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Insulin regulates arginine-stimulated insulin secretion in humans

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ABSTRACT

Aims: Insulin potentiates glucose-stimulated insulin secretion. These effects are attenuated in beta cell–specific insulin receptor knockout mice and insulin resistant humans. This investigation examines whether short duration insulin exposure regulates beta cell responsiveness to arginine, a non-glucose secretagogue, in healthy humans.

Materials and methods: Arginine-stimulated insulin secretion was studied in 10 healthy humans. In each subject arginine was administered as a bolus followed by continuous infusion on two occasions one month apart, after sham/saline or hyperinsulinemic-isoglycemic clamp, respectively providing low and high insulin pre-exposure conditions. Arginine-stimulated insulin secretion was measured by C-peptide deconvolution, and by a selective immunogenic (DAKO) assay for direct measurement of endogenous but not exogenous insulin.

Results: Pre-exposure to exogenous insulin augmented arginine-stimulated insulin secretion. The effect was seen acutely following arginine bolus (endogenous DAKO insulin incremental $AUC_{240\text{-}255min}$ 311.6 ± 208.1 (post-insulin exposure) *versus* 120.6 ± 42.2 μU/ml•min (sham/saline) (*t*-test $P = 0.021$)), as well as in response to continuous arginine infusion (DAKO insulin incremental $AUC_{260\text{-}290min}$ 1095.3 ± 592.1 (sham/saline) *versus* 564.8 ± 207.1 μU/ml•min (high insulin)($P = 0.009$)). Findings were similar when beta cell response was assessed using C-peptide, insulin secretion rates by deconvolution, and the C-peptide to glucose ratio.

Conclusions: We demonstrate a physiologic role of insulin in regulation of the beta cell secretory response to arginine.

1. Introduction

Type 2 diabetes is characterized by defective insulin action and secretion. Previous studies demonstrate pancreatic beta cells are responsive to insulin and insulin-like growth factor -1 (IGF-1). Insulin/IGF-1 receptors and their signaling proteins are present and participate in regulation of insulin secretion in rodent pancreatic beta cells and in humans [1–5]. *In vitro* insulin potentiates glucose-stimulated insulin secretion in mouse and human isolated beta cells [6]. In beta cell-specific insulin receptor knockout (βIRKO) mice, glucose-stimulated insulin secretion is defective, and animals develop progressive glucose intolerance [7]. We [8–10] and others [11,12] have demonstrated that pre-exposure to insulin potentiates glucose-stimulated insulin secretion in healthy humans and this effect is attenuated in impaired glucose tolerance and type 2 diabetes. Together, multiple lines of evidence now show beta cells are insulin/IGF-1 responsive in rodents and humans *in vivo*, supporting that diminished insulin secretory response to glucose in type 2 diabetes might be related to defective beta cell insulin/IGF-1 signaling.

Arginine is another potent physiologic stimulus for insulin secretion [13,14]. Arginine-stimulated insulin secretion provides a clinical measure of beta cell functional mass and secretory capacity [15–17].

Abbreviations: βIRKO, Beta cell-specific insulin receptor knockout; IGF-1, insulin-like growth factor -1; HPLC, high-performance liquid chromatography; FFA, free fatty acids; ISR, insulin secretion rate; HUVEC, human umbilical vein endothelium cells.

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Arginine-stimulated insulin secretion remains present in type 1 diabetes for a period of time after glucose stimulated insulin secretion is reduced [18]. Similarly, in type 2 diabetes arginine-stimulated insulin secretion is better preserved than that to glucose, with the response modulated by disease duration and anti-diabetic therapies [19]. Although glucose-stimulated insulin secretion is impaired in βIRKO islets, arginine-stimulated secretion is preserved [7], suggesting in rodents effects of arginine are independent of, or not mediated entirely by, insulin receptor signaling. *In vivo* interactions in humans have not previously been examined.

To evaluate the role of insulin to modulate the insulin secretory response to a non-glucose stimulus, we studied effects of pre-exposure to raised insulin concentrations on insulin secretory response to arginine in healthy humans.

2. Materials and methods

2.1. Study approval

The Joslin Committee on Human Studies approved these investigations. Written informed consent was obtained from all participants prior to study initiation.

