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

Florencia Halperin,[1,2,3] Ximena Lopez,[1,2,4] Raquel Manning,[1] C. Ronald Kahn,[1,3] Rohit N. Kulkarni,[1,3] and Allison B. Goldfine[1,2,3]

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

Type 2 diabetes (T2D) is characterized both by impaired insulin sensitivity and impaired insulin secretion. Multiple lines of evidence support the hypothesis that the pancreatic β-cell is an insulin-responsive tissue, and that insulin itself contributes to the regulation of insulin secretion. Insulin receptors and the major insulin receptor substrates (IRS-1 through IRS-4) are present and functional in rodent and human β-cells (1–7). In β-cell–specific insulin receptor knockout (βIRKO) mice, glucose-stimulated insulin secretion is defective, and animals develop progressive glucose intolerance and/or diabetes (8). In isolated human β-cells, insulin potentiates glucose-stimulated insulin secretion (9) and de novo insulin synthesis (2). In parallel, human pancreatic islets from patients with T2D have reduced RNA expression of multiple insulin signaling proteins including the insulin receptor IRS-2 and the serine/threonine protein kinase Akt2, which could impair insulin signal transduction and contribute to a diminished physiologic effect of insulin in patients with T2D (10).

Previous studies to evaluate whether insulin modulates its own secretion in humans in vivo have yielded inconsistent findings. Older studies, primarily assessing C-peptide levels, reported that administration of exogenous insulin at euglycemia inhibited or had no affect on insulin release (11–19). Using a novel paradigm with two immunologically distinct insulins, we recently demonstrated that pre-exposure to insulin potentiates glucose-stimulated insulin secretion (GSIS) in healthy subjects (20). Likewise, Anderwald et al. (21) reported insulin release during normoglycemia is modulated by exogenous insulin infusion, and the effect was lost in insulin-resistant persons. However, similar to previous studies, they did not have a direct measure of insulin secretion and calculated insulin secretion from plasma C-peptide concentrations by deconvolution, and the effect of glucose stimulation of insulin secretion at variable insulin was not assessed. We hypothesized that the effect of insulin to modulate the β-cell insulin response to glucose is attenuated in insulin-resistant persons, thereby providing a potential molecular link between the dual defects in insulin resistance and secretion manifest in T2D.

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 administration for 80 min (Fig. 1A) (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/kg per 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
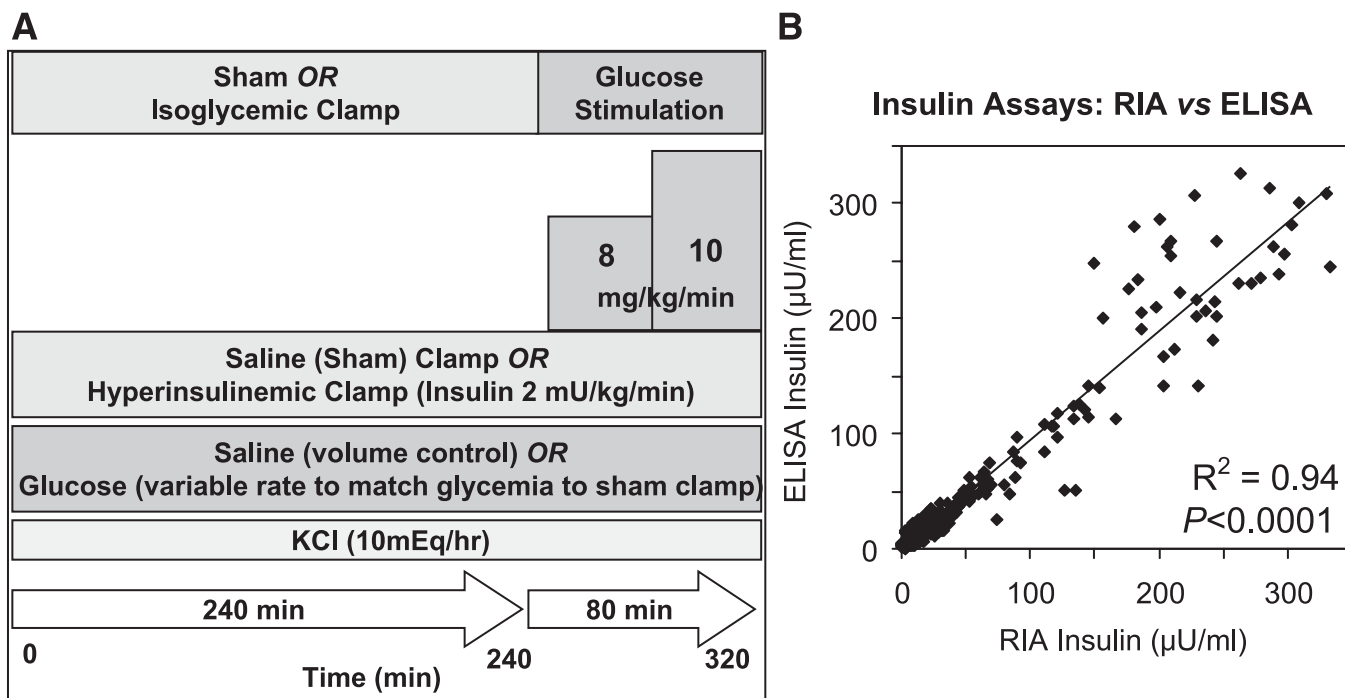
See accompanying commentary, p. 267.

