Reduced β-Cell Secretory Capacity in Pancreatic Insufficient, but Not Pancreatic Sufficient, Cystic Fibrosis Despite Normal Glucose Tolerance

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Abstract:

Patients with pancreatic insufficient cystic fibrosis (PI-CF) are at increased risk for developing diabetes. We determined β-cell secretory capacity and insulin secretory rates from glucose-potentiated arginine and mixed meal tolerance tests (MMTT), respectively, in pancreatic sufficient cystic fibrosis (PS-CF), PI-CF, and normal control subjects, all with normal glucose tolerance, in order to identify early pathophysiologic defects. Acute islet cell secretory responses were determined under fasting, 230 mg/dL, and 340 mg/dL hyperglycemia clamp conditions. PI-CF subjects had lower acute insulin, C-peptide and glucagon responses compared to PS-CF and normal, indicating reduced β-cell secretory capacity and α-cell function. Fasting proinsulin-to-C-peptide and proinsulin secretory ratios during glucose-potentiation were higher in PI-CF, suggesting impaired proinsulin processing. In the first 30 minutes of the MMTT, insulin secretion was lower in PI-CF compared to PS-CF and normal, and glucagon like peptide-1 and gastric inhibitory polypeptide were lower compared to PS-CF, and after 180 minutes, glucose was higher in PI-CF compared to controls. These findings indicate that despite “normal” glucose tolerance, adolescents and adults with PI-CF have impairments in functional islet mass and associated early phase insulin secretion, which with decreased incretin responses likely leads to the early development of post-prandial hyperglycemia in CF.
Cystic fibrosis (CF) is a life threatening autosomal recessive disorder in which the function of the cystic fibrosis transmembrane regulator (CFTR) is absent or severely reduced. More than 2000 CFTR mutations have been reported and alterations in CFTR function result in impaired bicarbonate and chloride transport across epithelial membranes that may result in impaired pancreatic exocrine secretion leading to pancreatic insufficiency (PI-CF). Additionally, impaired pulmonary secretion clearance leads to increased susceptibility to pulmonary infections, progressive decline in pulmonary function, and ultimately respiratory failure for many individuals with CF. Advances in CF nutrition and pulmonary care have resulted in improved median survival with which the development of cystic fibrosis-related diabetes (CFRD) has emerged as a major co-morbidity affecting roughly 40% of adults aged > 30 years (1). CFRD is associated with worse clinical outcomes including reduction in pulmonary function, worsening nutritional status, declining kidney function, and increased mortality (2).

Pancreatic insufficiency is associated with increased risk for developing CFRD (3), where pancreatogenic diabetes can result from pancreatic inflammation and the subsequent fibrosis and sclerosis disrupting pancreatic islet structure and function (4). Incretin secretion abnormalities arising from pancreatic insufficiency related maldigestion are also posited to contribute to insulin secretion abnormalities (5). While reduced functional β-cell mass is expected by the time diabetes is diagnosed, limited data are available to inform what early pathophysiologic mechanisms may be targeted therapeutically to prevent the development of CFRD.

To identify early defects affecting glucose homeostasis in CF, we recruited PI-CF subjects with normal glucose tolerance according to the Cystic Fibrosis Foundation (CFF) criteria that are more stringent than current American Diabetes Association criteria in requiring a
1-hour glucose < 200 mg/dL during the standard 75-gram oral glucose tolerance test (6,7). We hypothesized that participants with PI-CF, despite having normal glucose tolerance, would manifest impaired β-cell secretory capacity and insulin secretory rates (ISR) as derived from glucose-potentiated arginine (GPA) and mixed meal tolerance tests (MMTT), respectively, compared to non-CF healthy controls, findings that would support a primary islet defect as the earliest mechanism responsible for future risk of diabetes. To consider possible effects of diminished CFTR function on insulin and incretin secretion independent of pancreatic exocrine insufficiency, we also studied individuals with pancreatic sufficient CF (PS-CF) to serve as disease controls.