2.2. Study design

Subjects were recruited from newspapers, posted flyers, and web-based postings. Participants included 10 healthy persons, with no first-degree relative with diabetes, on no prescription medications other than oral contraceptives. Participants reported receiving contraception only for birth control, known menstrual disorders were exclusionary. Each participant underwent two study visits during which they underwent either a 4-hour saline infusion (sham/saline clamp providing conditions of low/physiologic insulin exposure, as a time and infusion volume control), or a hyperinsulinemic (high insulin exposure) iso-glycemic clamp (Fig. 1) in a crossover study design. All paired studies were conducted approximately four weeks apart to minimize hormonal cycle effects. Participants were masked to the order of the clamps, sham/saline or insulin. Prior to study visits participants were instructed to refrain from vigorous exercise and consume 250 g or more of carbohydrate per day for three days, and to fast overnight for 10–12 h. Upon presentation to the clinical research center, 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 heated box to ensure arterialization of venous blood [20,21]. Potassium chloride (KCl) was administered at 10 mEq/h to prevent hypokalemia during both clamps.

On the day of the first visit, for the sham/saline clamp, saline was infused at the volume rate hypothetically required to maintain euglycemia during a hyperinsulinemic clamp during which insulin at 14 pmol/kg per minute (2.0 mU/kg per minute) would be administered [8,22] in a person with similar insulin sensitivity, which corresponded to the volume calculated for a glucose utilization of 10 mg/kg/min. Saline infusion for 240 min was followed by intravenous administration of arginine, first as a 5 g bolus over 30 s (at time 240 min), followed by 500 mg/kg continuous infusion initiated at 260 min and administered over 30 min, to induce arginine-stimulated insulin secretion. The sham/saline clamp was performed first as there is a small but statistically significant decline in glucose concentrations with the prolonged fast and the timed glucose value from the first study was used as the glycemic target for the hyperinsulinemic clamp. The second visit (high insulin) occurred four weeks later. B28-Asp insulin (Novolog™, Novo Nordisk, Bagsvaerd, Denmark), with relative receptor binding and *in vitro* potency similar to regular human insulin [23] but immunologically distinguishable from endogenous insulin, was administered as a two-stepped primed (56 pmol/kg per minute followed by 28 pmol/kg per min, each for 5 min), continuous infusion (14 pmol/kg per minute (2.0 mU/kg per minute)) [8–10]. Infusion of 20% dextrose at variable rate was used to maintain isoglycemia, to match but not exceed individual plasma glucose concentrations during the prior sham/saline condition. After 240 min arginine was administered as before. During arginine administration insulin was continued, as well as 20% dextrose, adjusted as needed to match glycemia to the sham/saline clamp. Subjects were masked to whether the sham or insulin clamp was being performed on a given study day.

2.3. Assays

Glycohemoglobin was assessed by high-performance liquid chromatography (HPLC) (Tosho 2.2; Tosho Bioscience), and potassium, total cholesterol, high density lipoprotein, 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 (DRC) using commercial assay kits for total insulin, measuring both endogenous (secreted) and exogenous (administered) insulin, and C-peptide (both insulin and C-Peptide RIA; Diagnostic Systems Laboratories, Webster, TX, USA), with endogenous serum insulin assayed using an ELISA that would not detect the administered B28-Asp insulin (DAKO Insulin ELISA; DakoCytomation, Carpinteria, CA, USA).

2.4. Statistics and calculations

The primary study endpoint was the difference in rate of endogenous insulin secretion in response to arginine following pre-exposure to low (saline) *versus* high insulin conditions. Sample size estimates were based on the change in area under the curve for C-peptide with

