Review Article

Measuring β-cell function *in vivo* to understand the pathophysiology of type 2 diabetes

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Abstract. Diabetes arises when insulin secretion is inadequate for the prevailing metabolic conditions. As such appropriate measurement of β-cell function is necessary for a better understanding of the pathophysiology of prediabetes and diabetes. Unfortunately this is not a straightforward process and requires utilization of mathematical modelling to best appreciate its complexities. This is because insulin concentrations in the plasma represent a balance between the processes of secretion, hepatic extraction and clearance. In isolation such simple measures reveal very little about β-cell function. Moreover, since insulin lowers glucose accounting for the effect of the former on the latter it is a key part of understanding insulin action. The development of the minimal model has allowed simultaneous measurement of the dynamic relationship between insulin secretion and insulin action and produces a quantitative number – the Disposition Index – which quantifies β-cell function. At present this remains the best functional measure of islet health, however, it may not capture other phenotypes such as β-cell senescence or the effect of incretin hormones on β-cell function. Future ongoing development and interaction with other technologies, such as functional imaging, should enhance the contribution of this functional testing to the prevention, treatment and understanding of type 2 diabetes.

Keywords Insulin Secretion – Insulin Action – Incretin Effect – Glucagon-Like Peptide-1 – Minimal Model

1 Introduction

Diabetes is defined by the presence of elevated fasting and postprandial glucose concentrations, and, indeed, currently a fasting glucose of ≥ 126 mg/dL would qualify a patient as having diabetes. A fasting glucose (≤ 100 mg/dL) is considered to be normal, and the range in between these two parameters characterizes an intermediate, often transitory, state referred to as prediabetes. It is well recognized that individuals with a higher fasting glucose, have a higher risk of transitioning to diabetes. For example, in a population from Olmsted County, if fasting glucose is ≥ 110 mg/dL, but ≤ 126 mg/dL over 10 years, the risk is 40 % (i.e., 40 % of that particular population will transition to diabetes) (Dinneen et al., 1998). This may strike one as being a particularly high number, but conversely, 60 % of people in the range of fasting glucose never actually transition to diabetes. In essence, if you look at the people whose fasting glucose is ≤ 100 mg/dL, 10 % of those at 10 years would have transitioned to diabetes, again implying that fasting glucose by itself may not necessarily be the best marker for diabetes risk and also demonstrating that there is significant heterogeneity of diabetes risk at a given fasting glucose level. The factors driving the transition to diabetes are at present not well characterized.

2 Pathophysiology of Diabetes: Defects in Insulin Secretion and Action

As already mentioned, diabetes is characterized by a fasting glucose which is elevated after a meal challenge. Glucose rises to higher concentrations and stays elevated for a lot longer than it does in normal non-diabetic in-

dividuals. This elevation in glucose over time is what leads to diabetes complications. Why does this arise? It arises because fasting insulin concentrations are inappropriate for the prevailing degree of glycaemia. Also, in response to a meal challenge, insulin concentrations rise to a lower peak and take a long time getting to that peak compared to the ten-fold elevation in insulin concentrations that occurs within about 30-45 minutes of meal ingestion in non-diabetic individuals. In addition to delayed and defective insulin secretion, people with type 2 diabetes inappropriately suppress glucagon in response to meal ingestion. In fact, a paradoxical rise in glucagon is often present, in contrast to glucagon suppression observed in the first two hours after meal ingestion that is seen in non-diabetic individuals. This further exacerbates postprandial hyperglycemia (Butler and Rizza, 1991).

Diabetes is also characterized by defects in insulin action (i.e. the ability of insulin to suppress endogenous production of glucose and to stimulate the peripheral uptake of glucose in insulin-sensitive tissues is impaired when compared to non-diabetic individuals). How important is the interaction of defects in insulin secretion with defects in insulin action? To address this question, when people with varying degrees of insulin action are studied, worsening defects in insulin secretion cause far greater rises in glucose concentration than either defect alone, with such effects most marked in people with type 2 diabetes. For example, Basu et al. (1996) studied three groups of people with varying grades of insulin resistance (i.e. one group comprised insulin-sensitive, lean, non-diabetic individuals. Another group comprised insulin-resistant people with type 2 diabetes, and in a third intermediate group were obese non-diabetic individuals who had milder degrees of insulin resistance). These individuals were studied on two occasions in the presence of a pancreatic clamp with somatostatin to inhibit endogenous insulin secretion and with glucagon-growth hormone replacement at basal concentrations. Subjects then received a 'diabetic' insulin profile (i.e. a decrease in delayed insulin infusion which mimicked postprandial response of insulin seen in diabetic individuals) versus a normal non-diabetic insulin profile. In all subjects, infusion of a 'diabetic' insulin profile in comparison to the 'non-diabetic' profile resulted in higher glucose concentrations, but the effect was most marked in the more insulin-resistant individuals. This implies that, in reality, if insulin action is sufficient, defects in insulin secretion can be compensated for. However, with varying degrees of insulin resistance, the hyperglycemia that occurs in response to a diabetic insulin profile increases (Basu et al., 1996).

