Estimates of Insulin Secretory Function in Apparently Healthy Volunteers Vary as a Function of How the Relevant Variables Are Quantified

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**BACKGROUND:** Several surrogate estimates have been used to define relationships between insulin action and pancreatic β-cell function in healthy individuals. Because it is unclear how conclusions about insulin secretory function depend on specific estimates used, we evaluated the effect of different approaches to measurement of insulin action and secretion on observations of pancreatic β-cell function in individuals whose fasting plasma glucose (FPG) was <7.0 mmol/L (126 mg/dL).

**METHODS:** We determined 2 indices of insulin secretion [homeostasis model assessment of β-cell function (HOMA–β) and daylong insulin response to mixed meals], insulin action [homeostasis model assessment of insulin resistance (HOMA–IR) and steady-state plasma glucose (SSPG) concentration during the insulin suppression test], and degree of glycemia [fasting plasma glucose (FPG) and daylong glucose response to mixed meals] in 285 individuals with FPG <7.0 mmol/L. We compared the relationship between the 2 measures of insulin secretion as a function of the measures of insulin action and degree of glycemia.

**RESULTS:** Assessment of insulin secretion varied dramatically as a function of which of the 2 methods was used and which measure of insulin resistance or glycemia served as the independent variable. For example, the correlation between insulin secretion (HOMA–β) and insulin resistance varied from an r value of 0.74 (when HOMA–IR was used) to 0.22 (when SSPG concentration was used).

**CONCLUSIONS:** Conclusions about β-cell function in nondiabetic individuals depend on the measurements used to assess insulin action and insulin secretion. Viewing estimates of insulin secretion in relationship to measures of insulin resistance and/or degree of glycemia does not mean that an unequivocal measure of pancreatic β-cell function has been obtained.

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Multiple approaches have been used to study the role of the pancreatic β-cell in regulation of glucose homeostasis. The simplest of these is homeostasis model assessment of β-cell function (HOMA–β),1 which relies on a mathematical formula involving fasting plasma glucose (FPG) and insulin (FPI) concentrations (1). Using HOMA–β, the UK Prospective Diabetes Study (2) concluded that “progressively increasing hyperglycemia, associated with decreasing β-cell function, was a marked feature irrespective of the therapy used.” In the same context, a recent population-based, prospective study (3) demonstrated that the rapid increase in FPG concentration that heralded the onset of type 2 diabetes was preceded for a considerable time by a progressive loss of insulin secretory function as assessed by HOMA–β.

More recently, it has been suggested that to understand the physiological significance of values of HOMA–β, it is necessary to relate them to a relevant measure of insulin resistance (4). Homeostasis model assessment of insulin resistance (HOMA–IR) is often used for this purpose, as it is also based on a mathematical formula involving FPG and FPI concentrations (1). HOMA–IR provides a surrogate estimate of insulin action that is determined under basal conditions and accounts for no more than one-third of the variance of an estimate of insulin action based on the ability of insulin to limit the increase in plasma glucose concentration in response to continuous glucose infusion in apparently healthy individuals (5, 6). In addition, because HOMA–β is an estimate of insulin secretory function based on only FPG and FPI concentrations,
we thought it would prove interesting to compare HOMA-\(\beta\) with the day-long plasma insulin response to a test mixed meal, a variable that takes into account more than just FPG and FPI.

To address these issues, we determined HOMA-IR, HOMA-\(\beta\), the ability of insulin to limit the change in plasma glucose concentration that occurs in response to a constant infusion of glucose, and the plasma glucose and insulin responses to test mixed meals in 285 apparently healthy individuals whose FPG was <7.0 mmol/L (126 mg/dL).

Materials and Methods

The experimental population consisted of 285 volunteers (180 women and 105 men) who had responded to newspaper advertisements describing our interest in the role of insulin resistance in human disease. Participants were apparently healthy, with FPG concentrations <7.0 mmol/L (126 mg/dL), and without known vascular disease. In addition, subjects had a normal medical history and physical examination and were free of hematologic, liver, or kidney disease. Stanford University’s Human Subjects committee approved the study protocol, and all subjects gave written informed consent for participation.

