Outpatient versus inpatient mixed meal tolerance and arginine stimulation testing yields comparable measures of variability for assessment of beta cell function

Sudha S. Shankar\textsuperscript{a}, Douglas S. Lee\textsuperscript{b}, Ralph H. Raymond\textsuperscript{c}, Roberto A. Calle\textsuperscript{b}, Claudio Cobelli\textsuperscript{d}, Atalanta Ghosh\textsuperscript{e}, R. Paul Robertson\textsuperscript{f}, Hartmut Ruetten\textsuperscript{g}, Myrlene A. Staten\textsuperscript{h}, Darko Stefanovski\textsuperscript{i}, Adrian Vella\textsuperscript{j}, Sanya Whitaker\textsuperscript{k}, David A. Fryburg\textsuperscript{l},\textsuperscript{*} On behalf of the Foundation for the NIH Biomarkers Consortium Beta Cell Project Team

\textsuperscript{a} Eli Lilly and Company, United States
\textsuperscript{b} Pfizer, United States
\textsuperscript{c} R-Squared Solutions, United States
\textsuperscript{d} University of Padova, Italy
\textsuperscript{e} Janssen, Belgium
\textsuperscript{f} PNRI, Univ. Washington, United States
\textsuperscript{g} Sanofi, France
\textsuperscript{h} © Kelly Government Solutions for NIDDK, United States
\textsuperscript{i} University of Pennsylvania, United States
\textsuperscript{j} Mayo Clinic, United States
\textsuperscript{k} Foundation for the NIH, United States
\textsuperscript{l} ROI BioPharma Consulting, United States

ABSTRACT

Standard practice to minimize variability in beta cell function (BCF) measurement is to test in inpatient (IP) settings. IP testing strains trial subjects, investigators, and budgets. Outpatient (OP) testing may be a solution although there are few reports on OP BCF testing variability. We compared variability metrics between OP and IP from a standardized mixed meal tolerance test (MMTT) and arginine stimulation test (AST) in two separate type 2 diabetes (T2DM) cohorts (OP, n = 20; IP n = 22) in test-retest design. MMTT variables included: insulin sensitivity (Si); beta cell responsivity (Φtot); and disposition index (DItot = Si Φtot) following 470 kCal meal. AST variables included: acute insulin response to arginine (AIRarg) and during hyperglycemia (AIRargMAX).

Results: Baseline characteristics were well-matched. Between and within subject variance for each parameter across cohorts, and intraclass correlation coefficients (ICC—a measure of reproducibility) across parameters were generally comparable for OP to IP. Table summarizes the ICC results for each key parameter and cohort.

| Test/Parameter | Outpatient (95% CI) | Inpatient (95% CI) |
|----------------|---------------------|--------------------|
| MMTT: Si       | 0.49 (0.0, 0.69)    | 0.28 (0.0, 0.60)    |
| MMTT: Φtot     | 0.65 (0.16, 0.89)   | 0.81 (0.44, 0.93)   |
| MMTT: DI       | 0.67 (0.0, 0.83)    | 0.36 (0.0, 0.69)    |
| AST: AIR Arg   | 0.96 (0.88, 0.98)   | 0.84 (0.59, 0.94)   |
| AST: AIR Arg Max| 0.97 (0.90, 0.99)  | 0.95 (0.86, 0.97)   |
| AST: ISR       | 0.93 (0.77, 0.97)   | 0.93 (0.82, 0.96)   |

In conclusion, the variability (reproducibility) of BCF measures from standardized MMTT and AST is
1. Introduction

Emerging interest in characterizing diabetes disease progression, as well as the surge in diabetes therapies, requires more routine inclusion of beta cell function (BCF) assessments in clinical trials. However, BCF testing is seldom incorporated in longitudinal outpatient trials, partly because such tests are traditionally conducted in an inpatient (IP) setting.

There is particular interest in BCF methodologies that are technically robust and operationally feasible to enable repeat testing in longitudinal settings. We have recently reported that standardized Mixed Meal Tolerance (MMTT) and Arginine Stimulation tests (AST) are reliable and reproducible methodologies that provide complementary information on BCF [1,2]. Both tests have variability metrics that support reasonable sample sizes to detect clinically relevant differences in BCF. In that series [1], all experiments were conducted in an IP setting (after an overnight stay), with a goal to reduce sources of variability.