These observations, in a sense, dispute the previous prevailing theory of predisposition to diabetes, which hypothesized that people secrete insulin increasingly in response to increasing degrees of insulin resistance. However, there comes a time when the beta cells collectively cannot sustain this increased rate of insulin secretion and hyperglycemia develops (DeFronzo, 1988). This theory has also been called the Starling's curve of the pancreas, taking its cue from the Starling curve as it applies to the heart where increasing degrees of preload increase contractility of the myocardium up to the point of cardiac failure. In practice, this is unlikely to occur, because across populations of individuals with prediabetes, it seems that with worsening degrees of hyperglycemia insulin secretion and action both decline concurrently, rather than insulin secretion increasing, as insulin action decreases and then suddenly failing (Sathananthan et al., 2012)

3 Measuring Insulin Secretion *in vivo*

The original proposition of a Starling Curve of the pancreas is likely flawed because it is based on population cross-sectional data with no longitudinal follow-up. In addition, most of the calculations used to quantify insulin secretion in response to a standardized meal challenge used qualitative peripheral insulin concentrations. It is important to remember that insulin concentrations alone do not necessarily reflect insulin secretion. The insulin concentrations that appear in the peripheral circulation have already gone through hepatic extraction and therefore do not necessarily reflect the concentrations of insulin appearing in the portal circulation as a result of insulin secretion. Thus the insulin concentrations peripherally represent the net sum of two processes–insulin secretion and hepatic extraction (Rossell et al., 1983). Therefore, for quantitative measures of insulin secretion, current state-of-the art measurements utilize C-peptide which is secreted in a 1:1 molar ratio with insulin. The problem with this approach is that while insulin has a half-life of $\sim$ 5 minutes in the circulation, C-peptide has a half-life of $\sim$ 35 minutes and tends to accumulate over time. Therefore to calculate insulin secretion rates, insulin secretion needs to be deconvoluted from the C-peptide concentrations using known rates of clearance for C-peptide (Cobelli et al., 2014).

The other observation to consider is that insulin secretion in response to a physiologic or supraphysiologic challenge arises from two distinct compartments. This phenomenon was first observed in isolated islets and then subsequently in perfused pancreata, where it was clear that exposure to glucose acutely results in a rapid upstroke in insulin concentrations, which is then rapidly followed by a second upstroke which is more sustained than the first, but whose amplitude is lower. These are

collectively referred to as the first and second phases of insulin secretion. The first phase of insulin secretion represents a pool of insulin that is already present in granules during fasting and is released immediately in response to hyperglycemia. In contrast, the second phase of insulin secretion likely represents the synthesis and secretion of insulin in response to sustained hyperglycemia. This phenomenon has subsequently been shown in rodents, primates and in humans in response to intravenous glucose challenges. However, this biphasic pattern of insulin secretion is actually not observed in response to oral glucose challenges, likely because of the effects of hepatic extraction, the magnitude of which differs in response to the first and second phase of insulin secretion (Nesher and Cerasi, 2002).
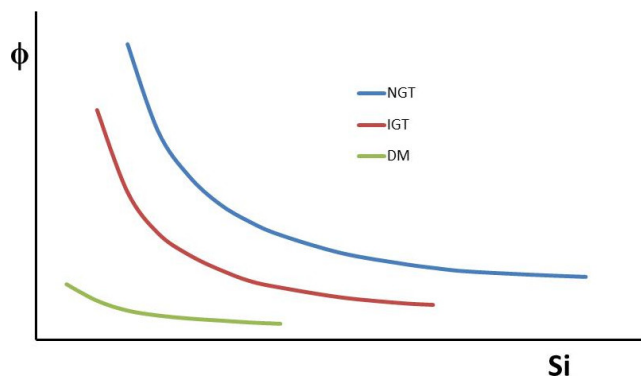

Figure 1: The nature of the hyperbolic relationship between β-cell responsivity (Φ) and Insulin action (S_i) depending on the glucose tolerance status of an individual. NGT = Normal Glucose Tolerance, IGT = Impaired Glucose Tolerance, DM = Diabetes Mellitus.