FIG. 1. *A*: Study protocol: participants underwent paired isoglycemic clamp studies under high insulin (B28-Asp-insulin, 2 mU/kg/min) and low insulin (saline-sham) conditions. Glucose (20% dextrose) was administered during insulin clamp to maintain isoglycemia and saline during sham 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.

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 (26). 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 μU/ml). B28-Asp-insulin (Novolog; Novo Nordisk, Bagsværd, Denmark) was used because it has relative receptor binding and in vitro potency similar to regular human insulin (27) but can be distinguished from endogenous insulin immunologically by a novel method our group has devised and previously reported (20). Infusion of 20% dextrose at a variable rate was used to match the plasma glucose concentrations to those observed for that individual during the saline/sham condition (i.e., maintain isoglycemia). After 4 h, additional 20% dextrose was infused at variable rates to match glycemia during the graded glucose infusion portion of the first visit and to stimulate endogenous insulin secretion. During this portion of the clamp both the insulin and variable-rate dextrose infusions were continued so that insulin concentration remained elevated while glucose concentrations observed in the subject during the sham condition were matched. This study evaluated GSIS at matched glycemia following pre-exposure to relatively high insulin conditions (compared with the sham clamp).

Assays. Glycohemoglobin was assessed by high-performance liquid chromatography (Tosho 2.2; Tosho Bioscience). Potassium, total cholesterol, HDL, and triglycerides were measured in the clinical laboratory of the Joslin Diabetes Center (Beckman Synchron CX9). Serum glucose was measured using the glucose oxidase method (YSI 2300 STAT). Immunoassays were performed in duplicate in Joslin's Specialized Assay Core Facility (Joslin Diabetes and Endocrinology Research Center [DERC]) using commercial assay kits for total insulin, measuring both endogenous (secreted) and exogenous (administered) insulin and C-peptide (radioimmunoassay [RIA]; Diagnostic Systems Laboratories), with endogenous serum insulin assayed using an ELISA that would not detect the administered B28-Asp-insulin (DAKO Insulin ELISA; DakoCytomation). Additional assays included serum free fatty acid (FFA) (NEFA FFA ELISA, Wako Chemicals), proinsulin (total proinsulin, Mercodia), cortisol (Diasorin), and glucagon (Millipore).

Statistical analysis. Subject characteristics and data are presented as mean ± SE. The primary study end point of the study was the difference in rate of insulin secretion, measured by endogenous insulin, in response to glucose following pre-exposure to low versus high insulin conditions. A linear regression of change in insulin per time was constructed for each subject during GSIS as the change in glycemic stimulus fit linear model assumptions despite the two steps of dextrose administration during clamps. The mean rate of increase on the insulin and sham days were compared by paired Student t test. ANOVA was used for multiple comparisons by group with Fisher exact test for between group comparisons. Student paired t tests were used for point comparisons within groups. Repeated-measures ANOVA was used for comparisons of multiple time points between sham and hyperinsulinemic clamps days. Results were considered significant with two-tailed P values <0.05. Analyses were done with StatView (SAS Institute).

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 ± 0.6 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 exogenous insulin, RIA and ELISA insulin measurements were strongly correlated ($R^2 = 0.94$, $P < 0.0001$) (Fig. 1*B*). Healthy (insulin

TABLE 1
Clinical and metabolic characteristics of study subjects

	Healthy control subjects	IGT	T2D	P value (ANOVA)
Age (years)	24 ± 2	40 ± 4**	55 ± 2**,##	<0.0001
Sex	3M/5F	3M/7F	5M/6F	NS†
BMI (kg/m^2)	22.5 ± 0.7	33.4 ± 4.4*	27.4 ± 1.3	0.06
Systolic blood pressure (mmHg)	113 ± 3	115 ± 6	130 ± 4*,#	0.04
Diastolic blood pressure (mmHg)	75 ± 3	75 ± 3	81 ± 3	0.3
HbA$_{1c}$ (%)	5.1 ± 0.1	5.5 ± 0.2	6.5 ± 0.2**,##	<0.0001
Glucose fasting (mmol/L)	4.44 ± 0.22	5.05 ± 0.17	6.55 ± 0.39**,##	<0.0001
Insulin fasting (pmol/L)	35.5 ± 4.9	91.7 ± 17.4**	60.4 ± 12.5	0.02
C-peptide (nmol/L)	0.57 ± 0.03	1.53 ± 0.27**	1.23 ± 0.20*	0.01
Cholesterol (mmol/L)	3.89 ± 0.21	4.30 ± 0.28	4.40 ± 0.26	0.4
Triglycerides (mmol/L)	0.55 ± 0.07	1.39 ± 0.40*	1.13 ± 0.19	0.2
HDL (mmol/L)	1.30 ± 0.10	1.01 ± 0.05*	1.22 ± 0.16	0.2
LDL (mg/dL)	2.33 ± 0.16	2.77 ± 0.23	2.64 ± 0.26	0.5
M$_{120–240\ min}$ (mg/kg/min)	10.3 ± 0.7	5.1 ± 0.9**	7.1 ± 0.4**,#	0.0001

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.

