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

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A B S T R A C T

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(0,0.69) | 0.28(0,0.60) |
| **MMTT: Φtot** | 0.65(0.16,0.89) | 0.81(0.44,0.93) |
| **MMTT: DI** | 0.67(0,0.83) | 0.36(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

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comparable between OP and IP settings. These observations have significant implications for complexity and cost of metabolic studies.

## 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: four separate visits completed within a 28-day period, with subjects undergoing MMTT at the first and third, and AST at the second and fourth visits. The interval between the two MMTTs and ASTs was approximately a week. Each MMTT or AST was separated from the previous test by about 3 days.

Subjects fasted overnight prior to the procedure. Metformin was withheld the morning of each procedure. Subjects arrived approximately two hours prior to initiation of testing. To minimize stress and ensure timely arrival, subjects were provided transportation as needed. Following arrival and after an hour's rest, subjects underwent brief physical examination and a glucose check. If glucose exceeded 270 mg/dL, testing was deferred to another day. If fasting glucose remained over 270 mg/dL, then the subject was discontinued from the study and referred to their physician.

***Procedures***: MMTT and AST procedures were identical to those employed in the previously published, inpatient cohort [1]. Samples for glucose, insulin and C-peptide were measured using commercially 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 (DItot = 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 ($0.05 \times 0.025$) 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/11women) [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.

**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/cm$^2$) | 32.7 (3.96) | 31.0 (3.78) |
| HbA1c (%) | 8.2 (0.85) | 8.2 (1.04) |

