Insulin Augmentation of Glucose-Stimulated Insulin Secretion Is Impaired in Insulin-Resistant Humans

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Type 2 diabetes (T2D) is characterized by insulin resistance and pancreatic β-cell dysfunction, the latter possibly caused by a defect in insulin signaling in β-cells. We hypothesized that insulin’s effect to potentiate glucose-stimulated insulin secretion (GSIS) would be diminished in insulin-resistant persons. To evaluate the effect of insulin to modulate GSIS in insulin-resistant compared with insulin-sensitive subjects, 10 participants with impaired glucose tolerance (IGT), 11 with T2D, and 8 healthy control subjects were studied on two occasions. The insulin secretory response was assessed by the administration of dextrose for 80 min following a 4-h clamp with either saline infusion (sham) or an isoglycemic-hyperinsulinemic clamp using B28-Asp-insulin (which can be distinguished immunologically from endogenous insulin) that raised insulin concentrations to high physiologic concentrations. Pre-exposure to insulin augmented GSIS in healthy persons. This effect was attenuated in insulin-resistant cohorts, both those with IGT and those with T2D. Insulin potentiates glucose-stimulated insulin secretion in insulin-resistant subjects to a lesser degree than in normal subjects. This is consistent with an effect of insulin to regulate β-cell function in humans in vivo with therapeutic implications. Diabetes 61:301–309, 2012

RESEARCH DESIGN AND METHODS

The Committee of Human Studies of the Joslin Diabetes Center approved the study. Written informed consent was obtained from all participants. Subjects included 10 subjects with impaired glucose tolerance (IGT) and 11 subjects with T2D as determined by a 75-g oral glucose tolerance test according to American Diabetes Association criteria (22). The T2D subjects were only included if they were newly diagnosed based on an oral glucose tolerance test, were on lifestyle therapy only, or were on one oral hypoglycemic medication that could safely be washed out for a period of 1 month. Blood pressure– and lipid-lowering therapies were permitted for insulin-resistant cohorts with stable dosing over 2 months. A third group of subjects included eight healthy persons with no first-degree relative with diabetes and on no prescription medications other than oral contraceptives. These healthy subjects have been previously reported (20).

Study design. Each participant underwent two study visits during which either a 4-h saline infusion (sham clamp) or an isoglycemic-hyperinsulinemic clamp was performed, both of which were immediately followed by graded glucose infusion for 80 min (Fig. L4) (20). Subjects were instructed to refrain from vigorous exercise and consume >250 g of carbohydrate per day for 3 days prior to each visit and were studied after a 10- to 12-h overnight fast. An intravenous catheter was inserted into each arm, one for infusions and the other for blood sampling. The arm used for phlebotomy was placed into a box heated to 60°C to ensure arterialization of venous blood (23,24). Subjects were blinded as to whether the sham or insulin clamp was being performed. Potassium chloride (KCl) was administered at 10 mEq/h to prevent hypokalemia during both clamps.

During the first visit (sham clamp), normal saline was infused for 4 h and was designed as a time and volume control for the isoglycemic-hyperinsulinemic clamp performed at the second visit. Saline was infused at the volume rate hypothetically required to maintain euglycemia during a hyperinsulinemic clamp during which insulin (14 pmol/L per kg/min [2.0 mU/kg/min]) would be administered (20,25) in a person with similar insulin resistance. For insulin resistant and healthy control subjects, this corresponded to the volume calculated for glucose utilization of 5 and 10 mg/kg/min, respectively. Saline infusion for 240 min was followed by intravenous infusion of 20% dextrose at the rates of 8 and 10 mg/kg/min for 40 min each (graded glucose infusion) to

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See accompanying commentary, p. 267.
For conversion to Scientific International units: insulin (μU/mL) × 6.945 pmol/L.

induce GSIS. As no insulin was administered during this first (sham) study, GSIS was evaluated following pre-exposure to low, endogenous insulin concentrations.

