The relationship between fasting hyperglycemia and insulin secretion in subjects with normal or impaired glucose tolerance

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Impaired insulin secretion plays a pivotal role in the progression from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) and to overt Type 2 diabetes mellitus (T2DM) (1–5). The impairment in β-cell function begins long before the onset of overt T2DM, and it is evident in the prediabetic state, e.g., IGT and impaired fasting glucose (IFG; Refs. 2–5). Previous studies (6–15) demonstrated that IGT is associated with a decrease in β-cell function. Importantly, the decline in β-cell function becomes evident with 2-h plasma glucose concentrations that are well within the range that is considered to represent NGT, i.e., 2-h plasma glucose (PG) = 100–139 mg/dl (13).

The mechanisms that regulate the plasma glucose concentration during the postabsorptive state, e.g., fasting plasma glucose (FPG), are very different from those that regulate the plasma glucose concentration after a meal (2). Thus the FPG is primarily determined by the rate of hepatic glucose production (16), while 2-h PG after a meal is determined by the rate of glucose-stimulated insulin secretion and skeletal muscle insulin sensitivity. Although hepatic insulin resistance (resulting in increases in both FPG and 2-h PG) is usually accompanied by insulin resistance in skeletal muscle and impaired insulin secretion, discordance between these measurements of glycemic control is often encountered (17). Thus the relationship between glucose-stimulated insulin secretion vs. the FPG and vs. 2-h PG may well differ. Thus while many previous studies (6–15) have examined the relationship between the 2-h PG and glucose-stimulated insulin secretion, few studies (18) have evaluated the relationship between FPG and glucose-stimulated insulin secretion.

Ferrannini et al. (14) demonstrated that an increase in FPG within the nondiabetic range (<126 mg/dl) is associated with an increase in basal insulin secretory rate (ISR), determined by deconvolution of plasma C-peptide concentration. However, when the ISR was related to FPG, the ratio of ISR to FPG was unchanged with increases in FPG between 70–125 mg/dl. These results suggest that the basal insulin secretion rate is not affected by the increase in FPG. In contrast, glucose-stimulated insulin secretion, measured with the acute insulin response during an intravenous glucose tolerance test, has been shown to decrease precipitously with increases in FPG in the nondiabetic range (18). Because there is no identifiable acute insulin response during glucose ingestion, the effect of an increase in FPG on β-cell function during regular daily life is unclear. The aim of this study was to assess the relationship between an increase in FPG concentration within the entire nondiabetic range (70–125 mg/dl) and glucose-stimulated insulin secretion during the more physiological oral route of glucose administration in subjects with normal and IGT. Because changes in insulin clearance, e.g., in diabetes, obesity, and other insulin resistance conditions (19–20), are known to affect the plasma insulin concentration independent of insulin secretion, we quantitated the ISR (deconvolution of plasma C-peptide concentration) during the OGTT to examine the effect of increasing FPG levels on insulin secretion in subjects with normal and IGT. We also studied two groups: Mexican Americans, who are characterized by severe insulin resistance (21), and Japanese, who are more characterized by β-cell failure (21), to determine whether the relationship between nondiabetic
plasma glucose elevations and insulin secretion vary amongst individuals with varying ethnic backgrounds.

**METHODS**

**Subjects.** The participants included 237 subjects of Mexican American descent studied on the General Clinical Research Center of the University of Texas Health Science Center (San Antonio, TX) and 303 Japanese subjects studied at the Diabetes and Endocrine Division, Kawasaki Medical School (Okayama-Ken, Japan). Based on the OGTT, the 532 subjects were classified as having NGT (2-h glucose <7.8 mmol/l, n = 293) or IGT (2-h glucose between 7.8 and 11.1 mmol/l, n = 238).

All subjects were recruited through advertisements in the medical center and local newspaper. All subjects had normal liver, cardiopulmonary, and kidney function as determined by medical history, physical examination, screening blood tests, electrocardiogram, and urinalysis. No subject was taking any medication known to affect glucose tolerance. Body weight was stable (±2 kg) for at least 3 mo before study in all subjects. The study protocol was approved by the Institutional Review Boards of the University of Texas Health Science Center at San Antonio and Kawasaki Medical School, and informed written consent was obtained from all subjects before their participation.

