
</tr>
<tr>
<td>AIRargSecRSRV vs. AIRglgnSecRSRV</td>
<td>0.84b</td>
</tr>
<tr>
<td>ACParg vs. ACPargMAX</td>
<td>0.75a</td>
</tr>
<tr>
<td>ACParg vs. ACPglgn</td>
<td>0.68a</td>
</tr>
<tr>
<td>ACParg vs. ACPglgnMAX</td>
<td>0.61a</td>
</tr>
<tr>
<td>ACPargMAX vs. ACPglgnMAX</td>
<td>0.80a</td>
</tr>
<tr>
<td>ACPargSecRSRV vs. ACPglgnSecRSRV</td>
<td>0.80a</td>
</tr>
</tbody>
</table>

ACR, acute C-peptide response; CPSRes, C-peptide secretory reserve. *Because responses are log-normally distributed, all results are summarized by geometric means (95% confidence interval) and CVs. **ICC reflects degree of repeatability of test (values closer to 1 indicate greater repeatability). ***Larger CV values (and lower ICC) driven by 1 subject during 1 visit (outlier test P < 0.001) for ACPglgnMAX ICC. Value in brackets reflects removal of that subject.

Table 4. Between and with maximal stimulation test parameter correlation coefficients

<table>
<thead>
<tr>
<th>Parameter Assessment</th>
<th>Correlation Coefficient (P &lt; 0.001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIRarg vs. AIRargMAX</td>
<td>0.90b</td>
</tr>
<tr>
<td>AIRglgn vs. AIRglgnMAX</td>
<td>0.76b</td>
</tr>
<tr>
<td>AIRarg vs. AIRglgn</td>
<td>0.57</td>
</tr>
<tr>
<td>AIRargMAX vs. AIRglgnMAX</td>
<td>0.84b</td>
</tr>
<tr>
<td>AIRargSecRSRV vs. AIRglgnSecRSRV</td>
<td>0.84b</td>
</tr>
<tr>
<td>ACParg vs. ACPargMAX</td>
<td>0.75a</td>
</tr>
<tr>
<td>ACParg vs. ACPglgn</td>
<td>0.68a</td>
</tr>
<tr>
<td>ACParg vs. ACPglgnMAX</td>
<td>0.61a</td>
</tr>
<tr>
<td>ACPargMAX vs. ACPglgnMAX</td>
<td>0.80a</td>
</tr>
<tr>
<td>ACPargSecRSRV vs. ACPglgnSecRSRV</td>
<td>0.80a</td>
</tr>
</tbody>
</table>

Table 5. Adverse events by severity

<table>
<thead>
<tr>
<th>Parameter Assessment</th>
<th>Correlation Coefficient (P &lt; 0.001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIRarg vs. AIRargMAX</td>
<td>0.90b</td>
</tr>
<tr>
<td>AIRglgn vs. AIRglgnMAX</td>
<td>0.76b</td>
</tr>
<tr>
<td>AIRarg vs. AIRglgn</td>
<td>0.57</td>
</tr>
<tr>
<td>AIRargMAX vs. AIRglgnMAX</td>
<td>0.84b</td>
</tr>
<tr>
<td>AIRargSecRSRV vs. AIRglgnSecRSRV</td>
<td>0.84b</td>
</tr>
<tr>
<td>ACParg vs. ACPargMAX</td>
<td>0.75a</td>
</tr>
<tr>
<td>ACParg vs. ACPglgn</td>
<td>0.68a</td>
</tr>
<tr>
<td>ACParg vs. ACPglgnMAX</td>
<td>0.61a</td>
</tr>
<tr>
<td>ACPargMAX vs. ACPglgnMAX</td>
<td>0.80a</td>
</tr>
<tr>
<td>ACPargSecRSRV vs. ACPglgnSecRSRV</td>
<td>0.80a</td>
</tr>
</tbody>
</table>

ACR, acute C-peptide response; CPSRes, C-peptide secretory reserve. *Because responses are log-normally distributed, all results are summarized by geometric means (95% confidence interval) and CVs. **ICC reflects degree of repeatability of test (values closer to 1 indicate greater repeatability). ***Larger CV values (and lower ICC) driven by 1 subject during 1 visit (outlier test P < 0.001) for ACPglgnMAX ICC. Value in brackets reflects removal of that subject.
This is the first report of the repeatability of the arginine stimulation test in an obese nondiabetic population and of a direct head-to-head comparison of the hormonal responses and tolerability of the arginine and glucagon stimulation tests in both men and women. Results from the above studies on the repeatability of two key methodologies, viz., the arginine and glucagon stimulation tests, add considerable value in the assessment of β-cell function. In particular, these data provide vital information on test characteristics that in turn should support quantitative assessments of changes in β-cell function over time both in the context of the natural history of disease as well as in the setting of novel and existing antihyperglycemic agents. As suggested previously (2, 7, 9, 12, 15, 17, 18, 19, 22, 24, 25, 26, 36), these methods should be useful for assessment of loss or preservation of β-cell functional reserve over time when used in conjunction with or independent of other, more resource-intensive methodologies such as pancreatic imaging (6, 33). Finally, the above results appear to indicate that of the two approaches, stimulation testing using arginine seems to be comparable with that of glucagon while being tolerated better. This would support an argument for arginine as the preferred probe for assessment of maximal insulin secretory response and β-cell function.