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
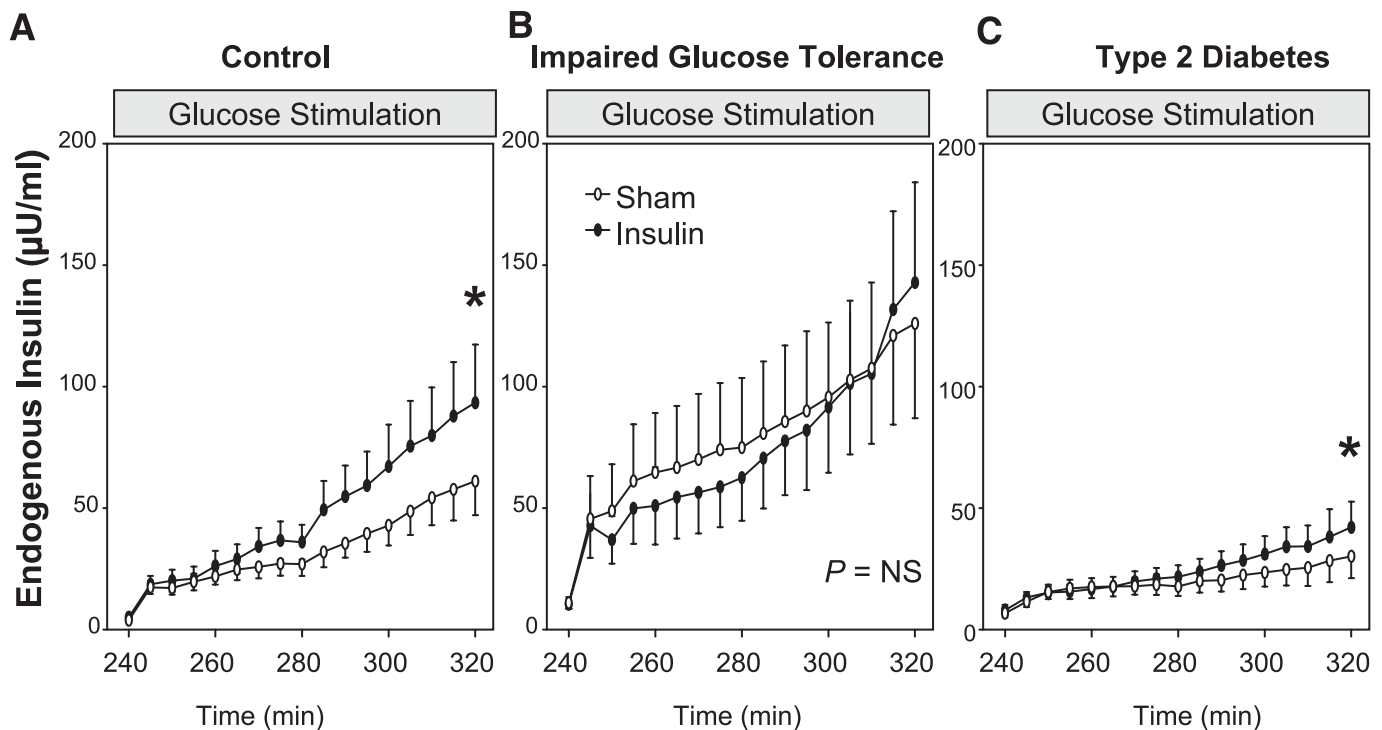

