

THE ASSESSMENT OF INSULIN SECRETION

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The assessment of insulin secretion is vital in both physiological and epidemiological studies of the pathophysiology of type 2 diabetes. The ultimate aim of treatment is to halt or reverse the progressive decline in β -cell function which characterizes type 2 diabetes, and tools with which to assess the impact of new interventions on the rate of β -cell failure are essential if this aim is to be realized. The information yielded by the various methods used to quantify β -cell function and the limitations of these tools need to be appreciated in order to interpret the findings appropriately. This issue is of particular relevance to type 2 diabetes as there is continuing debate whether the characteristic progressive β -cell functional loss is due to a loss of mass or of metabolic functional capacity.¹

Insulin is secreted by the β cell in response to a variety of stimuli including glucose, amino acids, glucagon-like peptide 1 (GLP-1), and drugs such as sulfonylureas. There are a number of components that contribute to overall β -cell function. These include the sensitivity of the β cell to secretagogues, the rate of the response (1st-phase insulin secretion), the capacity of response (ie, the maximal sustained response), and the character of the response in terms of pulsatile and oscillatory insulin release. The response of the β cell also varies according to whether a glucose stimulus is oral or intravenous. Oral stimuli have greater effects on the β cell because of the so-called "incretins," ie, hormones triggered from the gut that directly affect secretion.²



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The existence of an inverse relationship between β -cell function and insulin sensitivity is well established.^{3,4} Measures of insulin secretion cannot be interpreted in isolation as it is not possible to distinguish between pathologically reduced insulin secretion or a physiologically appropriately low insulin in response to a high degree of insulin sensitivity.

Molecular basis for insulin secretion

Insulin is secreted by the pancreatic β cell as a result of closure of adenosine triphosphate (ATP)-sensitive potassium channels, which leads to membrane depolarization, which in turn opens voltage-gated channels, allowing influx of calcium ions with subsequent exocytosis of preformed insulin granules

SELECTED ABBREVIATIONS AND ACRONYMS

CIGMA	continuous infusion of glucose with model assessment
GLP-1	glucagon-like peptide 1
HOMA	homeostatic model assessment
IVGTT	intravenous glucose tolerance test
OGTT	oral glucose tolerance test
SUR	sulfonylurea receptor
UKPDS	United Kingdom Prospective Diabetes Study

The assessment of insulin secretion is vital in both physiological and epidemiological studies of the pathophysiology of type 2 diabetes. The simplest surrogate measure of insulin secretion is the level of glycemia measured either by HbA_{1c} or fasting glucose concentration, but a more informative approach is that of the coefficient of failure. Direct hormone measures are appropriate for assessing β -cell function, and there are several mathematical techniques that can be applied to insulin and C-peptide data to yield evidence of function, and deconvolution techniques allow assessment of insulin secretion rate. Glucose clamps assess β -cell function under specific conditions that can be reliably compared between groups, but may yield results that are near maximal stimulation. Step clamps can describe a dose-response curve. Modeled results from glucose tolerance tests or from homeostatic model assessment (HOMA) analysis can be used in epidemiological studies. A number of other surrogate measures have been reported, including proinsulin concentrations, pres-

ence or absence of regular pulsatility, and a variety of nonglucose stimuli. *In vitro*, the electrophysiology of the cellular response is highly informative. No one method of assessment of β -cell function will demonstrate the range of activity—HOMA modeling assesses basal function, glucose tolerance tests assess dynamic function, and hyperglycemia clamps assess maximally, or at least chronically, stimulated β cells. The choice of test depends on the nature of the research—epidemiology has tended to use HOMA, and physiological studies on small numbers of subjects have often utilized clamps.

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(Figure 1). Under physiological circumstances, closure of the potassium channel occurs in response to the ATP generated from the metabolism of glucose in the β cell by glycolysis and from oxidative phosphorylation within the mitochondria. The β -cell potassium channel is a complex comprised of 4 potassium inwardly-rectifying subunits (Kir6.2 subunits) that form the pore of the channel, and an associated 140-kd regulatory protein, the sulfonylurea receptor (SUR1).⁵