The ability of insulin to modulate the change in plasma glucose concentration in response to a constant infusion of glucose was quantified by a modified version (7) of the insulin suppression test (IST) as described and validated by our research group (8, 9). After an overnight fast, an intravenous catheter was placed in each participant’s arm, 1 for the 3-h infusion of octreotide (0.27 \(\mu\)g/m\(^2\)/min), insulin (32 mU/m\(^2\)/min), and glucose (267 mg/m\(^2\)/min), and 1 for drawing blood. Plasma glucose and insulin concentrations were measured every 10 min from 150 to 180 min and averaged to obtain the steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations. Because SSPI concentrations are comparable in all individuals, and glucose infusion is identical, the SSPG concentration provides an estimate of the ability of insulin to limit the magnitude of the glucose concentration that occurs in response to the infused glucose load: the greater the SSPG concentration, the more “resistant” the individual is to the action of insulin. We have previously shown that estimates of degree of insulin resistance with the IST correlate highly \((r > 0.9\) SSPG concentration\) with measures of insulin action obtained using the hyperinsulinemic, euglycemic clamp method (9).

On another morning after an overnight fast, volunteers were admitted to the General Clinical Research Center for day-long measurements of plasma glucose and insulin concentrations before and after test breakfast and lunch meals (10, 11). Each meal had, as percent of daily calories, 15% protein, 42% carbohydrate, and 43% fat. Breakfast was at 0800 (20% of estimated daily caloric intake) and lunch was at noon (40% of estimated daily caloric intake). Blood was drawn at hourly intervals from 0800 to 1600, before and after the meals.

Data are presented as mean (SD). We used fasting plasma insulin (mU/L) and glucose (mmol/L) concentrations to calculate HOMA-\(\beta\) \((20 \times \text{insulin}/(\text{glucose} - 3.5))\) and HOMA-IR \((\text{insulin} \times \text{glucose}/22.5)\) as described by Matthews et al. (1). We calculated the total integrated day-long plasma glucose \([\text{glucose area under the curve (AUC)}]\) and insulin (insulin AUC) responses using the trapezoidal rule. Statistical calculations used SPSS Statistics 17.0. We evaluated relationships between variables of interest by calculating Pearson correlation coefficients and compared correlations using the Meng–Rosenthal–Rubin method (12) with Fisher’s \(r\)-to-\(z\) transformation and a 2-tailed significance test.

Results

The demographic and metabolic characteristics of the study population are given in Table 1. Of note, the group tended to be overweight/obese, with a mean body mass index of 30.4 kg/m\(^2\). All of the experimental variables varied by at least 2-fold, and of particular importance to this analysis was the wide range of values for insulin action (SSPG concentration and HOMA-IR varied by approximately 10-fold).

The relationship between estimates of insulin action provided by calculating HOMA-IR and those obtained by determining the SSPG concentration during the IST is illustrated in Fig. 1. These data demonstrate that although the 2 methods to quantify insulin action are significantly correlated \((r = 0.47, P < 0.001)\), the magnitude of the relationship is relatively modest.