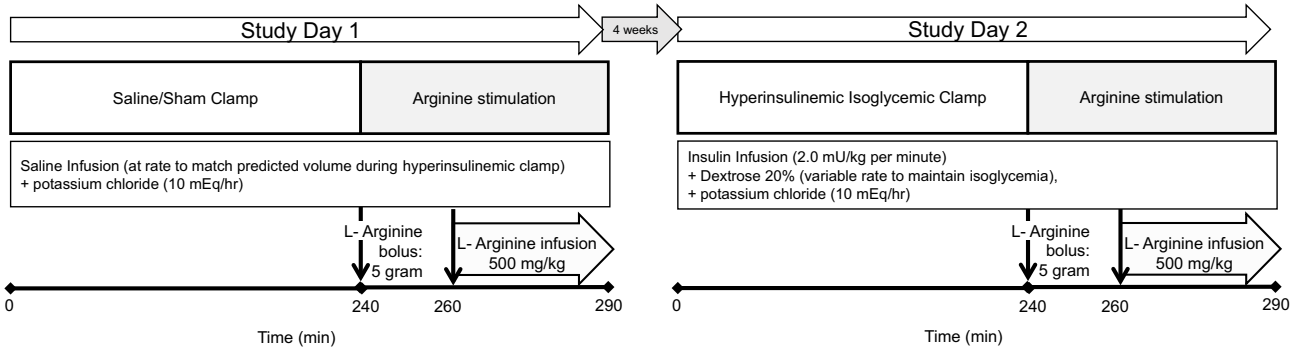

Fig. 1. Schematic of study protocol performed in healthy humans. Each participant underwent two study visits during which either a 4-hour sham/saline infusion (Study Day 1, left) or a hyperinsulinemic clamp (Study Day 2, right) was performed, and then on both occasions arginine was administered to stimulate endogenous insulin secretion as both a 5 g intravenous bolus (at time 240 min), and a 500 mg/kg continuous infusion (time 260–290 min).

a magnitude of change and coefficient of variation similar to changes in the studies to evaluate the effect of insulin to potentiate the beta cell response to glucose in healthy humans [8], and ten subjects per group would permit detection of a 40% change in C-peptide response to arginine, with 80% power and an alpha of 0.05. Endogenous insulin release in response to arginine in humans was assessed in four ways: 1) a direct measurement of endogenous insulin secretion using the DAKO insulin assay (DakoCytomation, Carpinteria, CA, USA) which detects endogenous insulin, but not the immunologically distinct B28-Asp insulin exogenously administered; 2) C-peptide, as a proxy for insulin secretion; 3) insulin secretion rate (ISR), calculated from plasma C-peptide by the deconvolution method, using I(nsulin-)SEC(retion) (ISEC, Version 3.4a, Hovorka, 1994) and population estimates of C-peptide kinetics [24]; and 4) to account for potential differences in glycemia between the two study conditions, which alone could account for any potentially observed difference in the insulin secretory response, C-peptide to glucose ratio was calculated at each study time point. Finally, because arginine bolus led to modest differences in glucose at the start of arginine administration, we calculated fold change in C-peptide to glucose ratios using average values between 230 and 240 min (prior to arginine bolus) and 260 min (prior to start of arginine continuous infusion).

For measures of insulin secretion, including as above direct endogenous insulin measurement (DAKO), C-peptide, ISR by deconvolution, and C-peptide to glucose ratio, we compared the response to arginine following pre-exposure to hyperinsulinemic or sham/saline conditions both in response to acute arginine bolus (at 240 min) and arginine infusion (260 to 290 min), using mixed model repeated-measures analysis, with autoregressive structure (AR(1)) as repeated covariance type, and both time and study condition (sham/saline or insulin) in the model. A *post hoc* analysis was performed to assess for potential heterogeneity in response between male and female subjects. Results are presented as mean ± standard error. Categorical data were analyzed using $\chi 2$-testing. *P*-values <0.05 were considered significant. Analysis was performed using SPSS (SPSS Inc., Version 17.0. Chicago, IL).

3. Results

3.1. Participant characteristics

Twenty subjects were evaluated for study participation. Ten were excluded, due to overweight ($n = 1$), large weight loss in the preceding year (n = 1), mother with gestational diabetes (n = 1), sister with polycystic ovarian disease (n = 1), diagnosed with dyslipidemia (n = 1), poor intravenous access (n = 1), and inability to schedule the long physiologic study visits ($n = 4$).

Clinical and metabolic characteristics of the ten subjects in the clamp studies are shown (Table 1). Schema of the infusion protocols are summarized in Fig. 1. Participants were insulin sensitive (M-value of glucose utilization during isoglycemic-hyperinsulinenic clamp at 240 min: 11.2 ± 1.5 mg/kg/min).

