A Review of Methods for Measuring β-Cell Function: Design Considerations from the Restoring Insulin Secretion (RISE) Consortium

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Running Title: Measurement of β-cell function

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Abstract

The Restoring Insulin Secretion (RISE) study was initiated to evaluate interventions to slow or reverse the progression of β-cell failure in type 2 diabetes (T2D). To design RISE, we undertook an evaluation of methods for measurement of β-cell function and changes in β-cell function in response to interventions. Here we present a review of approaches for measurement of β-cell function, focusing on methodologic and feasibility considerations. Methodologic considerations included 1) the utility of each technique for evaluating key aspects of ß-cell function (first- and second-phase insulin secretion, maximal insulin secretion, glucose sensitivity, incretin effects); and 2) tactics for incorporating a measurement of insulin sensitivity in order to appropriately adjust insulin secretion measures for insulin sensitivity. Of particular concern were the capacity to accurately measure β-cell function in those with poor function, as is seen in established T2D, and the capacity of each method for demonstrating treatment-induced changes in β-cell function. Feasibility considerations included staff burden including time and required methodological expertise; participant burden including time and number of study visits; and the ease of standardizing methods across a multi-center consortium. Following this evaluation, we selected a 2-day measurement procedure, combining a 3-hour 75g OGTT and a 2-stage hyperglycemic clamp procedure augmented with arginine.

Introduction

The study of progressive metabolic dysfunction in prediabetes and type 2 diabetes (T2D) has focused on the pancreatic islet ß-cell. [1] Cross-sectional studies in adult and adolescent populations have shown inferior ß-cell function in individuals with impaired glucose tolerance (IGT) and even poorer function in those with T2D. [2-5] Longitudinal assessments to-date demonstrate progressive worsening of β-cell function among individuals with genetic and metabolic risk factors for T2D, and in patients with increasing hyperglycemia. [6-8] Methodologies for measuring β-cell function range in complexity from fasting measures, to protocols involving intravenous infusion of multiple stimulators of insulin release [9-16]. Each method brings strengths and weaknesses, and selection of the optimal approach must not only reflect pertinent physiology, but also factors such as cost and participant burden.

The Restoring Insulin Secretion (RISE) study was designed to test interventions to slow or reverse the progression of β-cell failure in individuals at high risk of T2D, or with recent onset T2D. [17] Here we present a review of available techniques for measurement of β-cell function, focusing on the methodologic and feasibility considerations that informed the selection of approaches utilized in RISE.

Physiologic Considerations for the Measurement of β-cell Function

β-cell function can be defined as the ability of pancreatic β-cells to produce, store and release insulin in concentrations sufficient to maintain euglycemia. Under normal physiologic conditions, circulating insulin concentrations are reciprocally related to insulin sensitivity, expressed as the body's capacity for glucose disposal and ability to suppress hepatic glucose production in response to insulin. [10] When insulin sensitivity declines, the appropriate

physiologic response is for insulin secretion to increase in a compensatory manner. The

calculated line linking these factors, which exhibit a square hyperbolic relationship, is commonly

expressed as the 'disposition index' (DI, insulin sensitivity * first-phase insulin secretion; Figure

1). [10, 14] The need to incorporate a measurement of insulin sensitivity into assessments of β-

cell responses is widely accepted. With this in mind we will briefly review alternatives for

measuring insulin sensitivity before turning to methods for assessing β-cell responses.

Measuring Insulin Sensitivity

In order to appropriately adjust the β-cell reponse for the prevailing insulin sensitivity, a

concurrent measure of insulin sensitivity is required. Robust discussions of different approaches

to measuring insulin sensitivity have been previously published. [18-20] Here we present a brief

exposition of available methods with a focus on the technical limitations and participant burden.

Hyperinsulinemic-Euglycemic Clamp – This technique allows measurement of whole-

body and tissue sensitivity to a steady-state concentration of insulin while the plasma glucose

concentration is held constant (clamped), generally at physiologically normal (euglycemic)

levels. [21] Performing the procedure in the euglycemic state obviates the need to correct for the

impact of hyperglycemia on glucose disposal. This method produces measures of insulin-

stimulated glucose disposal (M), and insulin sensitivity (M /I, where I denotes steady state

plasma insulin concentration). Because this technique imposes plasma insulin and plasma

glucose concentrations at defined experimental levels, independent of insulin production or

release, it provides reliable measurements of insulin sensitivity in subjects across the full range

of β-cell function.

The limitations of this technique include the need for two intravenous lines (one for infusion of insulin and glucose, and the other for blood sampling), high-precision glucose measures every 5 minutes, and personnel with expertise to make adjustments in the glucose infusion rate in order to maintain the target level of glycemia. The participant considerations include the need for two intravenous lines, and the duration of the procedure. Overall, this method is relatively resource-intensive (requiring nursing and investigator time and expertise), and it provides a measurement of insulin sensitivity without a simultaneous measure of β-cell function (as is provided in some methods discussed below).

Hyperglycemic Clamp-Derived Insulin Sensitivity – The hyperglycemic clamp technique is discussed in detail below as a method for measurement of β-cell function. It also provides an indirect measure of insulin sensitivity, using the rate of glucose disposal under imposed steady-state hyperglycemic conditions, adjusted for the achieved endogenous insulin (or C-peptide) concentrations. [12, 22] Adjustments are needed for variations in achieved steady state glucose concentrations, and for urinary glucose losses. Typically, insulin sensitivity is calculated by dividing the glucose disposal rate by the plasma insulin (or C-peptide) concentration at steady state during the last 30 or 60 minutes of a 2-hr hyperglycemic clamp.