| Table 1. Demographic and metabolic characteristics of the study population (105 men, 180 women). |
|---------------------------------|-----------|-----------|
| **Age, years**                  | Mean (SD) | Range     |
| 51 (9)                          | 23–72     |
| **Body mass index, kg/m\(^2\)** | 30.4 (3.6) | 18.8–38.2 |
| **FPG, mmol/L**                 | 5.36 (0.65) | 3.97–6.94 |
| **FPI, pmol/L**                 | 119.4 (67.6) | 20.8–388.9 |
| **HOMA-IR**                     | 2.22 (1.23) | 0.38–6.85 |
| **SSPG, mmol/L**                | 10.43 (3.94) | 2.11–20.70 |
| **HOMA-\(\beta\)**             | 141.7 (55.0) | 27.2–382.6 |
| **Glucose AUC, mmol/L**         | 47.3 (5.7) | 35.8–67.1 |
| **Insulin AUC, pmol/L**         | 2705 (1697) | 413–10 527 |
Fig. 2A and B compare results when insulin secretory function, as estimated from HOMA-β, is related to the coexisting degree of insulin resistance, as estimated by either HOMA-IR or SSPG concentration. The measurements of HOMA-IR and HOMA-β are highly correlated ($r = 0.74, P < 0.001$) in this group of healthy individuals (Fig. 2A), a result that is not surprising considering that both estimates are derived from values of FPG and FPI concentrations. In contrast, there is a much weaker relationship ($r = 0.22, P < 0.001$) between degree of insulin resistance, as measured by SSPG concentration, and insulin secretory function as estimated by HOMA-β. These 2 correlations were statistically different from each other ($P < 0.001$).

Given the discrepancy between evaluations of insulin secretory function when viewed in relationship to the 2 methods for estimating insulin action, we were interested in what would happen if the total day-long insulin response to test mixed meals (insulin AUC) were viewed in the context of the HOMA-IR and SSPG concentration. The results of this comparison (Fig. 2C and D) show that when insulin secretory function is estimated by insulin AUC in response to mixed meals, it does not seem to matter if it is related to HOMA-IR ($r = 0.67, P < 0.001$) or SSPG concentration ($r = 0.59, P < 0.001$).

Pancreatic β-cell function is often evaluated by relating it to the coexisting plasma glucose concentration, in addition to degree of insulin resistance. We followed this approach by relating the total integrated day-long plasma glucose concentration (glucose AUC) in response to mixed meals and either HOMA-β (Fig. 3A) or the day-long insulin response (Fig. 3B). These data demonstrate that apparently healthy individuals are able to maintain plasma glucose concentrations within a relatively narrow range and maintain glucose homeostasis by varying their insulin secretory response severalfold. Furthermore, the results do not differ substantially depending on how the insulin secretory response is being assessed.
Finally, we evaluated the relationship of HOMA-\(\beta\) and the insulin AUC to the coexisting FPG concentration (Fig. 3C and D). In this instance, the physiological response of the \(\beta\)-cell does seem to vary as a function of how it is estimated. Thus, Fig. 3C is consistent with the conclusion that as FPG concentration increases throughout the nondiabetic range, insulin secretory function, as estimated by HOMA-\(\beta\), declines \((r = -0.33, P < 0.001)\). In contrast, as shown in Fig. 3D, insulin AUC increases modestly as FPG concentrations increase \((r = 0.23, P < 0.001)\). These 2 correlations are statistically different from each other \((P < 0.001)\).

**Discussion**

The results of this study emphasize that differences in experimental technique confound attempts to evaluate insulin secretory function, whether or not it is related to estimates of insulin action or level of glycemia. For example, discordant findings are observed when insulin secretory function, as assessed by HOMA-\(\beta\), is considered in light of 2 different estimates of insulin action, HOMA-IR or SSPG concentration. The more insulin resistant the subject, as assessed by HOMA-IR (a mathematical estimate of insulin action), the greater the insulin secretory response as estimated by HOMA-\(\beta\) \((r = 0.74)\) (Fig. 2A). In contrast, the magnitude of the relationship between insulin action and secretion is markedly attenuated \((r = 0.22)\) when viewed in terms of SSPG concentration (Fig. 2B).

The strong relationship between HOMA-IR and HOMA-\(\beta\) suggests that the pancreatic \(\beta\)-cells of these individuals compensate effectively for increasing degrees of insulin resistance. In contrast, the relationship between SSPG concentration and HOMA-\(\beta\) indicates that, in these same patients, increases in insulin secretory function do not effectively compensate for increasing degrees of insulin resistance.

Which of these conclusions regarding insulin secretory function in our group of apparently healthy individuals is the right one?

