Standardized Mixed-Meal Tolerance and Arginine Stimulation Tests Provide Reproducible and Complementary Measures of β-Cell Function: Results From the Foundation for the National Institutes of Health Biomarkers Consortium Investigative Series

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OBJECTIVE

Standardized, reproducible, and feasible quantification of β-cell function (BCF) is necessary for the evaluation of interventions to improve insulin secretion and important for comparison across studies. We therefore characterized the responses to, and reproducibility of, standardized methods of in vivo BCF across different glucose tolerance states.

RESEARCH DESIGN AND METHODS

Participants classified as having normal glucose tolerance (NGT; *n* = 23), prediabetes (PDM; *n* = 17), and type 2 diabetes mellitus (T2DM; *n* = 22) underwent two standardized mixed-meal tolerance tests (MMTT) and two standardized arginine stimulation tests (AST) in a test-retest paradigm and one frequently sampled intravenous glucose tolerance test (FSIGT).

RESULTS

From the MMTT, insulin secretion in T2DM was >86% lower compared with NGT or PDM ($P < 0.001$). Insulin sensitivity (Si) decreased from NGT to PDM (∼50%) to T2DM (93% lower [$P < 0.001$]). In the AST, insulin secretory response to arginine at basal glucose and during hyperglycemia was lower in T2DM compared with NGT and PDM (>58%; all $P < 0.001$). FSIGT showed decreases in both insulin secretion and Si across populations ($P < 0.001$), although Si did not differ significantly between PDM and T2DM populations. Reproducibility was generally good for the MMTT, with intraclass correlation coefficients (ICCs) ranging from ∼0.3 to ∼0.8 depending on population and variable. Reproducibility for the AST was very good, with ICC values >0.8 across all variables and populations.

CONCLUSIONS

Standardized MMTT and AST provide reproducible and complementary measures of BCF with characteristics favorable for longitudinal interventional trials use.

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The pathophysiologic hallmarks of type 2 diabetes mellitus (T2DM) are defects in insulin action and β-cell function (BCF) (1), with the latter manifesting as inadequate insulin secretion in response to hyperglycemia (2). Early intervention may ameliorate these defects (3). However, determination of whether a given intervention has clinically relevant effects on BCF requires long-term testing in large cohorts. Therefore, the ability to simply and reproducibly test BCF is critical. Quantification of BCF would enable evaluation of interventions tailored to specific functional β-cell defects in multiple populations, as well as their potential to alter disease progression.

It is readily apparent that assessment of BCF has been accomplished using different challenge routes (oral vs. intravenous), stimuli (e.g., arginine, glucagon, glucose, mixed meals), and sampling times, often in single-center studies (4–7). Even for the same method, such as the meal tolerance test, the composition and caloric load of the test meals often differ (5,8,9). Additionally, different approaches have been used concurrently to assess insulin secretion and sensitivity. Some reports use simple ratios or calculations for insulin sensitivity (Si) and secretion using measurements under basal (e.g., homeostasis model assessment of BCF and quantitative insulin sensitivity check index) or postchallenge conditions (e.g., Matsuda index and $\Delta I_{0–30}/\Delta G_{0–30}$). However, due to the complex physiology of appearance of the nutrient stimuli and the sites and timing of insulin action and clearance, indices obtained using these simple calculations may have limited utility (6,7). Some of the most widely used methods of measuring BCF are described in Table 1, along with a summary of the strengths and limitations of these methods.

Recognizing the need for more consensus methods for the measurement of BCF in humans, the multistakeholder β-Cell Project Team (BCPT) of the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium (http://www.biomarkersconsortium.org/) was formed to standardize and characterize select BCF tests. The goal of this consortium-driven work is to enable inclusion of such assessments in future longitudinal clinical trials examining response to therapeutic interventions and disease progression. Members of the BCPT are listed in the APPENDIX. In accordance with the principles of the FNIH partnership, project results are made publically available.

We sought to characterize the reproducibility of two methods that could be used in a multicenter clinical trial and that could quantify insulin secretion across the glucose tolerance spectrum. An important aspect of quantify insulin secretion is to do so as a function of prevailing insulin action. This is the basis of the minimal model as applied to data obtained from an intravenous (frequently sampled intravenous glucose tolerance test [FSIGT]) or oral challenge (mixed-meal tolerance test [MMTT] or oral glucose tolerance test [OGTT]) (10,11). Although the FSIGT is the only in vivo test that potentially replicates the in vitro finding of first-phase insulin secretion, it is more difficult to implement routinely in large multicenter interventional trials (12,13). For the BCPT, this spurred interest in alternative BCF tests, with particular emphasis on operational feasibility and in the context of a physiologically relevant challenge (e.g., meal ingestion) (14).

Additional considerations for BCF testing include attempts to elicit near-maximal stimulation (15) of insulin secretion and determination of insulin secretory reserve. Arginine and glucagon in pharmacologic doses have been used for these purposes, with both stimuli yielding similar responses, although arginine is better tolerated (16). The response to either secretagogue is potentiated by simultaneous administration of glucose. The arginine stimulation test (AST) has been used in islet autotransplantation studies in which the insulin secretory response to arginine correlates with the number of transplanted islets in this patient population (17,18). Although these various tests have been used to detect and quantify phenotypic differences across health and disease, their application to evaluate pharmacologic interventions has been demonstrated in a limited number of studies (19–21). In particular, there is scant information on their variability and reproducibility characteristics.

Following review and discussion of each method, the BCPT selected the MMTT (with minimal model calculations) and AST for further study. The selection was based on both scientific and practical reasons. The MMTT offers a simple-to-administer physiologic test that incorporates the incretin response. When analyzed with the minimal model, the MMTT provides a simultaneous estimate of insulin secretion and sensitivity. The AST generates a supraphysiologic insulin secretory response and, like the MMTT, is far less technically demanding than methodologies such as the hyperglycemic clamp. The BCPT also chose to include the FSIGT as a widely accepted comparator to allow for a reference method, especially as the FSIGT and MMTT both use minimal model approaches for data analysis.