**OGTT.** A 75-g OGTT was performed at 8:00 AM after a 10–12-h overnight fasting. Before the OGTT, a small polyethylene catheter was placed into an antecubital vein and blood samples were collected at −30, −15, 0, 30, 60, 90, and 120 min for measurement of plasma glucose, insulin, and C-peptide concentrations. On the day of OGTT, height and weight were measured.

**Calculations.** ISR during the OGTT was calculated from deconvolution of the plasma C-peptide concentration using ISEC software package developed by Hoverka et al. (22). ISR was related to the glucose stimulus by dividing the incremental area under the ISR curve by the incremental area under the plasma glucose curve. The insulin secretion/insulin resistance (disposition) index was determined by dividing ISR/G by the severity of insulin resistance [(ISR(AUC)/G(AUC)) − IR], as measured by the inverse of the Matsuda index (23). The Matsuda index incorporates both hepatic and muscle components of insulin resistance, correlates well with euglycemic insulin clamp, and was calculated as follows:

\[
\text{Matsuda index} = \frac{10,000}{\sqrt{\text{FPG} \times \text{FPI} \times (\text{mean PG} \times \text{mean PI})}} \tag{1}
\]

The incremental area under the ISR curve [ISR(AUC)] and the incremental area under the plasma glucose concentration curve [G(AUC)] were calculated according the trapezoid rule.

The insulin secretion/insulin resistance index was determined during the entire OGTT (0–120 min), during the initial 30 min of the OGTT (0–30 min; i.e., the early-phase insulin secretion), and during the second hour (60–120 min) of the OGTT (i.e., the late-phase insulin secretion).

**Analytical techniques.** Plasma glucose concentration was measured by the glucose oxidase reaction (Glucose Oxidase Analyzer, Beckman, Fullerton, CA). Plasma insulin and C-peptide concentrations were measured by radioimmunoassay (Linco Research, St. Louis, MO) in both Mexican American and Japanese subjects.

**Statistical analysis.** To determine the best fit for the relationship between insulin secretion/insulin resistance index and FPG concentration, hyperbolic and exponential models were tested. The insulin secretion/insulin resistance index was considered the dependent variable, and FPG concentration was the independent variable. The fitting procedure was performed on the raw data with Sigmaplot software package. The sum of square residuals was used to compare the two models. The sum of square residuals for exponential fit was significantly lower compared with hyperbolic relationship. Single exponential fit was also superior to double exponential fit. Therefore, a single exponential model, described by the following equation was used to quantitate the relationship between insulin secretion (measured with the insulin secretion/insulin resistance index) and FPG concentration:

\[
\text{IS/IR} = a \times \exp(-b \times \text{FPG}) \tag{2}
\]

where a and b are constants. The index 1/b was used to compare the relationship between insulin secretion and FPG concentration between the different glucose tolerance groups. A change in FPG equal to 1/b is associated with a 63% (1/e) change in IS/IR.

Data are means ± SD. For comparison between two groups, Student’s t-test was used. To compare the mean of more than two groups, ANOVA was used. Significant differences were confirmed by the Bonferroni test. Statistical significance was considered at P < 0.05.

**RESULTS**

There were 293 subjects with NGT and 238 with IGT studied. Table 1 presents the anthropometric and metabolic characteristics of the study population. Subjects with IGT were slightly older and had a higher body mass index. FPG, insulin, and C-peptide concentrations were significantly higher in IGT vs. NGT subjects. The basal ISR was slightly higher in IGT vs. NGT subjects. However, when basal ISR was related to FPG, both groups had a similar basal ISR. Insulin sensitivity, mea-
sured with Matsuda index, as expected, was significantly reduced in subjects with IGT compared with NGT.

The area under insulin secretion curve during the OGTT was similar in the two groups. However, because subjects with IGT had higher plasma glucose levels during the OGTT and lower insulin sensitivity, the insulin secretion/insulin resistance index \( \frac{\Delta ISR(AUC)_{0-120}/G(AUC)_{0-120}}{\Delta G(AUC)_{0-120}} \) was markedly reduced in subjects with IGT compared with NGT (Table 1).

