Assessment of β-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests

Claudio Cobelli,1 Gianna Maria Toffolo,1 Chiara Dalla Man,1 Marco Campioni,1 Paolo Dentì,1 Andrea Caumo,2 Peter Butler,3 and Robert Rizza4

1Department of Information Engineering, Univ. of Padua, Padua, Italy; 2San Raffaele Scientific Institute, Milan, Italy; 3Larry Hillblom Islet Research Center, David Geffen School of Medicine, University of California, Los Angeles, California; and 4Division of Endocrinology, Diabetes, Metabolism and Nutrition, Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, Minnesota

Submitted 16 August 2006; accepted in final form 27 February 2007

IT IS WELL ESTABLISHED that abnormalities in insulin secretion are an important determinant of diabetes mellitus and other states of glucose intolerance. However, assessment of β-cell function in humans under physiological conditions has been a challenge because of its complex interplay with insulin action and hepatic insulin extraction. The possibility of simultaneously assessing β-cell function, insulin sensitivity, and hepatic insulin extraction under physiological conditions using a simple protocol is appealing, since it has the potential to provide novel insights regarding the regulation of fasting and postprandial glucose metabolism in diabetic and nondiabetic humans. In this Perspective, we review data indicating that an oral glucose tolerance test (OGTT) or a meal test is able to accomplish this goal when interpreted with the oral β-cell minimal model. We begin by using the well-established intravenous minimal model to highlight how the oral minimal model was developed and how the oral assessment parallels that of an intravenous glucose tolerance test (IVGTT). We also point out the unique aspects of both approaches in relation to their ability to assess different aspects of the β-cell secretory cascade. We review the ability of the oral model to concurrently measure insulin sensitivity and hepatic insulin extraction, thereby enabling it to quantitatively portray the complex relationship among β-cell function, hepatic insulin extraction, and insulin action. In addition, data from 204 individuals (54 young and 159 elderly) who underwent both IVGTT and meal tolerance tests are used to illustrate how these different approaches provide complementary but differing insights regarding the regulation of β-cell function in humans.

Assessment of β-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests

AM J PHYSIOLOG ENDOCRINOL METAB 293: E1–E15, 2007. First published March 6, 2007; doi:10.1152/ajpendo.00421.2006.—Assessment of insulin secretion in humans under physiological conditions has been a challenge because of its complex interplay with insulin action and hepatic insulin extraction. The possibility of simultaneously assessing β-cell function, insulin sensitivity, and hepatic insulin extraction under physiological conditions using a simple protocol is appealing, since it has the potential to provide novel insights regarding the regulation of fasting and postprandial glucose metabolism in diabetic and nondiabetic humans. In this Perspective, we review data indicating that an oral glucose tolerance test (OGTT) or a meal test is able to accomplish this goal when interpreted with the oral β-cell minimal model. We begin by using the well-established intravenous minimal model to highlight how the oral minimal model was developed and how the oral assessment parallels that of an intravenous glucose tolerance test (IVGTT). We also point out the unique aspects of both approaches in relation to their ability to assess different aspects of the β-cell secretory cascade. We review the ability of the oral model to concurrently measure insulin sensitivity and hepatic insulin extraction, thereby enabling it to quantitatively portray the complex relationship among β-cell function, hepatic insulin extraction, and insulin action. In addition, data from 204 individuals (54 young and 159 elderly) who underwent both IVGTT and meal tolerance tests are used to illustrate how these different approaches provide complementary but differing insights regarding the regulation of β-cell function in humans.

Address for reprint requests and other correspondence: C. Cobelli, Dept. of Information Engineering, Univ. of Padua, Via Gradenigo 6/B, 35131 Padua, Italy (e-mail: cobelli@dei.unipd.it).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpendo.org 0193-1849/07 $8.00 Copyright © 2007 the American Physiological Society
than the oral ones, since the input into the circulation, i.e., the amount of glucose injected, is known. In contrast, measurement of insulin action during an oral protocol requires assessment of the systemic rate of appearance of the ingested glucose. It is for this reason that reliable models of the oral perturbation only recently have become available thanks to multiple tracer meal validation studies (21, 24).

Simultaneous assessment of β-cell function, hepatic insulin extraction, and insulin sensitivity under physiological conditions with the use of a simple protocol is appealing, since it has the potential to provide novel insights regarding the regulation of fasting and postprandial glucose metabolism in diabetic and nondiabetic humans and to evaluate different approaches to restore appropriate β-cell function by pharmacological means and to transplant or perhaps regenerative approaches in future. We review in this Perspective data indicating that an OGTT or a meal test can be used to simultaneously and quantitatively measure β-cell function, hepatic insulin extraction, and insulin sensitivity. We begin with a schematic representation of the cellular events involved in the regulation of insulin secretion and then put into context which aspects of the secretory pathway are likely being examined during in vivo measurement of β-cell function. Next, we employ the now widely used intravenous minimal model to highlight how the oral minimal model was developed and how the oral assessment parallels that of IVGTT. Finally, we point out the unique aspects of both approaches, particularly in relation to their ability to assess different parts of the β-cell secretory cascade. Because of space limitations, we do not discuss the additional mechanistic information that can be gained by adding glucose tracers to the intravenous or oral protocols (22, 24, 58). We present recently published data from 204 individuals (54 young and 159 elderly) who underwent both IVGTT and meal tolerance tests to illustrate how these different approaches provide complimentary but differing insights regarding the regulation of β-cell function in humans (2).