The methodologies for the MMTT, AST, and FSIGT were first standardized. Subsequently, experiments to estimate between and within subject variability (and reproducibility) as well as contrast the means and distributions of BCF parameters across the glucose tolerance spectrum were undertaken. To minimize differences in body composition for comparisons across glucose tolerance groups, all subjects were required to be obese, including those with normal glucose tolerance (NGT).

RESEARCH DESIGN AND METHODS

Subjects

Three groups of subjects classified by their fasting and postchallenge glucose tolerance status (2-h post–75-g OGTT) (and balanced for gender) were studied. NGT subjects had a fasting glucose $<$100 mg/dL and postchallenge $<$140 mg/dL; prediabetes mellitus (PDM) subjects had impaired fasting glucose ($\geq$100 and $<$126 mg/dL) and impaired glucose tolerance ($\geq$140 and $<$200 mg/dL); subjects with T2DM had fasting glucose values of 126–270 mg/dL and HbA_{1c} 6.5–10.0% on a stable dose of metformin monotherapy (500–2,000 mg/day).

Study Design

After obtaining Institutional Review Board approval, local advertisement was used to recruit for trials, conducted at two study sites (ICON Development Solutions in San Antonio, TX, and Omaha, NE). After written informed consent was obtained and the subjects were screened, all subjects underwent each procedure on separate days. The MMTT and AST were administered twice and the FSIGT once, grouped into three separate visits during which the subject resided at the research center from the evening before the first test until completion of all of the tests that were part

Table 1—Comparison of current methods for measuring BCF

Test	Description	Advantages	Limitations
Hyperglycemic clamp	A variable IV glucose infusion is administered to maintain the glucose level at a steady state Frequent blood sampling and minute-to-minute adjustments of glucose infusion rate at bedside are required	Provides measures of insulin secretion (first and second phases) and with modeling insulin action Does not require modeling of data for insulin secretion Widely reported and accepted	No GI incretin effects Requires continuous adjustment of IV glucose Technically challenging to conduct testing Expertise limited to select centers
Graded glucose infusion	IV glucose is administered at progressively increasing rates (each rate maintained for ~40 min) Requires frequent blood sampling	Provides measure of insulin secretion over a range of glucose levels Provides measure of β-cell glucose sensitivity	No GI incretin effects Not as widely studied and reported as hyperglycemic clamp, especially in the context of therapeutic interventions Data analyses often require expertise in model-based methods
FSIGT	Rapid IV injection of glucose is followed 20 min later by an IV injection of insulin Requires very frequent blood sampling	Provides insulin secretion and action during rapidly changing glucose levels Provides first-phase insulin release measures Insulin action results correlate well with those from euglycemic clamp Widely used and reported With C-peptide modeling, provides second-phase insulin release	No GI incretin effect Technically challenging to conduct Expertise to conduct limited to select centers Requires computer modeling for the outcome measures, requiring specialized expertise Requires IV administration of insulin
AST	IV arginine is administered followed by combined glucose/arginine infusions Frequent blood samplings over a short period of time are necessary	Measures of insulin secretion known to correlated with β-cell mass in islet transplant recipients Provides a measure of near-maximal insulin secretion (insulin secretory reserve)	Mixed effect on incretin response Requires IV administration of arginine and glucose Does not inform on insulin action
Glucagon stimulation test	IV glucagon is given twice sequentially (at baseline and after glucose has been infused to achieve elevated glucose)	Robust insulin secretory response similar to that of arginine but through different mechanism of action	No oral incretin effect Requires IV administration of glucagon Does not inform on insulin action Side effects of nausea and vomiting are common and potentially confounding
MMTT/OGTT	Oral meal or glucose solution is ingested MMTT physiologically highly relevant, mimicking oral challenges routinely encountered daily Blood samples taken at specified intervals up to 5 h postchallenge	Easy to administer Effect of incretins included Provides insulin secretion and action during changing glucose levels OGTT standardized and simple as single substrate Insulin action and secretion results correlate with those from hyperglycemic and euglycemic clamps	Assumptions must be made for rate of nutrient absorption into systemic circulation Technically challenging to model outcome measure of insulin secretion of sensitivity, requiring software and expert analysis Lack of standardized test meal MMTT with minimal modeling not as widely reported as the hyperglycemic and euglycemic clamps

GI, gastrointestinal; IV, intravenous.

of that visit. During each of the first two visits, the overall order of procedures was fixed, with an MMTT on day 1 and AST on day 2, after which the subject was discharged. At the third and separate visit, an FSIGT was performed. All testing was completed within 28 days with at least 5 days between visits.

Procedures

In those subjects with T2DM, metformin was held on the morning of the procedure. After a 10-h overnight fast, a single indwelling intravenous catheter was placed in the forearm for the MMTT, whereas for the AST and FSIGT, indwelling catheters were placed in both upper extremities for infusion and sample acquisition, respectively. The procedures are briefly described below with extensive detail in the *Study Operations Manual* (available at http://www.fnih.org/what-we-do/current-research-programs/biomarkers-consortium-beta-cell-project).

MMTT

Following baseline sampling (−30, −15, and 0 min), a test meal (470 kcal, ~66% carbohydrate, 18% fat, and 16% protein) composed of one 8-fluid-ounce Boost nutrition supplement drink (Nestlé Health Science) and one PowerBar (Nestlé Nutrition) was administered. The meal was consumed within 10 min, with the bar consumed first and serial sampling for analytes performed at 10, 15, 20, 30, 60, 90, 120, 180, and 240 min postmeal.

AST

Following baseline sampling (−10, −5, and 0 min), an intravenous injection of 5 g of arginine (given as 10% arginine HCl [as R-Gene; Pfizer]) was administered over 60 s followed by serial sampling at 2, 4, 5, 7, and 10 min. Subsequently, glucose levels were raised by a continuous infusion of glucose (20% dextrose at

900 mg/min) over 60 min with repeat sampling at 50, 55, and 60 min, followed by a second dose of 5 g of arginine at 60 min with sampling at 62, 64, 65, 67, and 70 min.