The insulin secretion/insulin resistance index correlated inversely with the FPG concentration. As the FPG concentration increased, the insulin secretion/insulin resistance index decreased precipitously (Fig. 1). The relationship between the insulin secretion/insulin resistance index and FPG concentration was highly nonlinear but with log-log transformation it became linear. The relationship between \( \Delta ISR(AUC)_{0-120}/G(AUC)_{0-120} \) (where IR = inverse of Matsuda index) and FPG was best fit with an exponential decay. Hyperbolic decay had a lower fit, and the difference between the sum of square residuals for the two fits was statistically significant \( (P = 0.05) \). The correlation coefficient of the exponential decay \( (r = 0.35) \) was significantly greater than that of the hyperbolic decay \( (r = 0.22; P = 0.02) \). Therefore, we used the exponential decay to quantify the relationship between the insulin secretion/insulin resistance index and FPG concentration. Table 2 presents the fitting parameter for the insulin secretion/insulin resistance index and FPG concentration. However, it should be noted that quantitatively similar results are obtained whether one uses the exponential or hyperbolic curve fit.

From the fitting parameters, we calculated that a 24 mg/dl increase in FPG concentration is associated with 63% decline in \( \frac{\Delta ISR(AUC)_{0-120}/G(AUC)_{0-120}}{\Delta G(AUC)_{0-120}} \). We (24) have previously shown that IFG differentially affects early and late-phase insulin secretion during the OGTT. Therefore, we examined the relationship between the rise in FPG concentration and the early and late phases of insulin secretion during OGTT. The insulin secretion/insulin resistance index during the first 30 min of the OGTT (0–30 min) was highly dependent on the FPG concentration and declined markedly as FPG increased (Fig. 2). Every 25 mg/dl rise in FPG concentration was associated with a 63% decline in \( \frac{\Delta ISR(AUC)_{0-30}/G(AUC)_{0-30}}{\Delta G(AUC)_{0-30}} \) (where IR) was more sensitive to the rise in FPG concentration during the second hour of the OGTT (60–120 min) was much less affected by the increase in FPG concentration (Fig. 3; Table 2); a 53 mg/dl rise in FPG was associated with a 63% decrease in \( \frac{\Delta ISR(AUC)_{60-120}/G(AUC)_{60-120}}{\Delta G(AUC)_{60-120}} \).

When subjects were divided according to glucose tolerance status, insulin secretion \( \frac{\Delta ISR(AUC)_{0-120}/G(AUC)_{0-120}}{\Delta G(AUC)_{0-120}} \) in subjects with NGT decreased more steeply in response to the rise in FPG concentration than in subjects with IGT (Table 2). The rate of decay of insulin secretion/insulin resistance index with the rise in FPG in subjects with NGT was double that of subjects with IGT (Table 2 and Fig. 2). At all FPG concentrations, NGT subjects had a higher rate of insulin secretion \( \frac{\Delta ISR(AUC)_{0-120}/G(AUC)_{0-120}}{\Delta G(AUC)_{0-120}} + \) than IGT subjects (Fig. 1). Early-phase insulin secretion \( \frac{\Delta ISR(AUC)_{0-30}/G(AUC)_{0-30}}{\Delta G(AUC)_{0-30}} \) was more sensitive to the rise in FPG concentration than the late-phase insulin secretion (Figs. 2B and 3B). No significant difference in the relationship between insulin secretion and FPG concentration was observed in Mexican Americans compared with Japanese subjects. The slope of the log transformation of the insulin secretion/insulin resistance index for early-phase insulin secretion (0–30 min) vs. FPG was \(-0.0143\) in Japanese subjects and \(-0.0141\) in Mexican Americans \( (P = NS) \), and the slope for the relation between the insulin secretion/insulin resistance index (0–120 min) vs. FPG was \(-0.0137\) for Japanese and \(-0.0147\) for Mexican Americans \( (P = NS) \).