A Control
B Impaired Glucose Tolerance
C Type 2 Diabetes

FIG. 2. Pre-exposure to isoglycemic hyperinslinemia 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 β-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, *arrows*). 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 ($P \geq 0.7$ 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
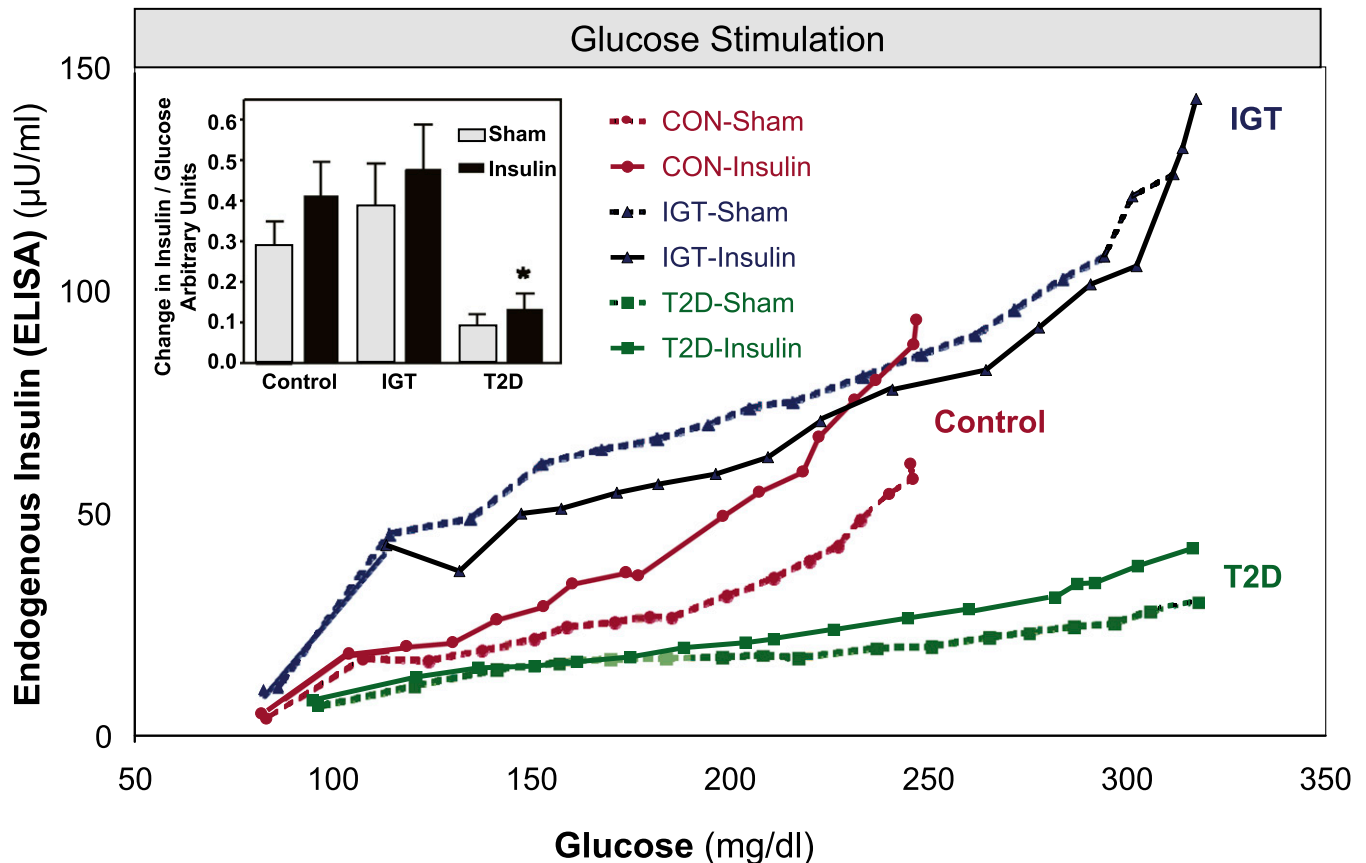

FIG. 3. Higher glucose concentrations were achieved in the insulin-resistant cohorts (IGT and T2D) compared with control subjects. β-cell glucose sensitivity tended to be higher following high compared with low insulin pre-exposure in control subjects. The effect of insulin pre-exposure on change in 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 (µU/mL) × 6.945 pmol/L. CON, control subjects. *$P = 0.04$ vs. control.
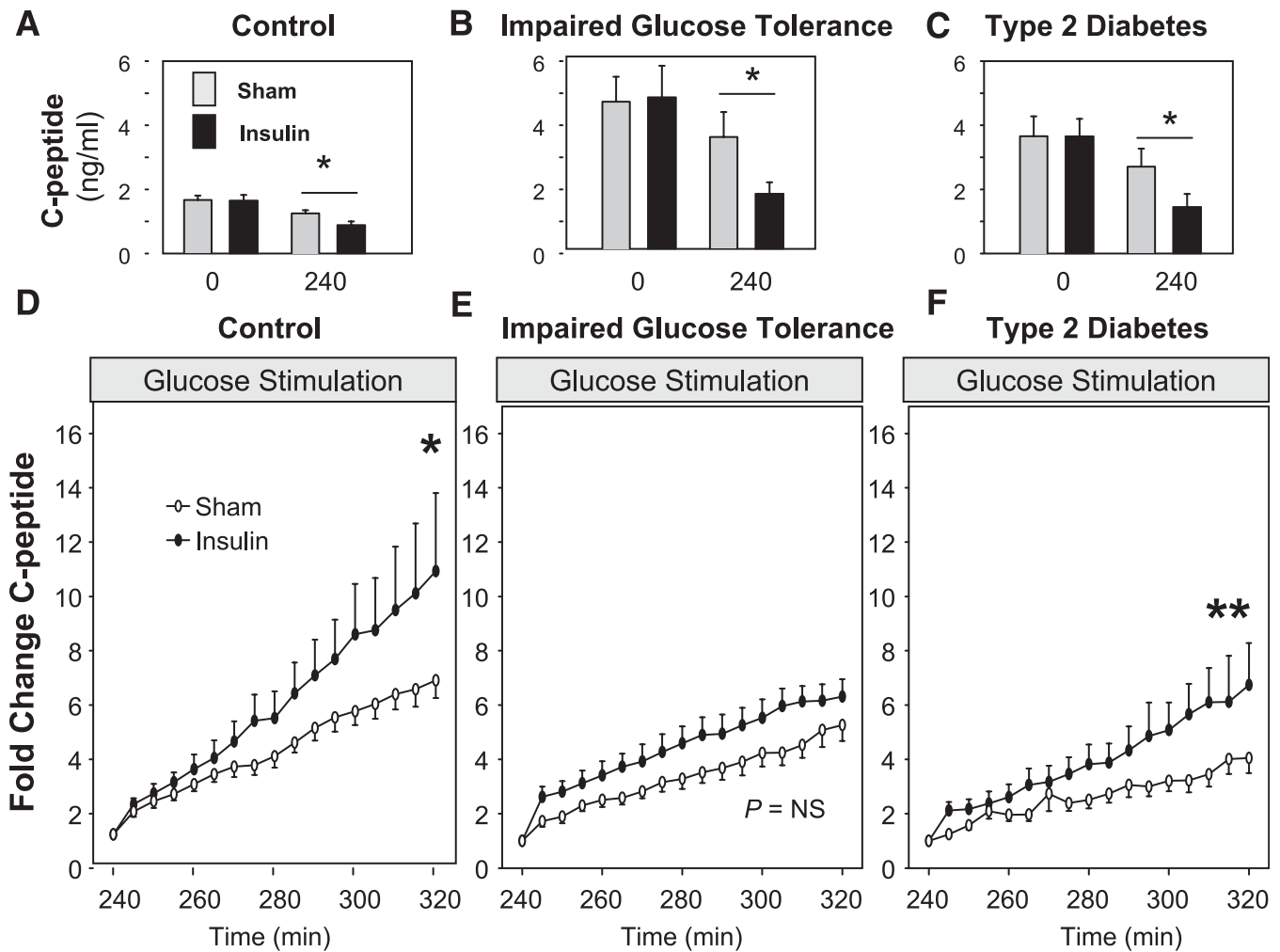
FIG. 4. *A–C*: Fasting C-peptide concentrations (time 0 min) were similar in control subjects, IGT, and T2D cohorts on both study days. C-peptide concentrations were lower in all groups following isoglycemic hyperinsulinemia (time 240 min). *D–F*: The fold increase in C-peptide in response to glucose stimulation was significantly increased following pre-exposure to insulin in control subjects and T2D. The magnitude of effect was diminished in the insulin-resistant groups. *$P < 0.05$, **$P < 0.01$. Black circles, isoglycemic-hyperinsulinemic clamp; white circles, sham clamp. For conversion to Scientific International units: C-peptide (ng/mL) × 0.333 for nmol/L.