However, the need to sequester subjects for an overnight stay places significant strain on trial execution, including hardship for volunteers; limiting trial execution to study sites with domicile capabilities; and increased cost. Furthermore, overnight confinement could be stressful for volunteers and impact overall quality of the test itself.

These considerations spurred interest in the conduct of these procedures in an outpatient (OP) setting, i.e., where subjects present to the clinical research unit on the morning of the procedure.

2. Methods

To address this question, we assessed variability and reproducibility of standardized MMTT and AST in an OP setting in a group of T2DM subjects using a test-retest paradigm that replicated the inpatient paradigm [1]. We compared these metrics against similar data previously reported in a separate, but similar cohort of IP T2DM subjects, using identical procedures and analytical methods [1].

Subjects: OP: 20 T2DM subjects were evaluated. Inclusion criteria included: fasting glucose of 126–270 mg/dL, HbA1c 6.5%–10.0% on stable metformin monotherapy (500–2000 mg/day) as described previously [1].

Study Design: After obtaining Institutional Review Board approval the study was conducted at two sites (ICON Development Solutions, San Antonio, Texas, and Celerion, Phoenix, Arizona). Following written informed consent and screening, all subjects underwent each procedure on separate days.

OP: Of 26 subjects recruited, 20 (10 men/10 women) completed the study (two subjects were removed from the study for persistent elevation of fasting glucose over 270 mg/dL; four subjects discontinued for reasons unrelated to study). IP: Comparison inpatient data were derived from the previously reported cohort of 22 subjects (11 men/11 women) [1]. Demographic and baseline characteristics for both cohorts are summarized in Table 1. Study cohorts were comparable with significant overlap in distributions of baseline characteristics.

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available assays described previously [1].

2.1. MMTT

BCF parameters were derived as described previously [1]. Glucose, insulin, and C-peptide profiles were used to fit the minimal model to derive estimates of insulin sensitivity (Si); beta cell responsivity (φtot); and disposition index (Di = Si+φtot) [3,4]. For the AST, the baseline corrected acute insulin response to arginine (AIRarg) was determined in the first 5 min post arginine infusion (5 gm IV) during the baseline glucose state or after the glucose infusion (AIRargMAX) [2,5]. Insulin secretory reserve (ISR) was calculated from AIRargMAX-AIRarg.

2.2. Statistical analyses

As described for the inpatient cohort [1] between- and within-subject variance component estimates across genders were derived using a mixed effects model on natural log transformed data, treating gender as a fixed effect, subjects grouped by gender as a random effect, and visits as a repeated effect. Results are reported as geometric coefficients of variation (GCVs) and respective asymptotic 90% confidence intervals. Model predicted adjusted geometric means (95% CI) for the inpatient and outpatient cohorts are provided. To characterize reproducibility of within subject measures of BCF, intra-class correlation coefficients (ICCs) and respective bootstrap 90% CIs were calculated.

Following the outpatient cohort analyses, a pooled analysis for assessment of variance component structures between- and within-subject across study cohorts was conducted. As the cohorts were composed of different individuals, comparability of cohorts was tested to allow adjustment for potential differences. Between- and within-subject variance components across cohorts were estimated using a mixed model analysis of covariance (ANCOVA) on the pooled results. Pooled analyses included fixed terms for study cohort and gender, as well as, age, BMI and HbA1cto adjust for minor covariate variation between cohorts. Sequential model reductions were performed testing different or common within- and between-subject variance component structures across genders. Subjects were grouped by cohort as a random effect and visits as a repeated effect. Likelihood ratio tests (LRTs) were used to assess variance component structures across cohorts to determine whether a common between- and within-subject variance structure across cohorts sufficiently described the data. A two-sided significance level equal to 0.00125 was pre-specified to protect against declaring spurious differences in variance component estimates across study cohorts in the model selection process. This alpha level corresponds to a replication p-value threshold (0.05 × 0.025) for detecting a difference in analysis models. If results indicated that a common within- and between-subject variance component structure across genders sufficiently described the data, then the pooled inpatient/outpatient results would be presented.

3. Results

OP: Of 26 subjects recruited, 20 (10 men/10 women) completed the study (two subjects were removed from the study for persistent elevation of fasting glucose over 270 mg/dL; four subjects discontinued for reasons unrelated to study). IP: Comparison inpatient data were derived from the previously reported cohort of 22 subjects (11 men/11 women) [1]. Demographic and baseline characteristics for both cohorts are summarized in Table 1. Study cohorts were comparable with significant overlap in distributions of baseline characteristics.
3.1. Measured parameters and derived indices from MMTT and AST

Profiles of glucose, insulin and C-peptide exhibited similar responses across study cohorts for both MMTT and AST Fig. 1 (Means ± SE). Visual inspection of glucose, insulin and C-peptide responses within the MMTT and AST in the outpatient cohort showed good reproducibility for both tests (Fig. 1).