The principal limitation of measuring insulin sensitivity with this method is the dependence of the insulin sensitivity measure on the endogenous late-phase β-cell response. This is primarily an issue where poor late-phase insulin release provides an insufficient stimulus to drive glucose disposal in the face of poor insulin sensitivity, limiting accuracy of measurement of insulin sensitivity.

IVGTT - Minimal Model-Derived Insulin Sensitivity- The minimal model of glucose kinetics developed by Bergman and colleagues allows insulin-mediated glucose disposal to be

calculated from intravenous glucose tolerance test (IVGTT) data, with derivations of a model-derived measure of insulin sensitivity (S_I). [23, 24] The minimal model has been extensively evaluated and widely adopted. In a modification of the original methodology, exogenous tolbutamide or (more commonly) insulin is administered after assessing the first-phase insulin response, to better characterize insulin dependent glucose disappearance where endogenous production is insufficient. [25, 26] However, in more severe insulin resistance, the standardized exogenous insulin bolus may be insufficient to produce data adequate for modeling.

Surrogate Measures of Insulin Sensitivity – Indices of insulin sensitivity have been developed using fasting blood samples (e.g. inverse fasting insulin, homeostasis model assessment (HOMA) [27], quantitative insulin sensitivity check index (QUICKI)) [28], or the combined glucose and insulin excursions of the OGTT (e.g. Matsuda index) [19]). In cross-sectional evaluations, these fasting and OGTT-derived measures correlate reasonably well with hyperinsulinemic-euglycemic clamp or minimal model-derived measures (r=0.6-0.7) [29]. The utility of surrogate indices for longitudinal use has not been extensively evaluated. Some reports have described concurrent changes in multiple indices over time [30, 31], but to date correlations between longitudinal changes in surrogate indices of insulin resistance and more direct measures have only been formally evaluated in one publication. [20] This paper evaluated a cohort of Mexican-American women followed after gestational diabetes, and found changes in the surrogate indices to be less strongly correlated to changes in IVGTT-derived S_I than is observed in cross-sectional settings.

Measuring β-cell Function

Glucose is the principal regulator of insulin secretion, via a well-described pathway linking β-cell glucose uptake to changes in ADP/ATP ratios and ultimately to changes in membrane potassium conductance and movement of insulin granules, producing a pulsatile and oscillatory pattern of insulin secretion in health. [32, 33]. Non-glucose β-cell stimuli include incretin hormones, acting through a cAMP system to potentiate the response to glucose [34, 35], and monobasic amino acids, fatty acids, and β-adrenergic agonists, which also act independent of the glucose sensing systems but converge on the same insulin secretion pathways. [36] These features are exploited in the many methods that have been developed for the measurement of β-cell function.

Hyperglycemic clamp - Under this method, an exogenous glucose infusion is applied to raise blood glucose to a specified target concentration, or to achieve an increment above the individual's fasting glucose. Both the magnitude and timing of the hyperglycemic stimulus are controlled, allowing for a precise and repeatable stimulus to insulin/C-peptide secretion and for clear separation of first- and second-phase responses to intravenous glucose (Figure 2). [21, 22]

The first-phase insulin/C-peptide response primarily consists of release of stored insulin and occurs in the first few minutes after circulating glucose concentrations increase, subsiding within 10 minutes. [37] The first-phase response is measurably diminished in individuals with only modest elevations in fasting glucose and/or IGT, more severely diminished in individuals with fasting glucose concentrations >115 mg/dL (6.4 mmol/L), and absent in T2D. [38-40]

The second-phase insulin/C-peptide response begins concurrent with the first-phase response, and consists of a slow and sustained increase in insulin/C-peptide concentrations, reflecting pools of insulin granules with varying kinetic properties. [37, 41, 42] The second-

phase response is not lost early in T2D, but declines over time with progressive reduction in β-cell function. [43, 44]

A DI can be calculated from data derived from the hyperglycemic clamp alone as long as a hyperbolic relationship exists between the measures of insulin/C-peptide secretion and insulin sensitivity. The DI is calculated using the measured insulin/C-peptide response and the indirect measure of insulin sensitivity (M/I) as explained above. [11]

Examples of application of the hyperglycemic clamp include understanding the progressive pathophysiology of β-cell dysfunction [12, 13, 30], and assessing the effects of pharmacologic interventions, weight loss and bariatric surgery on β-cell function. [44-46]

The ability to measure insulin/C-peptide secretion and insulin sensitivity in a single day is an advantage of the hyperglycemic clamp. Another advantage is that measures of β-cell function are accurate along the entire spectrum of NGT to prediabetes to diabetes, with reliable measures even in the low-response range seen in individuals with prediabetes and T2D. Despite impaired β-cell function, such individuals generally mount a sufficient second-phase response to provide a reliable measure of insulin-mediated glucose disposal. The principal technical limitations of the hyperglycemic clamp include the need for two intravenous lines (one for infusion of glucose and the other for blood sampling), rapid early sampling after the initial glucose bolus, high-precision glucose measures every 5-10 minutes, and personnel with expertise to make adjustments in the glucose infusion rate in order to maintain the target level of glycemia. The participant considerations include the need for two intravenous lines, and the 3-4 hour duration of the procedure (Table 1).