**WHY IT IS DIFFICULT**

Insulin secretion can be readily assessed during a glucose perturbation by measuring C-peptide concentrations and using deconvolution. This approach is simple and virtually model independent (28, 47). However, it is more difficult to measure β-cell function, since this requires the rate of secretion to be interpreted in relation to the prevailing glucose concentration, i.e., to take into account changing glucose concentration on β-cell response. To do so, the two time courses need to be related by some mechanistic causal relationship; in other words, an hypothesis, or as commonly phrased, a model, of β-cell function is required. β-cell function also needs to be interpreted in light of the prevailing insulin sensitivity. One possibility is to resort to a normalization of β-cell function based on the disposition index paradigm, first introduced in 1981 (4), where β-cell function is multiplied by insulin sensi-

![Fig. 1. β-Cell function is causally related to insulin sensitivity, since the glucose system is negatively feedback regulated.](image)

**Table 1. Protocols: their attributes, and information content**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal state</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes, but limited</td>
<td>Yes, but limited</td>
<td>No</td>
</tr>
<tr>
<td>Intravenous perturbation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemic clamp</td>
<td>No</td>
<td>No</td>
<td>Yes, but limited without a model</td>
<td>Yes, but requires a model</td>
<td>Yes, but requires a model</td>
</tr>
<tr>
<td>Euglycemic clamp</td>
<td>No</td>
<td>No</td>
<td>Yes, but limited without a model</td>
<td>Yes</td>
<td>Yes, but requires a model</td>
</tr>
<tr>
<td>IVGTT</td>
<td>No</td>
<td>No</td>
<td>Yes, but limited without a model</td>
<td>Yes, but requires a model</td>
<td>Yes, but requires a model</td>
</tr>
<tr>
<td>Graded infusion</td>
<td>No</td>
<td>No</td>
<td>Yes, but limited without a model</td>
<td>Yes, but requires a model</td>
<td>Yes, but requires a model</td>
</tr>
<tr>
<td>Oral perturbation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGTT</td>
<td>Yes, but no nutrients</td>
<td>Yes</td>
<td>Yes, but limited without a model</td>
<td>Yes, but requires a model</td>
<td>Yes, but requires a model</td>
</tr>
<tr>
<td>Meal</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes, but limited without a model</td>
<td>Yes, but requires a model</td>
<td>Yes, but requires a model</td>
</tr>
</tbody>
</table>

IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.
tivity. This concept, self-evident in Fig. 1, is more clearly illustrated in Fig. 2. Although regulation of carbohydrate tolerance is undoubtedly more complex, it is conceived that glucose tolerance of an individual is related to the product of β-cell function and insulin sensitivity. In essence, different values of tolerance are represented by different hyperbolas, i.e., DI = β-cell function × insulin sensitivity = constant, where DI is the disposition index. If an individual’s β-cells respond to a decrease in insulin sensitivity by adequately increasing insulin secretion (state II), the product of β-cell function and insulin sensitivity (the disposition index) is unchanged, and normal glucose tolerance is retained. In contrast, if there is not an adequate compensatory increase in β-cell function to the decreased insulin sensitivity (state 2), the individual develops glucose intolerance. Thanks to its intuitive and reasonable grounds, this measure of β-cell functionality, which was first introduced for IVGTT, has become the method of choice also with other tests, such as clamp and OGTT. However, the glucose-insulin feedback system is more complex than the hyperbola paradigm, i.e., DI = β-cell function × insulin sensitivity = constant. The relation between β-cell function and insulin sensitivity is certainly describable by a nonlinear inverse relationship but is in all likelihood more general than an hyperbola, e.g., DI = β-cell function × (insulin sensitivity)^α = constant. In addition, this seemingly simple concept hides several methodological issues that unless fully appreciated could lead to errors in interpretation, particularly when comparing different populations. Furthermore, since the effect of insulin on peripheral tissues is determined not only by the biological effect of insulin but also by the amount of insulin to which the tissue is exposed, changes in hepatic insulin extraction provide yet another dimension to the relationship between insulin secretion and action portrayed in Fig. 2.

CELLULAR EVENTS IN β-CELLS

The insulin secretory pathway is complex and regulated by multiple factors including glucose, protein, fat, and incretins (Fig. 3). Exposure of β-cells to an abrupt increment of glucose elicits biphasic insulin secretion (19), an observation that prompted interest in the intracellular events leading to insulin secretory vesicle discharge (31, 32), resulting in proposal of a two-pool model of insulin-filled vesicles, broadly defined as those available for immediate exocytosis (first phase) and a reserve pool from which granules needed to be mobilized to being available for secretion (second phase). Advances in live cell imaging of neuroendocrine cells have permitted insights on insulin vesicle trafficking and exocytosis (50). In β-cells a subpopulation of secretory vesicles is closely related to, and presumably docked to, the cell membrane (44). Upon stimulation with glucose, a small fraction of docked vesicles discharge their contents, implying that first-phase secretion is derived from a subset of docked vesicles that have been termed the primed docked vesicles (1).

With continued glucose stimulation, new insulin secretory vesicles are directed to the cell membrane (43, 48). Indirect and, more recently, direct evidence suggest that vesicles targeted to membrane docking are those that were most recently synthesized (27). Since the duration of fusion appears to be very brief and not all insulin within the granule is released before the granule closes and reenters the cytoplasm, this process has been referred to as “kiss and run” (60). Thus the intensity of the stimulus, e.g., glucose concentration, for secretion may direct the quantity of hormone secreted by exocytosis not only by recruiting the number of primed vesicles to undergo exocytosis but also the extent to which these vesicles discharge their cargo. Both nonprimed and previously fused granules can be recycled back into the cytoplasm, where insulin within the granule is degraded.