**DISCUSSION**

Previous studies from our laboratory and others have reported a decrease in glucose stimulated insulin secretion with the deterioration in glucose tolerance. Most of theses studies have related the decrease in β-cell function to the increase in 2-h plasma glucose. IFG was introduced by the American Diabetes Association in 1997 as an intermediate stage between NGT and Type 2 diabetes and it was meant to be analogous to IGT (25). Today, it is defined as FPG concentration = 100–
125 mg/dl (26). However, later studies have demonstrated that IFG and IGT are distinct states of glucose intolerance with distinct metabolic abnormalities. In this study, we demonstrate that in subjects with NGT (2-h PG < 140 mg/dl) the increase in FPG concentration is associated with a precipitous decrease in β-cell function. Prospective epidemiological studies (25–31) demonstrated that subjects with isolated IFG have an increased risk for progression to Type 2 diabetes despite having 2-h PG < 140 mg/dl. This observation indicates that a rise in FPG is an independent risk factor for progression to diabetes. The decrease in β-cell function associated with the increase in FPG reported in this study could explain the increased risk of subjects with isolated IFG for Type 2 diabetes. We previously have shown that the insulin secretion/insulin resistance index is the best predictor of future development of Type 2 diabetes in subjects with NGT compared with other predictive models, e.g., FPG and 2-h PG (32). Furthermore, addition of the insulin secretion/insulin resistance index to other predictive models improves their ability to predict future risk of diabetes (32). These observations emphasize the importance of the decline in insulin secretion/insulin resistance index for the development of future Type 2 diabetes.

Many previous studies (6, 8, 18) have demonstrated that subjects with IFG have a decline in acute insulin response during an intravenous glucose tolerance test. Our results extend these observations and provide strong evidence that, although a first-phase insulin response cannot be recognized during oral glucose ingestion, the rise in FPG concentration is associated with a precipitous decrease in the early (0 –30 min), as well as late (60 –120 min) phases of insulin secretion. Reduced early-phase insulin secretion (0 –30 min) during the OGTT is strongly associated with increased FPG levels within the nondiabetic range in both NGT and IGT individuals. The increase in FPG concentration in the non-diabetic range is associated with a precipitous decrease in β-cell function.
diabetic range (70–125 mg/dl) was associated with a marked exponential decrease in the insulin secretion/insulin resistance index. The rate of decrease in insulin secretion was $\sim 2.5\%$ for every 1 mg/dl increase in FPG concentration, and this rate of decrease was constant over the entire range of the nondiabetic FPG concentrations (70–125 mg/dl), beginning with FPG concentrations considered to be well within the normal range (70–99) and continuing through the range of impaired fasting glucose (100–125 mg/dl). Thus subjects with FPG between 95–100 mg/dl, which is considered to be normal, already have lost $\sim 60\%$ of their $\beta$-cell function compared with subjects with a FPG concentration of $\sim 70$ mg/dl.

The decline in early-phase (0–30 min) insulin secretion with increasing FPG levels was more pronounced than the decline in the late-phase (60–120 min) insulin secretion. Since insulin secretion during the first 30 min during the OGTT strongly correlates with first-phase insulin secretion, this observation indicates that first-phase insulin secretion is probably more sensitive to the rise in FPG compared with second-phase insulin secretion. This observation is consistent with previous studies that reported complete elimination of first-phase insulin secretion with acute small increase in plasma glucose concentration in lean healthy individuals.

A second important finding of the present study relates to the observation that, for any given increment in FPG concentration, the decline in the insulin secretion/insulin resistance index is more profound in NGT than IGT subjects. Thus the rate of decline in the insulin secretion/insulin resistance index in subjects with NGT was more than twice that in subjects with IGT (Table 2). Since the decrease in insulin secretion associated with the increase in FPG is primarily due to decrease in early-phase insulin secretion (0–30 min) and first-phase insulin secretion has been shown to be markedly decreased in subjects with IGT, the slower decrease in insulin secretion in subjects with IGT could simply be explained by the observation that NGT subjects start with a higher insulin secretion/insulin resistance index.