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 $2,275 \pm 33$; $1,528 \pm 23$; and $1,095 \pm 16$ 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. 5*D–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).

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.

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. 6*A*). Plasma FFAs were lower throughout the hyperinsulinemic clamps compared with sham clamps (Fig. 6*B*). 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. 6*C*). Although potassium chloride was coadministered during all clamp protocols to
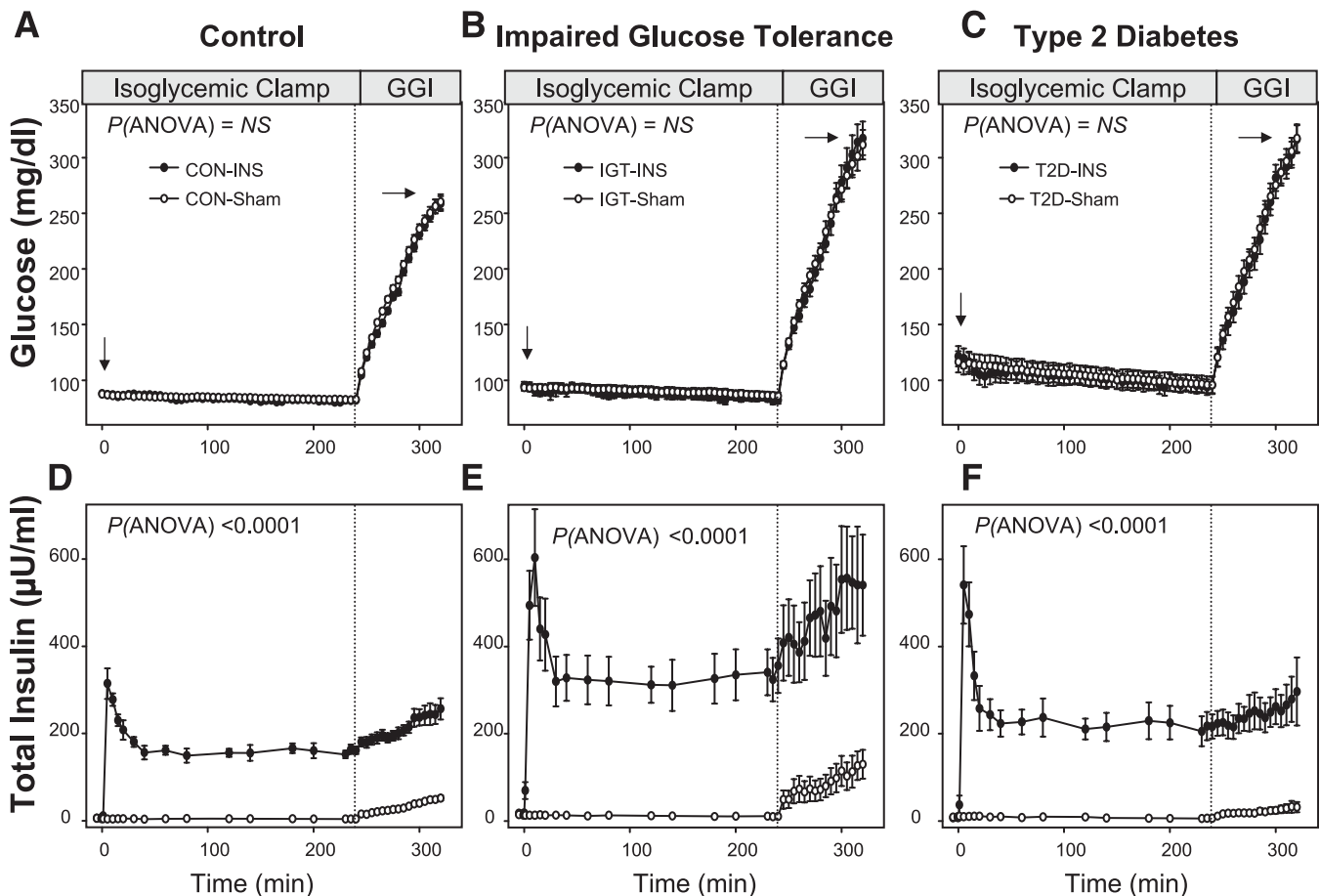
FIG. 5. Glucose concentrations were similar following pre-exposure to saline (sham clamp) or insulin (isoglycemic-hyperinsulinemic clamp) for control subjects (A), IGT (B), and T2D (C). Glucose levels achieved were greater in the IGT and T2D groups than in control subjects (→). Insulin levels were higher following exogenous insulin administration (isoglycemic-hyperinsulinemic clamp) than saline (sham clamp) as demonstrated by insulin RIA that detects both endogenous and exogenous insulin (D–F). The rise in total insulin can be seen in all cohorts following glucose administration following pre-exposure to insulin or saline due to endogenous insulin. Insulin concentrations achieved during hyperinsulinemic clamp were modestly higher in IGT and T2D cohorts compared with control subjects. Black circles, isoglycemic-hyperinsulinemic clamp; white circles, sham clamp. For conversion to Scientific International units: glucose (mg/dL) × 0.0555 for mmol/L; insulin (μU/mL) × 6.945 pmol/L. CON, control subjects; INS, insulin; GGl, graded glucose infusion.