**Research Design and Methods**

**Subjects**

Post-pubertal adolescents and adults with a confirmed diagnosis of CF including positive sweat test or CFTR mutation analysis (8) were invited to participate. Subjects were recruited based on their pancreatic exocrine insufficiency status; PI was determined by clinical diagnosis including symptoms of malabsorption, treatment with pancreatic enzyme replacement therapy, and if ambiguous, confirmed by fecal elastase testing (9). PS was defined by absence of malabsorption symptoms, absence of pancreatic enzyme replacement treatment, and previous fecal elastase levels > 200 µg/g. Subjects did not undergo fecal elastase testing at the time of enrollment. Normal glucose tolerance (NGT, 1-hour glucose < 200 mg/dL and 2-hour glucose < 140 mg/dL (6)) was documented by a standard 75-gram oral glucose tolerance test (OGTT) within 3 months prior to study. Individuals with acute illness requiring a change in antibiotics or administration of oral or intravenous glucocorticoids within the previous 4 weeks, clinically symptomatic
pancreatitis within the previous 12 months, prior lung or liver transplant, or significant kidney or liver dysfunction, as well as pregnant or nursing females were excluded.

Healthy individuals with NGT and of comparable sex, age, and BMI to CF participants served as controls. Control subjects for the GPA test \( n = 11 \) were derived from a recently reported study (10), and MMTT and continuous glucose monitoring (CGM) controls \( n = 10 \) were recruited prospectively with CF participants.

The Institutional Review Boards of the University of Pennsylvania (Penn) and Children's Hospital of Philadelphia (CHOP) approved the study, and all participants gave written informed consent and assent (when age-appropriate) to participate. Study procedures were performed over 2 study visits conducted in the Penn or CHOP Clinical and Translational Research Center (CTRC) after a 12-hour overnight fast.

**Glucose-potentiated arginine (GPA) test**

The glucose-potentiated arginine (GPA) test followed established methodology for evaluation of \( \beta \)-cell secretory capacity and sensitivity to glucose (11-13). At 0700, one catheter was placed in an antecubital vein for infusions, and one catheter was placed in a distal forearm or hand vein for blood sampling, with the hand placed in a heating pad to promote arterialization of the venous blood. After at least 20 min of acclimatization to the catheters, baseline blood samples were taken at \( t = -5 \) and \(-1 \) min before injection of 5 g of 10% arginine over 1-min starting at \( t = 0 \). Additional blood samples were collected at \( t = 2, 3, 4, \) and 5 min after arginine injection. Beginning at \( t = 10 \) min, a hyperglycemic clamp technique (14) using a variable rate infusion of 20% dextrose was performed to achieve a plasma glucose concentration of \( \sim 230 \) mg/dL. Blood samples were taken every 5 min to adjust the infusion rate and achieve the desired plasma
glucose concentration. After 45 min of glucose infusion (at $t = 55$ min), a second 5-g arginine pulse was injected with identical blood sampling. The first administration of arginine has no effect on the subsequent response to arginine using this protocol (15). A subsequent 2-hour period with no glucose infusion allowed plasma glucose to return to baseline. A second hyperglycemic clamp was then performed to achieve a plasma glucose concentration of ~340 mg/dL. After 45 min of glucose infusion, a third 5-g arginine pulse was injected with identical blood sampling.

*Mixed Meal Tolerance Test (MMTT)*

The MMTT has previously been used to differentiate insulin and incretin secretory responses (16). At 0700, an antecubital or forearm vein catheter was placed for blood sampling. After at least 20 min of acclimatization to the catheter, baseline blood samples were taken at $t = -10$ and -1 min before ingestion of an 820 kcal breakfast over 15 minutes starting at $t = 0$. The carbohydrate, fat, and protein composition of the meal were 47%, 40%, and 13% of the total energy content (16). Subjects with pancreatic exocrine insufficiency took their prescribed dose of pancreatic enzyme replacement with the test meal. Additional blood samples were obtained at $t = 10, 15, 20, 30, 60, 90, 120, 150, 180, 210$ and 240 min from the start of the meal.

*Biochemical analysis*

Plasma glucose was measured in duplicate by the glucose oxidase method using an automated glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Other samples were collected on ice into tubes containing EDTA and protease inhibitor cocktail (and for MMTT samples, DPP4 inhibitor; Sigma-Aldrich, St. Louis, MO), centrifuged at 4 °C, separated,
and frozen at -80 °C for subsequent analysis. Plasma insulin, C-peptide, proinsulin, and glucagon were measured in duplicate by double-antibody radioimmunoassays (Millipore, Billerica, MA). Plasma FFA levels were measured in duplicate using enzymatic colorimetrics (Wako Chemicals, Richmond, VA). Active glucagon like peptide (GLP-1) and total gastric inhibitory polypeptide (GIP) were measured in duplicate by enzyme-linked immunosorbent assays (Millipore).