Table 2 provides geometric mean point estimates for each cohort as well as the respective within- and between subject variance components and ICC estimates. As the cohorts were evenly balanced for gender and well matched for baseline age, BMI, and HbA1c; none of the covariates reached statistical significance in the ANCOVA. There was substantial overlap in the confidence intervals of the overall geometric mean point estimates for $\Phi_{tot}$ for the MMTT and measures of BCF in the AST. Although the outpatient cohort generally showed higher point estimates than the inpatient cohort, only $Si$ and $DI$ reached statistical significance.

Reproducibility in the previously reported inpatient cohort, as indexed by the ICC, ranged from weak to strong in the MMTT for all model-based parameters (Table 2). For the AST, reproducibility was strong across all parameters. In the outpatient cohort, all ICC values

| Table 1 | Summary baseline covariates by study cohort (mean, standard deviation). |
|---------|--------------------------------------------------|
|         | Inpatient                                       | Outpatient             |
| N       | 22 (11M, 11F)                                   | 20 (10M, 10F)          |
| Age (years) | 54.7 (8.11)                                   | 51.9 (9.18)            |
| Weight (kg) | 91.0 (14.11)                                   | 86.0 (16.61)          |
| BMI (kg/cm²) | 32.7 (3.96)                                    | 31.0 (3.78)           |
| HbA1c (%) | 8.2 (0.85)                                      | 8.2 (1.04)             |

Fig. 1. Glucose, insulin, and c-peptide profiles for the AST and MMTT tests by study visit and cohort. Values are means ± SE by study Visit. Open squares represent visit 1 results and open circles represent visit 2 results.
were numerically similar to, or higher than, those observed in the inpatient cohort.

Table 2 also provides the pooled variance estimates with common within- and between-subject variance components across cohorts. Of specific interest, magnitudes of the between- and within-subject variance components for each BCF parameter across cohorts sufficiently described the data. No LRTs reached the prespecified ($\leq 0.00125$) level of statistical significance. With the exception of AlRarg comparing within-subject variances, all were $p > 0.1$. The outpatient within subject variability of AlRarg was about 50% that of the inpatient (model selection $p = 0.004$), i.e., the outpatient test was less variable than the inpatient test.

4. Conclusions

The present findings demonstrate that variability and reproducibility metrics for the MMTT and AST appear at least comparable between outpatient and inpatient settings. Any differences observed in geometric mean estimates for variables such as Si and DI maybe attributable to the study of different cohorts of subjects. Taken together, these results support pooling of the inpatient and outpatient data to create common variance estimates, yielding greater estimate precision.

To the best of our knowledge, this is the first comparison of inpatient versus outpatient metabolic testing. There are reports of the variability of oral metabolic challenges, per se. In those studies ([6] [7]) there was slightly lower within subject variability likely due to a liquid meal. As it is simpler and faster to absorb, the liquid only meal may help counter any increase in variability arising from other sources as an outpatient. Although the sample size for this between cohort comparison was not based on statistical power considerations, these sample sizes are similar to those routinely used in interventional trials employing these methodologies.

Some potential sources of variability in an outpatient setting include an incomplete overnight fast as well as the stress of traveling to the study site on the morning of testing. Attention was paid to minimize these sources of variability. Conversely, it is possible that without an overnight stay, which could disrupt sleep and worsen metabolic outcomes [9,10], there may have been less metabolic stress. This may have helped counter any increase in variability arising from other sources as an outpatient.

Although the sample size for this between cohort comparison was not based on statistical power considerations, these sample sizes are similar to those routinely used in interventional trials employing these methodologies.

From a feasibility perspective, the outpatient study was more convenient for subjects and required fewer resources with lower study costs (∼15–20%) compared to the inpatient study. Finally, performing these procedures in an outpatient setting removes a major barrier to the inclusion of study sites unable to accommodate overnight stays, in turn enabling faster recruitment and shorter timelines.