The time course of the various steps involved in insulin secretory pathways is believed to differ substantially, with exocytosis occurring within seconds, docking and priming within 5–10 min, and synthesis and processing likely considerably longer. Therefore, different stimuli assess different aspects of this pathway. For example, the rapid increase in plasma insulin that occurs during the first few minutes after intravenous injection of glucose almost certainly is solely due to first-phase release of insulin from previously docked and primed granules. On the other hand, the rate of release of insulin during the first hour after ingestion of a mixed meal, i.e., when plasma glucose is rising (see below), is likely...
determined by multiple steps, e.g., processing, docking, and priming, involved in second-phase secretion, in addition to exocytosis.

β-CELL FUNCTION FROM BASAL MEASUREMENTS

The easiest and thus most popular assessment of β-cell function is the homeostatic responsivity index HOMA-B, derived from basal measurements of insulin and glucose (40). Recently, the use of C-peptide instead of insulin in HOMA-B has been encouraged to avoid the confounding effect of hepatic insulin extraction (63). Although HOMA-B is widely used because of its simplicity, it is worth pointing out that it reflects the release of insulin under nonstimulated conditions. The homeostatic model HOMA-S also provides an index of insulin sensitivity under nonstimulated conditions, thus permitting evaluation of β-cell function in relation to the prevailing insulin action. It should be noted that an hyperbolic relationship between the two indexes is inherent in the calculation, since HOMA-B/HOMA-S is a function of basal glucose concentration such that the two measurements are, by definition, in a given subject hyperbolically inversely related provided basal glucose [and also basal insulin if the computer model HOMA-S (63) is used] remains constant (16). Recently, another basal insulin sensitivity index, QUICKI, has been derived (34). QUICKI and HOMA-S are highly correlated \((R = 0.99)\) in our database of 204 young and elderly subjects. Also, HOMA-S and its computer-model version are highly correlated \((R = 0.99)\), whereas HOMA-B with insulin and C-peptide show \(R = 0.66\).

β-CELL FUNCTION FROM IVGTT

A variety of methods have been used to assess the β-cell response to a glucose stimulus. The IVGTT is probably the most common (Table 1).

First-Phase Index Based on Insulin

The acute insulin response (AIR) is perhaps the most popular index of insulin secretion. After injection, glucose rapidly increases from ~90 to ~350 mg/dl. Plasma insulin concentration is measured in the first 10 min, and its area under the curve is calculated. What does AIR measure? It almost certainly reflects a nearly instantaneous release of insulin from previously docked granules that then distributes in plasma to produce the subsequent rapid rise and then fall in plasma insulin due to processing and docking of new granules. However, being based on insulin concentration, AIR also reflects hepatic insulin extraction, and since extraction differs depending on pattern and amount of insulin release, AIR does not provide an independent assessment of insulin secretion (41).

As discussed previously, an accurate β-cell metric must account for insulin sensitivity. The IVGTT glucose minimal model Fig. 4, left, enables assessment of an index of insulin sensitivity, \(SI_{\text{IVGTT}}\) (4), which has been validated in numerous studies (see, for instance, Ref. 51). AIR is then multiplied by \(SI_{\text{IVGTT}}\) to calculate what commonly has been referred to as a disposition index, to determine whether insulin secretion is appropriate for the prevailing level of insulin action.

First- and Second-Phase Indexes and Delay Based on C-Peptide

AIR has two major limitations. First, it is a composite β-cell function and hepatic insulin extraction index, and second, it probes β-cell behavior in a very brief time window and immediately after a markedly supraphysiological glucose stimulus.

β-Cell function can be assessed from plasma glucose and C-peptide concentrations measured during a standard or insulin-modified IVGTT by using the minimal model shown in Fig. 5, left (59), which is based on the pioneering work of Grodsky and colleagues (31, 36). Use of C-peptide instead of insulin concentrations has the advantage that issues of hepatic insulin clearance are overcome. C-peptide kinetics is described by a two-compartment model whose parameters are usually determined, without loss of accuracy, with the population approach (62). Insulin secretion is made up of two components, first- and second-phase secretion. First-phase secretion portrays in the
model a rapidly turning over compartment (2 min) and likely represents exocytosis of previously primed insulin secretory granules (commonly called readily releasable). It introduces a derivative control, since it is proportional to the rate of increase of glucose from basal up to the maximum through the parameter $\Phi_1$, which defines the first responsivity index (in contrast to AIR, $\Phi_1$ is a pure $\beta$-cell function index; correlation between AIR and $\Phi_1$ in 204 individuals is $0.72$). Second-phase insulin secretion is believed to be derived from the provision and/or docking of new insulin secretory granules that occurs in response to (i.e. proportional to) a given glucose concentration through the parameter $\Phi_2$, which defines the second-phase responsivity index, and reaches the releasable pool with a delay time constant, $T$. The meaning of $\Phi_2$ and $T$ are readily envisioned by making reference to a thought experiment of an above-basal step increase of glucose: provision tends with time constant $T$ toward a steady state that is linearly related to the glucose step size through parameter $\Phi_2$. $T$ presumably represents the time required for new “readily releasable” granules to dock, be primed, and then be exocytosed. All these three ingredients, i.e. derivative term, delay, and presence of releasable pool, have been shown to be necessary for the model to accurately describe C-peptide data (59).