3.2. Glycemia during clamp studies

Overall, fasting and subsequent plasma glucose concentrations were comparable during sham/saline (low insulin) and hyperinsulinemic clamps from 0 to 240 min (mixed model repeated measures$_{0\text{-}240\text{min}}$ $P = 0.771$) (Fig. 2A), despite transiently lower levels following insulin exposure prior to achievement of steady state. On the sham/saline day, plasma glucose rose immediately following arginine bolus and infusion, consistent with prior reports of arginine effects on plasma glucose [25,26]. On the hyperinsulinemic study day, glucose levels were not fully matched to the sham/saline day during the arginine bolus and infusion consistent with the goal to match but not exceed individual plasma glucose during the sham/saline day, resulting in plasma glucose levels modestly lower from 240 to 255 min ($P = 0.002$) and 260 to 290 min ($P = 0.034$) (Figs. 2A and 3A). These differences were statistically significant, but it is critical to highlight that plasma glucose levels were *lower* after insulin pre-exposure compared to sham/saline, which would be expected to result in *reduced* beta cell response.

Table 1
Clinical and metabolic characteristics of human study subjects.

Age (years)	27.4 ± 2.6
Sex	5 Male/5 Female
Insulin Sensitivity ($M_{240\ mins}$ mg/kg/min)	11.2 ± 1.5
Systolic BP (mm Hg)	107.0 ± 5.8
Diastolic BP (mm Hg)	68.8 ± 4.2
Height (m)	1.7 ± 0.1
Weight (kg)	68.7 ± 6.4
Waist (cm)	80.8 ± 5.8
BMI (kg/m^2)	22.7 ± 1.1
Hematocrit (proportion of 1.0)	0.416 ± 0.023
(%)	(41.6 ± 2.3)
HbA_{1c} (mmol/mol)	36 ± 0.9
(%)	(5.4 ± 0.2)
Fasting insulin (μU/mL)	4.37 ± 1.41
(pmol/ml)	(30.3 ± 9.8)
Fasting C-peptide (ng/mL)	0.86 ± 0.35
(nmol/L)	(0.29 ± 0.12)
Fasting Glucose (mmol/L)	4.23 ± 0.31
(mg/dl)	(76.2 ± 5.6)
Cholesterol (mmol/L)	4.48 ± 0.36
(mg/dl)	(173.0 ± 13.8)
Triglycerides (mmol/L)	0.88 ± 0.18
(mg/dL)	78.2 ± 16.3
HDL (mmol/L)	1.85 ± 0.30
(mg/dL)	(71.3 ± 11.6)
Direct LDL (mmol/L)	2.28 ± 0.35
(mg/dL)	(88.2 ± 13.5)
TSH (IU/mL)	1.9 ± 0.5

Clinical and metabolic characteristics of human study subjects are presented as mean ± standard error. 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) ÷ 333 for ng/ml; cholesterol, HDL, and LDL (mmol/L) ÷ 0.0259 for mg/dl; triglycerides (mmol/L) ÷ 0.0113 for mg/dl. Body Mass Index (BMI), Blood Pressure (BP), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Thyroid Stimulating Hormone (TSH).

3.3. Arginine-stimulated insulin secretion

Beta cell function (insulin and C-peptide responses) to arginine bolus and infusion was assessed by the DAKO ELISA assay that recognizes only endogenous insulin, as well as by C-peptide secretion, and by Insulin Secretion Rate (ISR) as calculated by deconvolution [24].

Insulin response to a 5 g arginine bolus was higher after pre-exposure to 4 h of high physiologic insulin concentrations, compared to sham/saline, as assessed by direct measurement of endogenous insulin (DAKO assay) with insulin incremental $AUC_{240\text{-}255\text{min}}$ 120.6 ± 42.2 (sham/saline) *versus* 311.6 ± 208.1 μU/ml•min (insulin) (*t*-test $P = 0.021$) and mixed model repeated measures analysis for DAKO insulin values $_{240\text{-}255\text{min}}$ (sham/saline *versus* insulin pre-exposure $P < 0.001$) (Fig. 2B). Likewise, C-peptide concentrations after arginine bolus were higher following high insulin pre-exposure (mixed model repeated measures analysis of C-peptide values $_{240\text{-}255\text{min}}$, sham/saline *versus* insulin pre-exposure, $P = 0.005$) (Fig. 2C). Both insulin-to-glucose ratio (mixed model repeated measures comparing values $_{240\text{-}255\text{min}}$, $P < 0.001$) (Fig. 3B) and C-peptide-to-glucose ratio demonstrated increased response after high insulin compared with sham/saline pre-exposure (mixed model repeated measures analysis comparing values $_{240\text{-}255\text{min}}$, $P = 0.001$) (Fig. 3C). Fold change in C-peptide to glucose ratios from baseline (from minutes 230 to 240) was similarly augmented following insulin pre-exposure (mixed model repeated measures analysis comparing values 240–255 min, $P = 0.001$, not shown), consistent with modest differences in glucose after arginine not impacting the