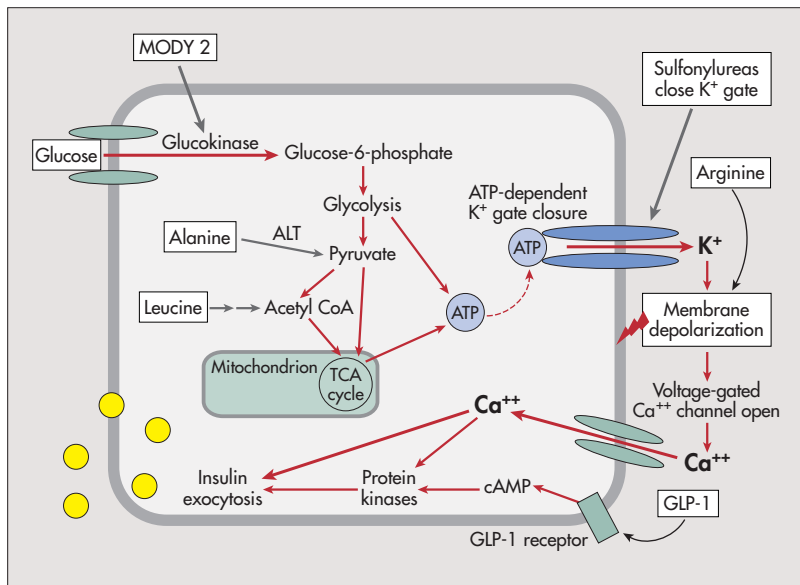


Figure 1. Mechanism of secretion of insulin from the β cell showing sites of action of various secretagogues.

Abbreviations: ALT, alanine aminotransferase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CoA, coenzyme A; GLP-1, glucagon-like peptide 1; MODY, maturity onset diabetes of the young; TCA cycle, tricarboxylic acid cycle.

Insulin secretion can now be measured from single islets, and the use of confocal microscopy means that in cellular β -cell preparations or insulin-secreting cell lines it is possible to detect and measure internal trafficking.⁶ The electrophysiology of β -cell function is now being intensively studied, and the effects of type 2 diabetes and the interrelationships of function and secretagogues is being delineated.⁷ Currently, however, there is no method for studying human β cells from living donors other than those obtained at, for example, pancreatic surgery. Nevertheless, the understanding of the electrophysiology allows insights into the mechanisms of and influences on β -cell secretory processes.

Glycemic measures

The simplest surrogate measure of insulin secretion is the level of glycemia measured either by HbA_{1c} or fasting glucose concentration. The iconic “tick-curve” of the United Kingdom Prospective Diabetes Study (UKPDS) glycemia data was one of the most definitive illustrations of β -cell failure, despite the fact that the data are presented without evidence of peptide secretion. However, glycemic measures represent an integrated measure of β -cell function and insulin resistance and thus collateral evidence for loss of function, as opposed to a reduction in insulin sensitivity, is required. In the case of the

UKPDS, evidence for β -cell failure came from the use of the homeostatic model assessment (HOMA) model,⁸ which is described in more detail below.

◆ Coefficient of failure

Progressive hyperglycemia in type 2 diabetes is now accepted as being a surrogate for β -cell failure. This means that “failure” of an agent with time may be measured by the extent to which glycemia in individual patients deteriorates to preset thresholds. Such methods were used in the UKPDS, and expressed as failure rates by Kaplan Meier plots.⁸

There are major problems inherent in using threshold methodologies to measure failure, however, and a more informative approach is that of the coefficient of failure which is determined from the slope of the deterioration of fasting plasma glucose or HbA_{1c} with time on monotherapy.⁹

Hormone measures

Direct hormone measures are appropriate for assessing β -cell function, and there are several mathematical techniques that can be applied.

◆ Insulin

Insulin is the hormone most often measured when assessing β -cell function.¹⁰ Peripheral insulin concentrations reflect the secretion of the β cells but quantitative estimation of insulin secretion rates cannot be made directly. Nor are insulin concentrations alone informative—they need to be interpreted in the context of the prevailing glucose concentration and usually require modeling of the data to be undertaken (such as HOMA or deconvolution, see below), before any robust interpretation can be made.

◆ C-peptide

C-peptide is produced in equimolar amounts to insulin.¹¹ Thus, C-peptide is an excellent measure of insulin secretion since it is not bound to somatic cells. It does not undergo hepatic extraction and so its concentration is directly proportional to insulin secretion rates. Because it has a longer half-life than insulin (26 minutes compared with 3.8 minutes for insulin),¹² the temporal pattern of secretion may not always be discernible and analysis depends on knowledge of the kinetics of C-peptide clearance from the plasma.¹³ The solution to this problem is the use of deconvolution techniques as discussed below.