4   The Minimal Model

Reproducible, optimal, and quantitative measures of insulin secretion require some form of modeling which takes into account the fact that insulin is secreted into a remote compartment (i.e. the portal vein which is not accessible for peripheral sampling) and that between its appearance in the portal vein and its appearance in the systemic circulation there is a time lag to account for the distribution of insulin across various tissues and compartments. This is required because sampling in the peripheral circulation does not necessarily represent what is occurring at that time in the portal circulation in response to a given challenge. The current minimal model assumes that the sample pool of insulin differs slightly but significantly from the actively circulating pool of insulin and, again, this differs from the pool of insulin which is actually released. The minimal model also takes into account the delay (otherwise known as time, T) between the first and second phase of insulin secretion which represents the time to stimulate insulin

synthesis in response to a given stimulus (Breda et al., 2001, 2002; Man et al., 2002).

The other consideration when measuring insulin secretion is to take into account the fact that insulin secretion occurs in the context of the prevailing insulin action. Just as it is unreasonable to talk about power in engineering terms without taking into account the amount of weight the said power needs to move, the same applies for beta cell function, and in such circumstances, insulin secretion should be expressed as a function of the prevailing insulin action. The resulting disposition index is currently considered the gold-standard measure of β-cell function. The disposition index tends to decrease across the spectrum of prediabetes, and the lower the disposition index, the likelier the risk of developing diabetes in the subsequent decade (Cobelli et al., 2014; Xiang et al., 2014).

Insulin secretion is related to insulin action via a hyperbolic relationship so that when glucose tolerance is maintained, even small decreases in insulin action are associated with a very significant increase in insulin secretion in an effort to maintain euglycaemia provided beta cell function is intact. The hyperbolic curve, which describes this relationship, will shift to the left as the beta cell fails and an individual becomes diabetic. Experimentally this paradigm has been proven in multiple situations, either by using pharmacotherapy or lifestyle intervention to improve insulin action with a commensurate offloading in insulin secretion or conversely making individuals acutely insulin resistant using various interventions such as free fatty acid elevation or niacin to increase insulin secretion, assuming euglycaemia is maintained in such circumstances (Bergman, 1989; Bergman et al., 2002).

The concept of the disposition index has been criticized more recently because of the realization that some individuals may not actually be able to respond by increasing insulin secretion in the presence of very significant changes in insulin action, and therefore, a hyperbolic relationship may not always exist. Indeed, efforts are now underway to perhaps better characterize the nature of the relationship between secretion and action in a given individual. How this relationship progresses over time, and whether genetic predisposition or disease itself actually accounts for a lot of these changes is, at present, uncertain (Ferrannini and Mari, 2004).

Another important reason for using an oral challenge over an intravenous glucose challenge to measure beta cell function is because it incorporates the incretin contribution to insulin secretion (Campioni et al., 2007).

5   What is the incretin effect?

It has long been known by physiologists that for a given glucose load administered intrajejunally as op-
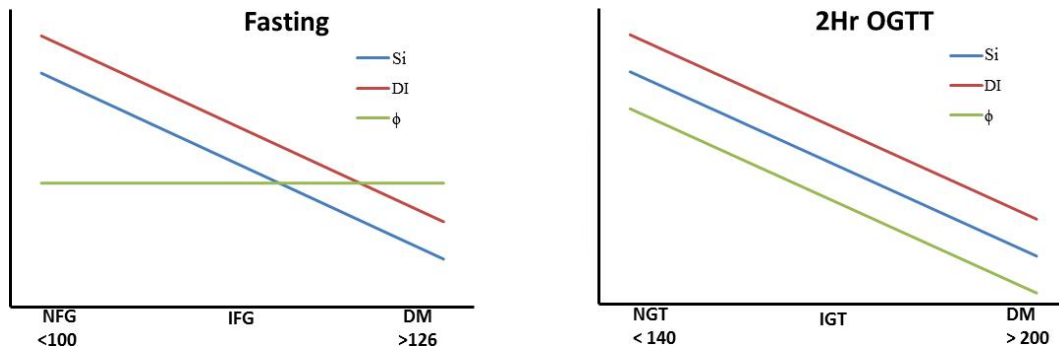
Figure 2: The relationship between β-cell responsivity (Φ) and Insulin action (S_i) and Disposition Index (DI) across the spectrum of impaired fasting glucose (left panel) and across the spectrum of impaired glucose tolerance (right panel). NFG = Normal Fasting Glucose, NGT = Normal Glucose Tolerance, IGT = Impaired Glucose Tolerance, DM = Diabetes Mellitus.