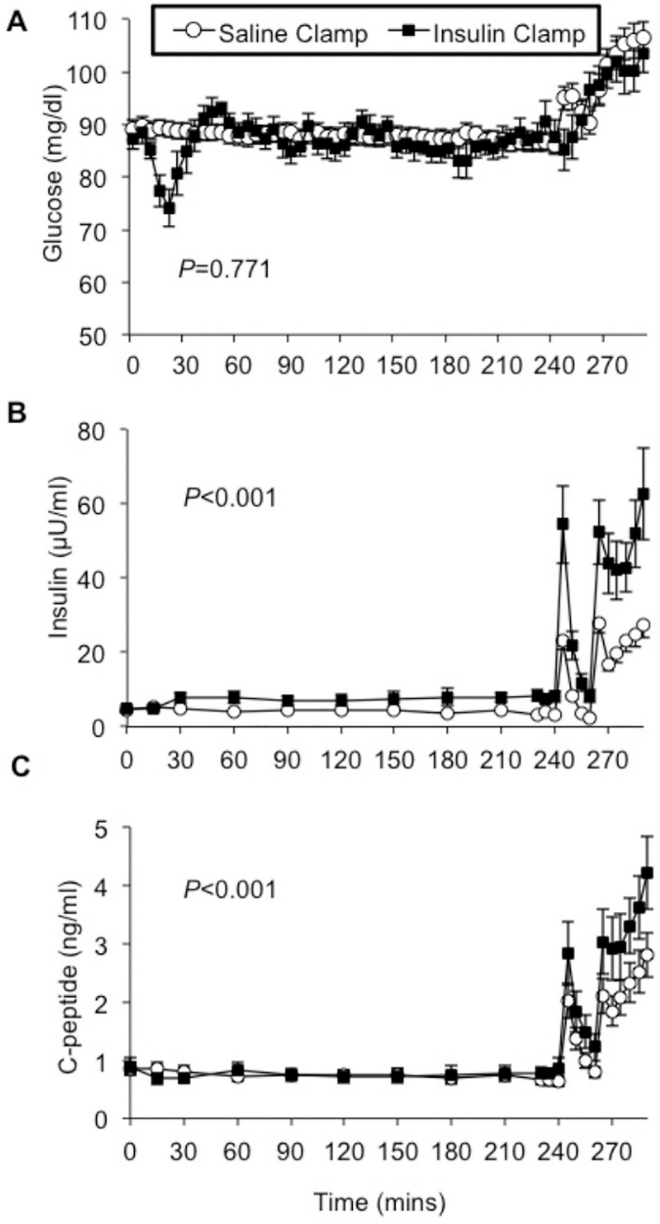

Fig. 2. Increased arginine-stimulated insulin secretion after insulin pre-exposure in healthy humans. Each participant underwent two study visits during which either a 4-hour sham/saline infusion or a hyperinsulinemic clamp was performed, and then arginine was administered to stimulate endogenous insulin secretion as both a 5 g intravenous bolus (at time 240 min), and a 500 mg/kg continuous infusion (time 260–290 min). Plasma glucose levels were overall well matched throughout both studies after stabilization and before arginine [A]. Arginine bolus and infusion increased endogenous insulin [B] and C-peptide [C] concentrations, and these responses were significantly augmented after insulin pre-exposure. Saline clamp (○), insulin clamp (•).