◆ Proinsulin and split products

Proinsulin concentration is raised in type 2 diabetes,¹⁴ and although it has only about 12% of the biological activity of insulin¹⁵ it may contribute to the assessed insulin concentration if this is measured using a nonspecific immunoassay.¹⁶ The relative proportions of proinsulin and insulin released from the β cell reflect the cell’s function: an increase in the ratio reflects the release of immature insulin granules, which is an indication of β -cell “stress” or deterioration.¹⁷⁻¹⁹ Elevated levels of fasting 32,33 split proinsulin have been linked to the de-

velopment of diabetes in a large prospective study,²⁰ but this association is not synonymous with prediction.

Proinsulin-mediated glucose disposal is about 7% that of insulin whereas proinsulin-mediated suppression of hepatic glucose output is approximately 12% that of insulin.^{15,21}

◆ Deconvolution

The mathematical technique of deconvolution is widely used to assess secretion from concentration data. It was first described by Turner et al in 1971, who described the principle of “curve stripping”²² (Figure 2). The technique is based on the concept that if the half-life of a hormone is known, then a single pulse of hormone should cause a rise in concentration followed by a fall. If the fall has time characteristics that exactly match the prediction of the half-life, then no further secretion has occurred. If the rate of fall is slower, then more hormone has been secreted. From this concept one can calculate moment-to-moment secretion rates for any hormone.

Eaton’s model assumes that C-peptide is secreted in a central compartment from which it is distributed into a peripheral extravascular compartment.¹³ The constants are estimated from a decay curve following an intravenous injection of biosynthetic C-peptide, while endogenous insulin production is suppressed by a somatostatin infusion. Polonsky et al initially suggested that the accuracy of this assessment might be improved by finding the individual half-life for every individual but later suggested that for most assessments the use of generic half-lives was adequate,²³ and this has now been widely accepted.

Clamps

◆ Hyperglycemic clamps

The hyperglycemic clamp is a technique used to measure β -cell secretory function. When infusing glucose at a variable rate to achieve and maintain steady state hyperglycemia, the secretory products can be compared in different groups under identical stimuli. This allows an assessment *independent*

of the glycemic stimulus. Glucose is administered intravenously to achieve and maintain a predetermined plasma glucose concentration.²⁴ A variety of algorithms for adjusting glucose infusion rates have been published.^{24,25} The duration of the study must be constant within an experiment, since insulin concentrations tend to augment with time. The analysis of the subject’s potential for insulin production is obtained from the plasma samples toward the end of the clamp which are assayed for insulin or C-peptide. This technique allows the comparison of normal subjects and those with type 2 diabetes under equivalent glycemic conditions.

Glucose clamps have sometimes been referred to as the “gold standard” method for establishing insulin secretion. However, clamp techniques are artificial, supraphysiological levels of glycemia are often used, there are limitations to clamp stability, and a relatively high coefficient of variation (CV) of the outcome measures (CV 40% for 1st-phase secretion and 47% for 2nd-phase secretion).²⁶

◆ Stepped clamps

Stepped clamps are similar to conventional hyperglycemic clamps except that here a series of predetermined glucose concentrations are achieved sequentially. Stepped clamps have been successfully used in assessing, for example, the combined effects of amino acids and glycemia on insulin production.²⁷ The technology is essentially identical to that for conventional hyperglycemic clamps except that the added secretagogue (nonglucose stimulus) is administered at each step of the process.

◆ Amino acid infusions

Amino acids trigger insulin release in several ways. Basic amino acids such as arginine cause direct depolarization of the β cell leading to insulin release via elevation of intracytosolic calcium concentration. Leucine, a branched chain amino acid, is actively metabolized in the β cell, the resultant elevation of intracytosolic calcium concentration leading to insulin secretion²⁸ (Figure 1). Amino acids may be used as secretagogues independent of the glycemic stimulus,²⁹ but are usually used in association with stepped-hyperglycemic clamps in order to elu-