Our observations are consistent with those of Godsland et al. (18), who also observed a decline in acute insulin response to intravenous glucose administration with increasing FPG concentrations within the normal range. In the study by Godsland et al, the 2-h PG status of the subjects was not known. Furthermore, the decline in acute insulin response was evident only when the FPG concentration increased $>95$ mg/dl. However, in that study (18) the acute insulin response was not related either to the incremental glucose response or to the prevailing level of insulin resistance.

An interesting question relates to the etiological significance of $\beta$-cell dysfunction that is associated with increasing fasting hyperglycemia: is the $\beta$-cell function primary or is it acquired? The results of this study do not favor one scenario over the other. Thus insulin resistance could lead to an increase in FPG that secondarily causes a defect in $\beta$-cell function. Alternatively, a primary decrease in $\beta$-cell function (either due to a functional defect to a loss in $\beta$-cell mass) could result in an increase in FPG, and the resultant increase in FPG could feed back to further impair $\beta$-cell function. Studies in experimental animals (33) have demonstrated that the FPG begins to rise only when $>80\%$ of $\beta$-cell mass is lost. Based upon recent studies in humans (34) that demonstrated that individuals with IFG have no more than a $50\%$ reduction in $\beta$-cell volume, it seems unlikely that decreased $\beta$-cell mass alone, in the absence of a concomitant reduction in $\beta$-cell function, could explain the rise in FPG observed in the present study in individuals with IFG (FPG = 100–125 mg/dl).

On the other hand, in vivo studies (35–37) in humans and animal studies using cell culture systems have shown that a small persistent rise in plasma glucose concentration has a deleterious effect on $\beta$-cell function, i.e., “glucotoxicity.” In partially pancreatectomized NGT rats, a 16 mg/dl increment in the mean day-long plasma glucose concentration was shown to markedly impair first-phase insulin secretion (36). A similar deleterious effect of elevated FPG on $\beta$-cell function in humans could explain the reduction in early-phase insulin secretion that is associated with increasing FPG concentrations within the nondiabetic range. Studies that have assessed the effect of elevations in FPG, with glucose infusion, on $\beta$-cell function have yield conflicting results. A small increase in plasma glucose concentration for 50 min has been shown to markedly inhibit first-phase insulin secretion measured with the hyperglycemic clamp (38). More prolonged elevation of FPG for 68 h decreased $\beta$-cell function by $36\%$ (39). Other studies (40) have reported increased $\beta$-cell function after 42 h of glucose infusion in healthy subjects. Methodological differences in the various protocols and differences in the study populations may, in part, explain these conflicting results. For example, in the latter study (40), plasma glucose concentration was allowed to return back to the preinfusion level for $\sim 2$ h before the repeat hyperglycemic change. Thus some of the glucotoxic effects of hyperglycemia may have been washed out. It is possible that the glucotoxic effect of chronic hyperglycemia only will manifest itself in genetically predisposed individuals (41). Other evidence in support of a glucotoxic effect of chronic hyperglycemia on $\beta$-cell function includes the improvement in insulin secretion in Type 2 diabetic subjects after correction of hyperglycemia with insulin (42), while acute hyperglycemia results in the loss of the normal oscillatory $\beta$-cell response to glucose in NGT subjects after short-term exposure to hyperglycemia (43).

In animal studies, correction of chronic hyperglycemia with phlorizin in partially pancreatectomized diabetic rats has been shown to restore the first and second phases of insulin secretion, indicating that in this animal model the reduction in insulin secretion results from chronic hyperglycemia and is reversible upon restoration of normoglycemia (35). A similar study in humans will be required to definitively establish the pathogenic role of chronic hyperglycemia in the development of impaired insulin secretion. Thus whether the decrease in $\beta$-cell function associated with the increase in fasting hyperglycemia is primary or secondary or both remains to be seen.

In summary, the present results demonstrate that in two diverse ethnic groups both the early (0–30 min) and late (60–120 min) phases of insulin secretion during the OGTT decrease markedly with increasing FPG concentrations within the nondiabetic range ($<26$ mg/dl). The decline in insulin secretion/insulin resistance index is steeper in individuals with NGT compared with those with IGT, and it is more pronounced during the first 30 min of the OGTT.

REFERENCES