Continuous glucose monitoring

72-hour CGM was performed as a dynamic assessment of glycemic control. The CGM system (CGMS iPro, Medtronic Minimed, Northridge, CA) measures interstitial glucose from a subcutaneously inserted sensor every ten seconds and records an average value every five minutes. Interstitial continuous glucose monitoring has previously been validated in CF (17). Subjects utilized a study glucometer (OneTouch Ultra, LifeScan, Milpitas, CA) to monitor blood glucose and calibrate the CGMS 4 times daily with no interval between readings exceeding 12 hours. Subjects removed the sensor after 72 hours. CGM data were excluded if < 36 hours in duration due to catheter dislodgement or if conducted with insufficient blood glucose calibration measurements.

Calculations and statistical analyses

GPA test: Acute insulin, C-peptide, proinsulin, and glucagon responses to arginine (AIR$_{arg}$, ACR$_{arg}$, APR$_{arg}$, and AGR$_{arg}$, respectively) were calculated as the mean of the 2-, 3-, 4-, and 5-min values minus the mean of the baseline values (14). Acute responses during the 230 mg/dL glucose clamp enable determination of glucose potentiation of arginine-induced insulin (AIR$_{pot}$), C-peptide (ACR$_{pot}$), and proinsulin (APR$_{pot}$) release, and glucose inhibition of arginine-induced
glucagon (AGR_{inh}) release. Acute responses during the 340mg/dL glucose clamp allow for
determination of the maximum arginine-induced insulin (AIR_{max}), C-peptide (ACR_{max}), and
proinsulin (APR_{max}) release, *i.e.* β-cell secretory capacity, and of the minimum arginine-induced
glucagon (AGR_{min}) release (11). The proinsulin-to-C-peptide ratio was calculated as the molar
centration of proinsulin divided by the molar concentration of C-peptide x 100 (18).
Estimation of the proinsulin-to-C-peptide ratio within the secretory granules of the β-cell is most
reliable after acute stimulation of secretion (19); therefore, we examined the proinsulin secretory
ratio (PISR) in response to each injection of arginine from the respective acute proinsulin:C-
peptide responses to arginine (12,18). PG_{50}, plasma glucose at which half-maximal insulin
secretion is achieved, was calculated to assess β-cell sensitivity to glucose as previously
described (11-13). Insulin sensitivity (M/I) was determined by dividing the mean glucose
infusion rate required during the 230 mg/dL glucose clamp (M) by the mean pre-stimulus insulin
level (I) between 40-45 min of the glucose infusion (12).

**MMTT:** Incremental areas under the curve (AUC) for glucose, FFA, insulin secretory rates
(ISR), glucagon, GLP-1, and GIP were calculated with baseline values subtracted by the
trapezoidal method using Origin software (Northampton, MA). The insulin secretory rates were
calculated from C-peptide values and derived by parametric deconvolution of C-peptide kinetics
using a two-compartment model (20) and insulin sensitivity (S_{I}) was derived using the oral
minimal model (21) in WinSAAM software 3.0.8 (University of Pennsylvania - New Bolton
Center, Kennett Square PA).
CGM: Interstitial glucose data were summarized to provide mean glucose, glucose standard deviation (SD), coefficient of variation (CV) and percent (%) time glucose > 140 mg/dL and > 180 mg/dL. CV for glucose was calculated from the glucose SD divided by mean glucose.

Statistical Analyses: All data are expressed as median and interquartile ranges (IQR), unless otherwise noted. Comparison of results between the PS-CF, PI-CF, and normal control subjects was performed with the Kruskall Wallis test and when significant differences at $P \leq 0.05$ were found, comparisons between groups were performed using the Mann-Whitney U test. Spearman’s rank correlation test was used to evaluate correlation between the OGTT and acute islet response measures. All analyses were performed using STATA 12 (StataCorp LP, College Station, Texas). Significance was considered at $P \leq 0.05$ (two-tailed).