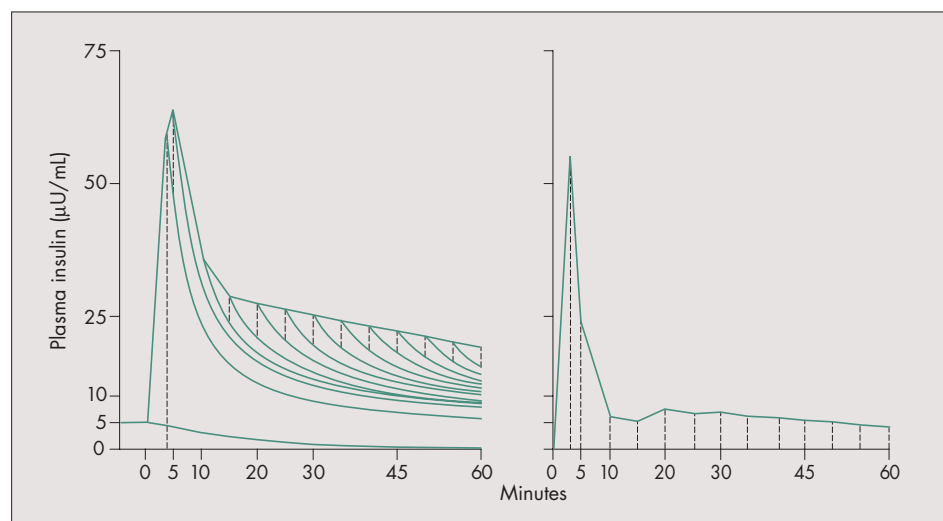


Figure 2. Diagrammatic representation of the curve stripping method used to assess insulin delivery rate. The figure on the left shows typical plasma levels following intravenous administration of glucose. The curved lines represent the fall in plasma insulin concentration, calculated from insulin disappearance rate, assuming no more insulin has entered the circulation since the last measurement of plasma insulin concentration. The dotted lines show the difference between the measured and calculated insulin levels; these have been redrawn on the right and represent an index of the insulin delivery rate.

Reproduced from reference 22: Turner RC, Grayburn JA, Newman GB, Nabarro JD. Measurement of the insulin delivery rate in man. *J Clin Endocrinol Metab.* 1971;33:279-286. Copyright © 1971, The Endocrine Society.

cidate the synergistic mechanisms by which the two stimuli act. Porte et al established the use of intravenous arginine boluses (5 g) superimposed on progressively higher, short hyperglycemic plateaus (fasting, 14 mmol/L, and >25 mmol/L).³⁰ This technique allows the calculation of the slope of acute plasma insulin responses versus plasma glucose concentrations, termed the glucose potentiation slope. The slope thus expresses the sensitivity of arginine-induced acute insulin release to preexisting glucose concentrations.

First- and second-phase insulin secretion

When glucose is given during an intravenous glucose tolerance test (IVGTT), insulin secretion shows a specific and well-described first and second phase.³¹ The insulin concentration reaches a peak within a few minutes of the glucose stimulus and then declines. The second phase is of gradually augmenting secretion continuing while hyperglycemia persists.

Secretagogues may have different effects on first- and second-phase insulin secretion,³² and it is likely that the cellular mechanisms underlying the 2 phases are different. In order to assess the effects separately, it has been customary to define the first phase as the area under the curve of insulin concentrations from 0 to 10 minutes, and the second phase as the area under the curve for the remainder of the assessment (typically 10 to 60 minutes). Measurements of phasic insulin concentrations are thus a convenient and repeatable method of assessing β -cell function, and can be carried out against a background of subjects being on or off a drug in a double-blind trial. No special equipment is necessary. The dose of glucose is not critical (provided it is the same throughout the series of experiments) and is typically 300 mg/kg body weight.²⁶

Oral glucose tolerance tests and mixed meal tests

The oral glucose tolerance test (OGTT) has traditionally been used in the diagnosis of diabetes but has also been used in the assessment of β -cell function. One of its drawbacks is the low reproducibility (CV 15% to 35%),^{33,34} which is partly due to the variable rate of absorption of glucose from the gut influencing the glucose profile; and also due to the fact that insulin secretion is influenced by the action of gut hormones (incretins²) secreted in response to an oral glucose stimulus. The accuracy of the OGTT can be improved by calculating the ratio of the increase in insulin to the increase in glucose 30 minutes after oral glucose loading ($\Delta I_{30}/\Delta G_{30}$). This measure correlates well with the peak (3-minute) insulin response during the IVGTT ($r=0.76^{35}$; $r=0.61^{36}$) but less well with steady-state measures obtained from the hyperglycemic clamp³⁷ and HOMA model ($r=0.44^{38}$; $r=0.38^{35}$). The measurement of $\Delta I_{30}/\Delta G_{30}$ is dependent on both insulin and glucose levels rising in response to an oral glucose stimulus and a value cannot be obtained if either parameter fails to increase. It has been proposed as a useful

method to assess insulin secretion in epidemiological studies involving large numbers of patients.³⁶ Several other OGTT-derived indices of insulin secretion have been proposed but are subject to the same limitations.^{39,40} Minimal modeling techniques have also been applied to the OGTT to yield a dynamic measure of β -cell function and insulin sensitivity but this approach has not been widely used.⁴¹

The minimal model has also been applied to mixed meal tests but although the steady-state component of β -cell function correlates well with the hyperglycemic clamp ($r=0.69$; $P=0.002$), the dynamic component does not correlate with first-phase insulin release from the clamp nor IVGTT.⁴² Other modeling approaches have reported good precision of β -cell responsiveness indices derived from meal tests (CV 15%)⁴³ and more recently the use of triple meal tests with sampling over a 24-hour period have been reported,⁴⁴ but these tests have not yet been widely validated. Mixed meal tests have the advantage of being more physiological than OGTTs.