$\beta$-Cell responsivity indexes can be usefully interpreted in terms of insulin secretion. To do this, insulin secretion during an IVGTT is decomposed (Fig. 6, left) into basal, first-phase, and second-phase components. Against this background it is easy to give an interpretation of the various indexes, summarized in Table 2. In addition to $\Phi_1$ and $\Phi_2$, basal responsivity, $\Phi_b$, also is of interest, since it gives a basal, nonstimulated index complementing the stimulated ones, $\Phi_1$ and $\Phi_2$. Finally, a single total index of stimulated $\beta$-cell responsivity, $\Phi_{ivgtt}$, can be derived by properly combining $\Phi_1$ and $\Phi_2$. Disposition indexes can also be calculated by multiplying responsivity
indices $\Phi_1$ and $\Phi_2$ by $S_{IVGTT}$ to determine whether that aspect of $\beta$-cell function is appropriate for the level of insulin action (Table 2). Disposition indexes $DI_1$ and $DI_2$ measure $\beta$-cell function in relation to insulin sensitivity under a glucose stimulus. This portrait can be usefully complemented by a basal disposition index, $DI_b$, obtained by multiplying $\Phi_b$ by HOMA-$S$ (or QUICKI).

**Hepatic Insulin Extraction**

Since plasma insulin concentrations are measured during the IVGTT for estimating insulin sensitivity, posthepatic insulin delivery rates can be calculated allowing estimation of hepatic insulin extraction during the test, $HE_{IVGTT}$, and in basal condition, $HE_b$ (57) (Table 2). To this end, the protocol of choice is an insulin-modified IVGTT, since the decay of insulin concentration observed after exogenous insulin administration facilitates a reliable estimation of insulin kinetics. However, a population approach, similar to that developed for C-peptide kinetics, is being developed for insulin kinetics, and preliminary results are very encouraging (13).

Figure 7, left, shows $\beta$-cell responsivity, insulin sensitivity, hepatic extraction, and disposition index in 54 young and 159 elderly subjects, whereas Fig. 8 shows their basal counterparts (the glucose minimal model does not provide a basal insulin sensitivity index, and thus one can use HOMA-$S$ or QUICKI to complete the basal picture). As is evident, the stimulated and basal index portraits provide important and complementary information that is discussed below. At this point we simply note that it is important to not just look at the relationship between $\beta$-cell function and insulin sensitivity but also to consider hepatic insulin extraction (41).

### $\beta$-CELL FUNCTION FROM AN OGGTT OR MIXED MEAL

An oral glucose test differs from IVGTT in several important ways. These include the oral route of delivery, the associated incretin hormone secretion, the physiologically smoother pattern of changes in glucose, insulin, and C-peptide concentrations, and the presence of nonglucose nutrients stimulation, e.g., amino acids and fat, during a mixed meal.

**$\beta$-Cell Responsivities and Hepatic Insulin Extraction**

$\beta$-Cell function after an oral glucose challenge can be assessed using the oral minimal model, which interprets plasma C-peptide concentrations in relation to the observed changes in glucose concentration (8). All of the previous model
ingredients employed in the IVGTT model were necessary with the exception of the fast-turning over insulin releasable pool (which is not evident under these conditions) to describe the data. In particular, both a rate of change of glucose component of insulin secretion and a delay between glucose stimulus and \(\beta\)-cell response were necessary to fit the data (9). The oral model thus features a dynamic component that senses the rate of change of glucose concentration and a static component that represents the release of insulin that, after a delay, occurs in proportion to prevailing glucose concentration. The oral \(\beta\)-cell model is shown in Fig. 5, right. The model has been successfully used by Steil et al. (52) for describing hyperglycemic clamp C-peptide data as well as meals, thus providing further independent evidence of its validity (see also comments in Ref. 14). Of interest, in contrast to the IVGTT model, where the derivative component of first-phase secretion was operative only during the first few minutes as the plasma glucose concentration increased from a “basal” to “maximal” concentration, the relatively gradual “bell-shaped” pattern in glucose concentrations observed during oral tests necessitated the presence of a secretion component proportional to the glucose rate of increase that contributed to the model for the first 60–90 min. In addition, and similar to the IVGTT, a component of insulin secretion proportional to glucose, characterized by a delay time \(T\) (presumably reflecting at least in part the time it takes for new granules to reach the releasable pool) that contributed throughout the experimental period was also necessary. The two components have been termed, respectively, dynamic and static, and the parameters responsible for them are termed dynamic (\(\Phi_d\)) and static (\(\Phi_s\)) responsivity indexes. As discussed above, this was markedly different from the IVGTT model, where the first-phase component only contributed during the first 4–6 min. Therefore, \(\Phi_d\), and \(\Phi_s\) indexes (during an oral glucose challenge) are numerically different albeit related to first-phase \(\Phi_1\) and second-phase \(\Phi_2\) indexes (following intravenous glucose injection); the correlation in the 204 individuals was only 0.52 for both indexes. Thus it is probable that they are assessing different aspects of the insulin secretory pathway. In particular, \(\Phi_d\) presumably also reflects multiple distal steps including the rate of granule docking, priming, and exocytosis, whereas \(\Phi_s\) also reflects earlier steps, e.g., synthesis, process, and granule maturation.
Oral β-cell responsivity indexes can be interpreted in terms of insulin secretion (Fig. 6, right), and they are summarized in Table 2. The basal responsivity index $\Phi_b$ and total responsivity index $\Phi_{oral}$, which combines $\Phi_d$ and $\Phi_s$, are also listed. The importance of $\Phi_b$ is often underappreciated. For example, $\Phi_b$ accounts for almost all of the differences when β-cell function was compared in obese subjects before and after weight loss (12). HOMA-B correlates poorly in the 204 individuals with $\Phi_b$ ($R = 0.42$, but HOMA-B with C-peptide improves to $R = 0.66$) and poorly with the stimulated indexes $\Phi_d$ and $\Phi_s$ (0.34 and 0.36, respectively); the same happens with IVGTT indexes 1 and 2 (0.39 and 0.40, respectively).