Results

Subject characteristics

Nine PS-CF, 11 PI-CF, 11 controls for the GPA test, and 10 controls for the MMTT participated (Table 1). Gender distribution, age, and BMI were comparable across the CF and control groups. The majority of PI-CF subjects (6/11) but no PS-CF subject was homozygous for the F508del CFTR mutation (Supplementary Table). Pulmonary function, as represented by percent-predicted forced expiratory volume in 1-second (FEV$_1$%_predicted), was not statistically different between PS-CF and PI-CF subjects (91 (26-119) vs. 80 (54-99)%).

PI-CF participants had higher HbA$_{1c}$ ($P < 0.001$ vs. controls) and OGTT 1-hour glucose ($P < 0.05$ vs. PS-CF and controls) even though all subjects had CFF-defined NGT (Table 1).
Compared to controls, PI-CF also had higher CGM mean glucose, glucose SD, glucose CV, and percentage of time glucose > 140 mg/dL and > 180 mg/dL (Table 2).

**Glucose, insulin, C-peptide and glucagon during the GPA test**

Fasting glucose prior to GPA testing was normal for all participants (93 (min-max: 89-97) mg/dL) and was not significantly different between groups (data not shown). Pre-stimulus glucose during the 230 mg/dL clamp was 226 (223-233) mg/dL and during the 340 mg/dL clamp was 325 (318-337) mg/dL; neither was different across groups.

Fasting islet cell hormone concentrations were comparable amongst the groups (Table 3). For insulin responses (Fig. 1A and Table 3), AIR\(_{\text{arg}}\) was greater in PS-CF compared to PI-CF and controls (\(P < 0.05\) for both), and AIR\(_{\text{pot}}\) was lower in PI-CF compared to PS-CF and controls (\(P < 0.01\) for both). Similarly for the C-peptide responses (Fig. 1B and Table 3), PS-CF had higher ACR\(_{\text{arg}}\) compared to PI-CF and controls (\(P \leq 0.05\) for both). PI-CF subjects demonstrated lower ACR\(_{\text{pot}}\) and ACR\(_{\text{max}}\) compared to PS-CF (\(P < 0.05\) for both) and to controls (\(P < 0.05\) for both).

For the glucagon responses, AGR\(_{\text{arg}}\), AGR\(_{\text{inh}}\), and AGR\(_{\text{min}}\) were lower in PI-CF than PS-CF (\(P \leq 0.05\) for all) and controls (\(P < 0.05\) for all; Fig. 1D and Table 3).

Data were reanalyzed after exclusion of CF subjects who were receiving ivacaftor for clinical indications (PS-CF, \(n = 2\); PI-CF, \(n = 1\); Supplementary Figure). AIR\(_{\text{arg}}\) and ACR\(_{\text{arg}}\) were now similar in PS-CF and controls, and ACR\(_{\text{max}}\) was no longer significantly different in PI-CF compared to PS-CF (\(P = 0.08\), although remained significantly less than control (\(P < 0.05\)).
**Proinsulin and proinsulin secretory ratios (PISRs)**

The fasting proinsulin-to-C-peptide ratio was elevated in PI-CF ($P = 0.01$ vs. controls; Table 3). Proinsulin responses, $\text{APR}_{\text{arg}}$, $\text{APR}_{\text{pot}}$ and $\text{APR}_{\text{max}}$ were not different across the PS-CF, PI-CF, and control groups (Fig. 1C and Table 3). However, because insulin and C-peptide secretory responses were decreased in PI-CF, the PISR was higher in PI-CF compared to PS-CF and controls during ~230 mg/dL hyperglycemic clamp conditions ($P < 0.05$ for both; Table 3). These differences persisted after exclusion of CF subjects treated with ivacaftor.

**β-cell sensitivity to glucose & insulin sensitivity during the GPA test**

$\text{PG}_{50}$, a measure of β-cell sensitivity to glucose, was not different amongst PS-CF, PI-CF, and control participants (125 (101-142) vs. 158 (131-193) vs. 148 (128-175); Fig. 2A). The glucose infusion rate ($M$), second phase insulin level ($I$), and the resulting estimates of insulin sensitivity ($M/I$; Fig. 2B) from the 230 mg/dL clamp were not different across the groups.