Models

There are a number of modeling techniques used in the assessment of β -cell function. β -Cell function, in such models, can be regarded as being synonymous with insulin secretory capacity or function. Models are mathematical abstractions that allow data to be interpreted—insulin and glucose concentration data are used to assess β -cell function and insulin resistance. There are, broadly speaking, 2 types of model—minimal models⁴⁵ and paradigm models.

Minimal models work by using individual patient data from IVGTT tests, and establishing the profile of both insulin and glucose. Since insulin causes glucose to fall and glucose causes insulin to rise, there is a complex feedback loop. The minimal model of Bergman and Cobelli⁴⁵ computes, for each profile, estimates of β -cell function (ϕ_1 and ϕ_2) and insulin resistance (S_I and S_G) by curve fitting techniques.

There are, however, problems with a simple monophasic response being modeled, as it is recognized that for some profiles there are no unique mathematical solutions for insulin resistance and β -cell function value. The lack of standardization resulting from the variation in the methodology for the IVGTT has sometimes made comparisons across studies difficult.⁴⁶ Later versions of the test have induced additional dynamic changes to the profile with, for example, tolbutamide so that the second-phase secretion is augmented.⁴⁷ Although it is still possible to measure ϕ_1 from the modified model, the ability to measure second-phase insulin secretion is lost unless analysis of multiple data sets is undertaken from the same individual, before and after physiological perturbation.

Paradigm models establish the normative response by computing all combinations of outcome from given combinations of insulin resistance and β -cell function. In this sense, they establish a paradigm or “idealized norm” against which the available data may be compared. Once the model has

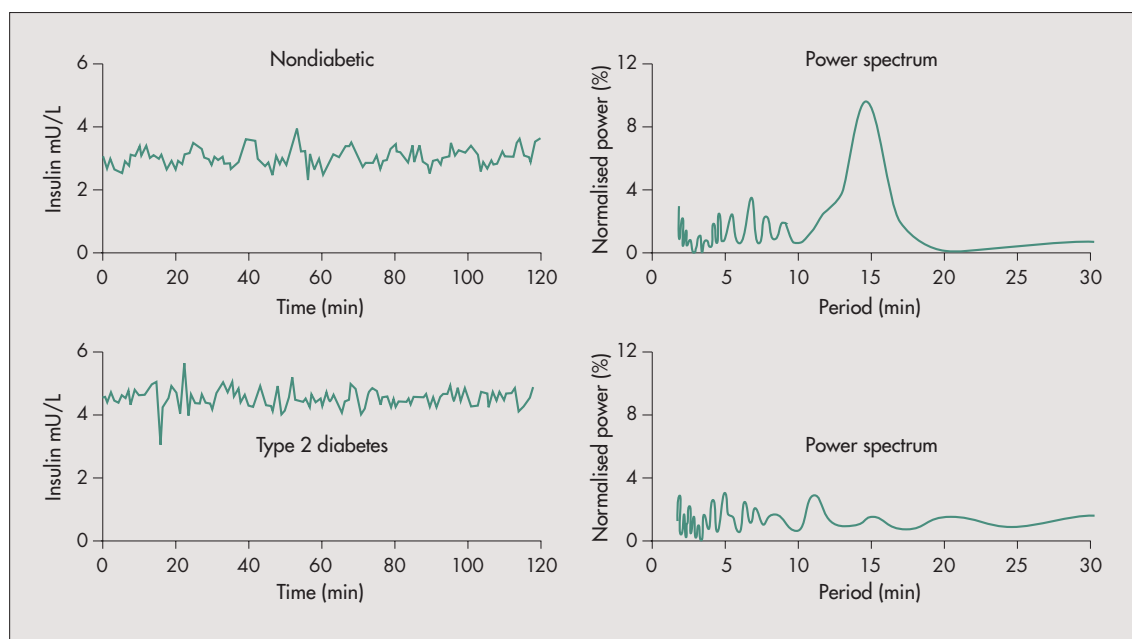


Figure 3. Left panels: Basal insulin concentrations showing oscillatory secretion. **Right panels:** Fourier transform analysis of the oscillations showing power spectra. **Upper panels** are from a normal subject; the **lower panels** are from a subject with type 2 diabetes. The oscillations at 15 minutes' periodicity in the normal subject are demonstrated.

been computed, results can be computed for any patient (www.dtu.ox.ac.uk/homa).⁴⁸ HOMA⁴⁸ and the continuous infusion of glucose with model assessment (CIGMA)⁴⁹ are both paradigm models. CIGMA, however, is rarely used.