During the second visit approximately 4 weeks later, subjects underwent an isoglycemic-hyperinsulinemic clamp for 4 h (20). Insulin was administered as a two-stepped primed (56 pmol/kg/min followed by 28 pmol/kg/min, each for 5 min), continuous infusion (14 pmol/L/kg per min [2.0 mU/kg/min]). This was anticipated to increase insulin concentrations to about 1,400 pmol/L (200 mU/mL). This was infused at variable rates to match glycemia during the graded glucose infusion portion of the clamp as a time and volume control. Potassium chloride (KCl) was administered during all clamps. At the start of the 4th h of the clamp, additional glucose was administered to investigate glucose-stimulated insulin secretion. During the sham clamp, the rate was 8 and 10 mg/kg/min for 40 min each step; on the hyperinsulinic day, dextrose administration rates were variable to match the glycemic challenge achieved during the sham clamp conditions. B: Correlation between the two insulin assays, RIA and ELISA, are demonstrated using samples obtained during the sham clamp study. For conversion to Scientific International units: insulin (μU/mL) × 6.945 pmol/L.

RESULTS

The clinical and metabolic characteristics of participants are shown in Table 1. As anticipated, the T2D cohort was modestly older (P = 0.0003), had higher fasting glucose (P < 0.0001), hemoglobin A1c (P = 0.002), and systolic blood pressure (P = 0.04), but unexpectedly tended to have lower fasting insulin concentrations and lower BMI than IGT and were more insulin sensitive (glucose utilization over the last 2 h of clamp T2D vs. IGT: 7.1 ± 1.3 vs. 5.1 ± 2.7 mg/kg/min, P < 0.04). Control subjects were younger, leaner, normoglycemic, and insulin sensitive (glucose utilization 10.3 ± 6.0 mg/kg/min P < 0.001, compared with both T2D and IGT) without dyslipidemia.

Effects of pre-exposure to exogenous insulin on GSIS GSIS. GSIS was measured during the 80 min of graded glucose infusion (240–320 min) using a DAKO ELISA assay that recognizes endogenous insulin but does not recognize exogenous B28-Asp-insulin (20). In the absence of endogenous insulin, RIA and ELISA insulin measurements were strongly correlated (R² = 0.94, P < 0.0001) (Fig. 1B). Healthy (insulin
sensitive) control subjects demonstrated increased GSIS as measured by the difference in rate of increase in endogenous insulin after pre-exposure to high compared with low insulin (saline/sham) clamp ($P = 0.04$) (Fig. 2A). The endogenous insulin area under the curve corrected for baseline increased about 40% ($P = 0.07$). The effect of insulin to potentiate GSIS was diminished in both insulin-resistant cohorts (IGT and T2D) (Fig. 2B and C). Whereas GSIS was increased in T2D following pre-exposure to high insulin ($P = 0.01$), the magnitude of 22% was less than in control subjects. More strikingly, GSIS was not increased at all after exogenous insulin exposure in the IGT cohort ($P = 0.14$), which manifested the greatest magnitude of insulin resistance. Likewise there was no change in endogenous insulin area under the curve in IGT.

Similarly, although glucose concentrations achieved during the graded glucose infusion were higher in the IGT and T2D cohorts compared with control subjects, the endogenous insulin concentrations achieved tended to be higher for glycemia following high compared with low insulin pre-exposure in control subjects; this was assessed by comparison of the slope of change in insulin to change in glucose during the graded glucose infusion.