Table 2

| Test: Parameter of Beta Cell Function | Study Cohort | Geometric Means (95% CI) | Geometric CV Between Subject (90% CI) | Geometric CV Within Subject (90% CI) | ICC (90%CI) |
|--------------------------------------|--------------|--------------------------|--------------------------------------|--------------------------------------|-------------|
| AST: AlRarg (μU/mL)                  | Inpatient    | 35.7 (29.0, 44.0)        | 54.2 (38.9, 77.5)                    | 22.8 (12.2, 43.8)                    | 0.84 (0.59, 0.94) |
|                                      | Outpatient   | 46.7 (37.5, 58.2)        | 60.3 (43.3, 86.8)                    | 11.7 (9, 15.3)                      | 0.96 (0.88, 0.98) |
|                                      | Pooled       | 51.7 (45.4, 72.9)        | 57.1 (45.4, 72.9)                    | 18.4 (15.3, 22)                     | 0.90 (0.75, 0.95) |
| AST: AlRargMAX (μU/mL)               | Inpatient    | 85.4 (67.5, 108.2)       | 62.1 (44.9, 88.7)                    | 13.8 (7.4, 25.8)                    | 0.95 (0.86, 0.97) |
|                                      | Outpatient   | 99.0 (77.2, 127.0)       | 71.6 (50.5, 106.1)                   | 11.2 (8.7, 14.6)                    | 0.97 (0.90, 0.99) |
|                                      | Pooled       | 96.6 (52.9, 85.2)        | 66.6 (37.4, 105.5)                   | 12.6 (10, 15.1)                     | 0.96 (0.91, 0.97) |
| AST: ISR (μU/mL)                     | Inpatient    | 47.3 (34.9, 63.9)        | 79.3 (55, 120.7)                     | 19.6 (10.5, 37.4)                   | 0.93 (0.82, 0.96) |
|                                      | Outpatient   | 47.3 (34.4, 65.0)        | 99.8 (66.1, 165.2)                   | 23.3 (17.9, 30.5)                   | 0.93 (0.77, 0.97) |
|                                      | Pooled       | 88.8 (68.9, 117.7)       | 73.2 (55, 120.7)                     | 21.5 (17.9, 25.8)                   | 0.93 (0.86, 0.95) |
| MMTT: $\Phi_{\text{total}}$ (10 $^{-3}$ min$^{-1}$) | Inpatient    | 15.5 (12.5, 19.2)        | 56.2 (39.6, 82.4)                    | 26.1 (13.9, 50.5)                   | 0.81 (0.44, 0.930) |
|                                      | Outpatient   | 18.9 (15.0, 23.7)        | 53.1 (34.7, 84.7)                    | 37.7 (28.7, 50.1)                   | 0.65 (0.16, 0.89) |
|                                      | Pooled       | 54.7 (42.3, 71.9)        | 32.1 (26.6, 38.8)                    | 0.73 (0.45, 0.86)                   | 0.73 (0.45, 0.86) |
| MMTT: Si (10 $^{-3}$ min$^{-1}$ X μU/mL$^{-1}$) | Inpatient    | 1.0* (0.8, 1.4)          | 83.2 (21.6, 124)                     | 9.0 (40, 234.1)                     | 0.28 (0.60) |
|                                      | Outpatient   | 1.9* (1.4, 2.5)          | 67.1 (39.9, 124.8)                   | 69.3 (51.2, 96.6)                   | 0.49 (0.69) |
|                                      | Pooled       | 58.1 (37.7, 94.1)        | 76.2 (60.9, 97.1)                    | 0.39 (0.58)                        | 0.39 (0.58) |
| MMTT: DI (10 $^{-3}$ min$^{-2}$ X μU/mL$^{-1}$) | Inpatient    | 17.2* (11.3, 26.0)       | 97.1 (31.7, 174.9)                   | 45.3 (39.8)                        | 0.36 (0.60) |
|                                      | Outpatient   | 37.1* (24.8, 55.6)       | 136.9 (77.8, 309.1)                  | 82.8 (60.3, 118.6)                  | 0.67 (0.83) |
|                                      | Pooled       | 101 (68.8, 160.8)        | 89.8 (70.9, 116.5)                   | 0.54 (0.14, 0.69)                  | 0.54 (0.14, 0.69) |
As both datasets were independently and sufficiently robust to enable comparison, we have presented pooled estimates for common variances across cohorts to provide the most robust and precise estimates for future study planning.