This issue gets more confounded when we compare the relationship between a very different estimate of insulin secretory function and the 2 measures of insulin action (SSPG concentration and HOMA-IR). To avoid the emphasis on fasting values, we chose the total day-long integrated insulin response to test mixed meals as an index of insulin secretory function (insulin AUC). The results of these comparisons (Fig. 2C and D) demonstrate that reasonably comparable relationships are seen when insulin secretory function as estimated by insulin AUC is viewed in light of increasing degrees of insulin resistance as assessed by either HOMA-IR \((r = 0.67, P < 0.001)\) or SSPG concentration \((r = 0.59, P < 0.001)\). The observation—that \(\beta\)-cell function, as estimated by insulin AUC, correlates

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**Fig. 3.** Associations between measures of plasma glucose and \(\beta\)-cell function. 
(A), Glucose AUC vs HOMA-\(\beta\). (B), Glucose AUC vs insulin AUC. (C), FPG vs HOMA-\(\beta\). (D), FPG vs insulin AUC.
with the 2 disparate measures of insulin action to a comparable degree—does not necessarily mean that insulin AUC, an estimate based on postprandial insulin measurements, provides a “better” estimate of overall insulin secretory function than HOMA-β, an estimate based on a mathematical formula involving fasting glucose and insulin concentrations. Rather, it emphasizes how conclusions concerning insulin secretory function vary with the methods used to assess it.

Insulin secretory function is also often evaluated in relationship to the plasma glucose concentration (13), and we have taken that approach to relate HOMA-β and insulin AUC to 2 measures of glyceemia. The results again emphasize that conclusions regarding β-cell function vary dramatically with how the relevant variables are defined. If total day-long integrated glucose response to mixed meals (glucose AUC) is used as the estimate of glyceemia, the apparently healthy population we studied were able to increase their insulin secretory response to mixed meals by approximately 6-fold (Fig. 3A and B), irrespective of how it was measured, thereby maintaining the glucose AUC within a narrow range (approximately 1.5-fold). When HOMA-β and insulin AUC are examined in relationship to FPG concentration, however, insulin secretory function as assessed by HOMA-β declines (Fig. 3C) as FPG concentrations increase ($r = -0.33, P < 0.001$), whereas values of insulin AUC increase (Fig. 3D) as FPG increases ($r = 0.23, P < 0.001$). These striking differences are particularly relevant in the context of the recent article by Tabak et al. (3), in which these authors interpreted measurements of HOMA-β to conclude that the development of significant fasting hyperglycemia is preceded by a period of declining insulin secretory function, the relationship seen in Fig. 3C. In contrast, if insulin AUC is used as a measure of the compensatory response to increasing levels of glyceemia, the data in Fig. 3D lead to an entirely different conclusion.

The goal of our study was not to decide how best to evaluate insulin secretory function in apparently healthy subjects. We wished to emphasize how important it is to address this issue in the future by demonstrating that physiological insights into insulin secretory function in these individuals will vary depending on (1) the method to assess insulin secretory function and (2) which measure of insulin action or glyceemia is used as the independent variable. This dilemma is not unique to the methods we used to assess insulin action and secretion and glyceemia, but will extend to other experimental approaches to evaluating insulin secretory function. One example of the many approaches that have been used for this purpose is to take the acute insulin response to intravenous glucose as the measure of insulin secretion and relate that value to insulin sensitivity as estimated by the frequently sampled intravenous glucose tolerance test (14). Another approach has been to take the graded glucose infusion method pioneered by Polkonsky and colleagues (15) to quantify insulin secretion rate and relate it to quantification of insulin action with the IST (16). Thus, stating that the estimate of insulin secretion used in a study has been viewed in relationship to a relevant physiological variable, e.g., insulin resistance and/or degree of glyceemia, does not ensure that a meaningful measure of pancreatic β-cell function has been obtained. In this context, it seems best to conclude by suggesting that it is incumbent on both (1) the investigator publishing studies on insulin secretory function to clearly state the potential limitations of the measurements used to quantify the independent and dependent variables and (2) the reader to evaluate the quality of the methods used and be aware of the impact that these choices can have on interpretation of the data.

**References**