### TABLE 1
Clinical and metabolic characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Healthy control subjects</th>
<th>IGT</th>
<th>T2D</th>
<th>$P$ value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24 ± 2</td>
<td>40 ± 4***</td>
<td>55 ± 2***</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>3M/5F</td>
<td>3M/7F</td>
<td>5M/6F</td>
<td>NS†</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5 ± 0.7</td>
<td>33.4 ± 4.4*</td>
<td>27.4 ± 1.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113 ± 3</td>
<td>115 ± 6</td>
<td>130 ± 4*</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75 ± 3</td>
<td>75 ± 3</td>
<td>81 ± 3</td>
<td>0.3</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.1 ± 0.1</td>
<td>5.5 ± 0.2</td>
<td>6.5 ± 0.2**</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose fasting (mmol/L)</td>
<td>4.44 ± 0.22</td>
<td>5.05 ± 0.17</td>
<td>6.55 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin fasting (pmol/L)</td>
<td>35.5 ± 4.9</td>
<td>91.7 ± 17.4**</td>
<td>60.4 ± 12.5</td>
<td>0.02</td>
</tr>
<tr>
<td>C-peptide (nmol/L)</td>
<td>0.57 ± 0.03</td>
<td>1.53 ± 0.27**</td>
<td>1.23 ± 0.20*</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.89 ± 0.21</td>
<td>4.30 ± 0.28</td>
<td>4.40 ± 0.26</td>
<td>0.4</td>
</tr>
<tr>
<td>Triglycerides (nmol/L)</td>
<td>0.55 ± 0.07</td>
<td>1.39 ± 0.40*</td>
<td>1.13 ± 0.19</td>
<td>0.2</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.30 ± 0.10</td>
<td>1.01 ± 0.05*</td>
<td>1.22 ± 0.16</td>
<td>0.2</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>2.33 ± 0.16</td>
<td>2.77 ± 0.23</td>
<td>2.64 ± 0.26</td>
<td>0.5</td>
</tr>
<tr>
<td>$M_{120-240}$ min (mg/kg/min)</td>
<td>10.3 ± 0.7</td>
<td>5.1 ± 0.9**</td>
<td>7.1 ± 0.4**</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are mean ± SE. Demographic information is provided for the three groups studied including IGT, T2D, and healthy control subjects. Conversions of Scientific International to conventional units: glucose (mmol/L) ÷ 0.0555 for mg/dL; insulin (pmol/L) ÷ 6.945 for μU/mL; C-peptide (nmol/L) ÷ 0.333 for ng/mL; cholesterol, HDL, and LDL (mmol/L) ÷ 0.0259 for mg/dL; triglycerides (mmol/L) ÷ 0.0113 for mg/dL. *$P$, 0.05 vs. controls; **$P$, 0.009 vs. controls; #P, 0.05 vs. IGT; ##P, 0.009 vs. IGT; †Chi square.

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**Fig. 2.** Pre-exposure to isoglycemic hyperinsulinemia potentiates glucose-induced insulin secretion in the insulin-sensitive persons (A), but the effect is attenuated in insulin-resistant cohorts with IGT (B) or T2D (C). *$P$, <0.05. Black circles, isoglycemic-hyperinsulinemic clamp; white circles, sham clamp.
in glucose (0.29 ± 0.06 vs. 0.41 ± 0.08 arbitrary units of change in insulin over change in glucose, sham vs. insulin, $P = 0.099$) (Fig. 3). The association was linear in all participants except one control subject in whom the best fit was logarithmic. We did not deem exclusion of this individual justifiable on clinical grounds for primary analysis, but analysis of the insulin-to-glucose relationship without this individual was statistically significant ($P = 0.02$). The change in insulin-for-glycemia effect was lower in magnitude in T2D than the control subjects but statistically significant (0.09 ± 0.03 vs. 0.13 ± 0.04, sham vs. insulin, $P = 0.04$) and absent in the IGT group (Fig. 3).