An accurate β-cell metric must account for insulin sensitivity. Insulin sensitivity can also be accurately measured with the oral minimal model of Fig. 4, right (22). This model provides an index, $SI_{oral}$ (Table 2), which has been validated both against a multiple tracer meal protocol (21) and in an OGTT vs. euglycemic clamp study (24), showing a correlation of 0.86 and 0.81, respectively. $SI_{oral}$ and $SI_{ivgtt}$ differ in that they are protocol dependent (26), but $SI_{oral}$ correlates well in the 204 individuals with $SI_{ivgtt}$ ($R = 0.74$), whereas correlation with nonstimulated indexes HOMA-S and QUICKI is 0.50.

It is also of interest to assess the reproducibility of oral β-cell function and insulin sensitivity indexes against that of IVGTT indexes reported previously (55). To do so, we have analyzed the meal data of Toffolo et al. (56). The results for $\Phi_d$, $\Phi_s$, and $SI_{oral}$ are shown in Table 3, where for the sake of comparison those of IVGTT (55) are also reported. The reproducibility of the indexes in the two tests is very similar, and one can safely assume a reproducibility between 20 and 30%.

The rich portrait of β-cell function provided by the oral minimal model carries over to the disposition index (DI) metric. Similarly to IVGTT, the oral model yields three disposition indexes, a dynamic (DId), a static (DIs), and an overall disposition index (DIoral) (Table 2), all probing different aspects of β-cell function. In addition, as with IVGTT, a basal disposition index (DIB) can be calculated. As expected, there is virtually no relation with between DId and DIs, and DIB correlates well in the 204 individuals with $SI_{oral}$ ($R = 0.39$ and 0.40, respectively).

Hepatic insulin extraction also can be estimated during an oral minimal test by following a strategy similar to that outlined for the IVGTT; an insulin model, where kinetics have been fixed by a population approach, can predict posthepatic delivery rate of insulin in plasma, which, combined with portal insulin delivery provided by the C-peptide model, allows the...
estimation of hepatic insulin extraction during the test, HEoral, and in basal conditions, HEb (Table 2).

Figure 7, right, documents the richness of information of the oral models by showing, respectively, indexes of β-cell function, insulin sensitivity, hepatic insulin extraction, and disposition index obtained in 54 young and 159 elderly subjects from a mixed-meal study. Both IVGTT and oral models reveal the same defective trend of metabolic indexes in the elderly subjects. The basal indexes of Fig. 8 complement the stimulated indexes of Fig. 7, right. As expected from theory, the two portraits provide very different information. In the basal state, elderly subjects have β-cell function, insulin sensitivity, and disposition index comparable, if not greater than, that of young subjects; only their hepatic extraction is higher. In contrast, the meal-stimulated state reveals the profound defects of all metabolic indexes in the elderly subjects. We comment further on this topic below.

Reduced Oral Protocol

An oral protocol that employs a reduced duration or number of samples while maintaining accuracy is potentially of considerable value, since it could facilitate the conduct of large-scale epidemiological and clinical trials. For the IVGTT minimal model (both standard and insulin modified), glucose and insulin concentrations must be measured for at least 3 h (and perhaps 4 h depending on glucose tolerance) to obtain a reliable measure of insulin action. In contrast, a reduced-sample oral minimal model enables insulin secretion and action to be reliably measured using a shorter protocol (2 h) and with only seven samples. This protocol was developed using a database of 100 OGTTs in which 11 samples were obtained over 5 h and 100 meal studies in which 21 samples were obtained over 7 h. These data established that a seven-sample protocol performed over 2 h after ingestion of either 75 g of glucose or a mixed meal enables accurate assessment of both β-cell function and insulin sensitivity in nondiabetic humans (20). The resultant indexes obtained with the 2-h protocol were not significantly different and were very well correlated to those of the full protocol; Fig. 9 shows the OGTT results, but similar results have also been obtained for the meal. The proposed 2-h duration is the same as that of the standard OGTT and therefore does not extend the period of study. It also retains the typical 0- and 120-min samples with the addition of samples at 10, 20, 30, 60, and 90 min. Of note, samples at 10 and 20 min are essential for an accurate and precise estimation of dynamic β-cell responsivity Φb, which by definition relies on the data derived when glucose is increasing. The reduced protocol was developed from a database comprising individuals who had a wide range of glucose tolerance. A first study employing the seven-sample OGTT was recently published (46). However, people with marked fasting hyperglycemia were not studied. Additional studies are required to determine whether a similar approach can be used in individuals with more severe impairment of insulin secretion and insulin action, e.g., diabetes.