FSIGT

Following baseline sampling (−30, −15, and 0 min), a 300 mg/kg glucose bolus was administered with sampling at 2, 4, 8, and 19 min. At 20 min, a single dose (0.03 units/kg) of U100 regular human insulin (Humulin R; Eli Lilly and Company, Indianapolis, IN) was administered intravenously with sampling at 22, 30, 40, 50, 70, 100, 180, and 240 min.

Analyte Assays

Samples were assayed in the Immunochemical Core Laboratory, Mayo Clinic (Rochester, MN). Glucose was measured on the Roche Cobas c311 (Roche Diagnostics, Indianapolis, IN) using a hexokinase reagent. C-peptide was measured by a two-site immunometric assay on the Roche Cobas e411 (Roche Diagnostics). Insulin (plasma) was measured by a two-site immunometric (sandwich) assay using electrochemiluminescence detection on the Roche Cobas e411 (Roche Diagnostics). All intra-assay coefficients of variation (CVs) were <3% and interassay CVs <6%.

Data and Statistical Analyses

MMTT

Baseline glucose, insulin, and C-peptide were calculated as the average of −30-, −15-, and 0-min values. Si was estimated using the oral glucose minimal model (22). β-Cell responsivity index (Φtot), a measure of insulin secretion, was estimated from the individual subject plasma glucose and C-peptide concentrations observed during the experiment using the oral C-peptide minimal model (23) incorporating C-peptide kinetics as reported by Van Cauter et al. (24). Disposition index (DI) was calculated as the product of Si and Φtot. For the purposes of this series, a standardized approach to modeling across glucose tolerance populations was used. MMTT analyses were performed using Matlab US R2010B (MathWorks, Natick, MA) with code provided by C. Cobelli. (also available through The Epsilon Group, Charlottesville, VA). No time points were excluded in the derivation of individual subject-level parameters.

FSIGT

Si was calculated by fitting the glucose profiles from the intravenous glucose tolerance tests using Minmod Millennium (Version 6.02; Minmod Inc., Los Angeles, CA). The acute insulin response to glucose (AIRg) was calculated as the area under the curve (AUC) of insulin concentration above the average basal value, from 0 to 10 min after the glucose injection. The DI was calculated as the product of the average Si and AIRg from each experiment.

AST

At basal glucose, the insulin secretory response to arginine at basal glucose (AIRarg) was derived as the mean of the three highest insulin values from minutes 2, 3, 4, and 5 minus baseline insulin at basal glucose (average of −10, −5, and 0 min). At elevated glucose, maximal insulin secretory response to arginine under hyperglycemic conditions (AIRargMAX) was derived as the mean of the three highest insulin values at minutes 62, 63, 64, and 65 minus baseline insulin at elevated glucose (average of 50, 55, and 60 min). Insulin secretory reserve, ISR, represents the difference between insulin secretion at elevated and at basal glucose (AIRargMAX − AIRarg).

Data Conventions and Handling

Analyses for AST and MMTT were performed on natural log-transformed data for subjects having matched pairs (both visits). Final results for transformed parameters were exponentiated and reported on the original scale. The Grubbs' test for a single outlier was used to assess extreme values, and, if found to be significant at the one-sided, 0.001 significance level, a secondary, sensitivity analysis was performed excluding these data for re-estimation of variance components and intraclass correlation coefficients (ICCs) and presented as secondary analyses. All other analyses, including tests for glucose tolerance population differences, and correlation analyses are presented with all evaluable subjects including extreme values.

Variance Component Estimation

Between- and within-subject variance component estimates were derived using a mixed-effects model, treating gender as a fixed effect, subjects grouped by gender as a random effect, and visits as a repeated effect. Initial modeling allowed for the potential of separate between- and within-subject variances across genders. Log-likelihood ratio tests at the two-sided 0.05 significance level were used to select the most appropriate model. As parameterized, the model accounts for any gender differences in mean response with simultaneous estimation of between- and within-subject variance components. Estimates (95% CI) for log-normally distributed data were reported as geometric CV and model predicted adjusted geometric means (95% CI). Tukey contrasts adjusting for multiplicity were used for comparison of means across glucose tolerance populations at the two-sided 0.05 significance level.

Using the estimated variance components, the ICC was calculated as (σ^2between/[σ^2between + σ^2within]). The ICC is a measure of the degree to which repeated measures within the same subject resemble each other, a measure of relative repeatability (25). ICC values >0.80 were considered highly reproducible. ICC values >0.50 were considered moderately reproducible, whereas those <0.50 were considered weakly reproducible. Overall correlations across populations were derived, including correlations within each population to make a general assessment of the consistency of results (concordance) within and across populations. Correlations among the different parameters are reported as Spearman rank correlations. The MMTT and AST values used were the subject averages.

RESULTS

The evaluable subjects included 23 NGT (12 men/11 women), 17 PDM (6 men/11 women), and 22 T2DM (11 men/11 women), for a total of 62 subjects. Complete demographic characteristics are summarized in Table 2. All groups, including those with NGT, were obese. No serious procedure-emergent adverse events were reported. A complete summary of procedure-emergent adverse events is reported in Supplementary Table 1.1 and 1.2.

Measured Parameters—Glucose, Insulin, and C-Peptide Concentrations

Summary plasma profiles of glucose, insulin, and C-peptide for the MMTT and the AST are shown in Fig. 1, reflecting the mean values of both pairs of tests.

Additional figures displaying the excursion of these analytes during each of the two MMTTs, as well as the two ASTs, can be found in Supplementary Fig. 1.1–1.6 (with additional information found in Supplementary Table 2.1–2.3). As expected, fasting glucose rose progressively across NGT, PDM, and T2DM. Fasting insulin and C-peptide were comparable in NGT and T2DM and higher in PDM. In the MMTT, following the meal challenge, glucose rose progressively across populations on both test days, achieving peak levels in T2DM > PDM > NGT, whereas insulin and C-peptide responses were highest in PDM, followed by NGT and then T2DM. AUC for glucose, insulin, and C-peptide all differed among the three groups (Supplementary Table 2.2).