**C-peptide.** Fasting C-peptide concentrations were higher in IGT and T2D compared with control subjects (Table 1). Previous studies demonstrate exogenous insulin reduces C-peptide concentrations (17), an effect that may be attributed in part to increased C-peptide clearance (20). We also demonstrated C-peptide concentrations to be reduced from baseline during the 240 min of the isoglycemic-hyperinsulinemic clamp compared with the sham clamp in all groups (Fig. 4A–C). In response to glucose stimulation, C-peptide concentrations increased. To adjust for the lower C-peptide concentrations at steady state following high compared with low (saline/sham) clamp conditions, we calculated the fold increase in C-peptide during the glucose stimulation. Similar to estimates of $\beta$-cell function from measures of endogenous insulin, the fold increase in C-peptide during glucose stimulation was increased most following pre-exposure to exogenous insulin in the insulin-sensitive cohort, intermediate in T2D (intermediate insulin resistant) cohort but not significant in the IGT cohort with the greatest magnitude of insulin resistance, which is consistent with an attenuation of the effect of insulin on GSIS in insulin-resistant states (Fig. 4D–F).

**Glycemia.** As expected, fasting glucose concentrations and glycemia achieved during the sham and hyperinsulinemic conditions were higher in the IGT and T2D cohorts than in control subjects (Fig. 5A–C). Importantly, however, during both the 240 min prior to and during the 80 min of the graded glucose infusion, the glucose concentrations achieved were similar during the sham and insulin clamps within each group for all three cohorts (Fig. 5A–C).

**Total insulin.** Fasting insulin concentrations (DAKO ELISA) were higher in the IGT and T2D cohorts compared with control subjects (Table 1) but similar within each group on the 2 study days ($P > 0.7$ for all comparisons). Fasting insulins and insulin concentrations throughout saline infusion, sham clamp without B28-Asp-insulin, did not differ by ELISA or RIA, and agreement between the two assays was very high ($R^2=0.94, P < 0.0001$) (Fig. 1B). We have previously demonstrated that the DAKO insulin ELISA does not detect B28-Asp-insulin (20), thus on the isoglycemic-hyperinsulinemic clamp study, DAKO insulin represents only endogenous insulin. In each cohort, endogenous insulin concentrations were similar at time 240 min.

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**Fig. 3.** Higher glucose concentrations were achieved in the insulin-resistant cohorts (IGT and T2D) compared with control subjects. $\beta$-cell glucose sensitivity tended to be higher following high compared with low insulin pre-exposure in control subjects. The effect of insulin pre-exposure on change in insulin for glycemia was lower in magnitude in T2D than in control subjects, but statically significant and absent in the IGT group. For conversion to Scientific International units: glucose (mg/dL) × 0.0555 for mmol/L; insulin (mU/mL) × 6.945 pmol/L. CON, control subjects. *$P = 0.04$ vs. control.
prior to starting the glucose stimulation for both sham and hyperinsulinemic clamps studies.

By design, total insulin levels (endogenous plus exogenous) by RIA (60–240 min) remained low on the day of the sham clamp (IGT: 76.4 ± 2.1 pmol/L [11.0 ± 0.3 μU/ml]; T2D: 48.6 ± 3.5 pmol/L [7.0 ± 0.5 μU/ml]; control subjects: 25.0 ± 1.4 pmol/L [3.6 ± 0.2 μU/ml]), but were markedly increased during the hyperinsulinemic clamp with steady-state concentrations in IGT, T2D, and control of 2,275 ± 33; 1,528 ± 23; and 1,095 ± 16 pmol/L [327.7 ± 4.8, 220.5 ± 3.3, and 157.6 ± 2.3 μU/ml], providing a high physiologic level of insulin pre-exposure before the glucose infusion (hyperinsulinemic clamps vs. sham clamps, P < 0.0001 in all cohorts) (Fig. 5D–F). Higher total insulin levels in the IGT cohort compared with the T2D cohort likely resulted because insulin dosing was weight-based (14 pmol/kg/min [2.0 mU/kg/min]), and the IGT cohort was heaviest (Table 1).