### 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 Φtot 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

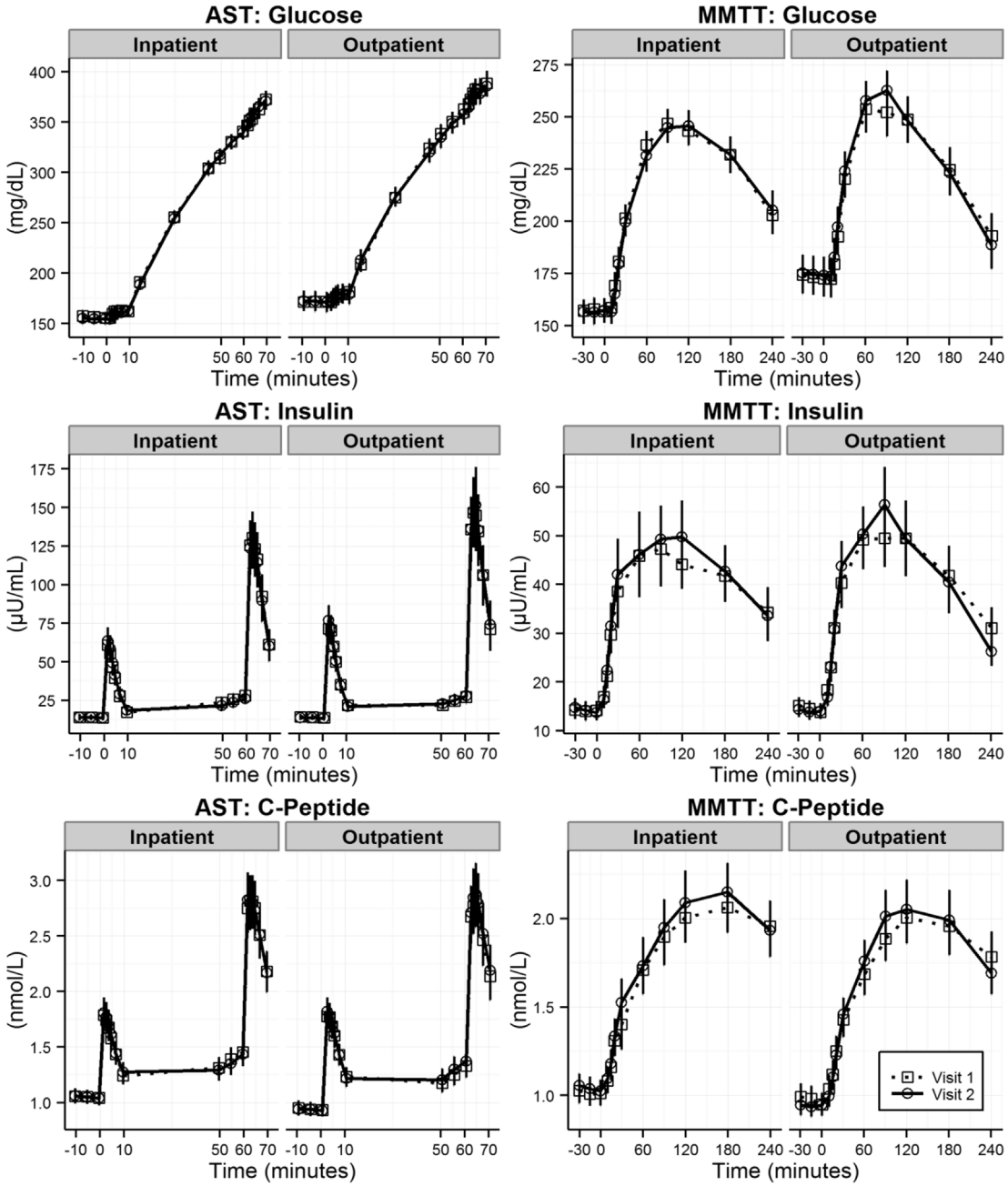


**Fig. 1.** Glucose, insulin, and c-peptide profiles for the AST and MMT tests by study visit and cohort. Values are means ± SE by study Visit. Open squares represent visit 1 results and open circles represent visit 2 results.

**Table 2**
Summary of beta cell function parameters, respective coefficients of variation, and intraclass correlation coefficients from AST and MMTT.

| 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: AIRarg (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 | | 57.1 (45.4, 72.9) | 18.4 (15.3, 22) | 0.90 (0.75, 0.95) |
| AST: AIRargMAX (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 | | 66.6 (52.9, 85.2) | 12.6 (10.5, 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) | 21.5 (17.9, 25.8) | 0.93 (0.86, 0.95) |
| MMTT: $\Phi_{total}$ ($10^{-9}min^{-1}$) | 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) |
| MMTT: Si ($10^{-4}min^{-1}$ X $(\mu U/mL)^{-1}$) | Inpatient | 1.0* (0.8, 1.4) | 47.9 (21.6, 124) | 83.2 (40, 234.1) | 0.28 (0, 0.60) |
| | Outpatient | 1.9* (1.4, 2.5) | 67.1 (39.9, 124.8) | 69.3 (51.2, 96.6) | 0.49 (0, 0.69) |
| | Pooled | | 58.1 (37.7, 94.1) | 76.2 (60.9, 97.1) | 0.39 (0, 0.58) |
| MMTT: DI ($10^{-13}min^{-2}$ X $\mu U/mL)^{-1}$) | Inpatient | 17.2* (11.3,, 26.0) | 66.5 (31.7, 174.9) | 97.1 (45.3, 309.8) | 0.36 (0, 0.69) |
| | Outpatient | 37.1* (24.8, 55.6) | 136.9 (77.8, 309.1) | 82.8 (60.3, 118.6) | 0.67 (0, 0.83) |
| | Pooled | | 101 (68.8, 160.8) | 89.8 (70.9, 116.5) | 0.54 (0.14, 0.69) |

Full Model ANCOVA Results (Age, BMI, and HbA1c as baseline covariates) with fixed terms for gender and study cohort and different within and between subject variances across study cohorts are reported for the Inpatient and Outpatient cohorts (*$p < 0.05$ for outpatient vs. inpatient geometric mean comparison). Pooled variance component estimates are derived from Full Model ANCOVA Results with common within- and between-subject variance component structure across study cohorts which sufficiently describe the study results. No pooled geometric mean estimates are provided, as modeling exercises are targeted towards variance component estimation.

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 AIRarg comparing within-subject variances, all were $p > 0.1$. The outpatient within subject variability of AIRarg 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 have led to lower variability estimates compared to the current meal which had solid and liquid components. From a physiologic perspective, a meal with both solid and liquid phases is a more relevant option [8].

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 such 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 (by ~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.

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.

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## 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.

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Sudha S. Shankar MD, PhD: Substantially contributed to study design, data interpretation, revising the article and has approved its submission.

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

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

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## 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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