**Correlations between OGTT glucose and acute islet responses on GPA test**

The OGTT 1-hour glucose was negatively correlated with $\text{AIR}_{\text{pot}}$ (rho = -0.43, $P = 0.02$), $\text{ACR}_{\text{arg}}$ (rho = -0.37, $P = 0.04$), $\text{ACR}_{\text{pot}}$ (rho = -0.41, $P = 0.03$), $\text{ACR}_{\text{max}}$ (rho = -0.40, $P = 0.03$), in all subjects. In PI-CF subjects, the OGTT 1-hour glucose was negatively correlated with $\text{AIR}_{\text{pot}}$ (rho = -0.65, $P = 0.03$), $\text{ACR}_{\text{max}}$ (rho = -0.67, $P = 0.02$), and $\text{APR}_{\text{pot}}$ (rho = -0.61, $P = 0.05$) and trended towards negative correlation with $\text{AIR}_{\text{arg}}$, $\text{AIR}_{\text{max}}$, $\text{ACR}_{\text{arg}}$, $\text{ACR}_{\text{pot}}$ ($P < 0.1$ for all). Similar correlations were not seen between either fasting glucose or 2-hour glucose and the acute β-cell responses. Acute glucagon responses did not significantly correlate with OGTT glucose.
Insulin and incretin secretion during the MMTT

During the first 30 minutes following meal ingestion, glucose (AUC_{glu}; Fig. 3A and Table 4) was not different across groups. However, insulin secretion (AUC_{ISR}) was lower in PI-CF compared to PS-CF and controls (P < 0.05 for both; Fig. 3B and Table 5), and the AUC_{ISR}/AUC_{glu} ratio was lower in PI-CF compared to PS-CF and controls (P < 0.01 for both; Table 5). GLP-1 (AUC_{GLP}) and GIP (AUC_{GIP}) were lower in PI-CF compared to PS-CF (P < 0.05 for both; Fig. 3E, F and Table 5).

Over the 180 minutes, AUC_{glu} was higher in PI-CF compared to controls (P < 0.01, Fig. 3A and Table 5) and AUC_{GIP} was lower (P < 0.01, Figure 3F and Table 5).

The oral minimal model derived $S_I$ was lower in PS-CF compared to controls (5.4 (3.9-7.3) vs. 14.1 (8.7-19.3) × 10^{-4} [(µU/mL)⁻¹·min⁻¹]; P = 0.005) but was not different for PI-CF compared to PS-CF or controls (9.8 (4.6-12.7) × 10^{-4} [(µU/mL)⁻¹·min⁻¹]; P = 0.16 & 0.13, respectively).

Discussion

This is the first study to assess β-cell secretory capacity and insulin secretory rates in PI-CF individuals with normal glucose tolerance and comparable healthy and disease controls. Our data suggest that PI-CF adolescents and adults, despite exhibiting “normal” glucose tolerance as defined by standard measures of glucose homeostasis, have impaired insulin and incretin secretion. The decreased β-cell secretory capacity and incretin secretion abnormalities likely explain impaired early phase insulin response and post-prandial hyperglycemia following meal ingestion, but their direct contribution to declines in CF outcomes has yet to be determined.
While all subjects included in this study met CFF criteria (6) for normal glucose tolerance (1-hour glucose < 200 and 2-hour glucose < 140 mg/dL), the 1-hour glucose was significantly higher within the “normal” range in the PI-CF group. Despite returning to normal glucose within two hours post OGTT, this same group also had both the highest post-prandial glucose during the MMTT and higher glucose and glucose variability during CGM under everyday life conditions. A higher 1-hour glucose many be clinically relevant, as glucose concentrations as low as 155 mg/dl are associated with increased risk of developing type 2 diabetes (22,23), and in CF, a 1-hour glucose as low as 160 mg/dL during an OGTT is associated with a 4-5 fold increased risk for developing CFRD over the subsequent 5 years (24). The correlation between acute insulin and C-peptide responses and 1-hour glucose seen here suggests that this measure may be useful for identifying PI-CF patients with impaired β-cell secretory capacity, who as a consequence are at higher risk for the development of diabetes.