Intravenous glucose tolerance test (IVGTT) - During the IVGTT, an intravenous bolus of dextrose is given and rapid sampling for measurement of glucose and insulin concentrations is

performed during the first 10 minutes of the test to measure the acute (first-phase) insulin and C-peptide responses. Subsequent measurements across the remainder of the test are used to derive the late- (second) phase responses. As noted above, under current usage a bolus of exogenous insulin is generally applied in order to successfully model insulin sensitivity (S_I).

Investigators have utilized the IVGTT minimal model to describe progressive loss of β-cell function in the development of diabetes [47-49], to describe the physiology of individuals at risk for diabetes [12, 47, 50-52], and to follow response to treatment. [53-55]

As with the hyperglycemic clamp, the IVGTT allows for derivation of measures of β-cell function and insulin sensitivity from a single testing day. This method works well when the endogenous β-cell response is sufficient to provide timely and effective control of the glucose excursion. However, important degrees of β-cell dysfunction are present earlier in the pathogenesis of diabetes than might be expected: In screen-detected T2D and in individuals with fasting glucose levels ≥115 mg/dL (6.4 mmol/L), the first-phase insulin response is characteristically low or unmeasureable. [38, 44] The insulin-modified protocol is intended to overcome this limitation, as discussed above, with its own pros and cons. This added insulin prevents assessment of the late-phase insulin secretion responses, as the exogenous insulin is measured together with the endogenous insulin. Because the test does not control for achieved levels of glycemia, the magnitude of the stimulus to β-cell secretion can differ between or within individuals. Overall, in the settings of low insulin release and/or low insulin sensitivity, the IVGTT methodology is less reliably able to provide accurate measures of β-cell function and insulin sensitivity than the hyperglycemic clamp.

The principal technical limitations of the IVGTT are the need for two intravenous lines, the need for rapid early sampling following the intravenous glucose bolus, the need for software

and expertise to undertake the modeling analyses for each individual IVGTT to derive the S_I. The participant considerations include the need for two intravenous lines, the 3-4 hour duration of the procedure, and the risks associated with insulin infusion (Table 1).

Graded glucose infusion – With this method, the insulin/C-peptide response to a prolonged intravenous infusion of glucose is measured. Rather than targeting a particular level of glycemia, the graded glucose infusion imposes a series of pre-set glucose infusion rates. This produces acute, stepwise increments in blood glucose, engendering stepped insulin secretory responses. [56-58] The initial bolus generally differs from that used in the hyperglycemic clamp, and therefore first-phase insulin/C-peptide response measurements are not directly comparable between hyperglycemic clamps and graded glucose infusion tests. [59]

The graded glucose infusion has been used across the spectrum of glucose tolerance, and has the advantage of allowing derivation of a slope reflecting the β-cell sensitivity to glucose. [58] An indirect measure of insulin sensitivity can be obtained by extending the methodology, using an up and down graded glucose procedure together with minimal modeling. [58] Unless this approach is used, a separate measure of insulin sensitivity is required to calculate a DI. Due to differences in the achieved glucose concentrations, this measurement incorporates degrees of glucose mass action (glucose-mediated glucose disposal) that are different from the other methods. Thus, the results are parallel but not strictly comparable to other approaches to measure insulin sensitivity. [60]

The graded glucose infusion has been used principally in exploring the pathophysiologic progression of β-cell dysfunction [57], and in assessing the effects of treatment interventions on β-cell function [61].

The principal technical limitations of the graded glucose infusion are the need for two intravenous lines, and expertise with the mathematical approaches needed for data extraction. The personnel burden is comparable in terms of time but this method requires less methodologic expertise than the hyperglycemic clamp, and the graded glucose infusion requires less frequent blood sampling overall. Participant considerations include the need for two intravenous lines, and a time commitment of 3-4 hours (Table 1).

Glucose-potentiated arginine stimulation test - L-arginine infused as a bolus while the participant is hyperglycemic at a level of 450 mg/dL (25 mmol/L) or greater produces a maximal insulin response considered to reflect the functional secretory capacity of β-cells. [36, 62-64] Individuals with blunted or absent first-phase insulin/C-peptide response to intravenous glucose maintain a brisk, although reduced response to arginine (Figure 3). [11]

Historically, stimulation with isoproterenol or glucagon was used to measure augmented insulin release, but resulted in unacceptable side effects. [63] Lower variability is observed in the insulin/C-peptide response with arginine versus glucagon; moreover, arginine stimulates glucagon release, allowing for a concurrent measure of α-cell function. [65] Recently, combinations of glucose with glucagon-like peptide 1 (GLP-1) or GLP-1 mimetics have also been used. [11, 34, 35] It is not clear whether these combination approaches offer an advantage in cost, safety, or measurement variability that may overcome the established experience with arginine.

A variation of the glucose-potentiated arginine stimulation test involves repeated applications of arginine under two or more achieved glucose concentrations. [66, 67] This approach measures stimulated responses at multiple levels of glycemia, allowing for derivation of slopes of glucose and arginine responsiveness. This provides complementary measures of β-

cell function with more physiologic glucose exposures, and provides the statistical advantage of repeated within-subject measurements. It is feasible to undertake a glucose-potentiated arginine response immediately after completing a hyperglycemic clamp procedure, functionally performing one procedure but measuring multiple aspects of β-cell function.