Funding and support

The methodological study described in this report was designed and implemented under the auspices of the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium. The Biomarkers Consortium is a public private partnership that develops and validates biological markers, which speed up the development of therapies for the detection, prevention, diagnosis and treatment of disease, and are ultimately aimed at improving patient care. For this study, the Consortium brought together diabetes experts from leading academic institutions, the Food and Drug Administration, The National Institutes of Health, the nonprofit sector, and the pharmaceutical industry to develop the project. The results of the partnership, discussed here, are important because they address a critical unmet medical need, and develop the project. The results of the partnership, discussed here, are important because they address a critical unmet medical need, and involve key stakeholders in the diabetes treatment field. FNIH has acted as a neutral convener for the partners and provided the project management expertise needed for the execution of the overall project. In the future, this type of partnership can be used as a model for establishing common standards for testing in other therapeutic areas as well.

Under the auspices of the FNIH, this project was jointly funded by the NIH (NIDDK) and FDA, and the following, participating pharmaceutical companies: Amylin (now AstraZeneca), Janssen, Eli Lilly, Merck, Novartis, Novo Nordisk, Pfizer, Sanofi, Takeda. An in-kind donation of 8800 tubes was made by Becton Dickinson (D. Craft). Additional support was also received from ADA and JDRF.

The authors also thank the staff and leadership of the FNIH for their continuous support in planning, executing, and managing these studies and the overall project.

Duality of interest disclosures

Sudha S. Shankar Former employee and shareholder of Eli Lilly and Co.
Douglas Lee: Employee and shareholder of Pfizer.
Ralph H. Raymond, none to report.
Roberto A. Calle, Employee and shareholder of Pfizer.
Claudio Cobelli, none to report.
Atalanta Ghosh, Employee and shareholder of Janssen Pharmaceuticals.
Hartmut Ruetten, Employee and shareholder of Sanofi-Aventis Deutschland GmbH.
R. Paul Robertson, none to report.
Myrlene A. Staten, none to report.
Darko Stefanovski, none to report.
Adrian Vella, none to report.
David A. Fryburg, Shareholder, Pfizer Inc.

Author contributions

Sudha S. Shankar MD, PhD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.
Douglas Lee, PhD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.
Ralph H. Raymond, MS: Substantially contributed to study design, data interpretation, drafting and revising the article and has approved its submission.
Roberto A. Calle, MD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.
Claudio Cobelli, PhD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.

Atalanta Ghosh, PhD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.
Hartmut Ruetten, MD, PhD: Substantially contributed to study design, data interpretation, drafting and revising the article and has approved its submission.
R. Paul Robertson, MD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.
Myrlene A. Staten, MD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.

Darko Stefanovski, PhD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.

Adrian Vella, MD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.

David A. Fryburg, MD: Substantially contributed to study design, data interpretation, drafting and revising the article and has approved its submission.

Acknowledgements

Current members of the β-Cell Project Team include:
Richard Bergman, PhD - Cedars-Sinai Diabetes & Obesity Research Institute.
Roberto Calle, MD - Pfizer.
Claudio Cobelli, PhD - University of Padova.
Stephanie Cush, PhD-FNIH.
David Fryburg, MD – FNIH, ROI BioPharma Consulting.
Atalanta Gosh, PhD – Janssen.
Ilan Irony, MD - CDER/FDA.
Frank Martin, PhD - JDRF.
Malene Hersloev MD - Novo Nordisk.
Kolaczynski, Jerzy, MD—Novo Nordisk.
Stephanie Moran, MD – Takeda Development Center Americas.
David Polidori, PhD - Janssen.
Ralph Raymond, MS – FNIH, R-Squared Solutions.
R. Paul Robertson, MD – Pacific Northwest Research Institute and Univ. Washington.
Hartmut Ruetten, MD - PhD – Sanofi.
Sudha Shankar, MD - Eli Lilly & Company.
Myrlene Staten, MD – Kelly Government Solutions on contract to NIH/NIDDK.
Darko Stefanovski, PhD – University of Pennsylvania.
Lilit Vardanian, PhD—FNIH.
Adrian Vella, MD - Mayo Clinic.
Gordon Weir, MD - Joslin Diabetes Center.
Marjorie Zakaria, MD - Novartis.
Previous members of the B-Cell Project Team who contributed to this work:
Mark Deeg, MD, PhD-formerly of Eli Lilly.
David Kelley, MD, formerly of Merck.
Peter Savage, MD - NIH/NIDDK.
Nicole Spear, MS, formerly of FNIH.
Maria Vassileva, PhD, formerly of FNIH.
Sanya Whitaker, PhD, formerly of FNIH.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.conctc.2018.03.009.
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