An important part of methodological studies such as the present one is to address whether or not there are differences in sex or racial responses. Especially for cross-sectional or unpaired studies, understanding the potential impact of subject selection is critically important in the qualification of an experimental method. In this study, when expressed in whole subject terms, there were no apparent sex differences in the responses to arginine and glucagon with respect to either the repeatability of the test or magnitude of the response. Because the study had a predominance of subjects of Hispanic descent, it is unknown at the present time whether there are racial/ethnic differences in the responses to arginine or glucagon. Our evidence from this small number of subjects of differing backgrounds suggests that there are not significant differences due to race and ethnicity.

In the present study, the insulin secretory responses to arginine and glucagon in the baseline euglycemic state were well correlated with the augmented response observed under hyperglycemic conditions. The correlated response for arginine (baseline fasted glucose to hyperglycemic state) is very similar to that of Teuscher et al. (34) in patients receiving autologous islet transplants. In that study, the basal glucose state correlated highly \((r = 0.97)\) with the glucose-potentiated state. Because the number of islets was quantified before transplantation, the investigators had direct means to analyze the relationship between the arginine responses and the number of transplanted islets. There were clear, close correlations between the responses to arginine alone or glucose-potentiated arginine and transplanted islet mass. Therefore, differences among subject responses in the present study could reflect differences in islet mass and provide an important estimation of relative islet mass for long-term studies. Similarly to the insulin responses to arginine, the responses to glucagon in the basal normoglycemic state were highly correlated with the glucagon response in the hyperglycemic state. This latter notion is also supported in the present study by the within-subject correlations between the responses to arginine and glucagon. Complicating interpretation of the response to arginine, at least in part, is that arginine also stimulates the secretion of glucagon in the present study as well as in a study reported elsewhere (23). Nevertheless, across stimuli, the β-cell responds in a consistent fashion to these stimuli.

In conclusion, arginine and glucagon provide highly repeatable tests of β-cell function. Because of differences in tolerability, we conclude that arginine is the preferred stimulus and can be used in multicenter trials.

ACKNOWLEDGMENTS

This project reflects the collective work of the Beta Cell Project Team of the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium. Present members of the Beta Cell Project Team: Dr. Richard Bergman, Cedars-Sinai Diabetes & Obesity Research Institute; Dr. Roberto Calle, Pfizer; Dr. Charlie Cao, Takeda; Dr. Danny Chen, Pfizer; Dr. Claudio Cobelli, University of Padua; Dr. Mark Deeg, Eli Lilly & Co.; Dr. Christian Djurhuus, Novo Nordisk; Dr. Mark Farnen, Eli Lilly & Co.; Dr. David Fryburg, FNIH, ROI BioPharma Consulting; Dr. Atalanta Ghosh, Johnson & Johnson; Dr. Ilan Irony, Center for Drug Evaluation and Research/Food and Drug Administration; Dr. David Kelley, Merck; Dr. Douglas Lee, Pfizer; Dr. Lori Mixson, Merck; Dr. Stephanie Moran, Takeda; Dr. Dave Polidori, Johnson & Johnson; Dr. Jessica Ratay, FNIH; Ralph Raymond, FNIH; Dr. R. Paul Robertson, Pacific Northwest Diabetes Research Institute; Dr. Hartmut Ruette, Sanofi; Dr. Peter Savage, National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases (NIH/NIDDK); Dr. Sudha Shankar, Eli Lilly & Co.; Dr. Myrline Staten, NIH/NIDDK; Dr. Darko Stefanovski, Cedars-Sinai Diabetes & Obesity Research Institute; Dr. Maria Vassileva, FNIH; Dr. Adrian Vella, Mayo Clinic; Dr. Gordon Weir, Joslin Diabetes Center; Dr. Adrienne Wong, JDRF; Dr. Marjorie Zakaria, Novartis.

Previous members of Beta Cell Project Team who contributed to this work: Dr. Richard Chen, NIH/NIDDK; Dr. Ying Ding, Eli Lilly & Co.; Dr. Cong Han, Takeda; Dr. David Maggs, formerly of Amylin; Dr. Mads Rasmussen, Novo Nordisk; Dr. Thomas Strack, formerly of Takeda; Dr. Krystyna Tatarkiewicz, formerly of Amylin.

DISCLOSURES

None of the authors report any conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS


REFERENCES