The glucose-potentiated arginine response has been applied in assessing the function of a pancreas or islet cell transplant [67], and in assessing pharmacologic effects on β-cell function in T2D [68, 69]

The principal technical limitations of the glucose-potentiated arginine response are the same as for the hyperglycemic clamp, with the addition of the clinical supplies needed for the L-arginine infusion. Participant considerations include lengthening the hyperglycemic clamp procedure, and approximately 40% of participants experience mild side-effects (brief flushing or metallic taste) when L-arginine is administered. [65]

Oral Glucose Tolerance Test (OGTT) - The OGTT can be employed to assess β-cell function. The relatively delayed appearance of glucose in the circulation prevents strict separation of first- and second-phase insulin responses; these components are therefore traditionally described as early and late insulin responses. The early response can be evaluated simply as the rise in insulin/C-peptide above basal at any time interval up to 30 minutes after commencing glucose ingestion, or as the "insulinogenic index" (the increment above basal insulin/C-peptide divided by the increment in glucose in the same time interval). [70] The early insulin response and the insulinogenic index are reduced in IGT and T2D. [71, 72] The late insulin or C-peptide response is generally evaluated as the integrated response over the entire sampled duration; this measure has been less widely used. [44, 71] The use of OGTT parameters to derive a DI is increasingly applied [72, 73], supported by mathematical evidence for an

underlying hyperbolic relationship between specific measures of insulin secretion and insulin sensitivity from the OGTT. [74, 75]

The OGTT has been widely applied in the evaluation of the pathophysiologic progression of β-cell dysfunction [75, 76], and in assessing the effects of treatment interventions on β-cell function. [77]

A core advantage of the OGTT is that it incorporates the physiologic contributions of the gut-pancreas axis in the measure of ß-cell responses. When an OGTT is combined with an intravenous test (IVGTT or hyperglycemic clamp), this allows for comparisons of responses to parenteral versus enteral stimulation, enabling assessment of the contributions of the incretin effect to the overall response to ingested glucose. [78] OGTT-derived measures of insulin response can be adjusted for insulin sensitivity using a surrogate measure such as fasting insulin concentration or HOMA%S, or using a separate direct measurement. Minimal model methodologies have been developed that allow insulin response and insulin sensitivity to be simultaneously assessed with a multiple-sample, extended OGTT. [79-81] These models have been utilized to assess β-cell function during physiologic testing in subjects with NGT, prediabetes, and T2D.

A disadvantage of OGTT methodology is that differences in the rate of glucose absorption can modify the observed response. Due to the involvement of more biological systems and less direct control of the glycemic stimulus to insulin secretion, the variability in measures of β-cell function is high compared to that seen with intravenous testing. [13, 82-84]

Mixed meal tolerance test (MMTT) - Analogous to the OGTT, a liquid or solid enteral stimulus consisting of a mixture of carbohydrate with other macronutrients can be delivered orally with subsequent sampling of blood glucose and insulin. As with OGTT, this method can

be applied to assess contributions of the incretin effect to the overall mixed meal response. [85] The mixed nutrient load provides a more physiologically relevant comparison to human meal consumption than an isolated glucose load. [86] The same directly calculated and model-derived measures of β-cell responses can be derived from the MMTT dataset, with parallel advantages and disadvantages. [79, 87, 88] Despite the improved physiologic relevance of this method, the delivery of multiple nutrients involved in stimulation of gut hormones and in β-cell stimulation contributes to relatively high variability for this method as with the OGTT [9, 13]. Differences in size and composition of the enteral load lead to differences in insulin and incretin responses [86, 89], although there are recent efforts to standardize the test meal. [9]

The MMTT is widely utilized at present to assess β-cell function in therapeutic trials in type 1 diabetes. In contrast to high variability observed in other populations, results in this population have been highly reproducible.[90]

Simple indices and model-based estimates of β-cell function have been reported to be quantitatively higher when measured via MMTT as compared with OGTT with equal carbohydrate quantity among dysglycemic subjects. [13, 87, 88] (Figure 4) To date, no published data have formally demonstrated a hyperbolic relationship between MMTT derived β-cell function and insulin sensitivity to fully support their combination into a DI. Importantly, the MMTT glucose data cannot be interpreted using OGTT-based diagnostic criteria that define IFG, IGT and DM.

Both the OGTT and MMTT require the placement of a single peripheral intravenous line for repeated blood sampling. Blood sampling frequency and timing is parallel to that for OGTT, reduced compared to glucose infusion based protocols, and there is no requirement for specific expertise in making adjustments to glucose infusions. Modeling methods require software and

expertise. Participant considerations include the need for one intravenous line, and a time commitment of 3-4 hours.

Fasting proinsulin to insulin (or C-peptide) ratio In subjects without diabetes, the molar proportion of circulating proinsulin to insulin is approximately 15% in the fasting state. [91, 92] As β-cell failure ensues, processing of proinsulin to insulin and C-peptide is impaired, and the fasting proinsulin to insulin ratio increases two- to three-fold in T2D. [91, 92] Interestingly, the ratio is not significantly increased in all individulas with IGT [93], possibly suggesting that an elevated ratio is an indicator of more established β-cell dysfunction or of increased β-cell demand.