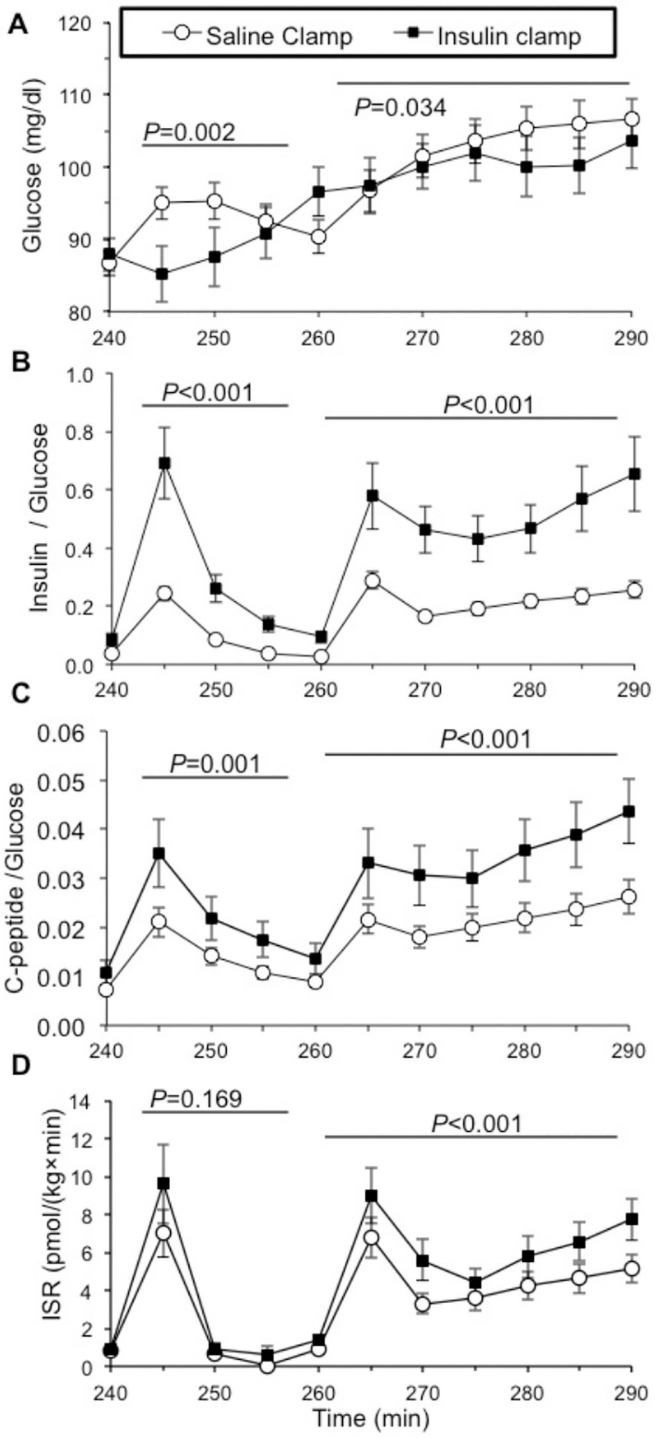

Fig. 3. Increased arginine-stimulated insulin secretion corrected for glycemia after insulin pre-exposure in healthy humans. During arginine stimulation (by intravenous bolus at time 240 min and continuous infusion time 260–290 min) the mean plasma glucose concentrations were lower following hyperinsulinemic compared with saline pre-exposure [A]. To account for potential confounding of different glucose concentrations achieved during the two study conditions, the insulin to glucose [B] and C-peptide-to-glucose [C] ratios were calculated, and found higher in response to arginine stimulation with hyperinsulinemia. The insulin secretion rate (ISR) estimated using the C-peptide deconvolution method was also higher in response to arginine bolus and infusion after insulin compared to saline pre-exposure [D]. After insulin pre-exposure there was a trend toward higher ISR in response to acute bolus arginine administration ($P = 0.169$), and a significantly higher ISR response to arginine infusion ($P < 0.001$). Saline clamp (○), insulin clamp (●).

augmenting effect of insulin pre-exposure. ISR by deconvolution in response to 5 g arginine bolus was numerically higher but did not reach statistical significance (mixed model repeated measures analysis comparing values 240–255 min, $P = 0.169$) (Fig. 3D).