In the AST, in response to arginine, increases over baseline in insulin and C-peptide were observed in all groups on both test days during basal and hyperglycemic conditions. The insulin secretory response to arginine was blunted in those with T2DM compared with PDM and NGT. In the FSIGT, following the glucose bolus, glucose rose progressively over baseline in all groups, achieving peak levels greater in T2DM than in PDM or NGT (Supplementary Fig. 1.7–1.9 and Supplementary Table 2.4). Endogenous insulin and C-peptide responses within the first 19 min were blunted in T2DM. Following exogenous insulin, return to baseline for glucose occurred within 50 min in NGT, but was more delayed in PDM and T2DM.

Indices of BCF From the MMTT, AST, and FSIGT

Overall, insulin secretion, as measured by either the MMTT or AST, was similar between the NGT and PDM groups, yet both differed significantly from the T2DM subjects. Fig. 2 summarizes the derived responses to the MMTT and AST, with the actual values presented in Table 3. In the MMTT, β-cell responsivity (Φtot, an index of insulin secretion) in the T2DM group was 86 and 87% lower compared with NGT and PDM ($P < 0.001$), respectively. Although Φtot was numerically higher in PDM compared with NGT, the difference was not statistically different. Si decreased progressively across glucose tolerance populations, with T2DM being 78 and 52% lower compared with NGT and PDM ($P < 0.001$), respectively, whereas PDM was 55% lower compared with NGT ($P < 0.01$). Correspondingly, DI was 51% lower in the PDM group compared with NGT ($P < 0.001$) and was 93% lower in T2DM compared with PDM ($P < 0.001$).

In 3 of the 44 (~7%) individual subject visits in the MMTT in the T2DM population, the standardized analytical approach yielded near-zero values for Si. As predefined in the analysis plan, the Si and DI data from these subjects were not included in the primary analyses. An alternate, slightly modified approach to the minimal model as described by Basu et al. (26) (see Supplementary Data) that allowed for inclusion of Si data from these three subjects did not yield values for the point estimates (geometric means and 95% CI) of Si or DI (with these three subjects, Si = 1.2 [0.9–1.6]; DI = 19 [13–38]) that were different from the original analysis (without these three subjects, Si = 1.2 [0.9–1.6] and DI = 21 [14–31]). This was true for the variance component estimates as well.

In the AST, AIRarg, AIRargMAX, and, most notably, the ISR were all lower ($P < 0.001$) in T2DM compared with NGT and PDM (58 and 63%, 79 and 78%, and 85 and 84% lower, respectively, for NGT and PDM). No significant differences were observed between NGT and PDM.

In the FSIGT, AIRg progressively and significantly decreased across glucose tolerance populations with the mean value for T2DM being nearly zero (Fig. 2). AIRg was statistically separable among the three glucose tolerance groups. Likewise, Si decreased progressively across glucose tolerance populations, although the difference between PDM and T2DM did not reach statistical significance. DI decreased significantly and progressively across groups.

Between- and Within-Subject Variability and Reproducibility for Measured Parameters and Indices From MMTT and AST

Glucose, insulin, and C-peptide responses within the MMTT and AST for each population had good reproducibility (Supplementary Fig. 1.1–1.6 and Supplementary Table 2.1–2.3). The AUCs (0–4 h) for glucose, insulin, and C-peptide from the MMTT generally displayed moderate to high reproducibility (as per ICC values), with the sole exception of glucose in the NGT. Reproducibility, as indexed by the ICC, ranged from weak to strong in the MMTT for all model-based parameters in all populations (Table 3). The inclusion of the three additional subjects with T2DM whose Si was numerically nonidentifiable did not affect that conclusion (not shown). For the AST, reproducibility

Table 2—Summary demographics and anthropometrics for evaluable subjects by glucose tolerance population

Parameter/population	NGT	PDM	T2DM
Number of evaluable (paired) observations [*N* (men/women)]	23 (12 men/11 women)	17 (6 men/11 women)	22 (11 men/11 women)
Age (years ± SD)	41.9 ± 8.4	44.8 ± 9.8	54.7 ± 8.1
Weight (kg ± SD)	85.8 ± 14.3	97.4 ± 12.9	91.1 ± 14.1
BMI (kg/m^2 ± SD)	31.5 ± 2.8	35.0 ± 3.8	32.8 ± 3.9
Ethnicity distribution			
White, not Hispanic or Latino	5	3	3
White, Hispanic, or Latino	14	10	16
Black, not Hispanic	4	3	2
Other		1	1
HbA_{1c}		5.7 ± 0.38% (39 mmol/mol)	8.28 ± 0.79% (67 mmol/mol)

For the PDM population, there were 16 evaluable subjects for the AST.