HOMA yields a measure of β -cell function and insulin sensitivity from paired fasting glucose and insulin concentrations. It is an appropriate method for assessing β -cell function longitudinally in individuals, but is not helpful as a "one-off" measure. It is a robust method for assessing β -cell function in large numbers of patients over long periods of time because the sampling and calculation is simple.⁵⁰ Estimates of β -cell function using HOMA have been shown to correlate well with estimates using CIGMA ($R_s=0.88$),⁵¹ hyperglycemic clamps ($R_s=0.61$; $P<0.01$),⁴⁸ and with the acute insulin response from the IVGTT ($R_s=0.63$).⁵¹

Pulsatility

Insulin is secreted in pulses of 8-to 13-minute periodicity.⁵² Regular pulsatility is a marker of normal β -cell function, and there are disorganized and irregular cycles found in those with type 2 diabetes and obesity (Figure 3).⁵³ Assessing pulsatility is labor-intensive (eg, 1-minute samples for 2 hours), and the mathematical techniques can be complex. However, the study of pulsatility has demonstrated that insulin cannot be assessed with single samples if its true signaling complexity is to be understood. Insulin secretion and action is a function both of the prevailing concentration and the antecedent time series. Secretagogues generally increase the amplitude of oscillations without altering the periodicity.⁵⁴

Oscillations of insulin in a feedback domain between insulin and glucose have also been studied—the period of this is always in excess of 40 minutes and the oscillations can be entrained by altering glucose concentrations in an oscillatory cycle.⁵⁵ Type 2 diabetic subjects show loss of entrainment.

Designing research protocols for assessment of secretagogues

No data should be collected in any trial before the methods of sampling, assay, and mathematical and statistical analysis have been established.

◆ Sampling

It is essential for valid assessments of secretagogues that hormone sampling is carefully managed. An adequate time series of data will be necessary for many analyses: for example, if phasic insulin secretion is to be studied, then it is galling to discover at the time of analysis that there are insufficient samples to assess this.

◆ Assays

Peptide hormones are susceptible to progressive degradation by peptidases if hemolysis of the sample occurs. This results in a significant fall in measured insulin concentrations.⁵⁶ Glucose concentrations in unpreserved samples decrease rapidly at room temperature due to glycolysis; ideally, samples should be centrifuged and the plasma stored at 4°C prior to analysis on the same day or, if this is not possible, the blood should be collected in a tube containing fluoride oxalate. In order to minimize assay problems, following collection, samples should be placed on ice, centrifuged immediately, and, unless analyzed in the next few hours, the plasma should be stored frozen. However, frozen plasma samples are also liable to some progressive loss, and many laboratories now recommend assay of freshly spun plasma within a few days of their collection.

In addition to these potential problems, insulin interassay variation is large and values can vary by as much as a factor of 3 between different laboratories.⁵⁷

◆ Common problems with assessments

Assessing insulin secretion is not straightforward, there are many problems of detail. One aspect relates to comparisons between secretagogues—a de-

cision is needed whether the comparison is, for example, to be made using molar equivalents of drugs, maximum therapeutic dosages, single or multiple dosages, and so on.

Another vexed problem is that of body weight. If stimuli such as glucose are used in different weight subjects, should one use the same stimuli for all or adjust the dose on the basis of body weight? Virtually all protocols use adjusted doses for intravenous stimuli, yet this is in marked contrast to oral stimuli where 75 g glucose has been nearly universally adopted. But on what basis should one adjust the stimulus? Some have used lean-body mass calculations, others ideal body weight, and yet others the body mass index. Most protocols have adopted glucose stimuli based on some assessment of lean body mass, since this is the closest related to the extracellular fluid space. It is clear that caution should be exercised when making comparisons between studies due to variations in infusion protocols, sampling, and hormone assays used in different studies. In addition, there is the issue of reproducibility (both within-subject and between-subject) inherent in all methods of assessment.