Other hormones and metabolites. Despite closely matched glycemia between the two experimental conditions, hyperinsulinemia can induce changes in other hormones and metabolites, which could also participate in the β-cell secretory response. Glucagon concentrations were lower during the first 240 min of hyperinsulinemic clamps compared with sham clamps (P < 0.01) in all cohorts, consistent with an effect of insulin on the α-cell (28), and glucagon concentrations were further lowered following endogenous insulin secretion during the GSIS phase in IGT (Fig. 6A). Plasma FFAs were lower throughout the hyperinsulinemic clamps compared with sham clamps (Fig. 6B). While cortisol was higher in the control subjects at euglycemic hyperinsulinemia (time 240 min), and lower in the two insulin resistant cohorts following hyperglycemic hyperinsulinemia (time 320 min), in general the effect of diurnal variation was more pronounced than the differences between clamp conditions (Fig. 6C).

Glucose utilization during GSIS. As expected, glucose requirements to maintain similar glycemia during the graded glucose infusion phase of the clamp were markedly higher following pre-exposure to insulin. To maintain equivalent plasma glucose levels after insulin compared with saline (sham) pre-exposure, an additional 83 ± 6 mmol · kg⁻¹ · min⁻¹ (15 ± 1 mg · kg⁻¹ · min⁻¹) were infused in control subjects, 89 ± 50 mmol · kg⁻¹ · min⁻¹ (16 ± 9 mg · kg⁻¹ · min⁻¹) in the IGT cohort, and 78 ± 28 mmol · kg⁻¹ · min⁻¹ (14 ± 5 mg · kg⁻¹ · min⁻¹) in T2D.
prevent insulin-induced hypokalemia, serum potassium concentrations were significantly lower during the hyperinsulinemic clamps compared with the sham clamps during graded glucose infusions in all groups (Fig. 6D).

DISCUSSION
Insulin resistance and β-cell dysfunction are largely regarded as two distinct processes participating in the pathogenesis of T2D. Recent data demonstrate that insulin itself augments GSIS in isolated human islets (2), rodent models (8,29), and healthy humans in vivo (20), suggesting an important physiologic role of insulin to modulate β-cell function in humans in vivo. Here we demonstrate that insulin’s affects to augment GSIS are diminished in insulin-resistant persons with IGT and T2D. Thus, insulin resistance at the level of the β-cell could represent a novel mechanism coupling insulin resistance and β-cell dysfunction.

Our findings in humans are consistent with the multiple genetic rodent studies demonstrating insulin’s positive physiologic affects on β-cell mass and function. Mice with βIRKO manifest diminished GSIS and progressive glucose intolerance progressing to overt diabetes in some animals (8,29). Likewise, deletion of insulin signaling proteins IRS-1, IRS-2, phosphoinositide 3-kinase (PI3K), or the serine/threonine protein kinase Akt2 alters glucose sensing and β-cell growth (1,30–32). Insulin and the insulin-mimetic compound L-783281 enhance insulin synthesis in isolated rodent islets (33,34). Exogenous insulin also leads to increased intracellular Ca2+, suggesting insulin may promote its own secretion by mobilizing Ca2+ from the endoplasmic reticulum (35). Higher insulin concentrations may induce β-cell glucokinase expression, potentiating glucose-stimulated insulin release. Moreover, glucose affects on β-cell growth and survival require activation of insulin signaling proteins (36), and hyperglycemia-induced reduction in expression of insulin receptor and activation of the proapoptotic cascade is physiologically antagonized by insulin signaling through the insulin signaling (IRS-PI3K-AKT-Bad) cascade (37,38). However, while multiple studies demonstrate an important role for the insulin signaling pathway in β-cell development and function, the physiologic role of insulin signaling on β-cell function has not previously been clearly demonstrated in humans in vivo.