Other Models

Other models have been proposed to assess β-cell function during OGTT and meal test. The model proposed by Hovorka et al. (33) assumes an instantaneous linear control of glucose on insulin secretion; i.e., there is no delay between glucose stimulus and β-cell response. The model proposed by Cretti et al. (18) describes insulin secretion with the static component of glucose control of the C-peptide minimal model; thus it is characterized by a delay but does not include any dynamic, i.e., rate of change, glucose control. Interestingly, the same authors have recently included a dynamic control to describe first-phase secretion in a subsequent report (64). The model proposed in by Mari et al. (38), like the oral model shown in Fig. 3, right, has both a proportional component and a component responsive to the rate of change of glucose, but there is no delay between glucose signaling and supply of new insulin to the circulation. The authors choose to account for the expected inability of a proportional plus derivative glucose control to account for C-peptide measurements with a time-varying term correcting only the static component of insulin secretion, which has been called the potentiation factor. In simple words, the potentiation factor is a time-varying correction term that mathematically compensates for the proportional plus derivative description deficiency. Given this framework, the physiological interpretation of this factor is difficult. The authors have hypothesized that this correction term is linked in some fashion to incretin- and/or glucose-mediated factors (37, 38). However, we have recently shown in an OGTT and matched intravenous study (14) that this potentiation factor (38) is not related to the incretin effect in that there was no difference between the potentiation factor during OGTT and matched intravenous.

Table 3. IVGTT and meal index reproducibility

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Difference (% of mean)</th>
<th>CV (%)</th>
<th>Parameters</th>
<th>Difference (% of mean)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVGTT</td>
<td></td>
<td></td>
<td>ORAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΦD</td>
<td>d = 1 − 4</td>
<td>19</td>
<td>dD</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>Φa</td>
<td>d = −17</td>
<td>22</td>
<td>aD</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>SImeal</td>
<td>d = 12</td>
<td>26</td>
<td>SImeal</td>
<td>8</td>
<td>23</td>
</tr>
</tbody>
</table>

Difference (% of mean) = (study 1 − study 2)/mean(study 1, study 2). Coefficient of variance (CV, %) = |study 1 − study 2|/mean(study 1, study 2).
study (Fig. 10). It remains possible that this time-varying factor, by sustaining insulin secretion after the glucose stimulus, is simply accounting for the physiological delay occurring between glucose signaling and provision of new insulin to the readily releasable pool. The model also deserves some comments in terms of modeling methodology. At variance with all the other models (8, 18, 33) that are numerically identified by standard nonlinear least squares and maximum likelihood methods (15), this model requires a less transparent strategy that also bears some degree of subjectivity. In fact, the reconstruction of the time course of this potentiation factor starting from the sole C-peptide measurements requires a smoothness deconvolution procedure governed by two unknown parameters to obtain the final solution. The criterion chosen for their estimation is the discrepancy criterion, which is known to provide oversmoothing (15). In addition, it is not uncommon when running this model to find multiple solutions in the same individual and, thus, different potentiation factor time courses.
DISPOSITION INDEX

It is important to revisit in the context of IVGTT, where it was first introduced (4), the classic measure of β-cell function in relation to insulin action provided by the disposition index. The disposition index was introduced as a conceptual surrogate of the efficiency of glucose homeostasis in a given individual, which is the ability of β-cells to upregulate insulin secretion in response to a decrease of insulin sensitivity, and is thus a measure of β-cell functionality. Numerous studies have confirmed the ability of β-cells to compensate for insulin resistance, and the disposition index relationship has recently received the status of the hyperbolic law of glucose tolerance (54). However, its conceptual simplicity hides some methodological issues which must be addressed in more depth (see also Ref. 29). More specifically, the questions we address are as follows: Is it true that the hyperbolic relationship $\text{DI}_1 = \Phi_1 \times \text{SI}_{\text{IVGTT}}$ and $\text{DI}_2 = \Phi_2 \times \text{SI}_{\text{IVGTT}}$ holds in a population? How should $\text{DI}_1$ and $\text{DI}_2$ be used in comparing populations, i.e., should the individual values be averaged, or should the disposition index relationship be estimated directly in the population? Should the disposition index be calculated from a single or different tests? Is insulin action the single normalization factor for β-cell function, or should hepatic insulin extraction, for example, come into play as an additional ingredient?

Methodology

We start by noting that there are some methodological issues behind these questions that have been dealt with differently in different studies, e.g., in Refs. 35 and 61 vs. Refs. 30 and 45. First, in testing whether the disposition index relationships are constant, it is not correct to use ordinary regression where one of the variables is assumed with no errors. This is done, for example, in Ref. 30, where $\text{SI}_{\text{IVGTT}}$ is assumed as the independent variable, and in Ref. 45, where the opposite is assumed. All indexes $\Phi_1$ or AIR, $\Phi_2$, and $\text{SI}_{\text{IVGTT}}$ have errors, and thus regression with error in two variables must be used, e.g., Refs. 10 and 17. Second, regression should be performed on the original variables, not on the log-transformed data with error in both AIR and $\text{SI}_{\text{IVGTT}}$ is considered.