Homeostatic model assessment (HOMA) – The HOMA provides estimates of basal β-cell function and insulin sensitivity. [27] Updates to the model take into account variations in hepatic and peripheral glucose resistance, and other whole-body determinants of metabolic physiology.[94] The original linear equations are simplified approximations of the original nonlinear solution. [27] With modern computing the direct calculation is widely accessible, particularly with the availability of an online calculator (https://www.dtu.ox.ac.uk/homacalculator/). The use of the online methodology is preferred. [27, 95]

Because the model requires only basal glucose and insulin/C-peptide concentrations, it has obvious advantages in terms of cost and ease of application, and has been widely utilized in large epidemiologic studies, longitudinal cohort studies, and clinical trials. The HOMA%B is correlated (r=0.6-0.9) with direct measures of β-cell function in cross-sectional studies of healthy populations with NGT, but may be less reliably related in progressive dysglycemia and diabetes.

[27, 83, 96]. Correlations with direct measures are weaker in longitudinal studies, even after accounting for increased variability of the measurements. [20]

These surrogate measures have been used primarily in epidemiologic studies, although in some instances they have been used to assess treatment effects on β-cell function in pharmacologic or surgical studies. [46, 77, 97]

These measures have advantages for cost and personnel burden, and require much less of individual participants. However, the compromises entailed make it an imperfect choice for studies primarily assessing β-cell function and response to interventions that may also improve insulin sensitivity.

A Case Study in Method Selection - The RISE Approach to Measuring ß-Cell Function

The Restoring Insulin Secretion (RISE) Consortium includes three studies assessing the hypothesis that glucose lowering will lead to sustained improvement in β-cell function in prediabetes and early T2D [17] :

1. RISE Adult Medication Study: Adult participants (ages 20-65) are randomized to one of the following treatment arms: (1) metformin alone, (2) early treatment with insulin glargine followed by metformin, (3) liraglutide plus metformin, or (4) placebo.

2. RISE Pediatric Medication Study: Pediatric participants (ages 10-19) are randomized to: 1) metformin alone, or 2) early treatment with insulin glargine followed by metformin.

3. RISE Adult Surgical Study (BetaFat Study): Adult participants (ages 20-65) are randomized to gastric banding or metformin.

The RISE Medication Studies will assess whether improvements in ß-cell function following 12 months of active treatment are maintained for 3 months following the withdrawal of therapy.

The BetaFat trial will assess the same outcome variables after 12- and 24-months of active treatment with metformin or following gastic banding surgery.

The RISE studies use a shared set of measurements. The selection of methods to be applied in RISE incorporated the need to carefully assess β-cell function at repeated intervals, while balancing considerations for participant burden and resource constraints. Also of importance was the capacity to demonstrate change on repeated testing performed longitudinally. Incorporating the considerations and comparisons of methodologies outlined above, the RISE Consortium elected to undertake, as the primary method for measurement of ß-cell function, a two-stage hyperglycemic clamp including arginine stimulation. The first stage uses an initial weight-based glucose bolus followed by a 2-hour continuous glucose infusion targeting a sustained plasma glucose concentration of 200 mg/dL (11.1 mmol/L), to allow derivation of first- and second-phase insulin/C-peptide responses to intravenous glucose and the measurement of insulin sensitivity at the end of this 2-hour clamp. The second stage incorporates a 30-45 minute increase in plasma glucose concentration to at least 450 mg/dL (25 mmol/L) followed by a bolus of 5g of L-arginine, to allow measurement of maximal β-cell secretory capacity.

RISE also chose to perform, a separate 3-hour OGTT with rapid early sampling (10/20/30 minutes following ingestion) to evaluate glucose tolerance and ß-cell responses in the context of an enterally delivered stimulus. This increases the subject burden by adding an additional testing day, but provides information on glycemic control and responses that incorporate the incretin contributions to ß-cell function. The incretins were of interest as both the gastric-banding surgery and GLP-1 receptor agonist therapies could have treatment-specific effects to modify responses to enterally delivered nutrients that may not be adequately assessed using the hyperglycemic clamp. Comparisons of clamp versus OGTT responses will be used to evaluate

whether changes in incretin response contribute to any observed effects of the RISE interventions. The OGTT was chosen over the MMTT to maximize standardization, and to allow a measure of glucose tolerance that can be evaluated against established clinical criteria.

The hyperglycemic clamp was chosen over the other methods described for the following reasons: 1) it allows for a controlled and repeatable hyperglycemic stimulus to the β-cell (minimizing variability), and allows the addition of arginine stimulation to measure β-cell secretory capacity; 2) the methodology is reproducible and amenable to standardization across study sites [21]; and 3) it simultaneously provides an indirect measure of insulin sensitivity. Including a hyperinsulinemic-euglycemic clamp would add considerable participant and staff burden over the course of a longitudinal study. We instead chose to evaluate insulin sensitivity using the insulin sensitivity index derived during the 200 mg/dL (11.1 mmol/L) steady-state period of the hyperglycemic clamp. Doing so allowed the inclusion of a second, less-intensive day of testing with an OGTT. Arginine-stimulated insulin secretion was included as a measure of the maximal ß–cell response, which could potentially show a different response to the various treatment approaches. Arginine was chosen as opposed to other available stimuli because it is an established method for this purpose, and it has superior technical performance.[65] Further, use of arginine would allow the evaluation of the glucagon response as a measure of α-cell function.