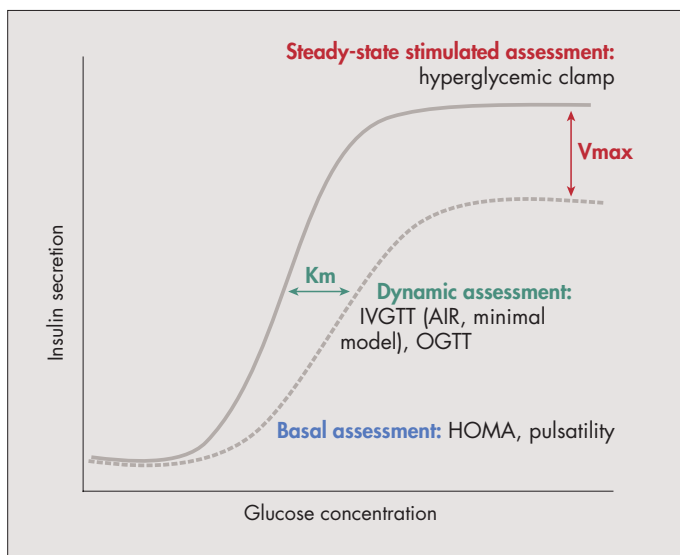


Figure 4. Dose response curve for insulin secretion. The domains of the curve assessed by various tests are shown.

Abbreviations: AIR, acute insulin response; HOMA, homeostatic model assessment; IVGTT, intravenous glucose tolerance test; Km, curve constant; OGTT, oral glucose tolerance test; Vmax, maximal secretory rate.

Studies should be designed to be parallel-group comparisons or longitudinal crossover. Pharmaceutical companies may favor the former, since they are interested in the generality of efficacy of a product. However, because of relatively large interindividual variation in metabolic function, physiologists often prefer crossover designs where the hope is that most biometric variables will remain unaffected. This is not always true, and since insulin secretagogues cause an increase in body weight, subsequent testing of function may be partially confounded by the conundrum discussed above.

There has also been a vigorous debate about wash-out periods in crossover studies. If substance A is tested for some weeks, and then substance B, should there be a period between taking the agents when

nothing is used, or is it acceptable to change immediately on to substance B? Some would point out that substance A will wash out anyway, and a period of no therapy might expose patients to unacceptable side effects of withdrawal of therapy. Wash-out periods extend trials, and some compensation for their omission can be made by order-effect analysis. Some researchers avoid this debate simply by insisting on parallel-group studies, but much larger numbers of patients are likely to be required to be studied, since they will all have disparate starting characteristics. This last problem has sometimes been addressed by stipulation of very tight entry criteria for selection, which leads to another problem, that of slow recruitment into a clinical trial.

Choice of method of assessment

There are numerous aspects of β -cell function. These include the sensitivity of the β cell to secretagogues, the rate of the response, the maximal sustained response, and the character of the response in terms of pulsatile and oscillatory insulin release. It is impractical to measure all of these elements in a single individual or a single study and so an a priori decision on which aspect is to be studied must be made and an appropriate method of assessment selected.

The fact that different methods used to assess β -cell function yield different estimates does not necessarily imply superiority of one method over another—the difference between measures derived from different tests may yield important information. While a degree of correlation between measures is to be expected as the various models have certain structural features in common, different models and techniques examine different aspects of function and sensitivity. Clamps, which are carried out at supraphysiological glucose levels, yield information about steady-state stimulated insulin secretion (Figure 4). In contrast, minimal model analysis of the response to intravenous glucose examines dynamic changes in insulin secretion and insulin sensitivity. HOMA gives information about β -cell function and insulin sensitivity in the unperturbed steady state and could thus be considered more “physiological” than clamps. All methods are an attempt to reduce an extremely complicated dynamic system to a simple mathematical approximation of the glucose/insulin dynamics. Any investigation of β -cell function needs to be clear about what information is being sought in order to utilize an appropriate test. Table I shows a comparison of the various methods used to assess β -cell function.