In response to the 30-min continuous arginine infusion, the beta cell functional response was also higher after pre-exposure to high insulin than after sham/saline, as assessed by direct measurement of endogenous insulin (DAKO assay, with DAKO insulin incremental $AUC_{260\text{-}290min}$ 564.8 ± 207.1 (sham/saline) *versus* 1095.3 ± 592.1 μU/ml•min (high insulin) ($P = 0.009$), and mixed model repeated measures comparing values 260–290 min, $P < 0.001$) (Fig. 2B). C-peptide concentrations were also increased following

high insulin compared with sham/saline pre-exposure with C-peptide incremental $AUC_{260\text{-}290min}$ 39.4 ± 19.6 (sham/saline) *versus* 55.9 ± 28.1 ng/ml•min (high insulin) ($P = 0.071$) and compared by mixed model repeated measures (values $_{260\text{-}290min}$, $P < 0.001$) (Fig. 2C). C-peptide to glucose ratio was also higher after insulin infusion (mixed model repeated measures comparing sham/saline *versus* insulin exposure values $_{260\text{-}290min}$, $P < 0.001$) (Fig. 3C), as was fold change in C-peptide to glucose ratio, compared to the baseline value of the mean C-peptide to glucose ratio at 260 min ($P < 0.001$, not shown). The calculated ISR by deconvolution in response to continuous arginine infusion was greater after pre-exposure to insulin compared to saline (comparison of sham/saline *vs*. insulin exposure values $_{260\text{-}290min}$, $P < 0.001$) (Fig. 3D). Finally, *post hoc* analysis by sex suggests all beta cell responses to arginine following insulin pre-exposure are more robust in male compared to female subjects.

4. Discussion

Multiple lines of evidence now support altered insulin/IGF-1 signaling within the beta cell itself contributes to beta cell dysfunction and type 2 diabetes pathogenesis. *In vitro* and *in vivo* studies demonstrate insulin/IGF-1 signaling pathways regulate beta cell insulin processing [27] and modulate glucose-stimulated insulin secretion in mouse and man. Insulin exposure augments glucose-stimulated insulin secretion in rodents [7,28], isolated human islets [3], and healthy humans *in vivo* [8,10], but is impaired in βIRKO mice [7] and in humans with impaired glucose tolerance and type 2 diabetes [9,11,12]. Indeed, insulin exhibits diverse effects by regulating signaling proteins such as the aryl hydrocarbon receptor nuclear translocator-hypoxia inducible factor-1 alpha to regulate gene expression, the forkhead box M1-polo-like kinase 1-centromere protein-A pathway to modulate adaptive beta cell proliferation and m^6A mRNA methylation to modulate epigenetic changes in beta cells. [31–33]. These observations are further supported by the recent identification of inceptor, a protein that is able to calibrate insulin action selectively in beta cells [29]. Whether insulin directly regulates beta cell secretory response to other physiologic stimuli was previously unknown. We now show pre-exposure to insulin potentiates beta cell insulin secretory response to arginine in humans with normal glucose tolerance.

Variations in glycemia strongly effect insulin secretion, so consideration of plasma glucose differences between the sham/saline and high insulin conditions is important. In our studies, plasma glucoses were well matched prior to arginine stimulation. Following arginine infusion, there was a rapid rise in plasma glucose during sham/saline clamp (Fig. 3A), in contrast to modestly *lower* glucose concentrations during high insulin infusion; therefore this difference in glucose concentrations does not account for *increased* arginine-stimulated insulin secretion observed after insulin pre-exposure.

To confirm our findings we assessed *in vivo* insulin secretion by multiple methodologies, and found that all of them supported insulin augments arginine-stimulated insulin secretion. We previously described methodology to distinguish endogenous insulin in the presence of biologically equivalent but immunologically distinct analog insulin, using a selective immunoassay [8]. We also present data on change in C-peptide concentrations and insulin secretion rates by deconvolution, as most studies use C-peptide to estimate beta cell function, with caveats that C-peptide clearance is modestly increased during hyperinsulinemia [8,10] and intracellular insulin processing may alter insulin to C-peptide secretion rates [27,30], which could introduce bias. Finally, to account for potential differences in glycemia between study conditions, we calculated insulin-to-glucose and C-peptide-to-glucose ratios at each study time point. Estimates of insulin secretion using ISR by deconvolution differences are somewhat more modest than those using insulin or C-peptide measures alone, and while our methods do not specifically address an explanation for this, it could represent that the experimental conditions change hepatic insulin extraction. However, despite some differences in magnitude of effect, our findings of increased beta cell response to arginine following exposure to high insulin compared to sham/saline were consistent across all of the approaches used to quantify the beta cell response.