posed to intravenously, despite lower glucose concentrations, insulin secretion is far higher. Because of this, the existence of a gut hormone that stimulates insulin secretion and glucose disposal was hypothesized. The observed effect has been termed the 'incretin effect' because of its effect in assimilating an oral challenge (Mcintyre et al., 1964). The subsequent observation that several segments of the intestine stained for glucagon and the realization that this was confined to enteroendocrine cells helped propel the discovery of Glucagon-like Peptide-1 (GLP-1). The intestine is characterized by cellular heterogeneity and a distinct population of cells, characterized as APUD cells (since they take up amine precursors and decarboxylate them) that actively secrete multiple gut hormones. The glucagon-staining cells today are called L cells, and in actual fact, the immunoreactivity for glucagon arises not because they contain glucagon but because they contain a fragment of proglucagon, GLP-1. GLP-1 arises from transcription and translation of the glucagon gene to produce proglucagon but, whereas in the alpha-cell prohormone convertase 2 converts proglucagon to glucagon, in the L-cells of the gut prohomone convertase 1 converts proglucagon to GLP-1. Another product of proglucagon is glucagon-like peptide-2 (GLP-2). GLP-2 is actually a growth factor for intestinal epithelial cells and has no effect on insulin secretion or glucose metabolism. A larger fragment of proglucagon, oxyntomodulin, has effects on appetite, but its effects are probably not very significant in normal physiologic circumstances (Holst et al., 1994; Holst and Orskov, 2001). Nevertheless, GLP-1 has excited the interest of endocrinologists because it is a powerful insulin secretagogue and it stimulates insulin secretion in a glucose-dependent manner (i.e. insulin secretion will not be stimulated if glucose concentrations are within normal limits and at fasting concentrations). Unfortunately, GLP-1 has a very short half-life in the circulation. Therefore, if GLP-1 is to have therapeutic effect in people with diabetes, it will

need to be infused on a continuous basis, given that its half-life is on the order of 1-2 minutes. GLP-1 activity depends on the integrity of the two end-terminal amino acids, loss of which inactivates GLP-1. An alternative is to inhibit the main enzyme responsible for its degradation. This enzyme is dipeptidyl-peptidase 4 and, indeed, dipeptidyl peptidase 4 inhibitors form a new class of glucose-lowering agents used to treat diabetes (Drucker and Nauck, 2006).

The third alternative is to use another analog of GLP-1 capable of stimulating the seven transmembrane helix GLP-1 receptor, yet resistant to the degradative effects of DPP-4. Such analogs are often called GLP-1 receptor agonists and, again, have been shown to have significant therapeutic viability in the treatment of diabetes.

There are significant differences between these two classes of agents which help to inform some of the underlying physiology that predisposes to diabetes. DPP-4 inhibitors raise endogenous GLP-1 concentrations, and in fact, active concentrations of GLP-1 are raised to high physiologic concentrations with a peak in the 12-20 pM range. In contrast, the equivalent activity of GLP-1 receptor agonists is probably achieved at GLP-1 concentrations of 25-30 pM. Both DPP-4 inhibitors and GLP-1 receptor agonists lower fasting and postprandial glucose concentrations, which is done by improving beta-cell function and simulating insulin secretion for a given glucose concentration. Intriguingly, GLP-1 receptor agonists also have powerful effects on intestinal motility, delaying gastric emptying, stimulating the nausea centers in the hypothalamus, and decreasing appetite. These effects are not observed with DPP-4 inhibitors (Vella et al., 2007).

6 The role of genetics in GLP-1 responsiveness

There are individuals who exhibit differential responsiveness to GLP-1-based therapy or GLP-1 infusion.