Measures of insulin secretion cannot be interpreted in isolation and β -cell function should be interpreted in the light of the prevailing insulin sensitivity. One formalized method for reporting the ability of β cells to compensate for insulin resistance is the disposition index. Originally described by Bergman in 1981 using data derived from the minimal model, the disposition index is the mathematical product of insulin secretion and insulin sensitivity (ϕ_1 and S_1).³ This relationship has since been shown to be best described by a hyperbolic function

	Labor intensity	Method	Reproducibility	Limitations	Interpretations of results
HOMA	3 basal samples at 5-min intervals (1 sample often used)	Overnight fast	CV 11%-32% ^{48,58}	Caution in subjects taking exogenous insulin ⁵⁰	Available from www.dtu.ox.ac.uk
Minimal modeling of IVGTT	>20 samples over 3 hours	Intravenous glucose bolus 0.3 g/kg	Low comparability across studies as workers adopt different criteria	Dependent on the presence of insulin response to glucose—may be absent in type 2 diabetes	Requires computer analysis of results
AIR (IVGTT)	9 samples over 10 minutes	Intravenous glucose bolus 0.3 g/kg	CV 10.5%-18% ^{59,60}	AIR often absent in type 2 diabetes	AUC usually calculated
OGTT	3-11 samples over 3 hours	Oral glucose 75 g	CV 15%-35% ³⁴	Absent response to glucose in type 2 diabetes often leads to $\Delta I_{30}/\Delta G_{30} \leq 0$	Simple ratios or AUC usually used. Minimal modeling has been applied but its use has not been validated
Meal tests	12 samples over 3 hours, or 27 samples over 24 hours	Single or triple mixed meals	Limited data available	Relatively new approach thus not yet well validated	Various models applied to insulin and glucose data ^{43,44,61}
Hyperglycemic clamp	Monitoring of glucose at 5-min intervals over 2-3 hours; samples at 5-min intervals during last 30 min.	Variable rate intravenous infusion of glucose	CV 10%-40% ^{26,62}	Supraphysiological glucose levels achieved	Insulin concentrations during steady state represent insulin secretion

Table I. Comparison of various methods used to assess β -cell function.

Abbreviations: AIR, acute insulin response; AUC, area under the curve; CV, coefficient of variation; HOMA, homeostatic model assessment; $\Delta I_{30}/\Delta G_{30}$, ratio of the increase in insulin to the increase in glucose 30 minutes after a 75-g oral glucose load; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

(ie, $\phi_1 = a \cdot S_1^{-1}$).⁴ More recently, the disposition index has been applied to data derived from OGTT, euglycemic clamps, and HOMA.⁶³⁻⁶⁵

Conclusion

The assessment of insulin secretion is relatively complex. There are no methods that fulfill all criteria, and most studies have utilized multiple methods. Sometimes a simple method can be used to study large numbers of subjects, a small proportion

of whom undergo more intensive examination to elucidate the mechanisms. There is no single test that can yield a complete description of β -cell function: the choice of method depends on the problem to be addressed, the aims of the investigation, and the experimental limitations. \square

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ÉVALUATION DE LA SÉCRÉTION D'INSULINE

L'évaluation de la sécrétion d'insuline est vitale dans les études physiologiques et épidémiologiques de la physiopathologie du diabète de type 2. La technique de mesure de remplacement la plus simple de la sécrétion d'insuline est la glycémie mesurée soit par l'HbA_{1c} soit par la glycémie à jeun, mais le taux de dysfonction des cellules β est une approche beaucoup plus intéressante. Il existe des mesures directes des hormones adaptées pour l'évaluation de la fonction β-cellulaire. Les résultats de l'insuline et du peptide C peuvent être traités mathématiquement afin d'intégrer les techniques fonctionnelles tandis que les techniques de déconvolution permettent l'évaluation des taux de sécrétion d'insuline. Des clamps de glucose évaluent la fonction cellulaire β sous certaines conditions qui peuvent être fidèlement comparées entre groupes, mais qui peuvent donner des résultats proches de la stimulation maximale. Des clamps par paliers peuvent former une courbe dose-réponse. Des résultats

modélisés issus de tests de tolérance au glucose ou d'analyse d'évaluation de modèle homéostatique (HOMA) peuvent être utilisés dans des études épidémiologiques. D'autres mesures de substitution ont été mentionnées, comprenant les concentrations de pro-insuline, la présence ou l'absence de pulsativité régulière et plusieurs stimuli non glucosés. L'électrophysiologie de la réponse cellulaire est riche de renseignements in vitro. Aucune méthode d'évaluation de la fonction cellulaire β ne démontrera l'ensemble de l'activité – le modèle HOMA évalue la fonction basale, les tests de tolérance au glucose évaluent la fonction dynamique et les clamps hyperglycémiques évaluent la stimulation β-cellulaire de façon maximale ou au moins chronique. Le choix du test dépend de la nature de la recherche : la recherche épidémiologique utilise plutôt le modèle HOMA et les études physiologiques sur un petit nombre de sujets utilisent souvent les clamps.