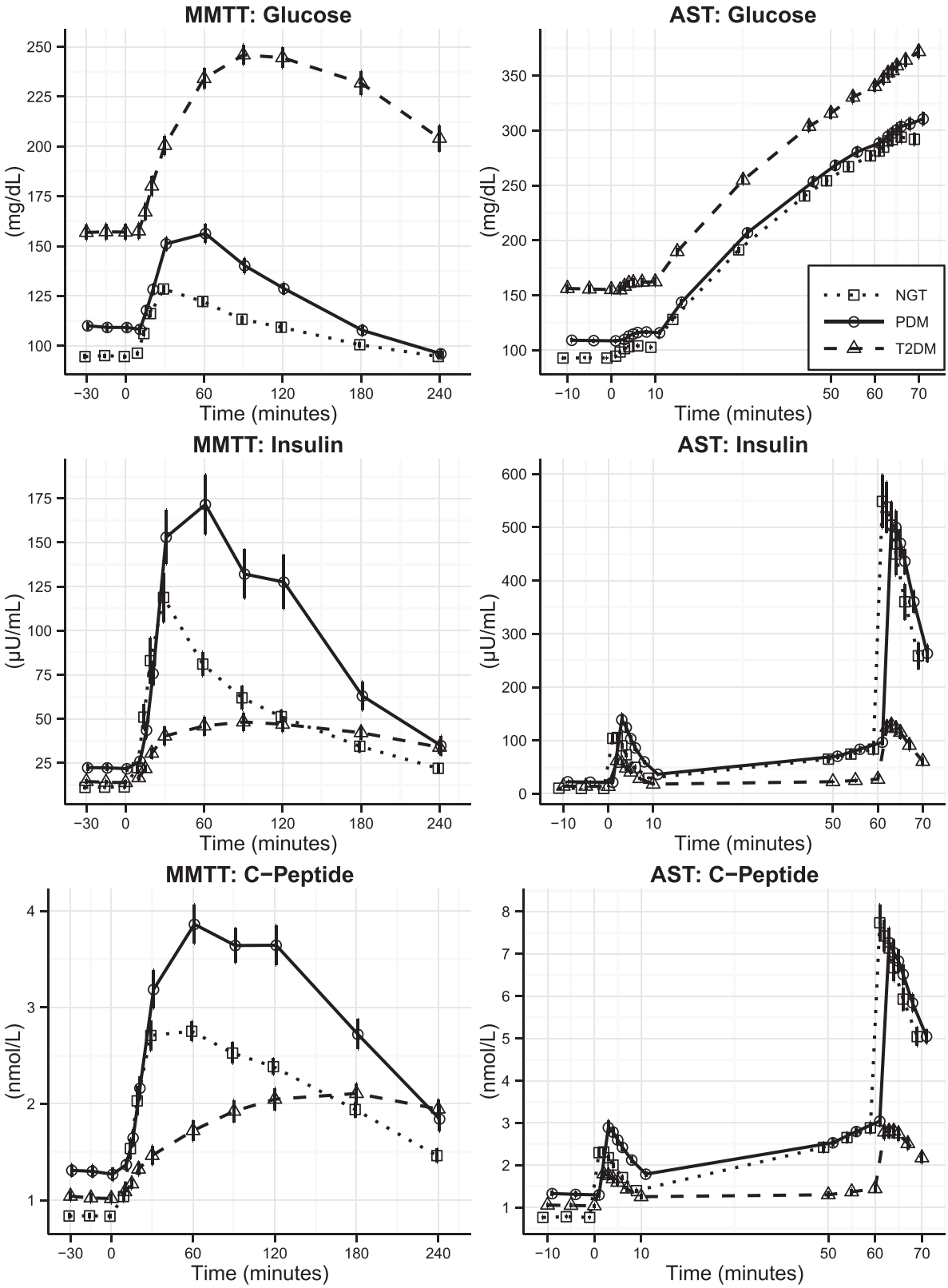

Figure 1—Mean ± SEM glucose, insulin, and C-peptide concentration time course profiles by stimulation test and glucose tolerance group. Squares, NGT; circles, PDM; triangles, T2DM.

was strong across all parameters/populations (all values >0.8).

Four extreme values from one of the two visits for two subjects (Φtot and DI in NGT and Si and DI in T2DM) for MMTT parameters were flagged during outlier analyses ($P < 0.001$). ICCs excluding these points as secondary, sensitivity analyses are provided in the legend for Table 3. When these outliers were excluded, reproducibility for the MMTT (per ICC values) rose considerably.