The earliest suggestion that this may be genetic in origin came from Beinborn et al. (2005) who identified a GLP-1 receptor mutation which altered responsivity of the GLP-1 receptor to GLP-1 by about 50 % (Beinborn et al., 2005). Subsequently, the suggestion that common genetic polymorphisms in the GLP-1 receptor may affect responsiveness to infused GLP-1 was tested in a population of healthy individuals and indeed, two polymorphisms were identified which significantly altered GLP-1 responsivity. Of the two SNPs identified, the most profound action was observed for RS3765467, which is an arginine to glutamine change at position 131 of the GLP-1 receptor in axon 4. This has a minor allele frequency of 5 % in Caucasians and, in the particular experiment, had a very profound effect on insulin secretion in response to infused GLP-1 and glucose. In contrast, RS6923761 results in a glycine to serine substitution at position 168 in axon 5. This has a minor allele frequency of about 30 % in Caucasian and, indeed, the individuals who are homozygous for serine at that position have decreased responsiveness to GLP-1 compared to the more common form where individuals are homozygous for glycine (Sathananthan et al., 2010).

7 GLP-1 and bariatric surgery

Another way that GLP-1 may be potentially important in diabetes is as it pertains to bariatric surgery. The commonest form of bariatric surgery undertaken, at least in North America, currently is Roux-en-Y gastric bypass, which creates a small restrictive gastric pouch and diverts nutrients to the distal intestine. The effect of distal delivery of calories results in stimulation of GLP-1 secretion. Individuals after gastric bypass have high concentrations of GLP-1 in response to meal ingestion (Laferrère et al., 2008). Roux-en-Y gastric bypass has aroused interest recently because bypass operations are associated with remission of diabetes in about 40 % of all individuals with type 2 diabetes, and it has been hypothesized that GLP-1 may play a role in this remission. In such circumstances, one of the experimental paradigms used to investigate the contribution of endogenous GLP-1 to insulin secretion in the postprandial period has been to use competitive antagonist of GLP-1 at its cognate receptor. This antagonist is exendin-(9,39) and is actually a derivative of a GLP-1 receptor agonist which is truncated and, therefore, has no significant metabolic effect when it binds the GLP-1 receptor.

In non-diabetic patients after Roux-en-Y gastric bypass, infusion of exendin-(9,39) results in decreased insulin and C-peptide concentrations compared to age-, weight-, and gender-matched controls. Indeed, this results in a small but significant rise in peak and integrated glucose concentrations. These effects are due to small effects on beta-cell function, as manifested by decreases in beta-cell responsivity. What is interesting is that the component of beta-cell responsivity that is affected is the one that is dependent upon insulin synthesis and secretion in keeping with the known effects of GLP-1 on insulin synthesis. The converse experimental administration of GLP-1 or GLP-1-based therapies raise the component of insulin secretion that is associated with synthesis and secretion (Shah et al., 2014).

A neglected part of the physiology of insulin secretion is the fact that insulin is actually secreted into the portal vein in a pulsatile fashion, and the amplitude and frequency of insulin pulses are decreased in pathologic states, such as aging, obesity, and type 2 diabetes. GLP-1 may be important when secreted into the portal vein because it may help maintain beta-cell competence as well as beta-cell pulsatility and amplitude. Whether fasting and postprandial insulin secretion or its pulsatility has effects on, for example, hepatic insulin action, thereby explaining the relationship between insulin secretion and action observed in prediabetes, is as yet unknown but is the subject of active investigation (Meier et al., 2005; Matveyenko et al., 2008).

8 Measuring β-cell mass

The final aspect as pertains to beta-cell function is the quest to try and quantify beta-cell mass. At present, this subject is problematic. Initial attempts of quantifying beta-cell mass were quite simple and qualitative as they involved counting islets in autopsy specimens. Since then attempts have been made to undertake invasive testing in humans using pancreatic biopsy. Unfortunately, this method is flawed with complications and rarely provides useful information, therefore it has almost been abandoned completely. Efforts have been made to try and quantify islet mass by extrapolating from pancreatic volume obtained by conventional, non-invasive imaging such as CT scanning of magnetic resonance imaging and subsequent extrapolation of islet number. More recently, efforts have been made to use ligands specific to the islets which then can be labeled either with a fluorescent marker detectable by noninvasive imaging or with a radio-labeled ligand. However, to date there has been no compound identified which binds specifically to the islets.

At the current time, the best paradigm for beta-cell function in vivo is ultimately reductionist in approach; currently islet numbers and islet morphology are less important than how they function in response to a given challenge. It is in this circumstance that a functional test such as an oral glucose tolerance test or a mixed meal test and subsequent mathematical extrapolation of beta-cell function may be important clinically. Our current therapeutic interventions are intended to improve glycaemic control not improving beta-cell health.

Nevertheless, using the current mathematical models to quantify beta-cell function over time may have therapeutic application in the near future with the development of novel therapeutics intended to increase beta-cell mass and function.

Acknowledgments

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