The IVGTT was ultimately not chosen because we anticipated very poor or absent first-phase insulin responses in the population to be evaluated, with attendant difficulties in modeling first-phase responses and missing data. We also considered graded-glucose infusion tests, given the unique advantage of directly quantifying β-cell glucose sensitivity, and the advantage of precise regulation of the glucose stimulus. However this method does not produce traditional measures of first- and second- phase insulin responses. In order to have a measure of insulin

sensitivity, we also would have needed to use the model-derived measure from the up-down graded procedure, or perform a hyperinsulinemic clamp on a separate day. [58] Here again, there was a concern that modeling for individuals with poor β-cell function and poor insulin sensitivity would prove difficult, with loss of data and incomplete datasets even with this detailed method of measuring ß-cell glucose responses.

Conclusion

Many different methods have been developed for *in vivo* measurement of human β-cell function, each with strengths and weaknesses. The optimal selection of methods will be determined by the particular focus of study. Table 1 provides an overview of the main strengths and weaknesses of the methods discussed.

The RISE study is evaluating the effects of interventions including pharmacotherapeutics and metabolic surgery on β-cell function, in populations spanning from pediatrics to adults. We elected to measure our β-cell outcomes using a 2-day measurement procedure, namely a 3-hour 75g OGTT and a 2-stage hyperglycemic clamp with arginine. This combination of methods provides an assessment of: 1) first- and second-phase insulin/C-peptide responses; 2) insulin sensitivity; 3) maximal β-cell secretory capacity; 4) early and late insulin response to an enteral glucose stimulus. This protocol has been successfully implemented in a multi-center consortium, highlighting the feasibility of using these methods in treatment studies with multiple participating study sites.

Acknowledgements

Table 1. Methods of Assessing Human β-Cell Function *In Vivo*

Method	Time* (hr)	Staff & Participant Burden	Directly Measures First- and Second-phase Insulin	Concurrent Measure of Insulin Sensitivity	Features of Note
Hyperglycemic Clamp	2-3	+++	Yes	Yes	Direct measurement of traditional beta-cell function plus a measure of insulin sensitivity
IVGTT	3-4	+++	Yes	Yes	Simpler procedure for combined measurement of beta-cell and insulin sensitvity
Graded Glucose Infusion	2-4	++	No	No	Direct measurement of beta-cell glucose sensitivity

Glucose-Potentiated Arginine Stimulation	1-2	++	No	No	Complementary, glucose-independent measurement of beta-cell function; may reflect beta-cell mass
OGTT or MMTT	2-4	+	No	Yes	Measuring physiologic response including incretin axis; OGTT can provide clinically diagnostic results
Fasting Measures	<1	+/-	No	Yes	Simplest and least expensive, useful for epidemiologic studies

*Times do not include time necessary for catheter insertion and study preparation. The time for glucose-potentiated arginine secretion is in addition to the time needed for any preparatory procedures.

Figure 1. The hyperbolic relationship between insulin sensitivity (*S*I) and the first phase (acute) insulin response to glucose (AIRglucose) (a) and the maximal acute insulin response to arginine (AIRmax) (b) in a cohort of healthy individuals. The solid line depicts the best-fit relationship (50th percentile), while the broken lines represent the 5th, 25th, 75th, and 95th percentiles. A reduction in insulin sensitivity, as measured by a decrease in *S*i, results in a compensatory reciprocal and proportionate increase in glucose-stimulated insulin secretion and an increase in maximal acute insulin response to arginine, the latter a measure of β-cell secretory capacity. (Copyright 1993 American Diabetes Association. From Reference [10] Reprinted with permission from *The American Diabetes Association*)

Figure 2. Plasma insulin response to a 20g intravenous glucose bolus in NGT (a) and T2D (b). The first-phase insulin response is absent in the subjects with diabetes while the second-phase response is relatively preserved, with a delayed maximal insulin response. (Reprinted from Reference [98], with permission from Excerpta Medica Inc.)

Figure 3. Comparison of the acute insulin response to a 5g intravenous L-arginine injection at different glucose levels in NGT participants compared with participants with T2D, with simiar age and body weight. (Reprinted with permission from Reference [39]).

Figure 4: (A) DI in obese adolescents across the spectrum of glycemia. Letters indicate significant post hoc analyses (a: T2D vs. NGT; b: T2D vs. IFG; c: T2D vs. IGT; e: NGT vs. IFG/IGT; f: NGT vs. IGT). Adapted with permission from reference [99]. (B) Incretin effect in

obese youth. Letters indicate significant post hoc analyses (a: NGT vs. IGT; b: NGT vs. T2D).

Adapted with permission from reference [78].