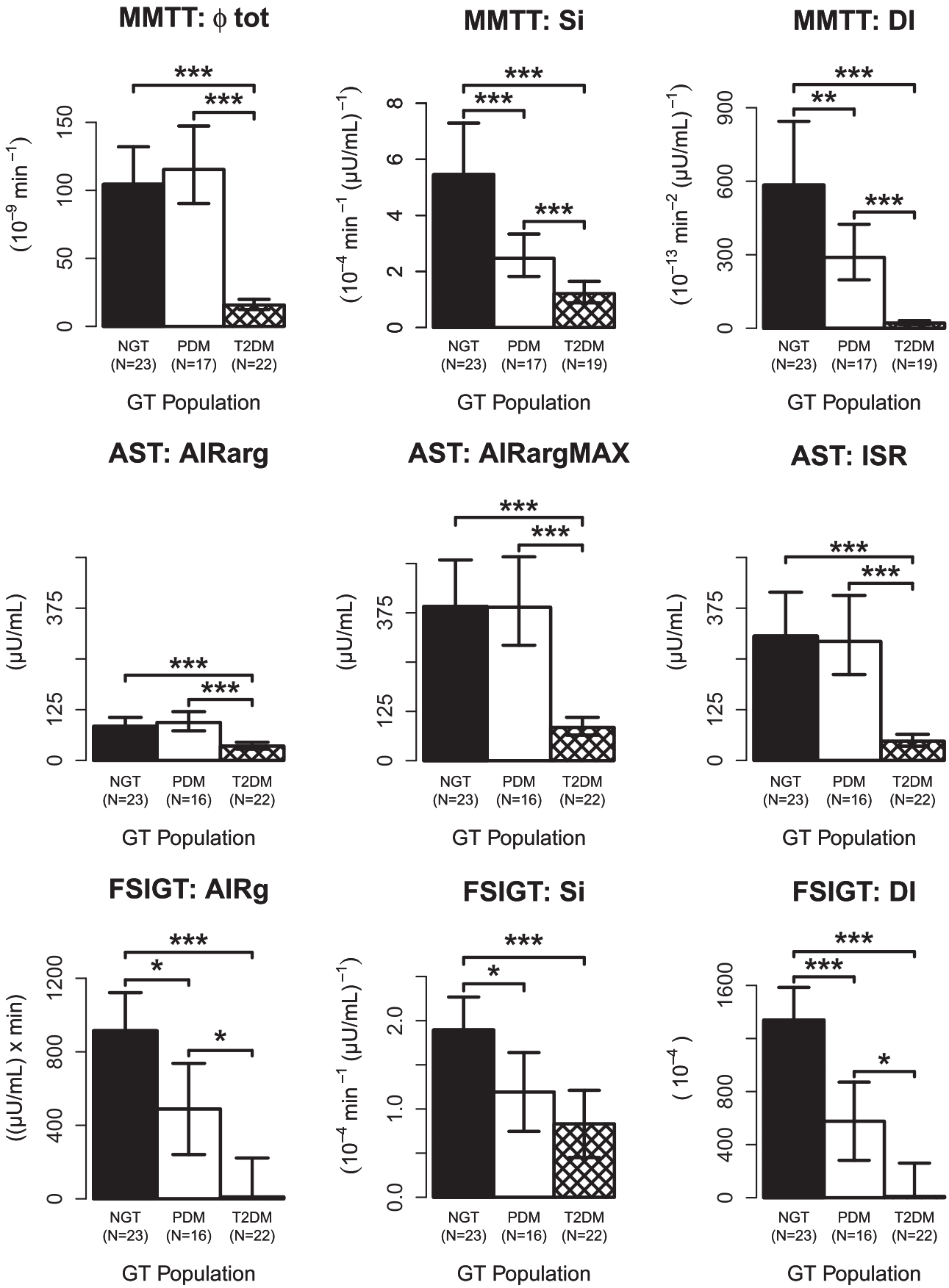

Figure 2—Indices of BCF. Model predicted geometric means (95% CI) for AST and MMTT and arithmetic means (95% CI) for FSIGT. Note: lower CI suppressed for graphical purposes for FSIGT AIRg and DI in T2DM. GT, glucose tolerance. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3—Variability and reproducibility metrics: geometric means, between- and within-subject geometric CVs, and ICCs for MMTT and AST and arithmetic means and total CVs for FSIGT

Stimulation test parameter	Population (*N*)	Model predicted geometric mean (95% CI)	Geometric CV% between subjects (90% CI)	Geometric CV% within subjects (90% CI)	ICC (90% CI)
MMTT: Φtot (10^{-9}min^{-1})	NGT (*N* = 23)	104 (83–132)	27.1 (14.7–51.6)	41.4 (32–54.1)	0.31* (0.30–0.77)
	PDM (*N* = 17)	115 (90–147)	34.8 (24.1–51.1)	19.6 (14.7– 26.2)	0.753 (0.70–0.9)
	T2DM (*N* = 22)	15 (12–20)	57.8 (41.8–82.1)	26.1 (20.2–33.8)	0.814 (0.69–0.96)
MMTT: Si (10^{-4}min^{-1} [μU/mL]$^{-1}$)	NGT (*N* = 23)	5.5 (4.1–7.3)	48.2 (34.1–69.8)	32.6 (25.4–42.3)	0.674 (0.521–0.874)
	PDM (*N* = 17)	2.5 (1.8–3.3)	44 (28.4–70.5)	35.5 (26.5–48.2)	0.598 (0.469–0.837)
	T2DM (*N* = 19)	1.2 (0.9–1.6)	53.5 (26.9–121.5)	83.2 (60.1–120.6)	0.323† (0.034–0.772)
MMTT: DI (10^{-13}min^{-2} [μU/mL]$^{-1}$)	NGT (*N* = 23)	585 (405–845)	42.3 (22.7–84.9)	66.1 (50–89.6)	0.312‡ (0.264–0.769)
	PDM (*N* = 17)	289 (197–424)	54.4 (35.3–87.9)	40.3 (29.9–55)	0.633 (0.543–0.852)
	T2DM (*N* = 19)	21 (14–31)	67.3 (35.1–152.2)	97.1 (69–145)	0.36§ (0.254–0.754)
AST: AIRarg (μU/mL)	NGT (*N* = 23)	84 (67–106)	38.9 (29.4–52.1)	11.6 (9.1–14.8)	0.914 (0.902–0.961)
	PDM (*N* = 16)	94 (73–120)	38.9 (27.6–55.7)	11.5 (8.6–15.4)	0.915 (0.874–0.972)
	T2DM (*N* = 22)	35 (28–45)	53.7 (39.1–75.4)	22.8 (17.7–29.5)	0.833 (0.734–0.966)
AST: AIRargMAX (μU/mL)	NGT (*N* = 23)	391 (301–509)	57.5 (42.8–79.2)	16.6 (13–21.2)	0.914 (0.858–0.967)
	PDM (*N* = 16)	389 (293–517)	31.2 (22.1–44.5)	11.2 (8.3–15)	0.882 (0.815–0.969)
	T2DM (*N* = 22)	84 (65–110)	63.1 (46.6–87.9)	13.8 (10.7–17.7)	0.947 (0.931–0.982)
AST: ISR (μU/mL)	NGT (*N* = 23)	306 (226–414)	64.1 (47.2–89.6)	20.1 (15.7–25.7)	0.897 (0.83–0.96)
	PDM (*N* = 16)	293 (212–406)	34.4 (24.3–49.3)	12.4 (9.2–16.6)	0.88 (0.759–0.976)
	T2DM (*N* = 22)	47 (35–64)	79.3 (57.3–114.5)	19.6 (15.3–25.3)	0.928 (0.916–0.968)

FSIGT: no within-subject variability estimable, total variance estimated, and analyses on arithmetic scale.

- Mean (95% CI) CV% AIRg ([μU/mL]) × min): NGT, 915 (708–1,122), 81%; PDM, 489 (241–737), 77%; and T2DM, 10 (−201 to 222), 413%.
- Mean (95% CI) CV% Si (10^{-4}min^{-1} [μU/mL]$^{-1}$): NGT, 1.9 (1.5–2.3), 60%; PDM, 1.2 (0.7–1.6), 37%; and T2DM, 0.8 (0.4–1.2) 101%.
- Mean (95% CI) CV% DI (10^{-4}): NGT, 1,339 (1,093–1,585), 64%; PDM, 576 (282–871), 90%; and T2DM, 9 (−242 to 261), 557%.