Recently, we developed a novel methodology for direct measurement of endogenous insulin secretion in the presence of exogenous insulin by infusing an insulin analog that is biologically equivalent to—but can be discriminated
immunologically from—endogenous insulin. In this way we can use a selective immunoassay to accurately distinguish endogenous insulin from the infused product (20). We have previously reported an effect of insulin to augment GSIS in healthy humans (20). To our knowledge, our dynamic study is the first to demonstrate that the effect of insulin to augment secretion following glucose stimulation is diminished in insulin-resistant persons. Our data are consistent with the recent findings of Anderwald et al. (21), which demonstrated at euglycemia, in the absence of additional glucose stimulation, insulin infusion leads to increased insulin secretion in insulin-sensitive—but not insulin-resistant—subjects. However, previous studies have not yielded consistent findings. Our findings were most apparent during the highest rate of glucose infusion, which could be due to either glucose dose or duration of β-cell exposure. Several factors could account for the largely negative findings of older studies (11–16,18,19,39). First, most prior studies were performed at euglycemia and therefore did not evaluate the effect of glucose-stimulated insulin secretion. Second, the presence or magnitude of insulin resistance in subjects was not accounted for in many prior studies, which may have influenced the results given our findings that the effect is altered in insulin resistance. Third, most studies relied on C-peptide measures to estimate β-cell function as a substantial portion of endogenously secreted insulin is sequestered and/or cleared by insulin receptor–mediated mechanism(s) in the liver (40), but C-peptide clearance measured using stable isotopic infusion methods is increased during hyperinsulinemia (20), and intracellular insulin processing may lead to altered insulin to C-peptide secretion rates (41), which could bias conclusions toward an underestimation of change in β-cell response. Finally, most but not all prior studies (42) were unable to distinguish exogenous from endogenous insulin that may have masked the accurate interpretation of dynamic secretion.

We find that both insulin-resistant cohorts have reduced insulin potentiation of GSIS compared with healthy control subjects, but it is worth distinguishing that the potentiation effect was diminished in the T2D group and altogether absent in the IGT cohort. This was unanticipated as the
T2D group, with a more advanced stage of disease, had been predicted to have the greatest effect reduction. The IGT group was more insulin resistant and tended to be more overweight than the T2D group. The T2D group also had relatively mild disease as evidenced by treatment by lifestyle without pharmacologic intervention. Consequently, insulin augmentation of the β-cell response to glucose in our study was in fact maximally attenuated in the most insulin-resistant group (IGT). We speculate additional lifestyle or other environmental factors could contribute to between-group differences. Multiple physiologic and pathophysiologic processes regulate β-cell function and mass. For example, it is possible that insulin signaling pathways cross-talk with incretin signaling, and additional studies will reveal whether this cross-talk is differentially regulated in patients with IGT versus those with T2D. It is also possible that T2D could have reduced β-cell mass or greater magnitude insulin resistance in peripheral tissues relative to that of the β-cell and, thus, manifest more advanced disease (dysglycemia) with lesser magnitude of β-cell insulin resistance. However, we demonstrate in two independent insulin-resistant cohorts that the effect of insulin to potentiate glucose-stimulated insulin secretion is reduced.

Since the T2D group was more dysglycemic, our findings further suggest that the attenuation of insulin potentiation of glucose-stimulated insulin secretion is not due to early glucose dysregulation. Furthermore, if the effect of insulin to augment β-cell function was mediated indirectly by providing β-cell rest, one might have anticipated the effect to be similar in the IGT and T2D cohorts, which was not seen.

Moreover, the IGT cohort demonstrated significantly higher insulin levels at each glucose concentration achieved during the clamp, suggesting that this cohort had relatively preserved β-cell function with retained capacity to compensate for insulin resistance by augmenting insulin secretion. In contrast, the T2D cohort exhibited more profound β-cell dysfunction despite better peripheral insulin sensitivity. We hypothesize that the increased insulin concentrations manifest in the IGT cohort already provided a maximum stimulatory effect on the β-cell such that no further augmentation could be demonstrated during a hyperinsulinemic clamp. The preserved, though diminished, effect observed in the T2D cohort may suggest that the effect of insulin to stimulate GSIS is decreased but not entirely lost as β-cell dysfunction progresses, and there is loss of glucose sensitivity.