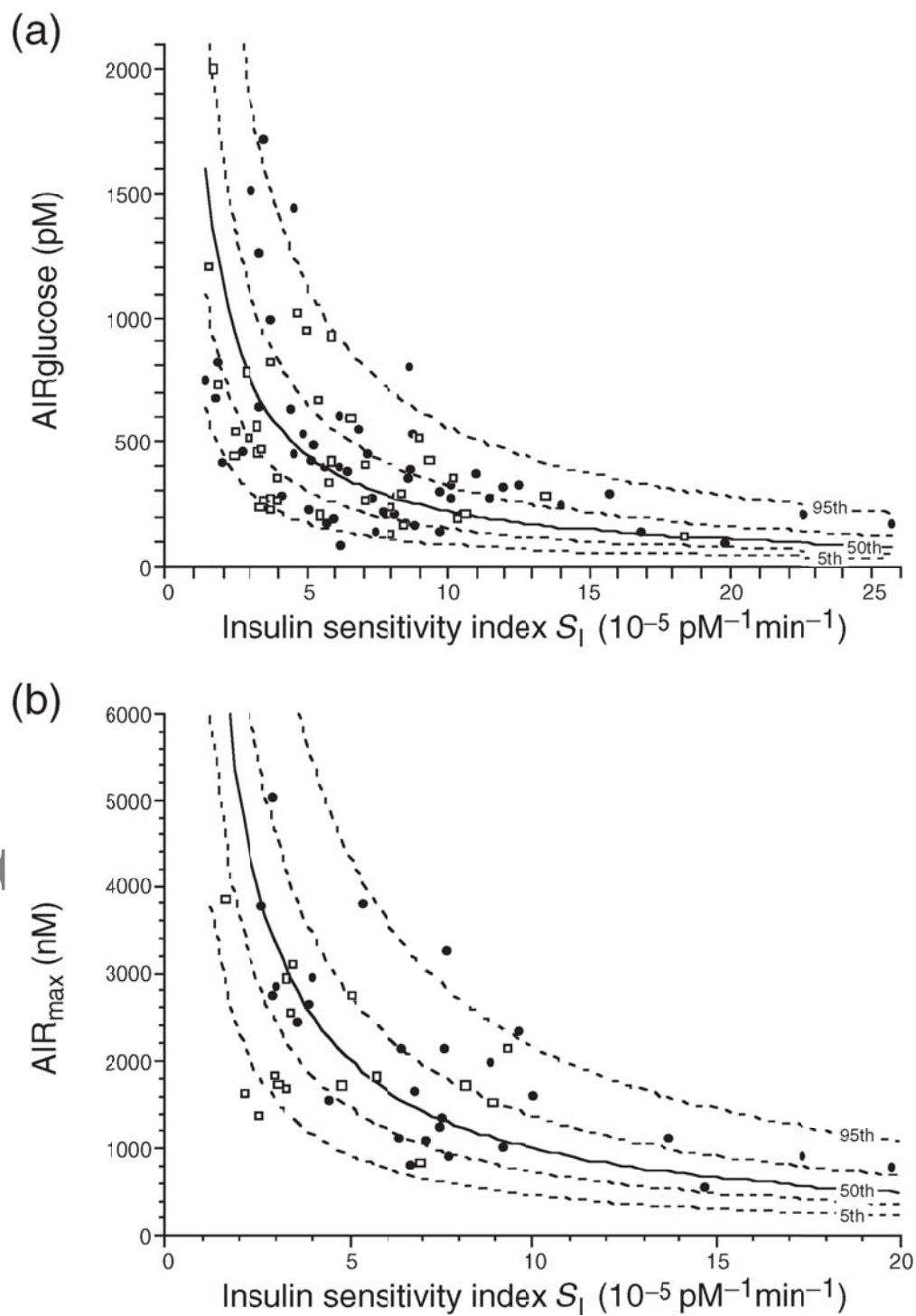Accepted Article

Figure 1.

(a)

AIRglucose (pM) vs Insulin sensitivity index S_I (10^{-5} pM^{-1}min^{-1})

95th
50th
5th

(b)

AIR$_{max}$ (nM) vs Insulin sensitivity index S_I (10^{-5} pM^{-1}min^{-1})

95th
50th
5th

Figure 2.

(a)

(b)

Plasma insulin (µU/ml)