Figures 11 and 12 portray our IVGTT β-cell responsivities $\Phi_1$ and $\Phi_2$ and insulin sensitivity $\text{SI}_{\text{IVGTT}}$ in 59 young and 145 elderly subjects, respectively. We have fitted these data with the nonlinear inverse relationships $\text{DI}_1 = \Phi_1 \times \text{SI}_{\text{IVGTT}}$ and $\text{DI}_2 = \Phi_2 \times \text{SI}_{\text{IVGTT}}$: if $\alpha_1, \alpha_2 = 1$, one has the classic disposition index hyperbola, i.e., $\text{DI} = \beta$-cell function $\times$ insulin sensitivity $= constant$; if $\alpha_1, \alpha_2 \neq 1$, this is no longer true, and glucose tolerance is characterized by two ($\text{DI}_1$ and $\alpha$) instead of one ($\text{DI}$) parameter. Parameters $\text{DI}$ and $\alpha$ were estimated by nonlinear regression in the original variables. Instead of using the cost function proposed in Ref. 49, which was developed for linear regression, we implemented the following. For each data point, the minimum weighted distance to the equation $y = \text{DI}/(\alpha^x)$ was calculated. The $x$ component of this distance was weighted according to error in the $x$ variable (e.g., $\text{SI}_{\text{IVGTT}}$), and the $y$ component according to that in the $y$ variable (e.g., $\Phi_1$), obtained from the numerical identification of glucose and C-peptide models, respectively. The sum of squares of these weighted distances was minimized, and estimates of $\alpha$ and $\text{DI}$ were obtained. Confidence intervals were also calculated to take into account the uncertainty of the estimates of both $\alpha$ and $\text{DI}$. Our results show that the values of $\alpha$ obtained for first-phase $\text{DI}$ in elderly subjects and second-phase $\text{DI}$ in young and linear regression on log-transformed data with error in both AIR and $\text{SI}_{\text{IVGTT}}$ is considered.

Figures 11 and 12 portray our IVGTT β-cell responsivities $\Phi_1$ and $\Phi_2$ and insulin sensitivity $\text{SI}_{\text{IVGTT}}$ in 59 young and 145 elderly subjects, respectively. We have fitted these data with the nonlinear inverse relationships $\text{DI}_1 = \Phi_1 \times \text{SI}_{\text{IVGTT}}$ and $\text{DI}_2 = \Phi_2 \times \text{SI}_{\text{IVGTT}}$: if $\alpha_1, \alpha_2 = 1$, one has the classic disposition index hyperbola, i.e., $\text{DI} = \beta$-cell function $\times$ insulin sensitivity $= constant$; if $\alpha_1, \alpha_2 \neq 1$, this is no longer true, and glucose tolerance is characterized by two ($\text{DI}_1$ and $\alpha$) instead of one ($\text{DI}$) parameter. Parameters $\text{DI}$ and $\alpha$ were estimated by nonlinear regression in the original variables. Instead of using the cost function proposed in Ref. 49, which was developed for linear regression, we implemented the following. For each data point, the minimum weighted distance to the equation $y = \text{DI}/(\alpha^x)$ was calculated. The $x$ component of this distance was weighted according to error in the $x$ variable (e.g., $\text{SI}_{\text{IVGTT}}$), and the $y$ component according to that in the $y$ variable (e.g., $\Phi_1$), obtained from the numerical identification of glucose and C-peptide models, respectively. The sum of squares of these weighted distances was minimized, and estimates of $\alpha$ and $\text{DI}$ were obtained. Confidence intervals were also calculated to take into account the uncertainty of the estimates of both $\alpha$ and $\text{DI}$. Our results show that the values of $\alpha$ obtained for first-phase $\text{DI}$ in elderly subjects and second-phase $\text{DI}$ in young and
elderly subjects are significantly different from 1, whereas the value of first-phase DI in young subjects is not.

The methodology for estimating DI\textsubscript{1}, α\textsubscript{1} and DI\textsubscript{2}, α\textsubscript{2} is of crucial importance. For instance, in elderly subjects we have α\textsubscript{1} = 0.68 (0.48–0.88) with DI\textsubscript{1} = 332 (208–455) (Fig. 11, top). If we had used regression with error in two variables but on log-transformed data, α\textsubscript{1} would have been 0.76 (0.56–0.96) with DI\textsubscript{1} = 559 (328–790). If we had performed a regression on original data by considering SI\textsubscript{ivgtt} as an independent variable, α\textsubscript{1} would have been 0.19 (0.05–0.33) with DI\textsubscript{1} = 135 (107–164).

Finally, the importance of also obtaining reliable confidence intervals in the population is worth emphasizing by looking back to Fig. 2. The normality range in the young and elderly subjects with impaired fasting glucose/tolerance can be compared for a targeted treatment.

**Individual vs. Population Estimation**

Two comments are in order: first, populations can be described by an α value that is not equal to 1; second, as expected from theory, the value of the disposition index obtained by averaging individuals is different from that obtained by estimating disposition index parameters DI\textsubscript{1}, α\textsubscript{1} and DI\textsubscript{2}, α\textsubscript{2} in the population, even when α\textsubscript{1} and α\textsubscript{2} are close to 1. Therefore, the correct approach for comparing different populations is to estimate the two disposition index parameters (α and DI) in each population. This was possible because of the availability of two large and homogeneous populations, i.e., young and elderly subjects are two normoglycemic homogenous populations (4.8 ± 0.1 vs. 5.2 ± 0.1 mM) with age being the only discriminatory variable (23 ± 0.4 vs. 69 ± 0.5 yr). In our young vs. elderly subjects, the statistical differences between disposition indexes observed by the population and individual averaging strategy are the same (Figs. 11 and 12), but this may be due to the homogeneity and numerosness of the studied population.