Glucose could play an important role in modulating the effect of insulin to potentiate β-cell function. Lower magnitude of glycemic stimulation could result in reduced β-cell responsiveness to insulin. However, this does not appear to have been the case in this study as glucose concentrations achieved during graded glucose infusions were highest in the insulin-resistant cohorts. In addition, glucotoxicity could diminish the effects of insulin to potentiate GSIS. As expected, our insulin-resistant cohorts had higher fasting and clamp glycoses than healthy control subjects. However, the IGT cohort had a greater reduction in insulin potentiation of GSIS but lower glycemia compared with T2D, so glucotoxicity does not accurately explain our study findings.

Multiple other metabolic factors could be significant in mediating a diminished effect of insulin on β-cell function. We have studied the effect of FFAs, and insulin augmentation of GSIS cannot be attributed to insulin suppression of FFA in healthy persons (43). Glucagon is another potent nonglucose secretagogue that promotes insulin secretion independent of the effects on glucose (44) and could have altered β-cell response. However, glucagon concentrations were reduced during hyperinsulinemic clamps, suggesting physiologic insulin action at the level of the α-cell (28,45). Likewise, low serum potassium has been associated with attenuated insulin secretion (46), but it is unlikely that the small differences in serum potassium seen in our cohorts explain the observed results because concentrations were similarly modestly reduced in the control subjects. Based on our findings, we cannot exclude the possibility that insulin potentiation of GSIS could be secondary to short β-cell rest with insulin administration during the period of iso-glycemic-hyperinsulinemic pre-exposure rather than directly modulated by insulin signaling, and that the interval of rest was insufficient to promote potentiation of GSIS in the insulin-resistant cohorts. Additional important factors that were not assessed in our studies could also contribute to β-cell dysfunction, including inflammation (47) and islet amyloid deposition (48,49).

We recognize our human in vivo experiments are limited because we cannot demonstrate dynamic changes in insulin signaling in β-cells directly. The differences in weight and degree of insulin resistance in the IGT versus T2D cohorts are additional study limitations. Finally, the two insulin-resistant cohorts were older than the healthy control subjects, and we cannot exclude confounding effects of age on insulin dynamics. Further studies are warranted to better understand how insulin modulates β-cell function in insulin-sensitive and -resistant populations.

In conclusion, we have demonstrated that while the β-cell is insulin responsive and pre-exposure to insulin enhances glucose-stimulated insulin secretion in healthy humans, this effect is reduced in states of insulin resistance. Thus, we demonstrate physiologically that the β-cell is an insulin-responsive tissue in humans in vivo. Our findings are consistent with a growing body of literature that suggests that pancreatic β-cell dysfunction could be caused by a defect in insulin signaling within β-cells, and this β-cell insulin resistance may lead to a loss of β-cell function and/or mass, contributing to relative hypoinsulinemia in response to glucose stimulation and subsequent hyperglycemia. Our findings also suggest that therapies for type 2 diabetes, particularly insulin-sensitizing agents, may exert their effect at least in part by restoring β-cell insulin sensitivity, therefore enhancing the capacity of the β-cell to secrete insulin as a response to glucose stimulation.

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P.H. participated in the research, was a discussion contributor, and wrote and edited the manuscript. X.L. participated in the research and reviewed and edited the manuscript. R.M. helped research the data and was a discussion participant. C.R.K. was a discussion contributor and edited the manuscript. R.N.K. was a participant in both discussion and manuscript composition. A.B.G. participated in the research, was a discussion contributor, and wrote and edited the manuscript. A.B.G. and R.N.K. are the
guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The authors acknowledge the support of the Joslin General Clinical Research Center and thank its philanthropic donors.

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