I = Mean ± SEM

$\downarrow$ = 20-g glucose pulse

-30 0 30 60 90 120

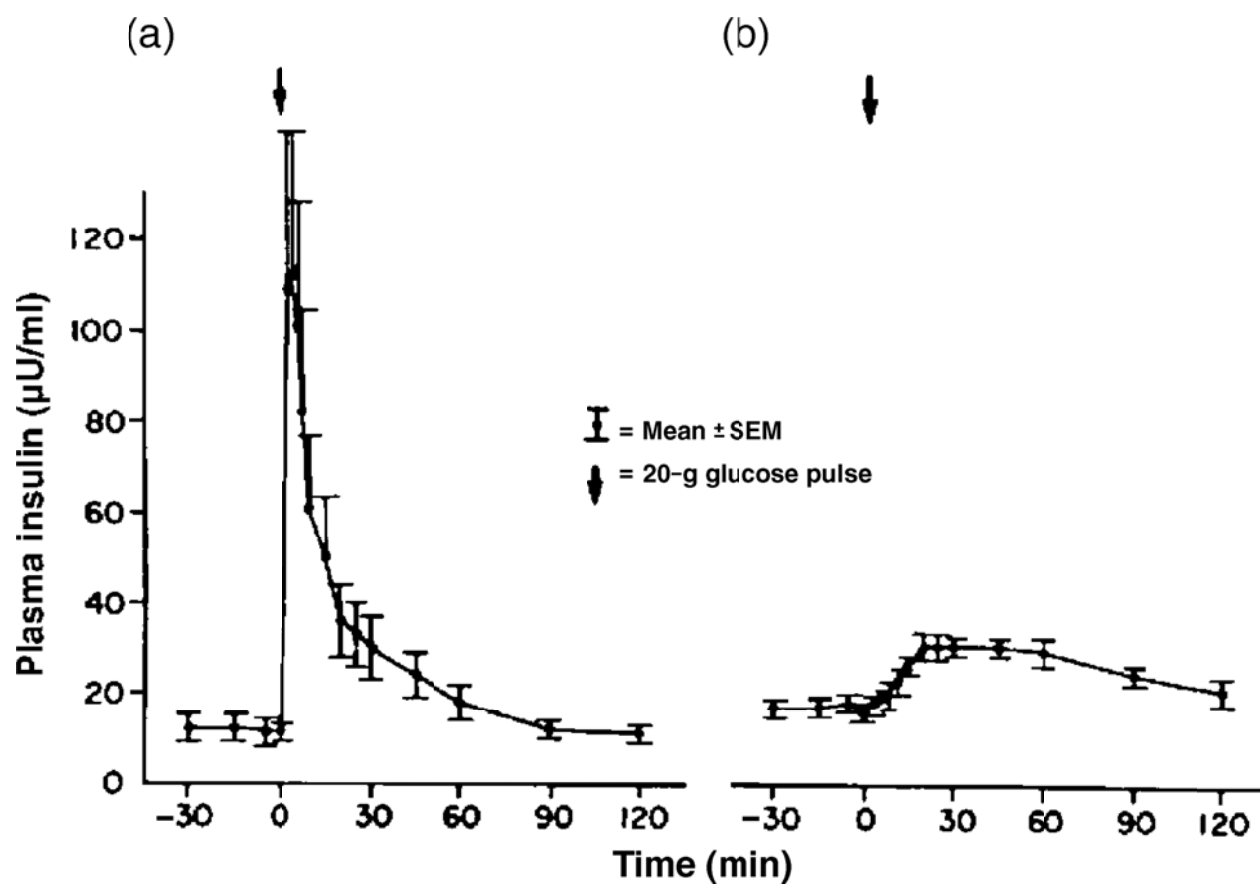-30 0 30 60 90 120
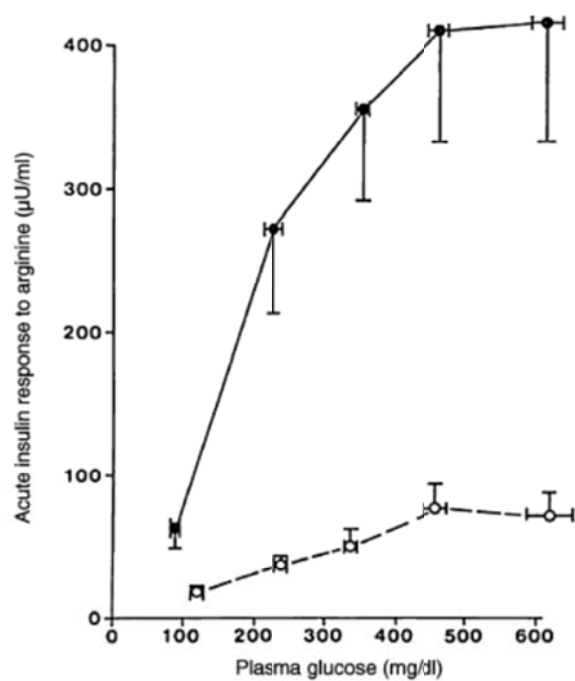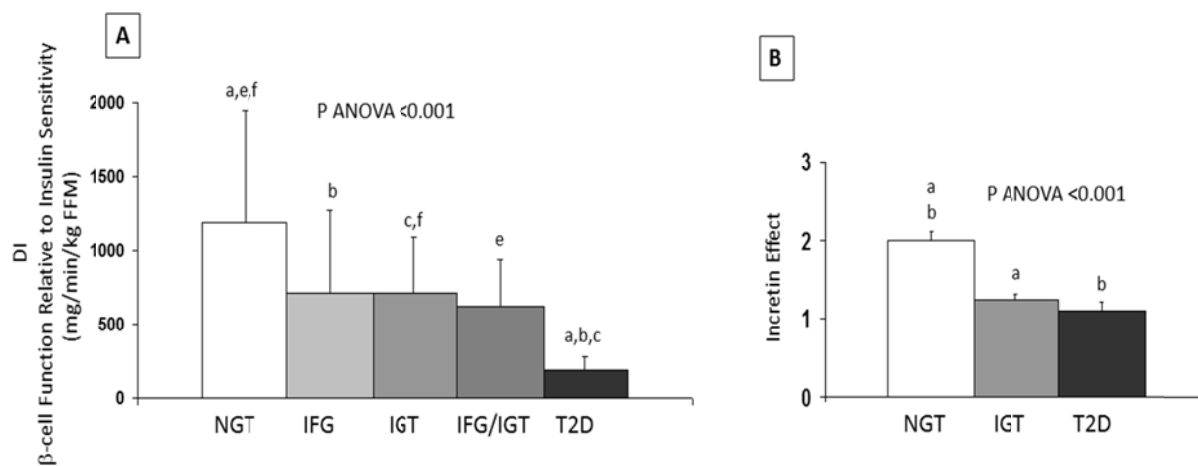
Time (min)

Figure 3.

Figure 4.

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