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 Ca^{2+}, suggesting insulin may promote its own secretion by mobilizing Ca^{2+} 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
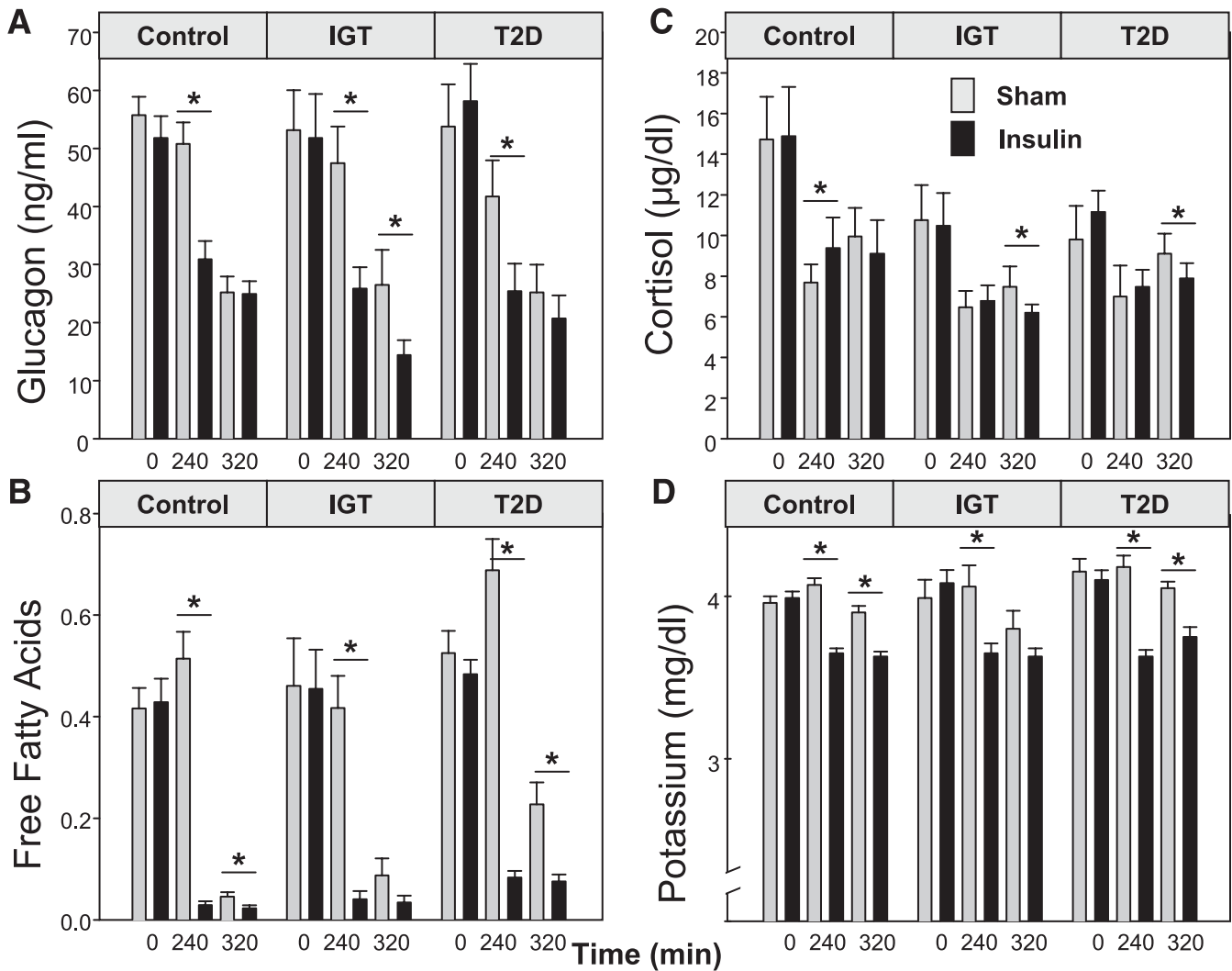
FIG. 6. Hormone and metabolites during isoglycemic-hyperinsulinemic and sham clamps are shown for glucagon (*A*), FFAs (*B*), cortisol (*C*), and potassium (*D*) at baseline (time = 0 min) and following insulin (black bars) or sham (gray bars) clamp, before (time 240 min) or after (time 320 min) glucose-induced insulin secretion. *$P < 0.05$. Black bars, isoglycemic-hyperinsulinemic clamp; gray bars, sham clamp. For conversion to Scientific International units: glucagon (pg/mL) × 1.0 for ng/L; FFA (mEq/L) × 1.0 for mmol/L; cortisol (μg/dL) × 27.59 for nmol/L; potassium (mg/dL) × 1.0 for mmol/L.