Interpretation of our findings must take into account several aspects of experimental design. Four to 5 g of arginine can be found in common food portions including 4 oz of chicken breast or one cup of soybeans, so the arginine exposure used in our study is generally physiologically relevant. High doses of insulin were chosen for these investigations based on doses previously shown to augment glucose-stimulated insulin secretion [8–10]; effects of lower insulin doses or shorter exposures on insulin secretion remain unknown. Study limitations include the recognition that peripheral blood sampling provides only indirect assessment of insulin secretion, and that our findings do not fully exclude the possibility that an augmented response to arginine after exogenous insulin was attributable to beta cell rest during insulin infusion, subsequently permitting a more exuberant response to the secretory stimulus. However, achieving isoglycemia, and including a volume control, prior to arginine stimulation is the best way to match the metabolic milieu that beta cells were exposed to prior to arginine stimulation. Finally, the study population intended to reflect the general population but *post hoc* analysis suggest the beta cell response to arginine following insulin pre-exposure is more robust in male compared to female subjects. This observation could be the result of chance, given no *a priori* hypothesis or underlying mechanism, and the two subsets are underpowered to confirm a difference by sex, thus additional studies would be warranted to examine this potential heterogeneity.

Various *in vitro* and *in vivo* studies have supported arginine's beneficial effects on glucose metabolism and insulin sensitivity [3,40,41]. Insulin increases L-arginine transport *in vitro* in human umbilical vein endothelium cells (HUVEC) by increasing solute carrier family 7 member 1 promoter activity [34] and cationic amino acid transporter 1 activity and expression [35,36]. Increased L-arginine transport has been proposed as the mechanism that underlies insulin-induced HUVEC relaxation [34]. In HUVEC from women with gestational diabetes or preeclampsia, increased L-arginine transport [37,38] is reported to maintain effects of insulin on HUVEC vasodilation [39]. Whether insulin increases arginine transporter expression or function in beta cells remains unknown. Furthermore, L-arginine supplementation improves insulin sensitivity (and insulin-mediated vasodilation) in healthy persons and in obesity, type 2 diabetes, and coronary artery disease [3,40,41]. L-Arginine may improve glucose transport and glycogen synthesis through enhanced signal transduction and direct activation of AKT and AMPK pathways in rat skeletal muscle. Plasma arginine levels are reduced in diabetes [42]. Our detailed human physiology studies expand our understanding to the *in vivo* effects of arginine on insulin secretion.

The scientific question addressed in this study is whether insulin impacts the beta cell secretory response to the non-glucose secretagogue, arginine, in healthy humans. In a future study the evaluation of insulin resistant individuals such as those with obesity or type 2 diabetes would be necessary to address whether this effect is diminished in insulin resistant states, as has been seen with diminished insulin potentiation of the beta cell response to glucose in insulin resistant compared to insulin sensitive persons [8–12]. Future studies that directly address potential differences between sexes and between species will provide further insights into the ability of insulin to regulate amino-acid effects on beta cell biology in physiological and pathophysiological states.

In summary, we demonstrate that in healthy humans insulin itself plays a role in regulation of the beta cell response to arginine as a secretory stimulus. Our findings provide continued support for a physiologically important role of insulin in the regulation of beta cell function for a secretagogue beyond glucose. Consequences of diminished insulin effects at the level of the beta cell in pathogenic states could be an important contributing mechanism to progressive beta cell dysfunction underlying type 2 diabetes.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Florencia Halperin: Conceptualization, Methodology, Software, Writing – original draft. **Teresa Mezza:** Data curation, Writing – original draft. **Ping Li:** Software, Validation. **Jun Shirakawa:** Visualization, Investigation. **Rohit N. Kulkarni:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Allison B. Goldfine:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors have declared that no conflict of interest exists. Dr. Goldfine completed the work when employed at the Joslin Diabetes Center and is now an employee of Novartis Institutes of Biomedical Research. Dr. Halperin completed the work when employed at Brigham and Women's Hospital and Joslin Diabetes Center and now works at Brigham and Women's Hospital and Form Health, Inc.

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