Exclusion of single-visit extreme value produces good reproducibility results for MMTT across all GT populations: *ICC = 0.59 (0.551–0.801) excluding single-visit extreme value ($P < 0.001$) from NGT ($N = 22$); †ICC = 0.559 (0.387–0.795) excluding single-visit extreme value ($P < 0.001$) from T2DM ($N = 18$); ‡0.527 (0.476–0.797) excluding single-visit extreme value ($P < 0.001$) from T2DM ($N = 18$); §ICC = 0.527 (0.476–0.797) excluding single-visit extreme value ($P < 0.001$) from T2DM ($N = 18$).

Comparative Assessment of Model-Based Indices of BCF Across Tests and Metabolic Spectrum

Correlation analyses were undertaken to better understand how these different measures tracked with one another within the same subject populations and across populations. Within the AST, the overall correlation between AIRarg and AIRargMAX was notably high across (0.923) and within (0.794–0.930) all populations (Table 4), indicating concordance in results across populations. In addition, the overall correlation between the AST-derived measures (AIRarg and AIRargMAX) and Φtot from the MMTT was high (0.858) and statistically significant across and within all populations. Overall correlations across glucose tolerance between AIRg from the FSIGT and Φtot from the MMTT as well as that between AIRg and AIRargMAX were high (0.753 and 0.826), whereas within-population correlation was less so, especially in T2DM. Si estimated in the MMTT and FSIGT showed a high overall correlation (0.695), consistent within the three populations. The overall correlation between DI from the MMTT and FSIGT was high (0.779), whereas no relationship for this parameter was observed within groups.

CONCLUSIONS

The current series of studies was undertaken to characterize the responses to and reproducibility of a standardized MMTT and AST for assessment of BCF in subjects with NGT, PDM, and T2DM. We report that the MMTT and AST are able to detect differences in BCF across the metabolic spectrum. The reproducibility of the MMTT is, in general, moderate (ranging from weak to strong), depending on parameter and population. The reproducibility of the AST is very strong across all populations. Importantly, for both MMTT and AST, the observed variability predicts reasonably sized clinical studies to detect clinically meaningful changes in insulin secretion. The MMTT- and AST-based measures of BCF are directionally and proportionally concordant and generally concur with indices derived from the reference FSIGT. It should be noted that despite these tests having been in use for quite some time, this is the first report of within- and between-subject variability for outcome parameters from the standardized MMTT and AST, especially in subjects across the metabolic spectrum. These data should be of value for the computation of sample size in interventional studies in relevant populations.

The standardized test meal used in the current series was able to elicit responses in glucose, insulin, and C-peptide during the MMTT that were similar to prior reports in NGT, PDM, and T2DM (27,28). Our findings for between-group differences for model-based estimates of BCF (Φtot) in those with and without diabetes are generally consistent with those reported by Bock et al. (27) and Ferrannini et al. (29), as well as with other methods such as the graded glucose infusion (30), OGTT (29), and hyperglycemic clamp (31). The significant progressive decrease in Si from NGT to T2DM is concordant with prior reports using clamp-based assessments (29). Notably, the DI, a measure of the appropriateness of insulin secretion to prevailing levels of insulin action,

Table 4—Spearman correlations and significance tests within and across stimulation tests for key parameters

Parameter association	Across GT populations	Within NGT population	Within PDM population	Within T2DM population
MMTT: Φtot (10^{-9}min^{-1}) vs. AST: AIRargMAX (μU/mL)	0.858***	0.492*	0.638**	0.799***
AST: AIRarg (μU/mL) vs. AST: AIRargMAX (μU/mL)	0.923***	0.914***	0.794***	0.930***
MMTT: Φtot (10^{-9}min^{-1}) vs. AST: ISR (μU/mL)	0.853***	0.482*	0.553*	0.744***
MMTT: Φtot (10^{-9}min^{-1}) vs. FSIGT: AIRg ([μU/mL] × min)	0.753***	0.394#	0.279 (NS)	0.010 (NS)
AST: AIRargMAX (μU/mL) vs. FSIGT: AIRg ([μU/mL] × min)	0.826***	0.779***	0.379 (NS)	0.082 (NS)
MMTT: Si (10^{-4}min^{-1} [μU/mL]$^{-1}$) vs. FSIGT: Si (10^{-4}min^{-1} [μU/mL]$^{-1}$)	0.695***	0.827***	0.650**	0.486*
MMTT: DI (10^{-13}min^{-2} [μU/mL]$^{-1}$) vs. FSIGT: DI (10^{-4})	0.779***	0.179 (NS)	0.085 (NS)	−0.284 (NS)
AST: ISR (μU/mL) vs. FSIGT: AIRg ([μU/mL] × min)	0.854***	0.824***	0.476#	0.156 (NS)

GT, glucose tolerance. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; #$P < 0.1$; NS, $P \geq 0.1$.

decreased across populations, indicating that the methodology was able to detect a progressive decrease in BCF. Together, these observations suggest that the MMTT used in this series recapitulated prior reports of BCF and insulin action measured using different tests.

The AST was able to elicit a β-cell response at baseline glycemia (AIRarg) and hyperglycemia (AIRmax) in all three populations. The responses were similar to those previously reported separately in each population (32,33). AIRarg, AIRmax, and ISR were highly reproducible across populations. The ability of β-cells to respond to arginine appears to be preserved in prediabetes.

An approach to better understand the utility of the reproducibility metrics of the MMTT and AST is to compute the sample sizes required to detect predetermined differences using parameters derived from the current trial. For example, in the MMTT, to detect a 25% increase in Φtot, 18 subjects per group are required for 80% power at a one-sided, 0.05 significance level. Although the variability observed in Si and DI is greater than with Φtot, in the current study, the MMTT detected differences across groups in Si and DI with modest numbers of subjects (~20) per group. It should be noted that the weaker reproducibility observed in some cases was driven by a single outlier, as shown by the sensitivity analyses. For the AST, five subjects per group are needed at the same power and significance level. Thus, both tests exhibit variances that permit reasonably sized clinical trials in relevant populations.