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 crosstalk 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 glucoses 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 isoglycemic-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.

REFERENCES

1. Kulkarni RN, Winnay JN, Daniels M, et al. Altered function of insulin receptor substrate-1–deficient mouse islets and cultured beta-cell lines. J Clin Invest 1999;104:R69–R75

2. Luciani DS, Johnson JD. Acute effects of insulin on beta-cells from transplantable human islets. Mol Cell Endocrinol 2005;241:88–98

3. Muller D, Huang GC, Amiel S, Jones PM, Persaud SJ. Identification of insulin signaling elements in human beta-cells: autocrine regulation of insulin gene expression. Diabetes 2006;55:2835–2842

4. Lingohr MK, Dickson LM, Wrede CE, McCuaig JF, Myers MG Jr, Rhodes CJ. IRS-3 inhibits IRS-2–mediated signaling in pancreatic beta-cells. Mol Cell Endocrinol 2003;204:85–99

5. Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA. Islet beta cell expression of constitutively active Akt1/PKB alpha induces striking hypertrophy, hyperplasia, and hyperinsulinemia. J Clin Invest 2001;108: 1631–1638

6. Hashimoto N, Kido Y, Uchida T, et al. Ablation of PDK1 in pancreatic beta cells induces diabetes as a result of loss of beta cell mass. Nat Genet 2006; 38:589–593

7. Tuttle RL, Gill NS, Pugh W, et al. Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha. Nat Med 2001;7:1133–1137

8. Kulkarni RN, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 1999;96:329–339

9. Aspinwall CA, Lakey JR, Kennedy RT. Insulin-stimulated insulin secretion in single pancreatic beta cells. J Biol Chem 1999;274:6360–6365

10. Gunton JE, Kulkarni RN, Yim S, et al. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 2005;122:337–349

11. DeFronzo RA, Binder C, Wahren J, Felig P, Ferrannini E, Faber OK. Sensitivity of insulin secretion to feedback inhibition by hyperinsulinaemia. Acta Endocrinol (Copenh) 1981;98:81–86

12. Waldhäusl WK, Gasić S, Bratusch-Marrain P, Korn A, Nowotny P. Feedback inhibition by biosynthetic human insulin of insulin release in healthy human subjects. Am J Physiol 1982;243:E476–E482

13. Garvey WT, Revers RR, Kolterman OG, Rubenstein AH, Olefsky JM. Modulation of insulin secretion by insulin and glucose in type II diabetes mellitus. J Clin Endocrinol Metab 1985;60:559–568

14. Argoud GM, Schade DS, Eaton RP. Insulin suppresses its own secretion in vivo. Diabetes 1987;36:959–962

15. Zilker T, Paterek K, Ermler R, Bottermann P. Studies on the question of a possible autoregulation of insulin secretion (author's translation). Klin Wochenschr 1977;55:475–479 [in German]

16. Shima K, Morishta S, Sawazaki N, Tanaka R, Tarui S. Failure of exogenous insulin to inhibit insulin secretion in man. Horm Metab Res 1977;9:441–443

17. Elahi D, Nagulesparan M, Hershcopf RJ, et al. Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. N Engl J Med 1982;306:1196–1202

18. Kraegen EW, Lazarus L, Campbell LV. Failure of insulin infusion during euglycemia to influence endogenous basal insulin secretion. Metabolism 1983;32:622–627

19. Best CH, Haist RE. The effect of insulin administration on the insulin content of the pancreas. J Physiol 1941;100:142–146

20. Bouche C, Lopez X, Fleischman A, et al. Insulin enhances glucose-stimulated insulin secretion in healthy humans. Proc Natl Acad Sci USA 2010;107:4770–4775