Is the population estimation strategy also the way to go when the numerosness of the population is small, or is the individual averaging the only possibility? To address this question, we created two situations in which only n = 15 or n = 30 young vs. elderly subjects were compared, randomly extracted from our database of young and elderly subjects. We tested the n = 15 and n = 30 young vs. elderly subjects against the 54 young vs. 159 elderly subject “truth” by considering significantly different first- and second-phase disposition indexes if at least one of the two parameters DI\textsubscript{1}, α\textsubscript{1} or DI\textsubscript{2}, α\textsubscript{2} were significantly different. Our results show that the population performs better than the individual averaging strategy in recovering the true significant difference: for first-phase disposition index, 97 vs. 95% and 99 vs. 100% tests (out of 1,000 random sets) agree with truth for n = 15 and n = 30, respectively, whereas for...
second-phase disposition index, 37 vs. 33% and 77 vs. 66% for \( n = 15 \) and \( n = 30 \), respectively. These results also indicate that the numerosness of the population is critical, particularly for second-phase disposition index, since with \( n = 30 \) the population strategy performed two times better than with \( n = 15 \), but there was still 25% probability of error (probably due to the smaller difference in second-phase with respect to first-phase disposition index).

**Same vs. Different Test**

Another relevant issue concerns the importance of measuring insulin secretion and action with the same test when comparing populations. This is often not the case. For instance, insulin secretion is often assessed by IVGTT AIR or oral test insulinoenic index (53), and insulin action via clamp or oral composite insulin sensitivity index (39). However, albeit some of these \( \beta \)-cell function and insulin sensitivity indexes, e.g., AIR and clamp insulin sensitivity, are well correlated with IVGTT \( \Phi_b \) and SI_IVGTT, their actual values differ and are protocol dependent (see Ref. 26 for insulin action). This means that the disposition index will be different with different metrics and thus impossible to compare. This problem is avoided when the same test (e.g., IVGTT or oral test) is used to simultaneously measure both \( \beta \)-cell function and insulin sensitivity with two different models, the C-peptide and glucose minimal models, from C-peptide and glucose data and with insulin and glucose data, respectively.

**Hepatic Extraction Dimension**

The results of Fig. 7 support consideration of hepatic insulin extraction in addition to the \( \beta \)-cell function and insulin sensitivity glucose tolerance picture. We have examined the interrelationship of \( \beta \)-cell responsivity and insulin sensitivity with hepatic insulin extraction, where this last is expressed as \( 1 - \text{HE}_{IVGTT} \), i.e., the fraction that reaches peripheral tissues. We observed a nonpatterned relation of hepatic insulin extraction with both \( \beta \)-cell function and insulin sensitivity in both young and elderly subjects (data not shown). Of note, however, this relationship differs with age, i.e., in elderly subjects a smaller proportion of secreted insulin reaches peripheral tissues (see below).

**Basal Complement**

All the above portrays \( \beta \)-cell function in relation to insulin sensitivity under glucose-stimulated conditions. However, it is important to complement this picture with the basal one by examining the basal disposition index: \( \text{DI}_b = \Phi_b \times \text{HOMA-S} \) (or QUICKI). Figure 13 shows \( \text{DI}_b \) for young (top) and elderly subjects (bottom), obtained with the population estimation assuming both \( \Phi_b \) and HOMA-S have errors (reflecting plasma concentration measurement error). In the basal state, elderly individuals show, in agreement with Fig. 8, a better ability of \( \beta \)-cell function to compensate for insulin resistance compared with young subjects. As under the IVGTT stimulated condition, we observed a nonpatterned relation between \( 1 - \text{HE}_b \) and HOMA-S (or QUICKI) while confirming results of Fig. 8, i.e., in elderly subjects a smaller proportion of insulin secretion reaches peripheral tissues in the basal state as well.

**SUMMARY**

Insulin secretion as well as insulin action and hepatic insulin extraction are important determinants of carbohydrate, fat, and protein metabolism. Ideally, these should be assessed using a single, simple physiological test in the presence of glucose, amino acids, incretins, and neural signals. As discussed in this Perspective, the oral minimal model using an OGTT or a mixed meal can be used to simultaneously and quantitatively measure \( \beta \)-cell function, insulin sensitivity, and hepatic insulin extraction. The widely used IVGTT minimal model, although nonphysiological, provides useful insight into the regulation of insulin secretion and action. We have attempted to highlight the unique aspects of both approaches, particularly in relation to their ability to assess important but differing cellular events in the \( \beta \)-cell secretory cascade. The oral method, by quantitatively portraying the complex relationships among \( \beta \)-cell function, insulin sensitivity, and hepatic insulin extraction, has the potential to provide novel insights regarding the regulation of fasting and postprandial glucose metabolism in nondiabetic and diabetic humans and has been recently employed in several studies (3, 6, 7, 42, 46).

**ACKNOWLEDGMENTS**

We thank Dr. Rita Basu and Ananda Basu for helpful discussions on modeling results of the 204 subjects who underwent both IVGTT and meal tolerance tests. We offer additional thanks to Dr. Gianluigi Pillonetto for thoughtful comments on literature models.

**GRANTS**

This study was supported by National Institutes of Health Grants EB-01975, AG-14383, DK-29953, and RR-00585 and by Ministero dell’Università e della Ricerca Scientifica e Tecnologica, Italy.

**REFERENCES**

Perspectives