The glycemic excursions and insulin secretory responses in the FSIGT detected differences in insulin secretion across the metabolic spectrum, which recapitulated previous experimental results (10,34,35). An interesting observation in the current series was that although Φtot in the MMTT did not differ between NGT and PDM, AIRg in the FSIGT was decreased in PDM subjects compared with the NGT subjects. There are two salient points to recognize, however. First, previously published studies have generally made similar observations regarding the MMTT. Previous work from Bock et al. (27) and Ferrannini et al. (29) reported that insulin secretion in subjects with IGT is similar to that of obese subjects with NGT. Distinctions in insulin secretion are detectable, in general, only when contrasted to lean subjects with NGT (29). The subjects with NGT in the current series had a BMI that was close to those of the PDM group and had high normal fasting plasma glucose and insulin levels; they likely had overlap of some aspects of BCF with PDM.

Second, it should be noted that although the MMTT and the FSIGT are different tests, they arrived at the same conclusion—i.e., a progressive decrease in DI from NGT to PDM to T2DM. Ultimately, the DI provided by the minimal model yields an integrated estimate of insulin secretion in the context of insulin action. In the context of a meal, enteral delivery of substrate to subjects with PDM elicits an increase in insulin secretion that, on an absolute basis, is similar to that of obese subjects with NGT. However, insulin secretion is inadequate for the prevailing insulin action, as quantified by the DI and as expected by inspection of the glucose profiles in each group. The FSIGT, in contrast to the MMTT's mixed substrate delivery and elicitation of incretin (and other) responses, uses intravenous glucose as the sole stimulus that results in a lesser insulin secretory response in PDM than NGT subjects. Consistent with prior reports, the FSIGT in the current series was able to detect differences in acute insulin secretion in response to glucose across the metabolic spectrum (10,34,35). At the same time, the integrated parameter represented in DI showed a progressive decrease from NGT to PDM to T2DM. Thus, with some differences noted, the MMTT and the FSIGT provided the same overall conclusion.

Given the fairly unique circumstance of having access to data from multiple tests of BCF in the same subjects and across the glucose tolerance spectrum, it was informative to ask how the measures of BCF related to one another. Several key findings emerged across tests and within and across populations. In general, correlation across all three glucose tolerance populations was high for every comparison, and comparisons of parameters of BCF obtained with the FSIGT and MMTT showed correlation characteristics consistent with previous reports (36). However, in some instances, within each

population, the associations were less prominent. This suggests that some associations may in part be due to significant differences between populations and less so to a correlation of the tests, per se, within a subject. A notable example would be comparisons between Φtot (MMTT) and AIRg (FSIGT), as well as between AIRarg/AIRmax (AST) and AIRg. Although these showed reasonable overall correlations across glucose tolerance groups, they were weaker within each group, especially within T2DM. Taken together, the above observations suggest that although the various indices from each of these tests likely quantify different facets of BCF, there is concordance in the directionality and proportionality across tests (29).

Operationally, the MMTT is simple enough to routinely perform at most clinical research sites and centers. The test meal used in this series had a balanced macronutrient composition, with solid and liquid components, and is readily available from a manufacturer, assuring consistency of the test meal across sites. For similar operational reasons, the AST can be performed at most clinical research sites. Some procedural choices were made to simplify the testing, including sampling of nonarterialized venous blood (i.e., no hot hand). We recognize that the use of the hot hand method may have improved reproducibility. Our approach, however, is similar to previous studies that did not obtain arterialized samples for assessment of insulin secretion and sensitivity (29,37) and avoids potential issues due to the technique (38,39).

Analytically, in contrast to the AST, the MMTT and FSIGT require model-based analyses and occasionally need operator intervention to reconcile near-zero or negative values, respectively, for some parameters (35,40,41). In the current series, we opted for a more standardized analysis strategy that led to nonidentifiable Si parameters for MMTT in three subject visits in the T2DM population. Various methods can mitigate such model-related vulnerabilities (42,43), although inclusion of these three subjects did not change the conclusions. Furthermore, a novel method for assessing parameter reproducibility applied to minimal model data supports the reliability of the MMTT-derived indices (44). Although this series used the Cobelli oral minimal model for MMTT (12), it is important to recognize that there are other methods for estimating BCF from MMTT or OGTT data (37).

In summary, the current series of experiments shows that the variability and reproducibility characteristics of the MMTT and AST are sufficient to detect the types of changes in measured and modeled parameters of BCF that are generally expected in response to therapeutic interventions in relevant patient populations. Although the choice of testing methodology will ultimately be determined by the scientific question, the standardized MMTT and AST can provide reliable, reproducible, and complementary measures of BCF, with characteristics favorable for use in large, longitudinal interventional studies. Further studies are required to evaluate these tests' performance characteristics in response to a specific pharmacotherapy or lifestyle intervention.

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Duality of Interest. S.S.S. is an employee and shareholder of Eli Lilly and Company. R.H.R. is a shareholder of Bristol-Myers Squibb. R.A.C., D.C., J.Q.D., and D.S.L. are employees and shareholders of Pfizer. R.N.B. is responsible for the MinMod program, which is also marketed. C.Ca. is an employee and shareholder of Takeda Pharmaceuticals. M.D. is an employee and shareholder of Eli Lilly and Company and a member of the American Diabetes Association grant review panel. D.P. is an employee and shareholder of Johnson & Johnson. H.R. is an employee and shareholder of Sanofi. D.A.F. is a shareholder of Pfizer. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. S.S.S., A.V., and R.H.R. designed the experiment, researched data, wrote, edited, and reviewed the manuscript, and coordinated overall integration of the manuscript. M.A.S., R.A.C., R.N.B., C.Ca., D.C., C.Co., M.D., D.S.L., D.P., R.P.R., H.R., D.S., M.T.V., and G.C.W. designed the experiment, researched data, and wrote, edited, and reviewed the manuscript. C.D.M. and J.Q.D. researched data and reviewed the manuscript. D.A.F. designed the experiment, researched data, oversaw study execution, edited and reviewed the manuscript, and coordinated overall integration of the manuscript. D.A.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix

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