21. Anderwald C, Tura A, Grassi A, et al. Insulin infusion during normoglycemia modulates insulin secretion according to whole-body insulin sensitivity. Diabetes Care 2011;34:437–441

22. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2011;34(Suppl. 1):S62–S69

23. McGuire EA, Helderman JH, Tobin JD, Andres R, Berman M. Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol 1976;41:565–573

24. Goldfine AB, Ebbeling CB, Ludwig DS. Antegrade intravenous catheterization for metabolic studies in man. Diabetologia 2002;45:1742–1743

25. Rizza RA, Mandarino LJ, Gerich JE. Mechanisms of insulin resistance in man. Assessment using the insulin dose-response curve in conjunction with insulin-receptor binding. Am J Med 1981;70:169–176

26. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–E223

27. Brange J, Owens DR, Kang S, Vølund A. Monomeric insulins and their experimental and clinical implications. Diabetes Care 1990;13:923–954

28. Kawamori D, Kurpad AJ, Hu J, et al. Insulin signaling in alpha cells modulates glucagon secretion in vivo. Cell Metab 2009;9:350–361

29. Assmann A, Hinault C, Kulkarni RN. Growth factor control of pancreatic islet regeneration and function. Pediatr Diabetes 2009;10:14–32

30. Withers DJ, Gutierrez JS, Towery H, et al. Disruption of IRS-2 causes type 2 diabetes in mice. Nature 1998;391:900–904

31. Cho H, Mu J, Kim JK, et al. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 2001;292:1728–1731

32. Kaneko K, Ueki K, Takahashi N, et al. Class IA phosphatidylinositol 3-kinase in pancreatic β cells controls insulin secretion by multiple mechanisms. Cell Metab 2010;12:619–632

33. Leibiger B, Wahlander K, Berggren PO, Leibiger IB. Glucose-stimulated insulin biosynthesis depends on insulin-stimulated insulin gene transcription. J Biol Chem 2000;275:30153–30156

34. Westerlund J, Wolf BA, Bergsten P. Glucose-dependent promotion of insulin release from mouse pancreatic islets by the insulin-mimetic compound L-783,281. Diabetes 2002;51(Suppl. 1):S50–S52

35. Aspinwall CA, Qian WJ, Roper MG, Kulkarni RN, Kahn CR, Kennedy RT. Roles of insulin receptor substrate-1, phosphatidylinositol 3-kinase, and release of intracellular Ca2+ stores in insulin-stimulated insulin secretion in beta-cells. J Biol Chem 2000;275:22331–22338

36. Assmann A, Ueki K, Winnay JN, Kadowaki T, Kulkarni RN. Glucose effects on beta-cell growth and survival require activation of insulin receptors and insulin receptor substrate 2. Mol Cell Biol 2009;29:3219–3228

37. Federici M, Hribal M, Perego L, et al. High glucose causes apoptosis in cultured human pancreatic islets of Langerhans: a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. Diabetes 2001;50:1290–1301

38. Hribal ML, Perego L, Lovari S, et al. Chronic hyperglycemia impairs insulin secretion by affecting insulin receptor expression, splicing, and signaling in RIN beta cell line and human islets of Langerhans. FASEB J 2003;17: 1340–1342

39. Ratzmann KP, Schulz B. Further support for inhibition of endogenous insulin secretion by exogenous insulin. Exp Clin Endocrinol 1985;85:75–80

40. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41:368–377

41. Neerman-Arbez M, Halban PA. Novel, non-crinophagic, degradation of connecting peptide in transformed pancreatic beta cells. J Biol Chem 1993; 268:16248–16252

42. Ohgawara H, Tasaka Y, Kosaka K, Shizume K. Effect of exogenous bonito insulin on endogenous insulin secretion in dogs. Endocrinol Jpn 1974;21: 147–156

43. Lopez X, Cypess A, Manning R, O'Shea S, Kulkarni RN, Goldfine AB. Exogenous insulin enhances glucose-stimulated insulin response in healthy humans independent of changes in free fatty acids. J Clin Endocrinol Metab 2011;96:3811–3821

44. Samols E, Marri G, Marks V. Promotion of insulin secretion by glucagon. Lancet 1965;2:415–416

45. Cooperberg BA, Cryer PE. Insulin reciprocally regulates glucagon secretion in humans. Diabetes 2010;59:2936–2940

46. Helderman JH, Elahi D, Andersen DK, et al. Prevention of the glucose intolerance of thiazide diuretics by maintenance of body potassium. Diabetes 1983;32:106–111

47. Donath MY, Böni-Schnetzler M, Ellingsgaard H, Ehses JA. Islet inflammation impairs the pancreatic beta-cell in type 2 diabetes. Physiology (Bethesda) 2009;24:325–331

48. Guardado-Mendoza R, Davalli AM, Chavez AO, et al. Pancreatic islet amyloidosis, beta-cell apoptosis, and alpha-cell proliferation are determinants of islet remodeling in type-2 diabetic baboons. Proc Natl Acad Sci USA 2009;106:13992–13997

49. Jurgens CA, Toukatly MN, Fligner CL, et al. β-cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. Am J Pathol 2011;178:2632–2640