While CFRD is associated with worse nutritional status and pulmonary function, declines in these CF outcomes occur within the several years prior to CFRD development (2). These declines suggest that the early insulin secretion defects demonstrated here are indeed clinically relevant. Potentially compounding insulin deficiency is impaired suppression of protein catabolism that has been reported at least in the setting of CFRD (25). Indeed lean body mass correlates with pulmonary function in CF, higher 1-hour glucose is associated with worse lung function (24), and a lower 1-hour insulin is associated with lower BMI% (26)

Little is known about the function of the endocrine pancreas in CF prior to the development of glucose intolerance. Neither the onset of nor the mechanisms underlying the earliest insulin secretion abnormalities can be gleaned from an OGTT. Therefore, the more sophisticated and sensitive techniques of glucose-potentiated arginine and deconvolution of C-
peptide were employed to identify differences in β-cell secretory capacity and insulin secretory rates in adolescents and young adults with “normal” glucose tolerance based on CFF criteria. β-cell secretory capacity derived from glucose-potentiation of arginine-induced insulin secretion represents the best in vivo estimate of functional β-cell mass (27). Here, we report data that indicate a reduced functional β-cell mass is a key early defect responsible for the future risk of diabetes in PI-CF. Previous studies have shown that subjects with PI-CF demonstrate impaired β-, α-, and pancreatic polypeptide cell function compared to PS-CF and normal (28-30) and that the β-cell defect is worse in the setting of overtly impaired or diabetic glucose tolerance (30-32). These earlier reports, however, did not exclude PI-CF subjects with indeterminate glucose tolerance (1-hour ≥ 200 mg/dL) and employed surrogate measures of insulin secretion and secretory capacity, and so have not addressed the initial mechanisms preceding the development of impaired glucose tolerance in CF.

The confirmation of decreased β-cell secretory capacity with normal β-cell sensitivity to glucose (PG_{50}) supports the hypothesis that the impaired insulin responses are at least partly a consequence of reduced β-cell mass, rather than a defect in the functional response of the β-cell. Decreased β-cell mass may be explained by islet loss in PI-CF due to extension of pancreatic exocrine fibrosis (33-35), disruption and loss of pancreatic vascularity (4), and/or related effects on pancreatic islet progenitor cells that preclude the development of a “normal” islet mass. Previous work supports a close correlation of pancreatic insufficiency and the development of CFRD (36). Such hypothesized “collateral damage” effects are anticipated to affect non-β islet cells as well, and indeed, here we report lower glucagon responses in PI-CF participants but preserved glucagon responses in PS-CF; these data are consistent with generalized islet loss present in those with pancreatic insufficiency. That the reduced functional β-cell mass in PI-CF
is experiencing a relative increased demand for insulin secretion is supported by the increased fasting proinsulin-to-C-peptide and proinsulin secretory ratios during hyperglycemia in PI-CF. Increased β-cell secretory demand results in the recruitment of immature secretory granules containing an abundance of incompletely processed proinsulin; however, the contribution of defective CFTR functioning on post-translational processing of proinsulin may also be possible.

A direct role for CFTR in islet function has been demonstrated in the ferret and pig models of CF (37,38). In mice, attenuation of glucose stimulated insulin secretion is present in F508del CFTR mice compared with wild-type mice, and VX-809 (lumacaftor), a corrector of F508del trafficking, can reverse this dampened response (39). Moreover, CFTR may be important for β-cell recovery from injury (40) as likely occurs during the fibrosis of the pancreas and development of PI. Reported improvement in glycemic status affecting patients with at least one of ten rare mutations treated with the CFTR modulator, ivacaftor, are prompting further human investigations of the underlying mechanisms of possible CFTR effects in the islet (41,42). Three subjects (two with PS-CF and one with PI-CF) in the present study were receiving ivacaftor therapy for approved clinical indications. Such treatment may have affected insulin secretion results (Supplemental figure), but excluding these subjects from the analyses did not affect the significance of the impaired insulin and incretin secretion comparisons for the PI-CF group.

Disruption of the enteroinsular axis, which is important in post-prandial glucose homeostasis, is hypothesized to contribute to the delayed insulin response and post-prandial hyperglycemia in CF. Active and total levels of postprandial GLP-1 and GIP have been studied in PI-CF with conflicting results (5,32,43), although pancreatic enzyme supplementation improves secretion of these incretins in PI-CF (44). All PI-CF subjects took pancreatic enzymes
before the MMTT, yet our PI-CF subjects exhibited lower early incretin responses than those with PS-CF, and over 180 minutes the AUC$_{\text{GIP}}$ was impaired in our PI-CF group. These data are consistent with earlier work in young adults (5). Thus, defects in the enteroinsular axis likely contribute to post-prandial hyperglycemia in CF.

Insulin resistance in the absence of systemic glucocorticoid treatment or illness, is not a prominent feature during the development of CFRD (45), and assessment of peripheral insulin sensitivity (M/I during GPA testing and S$_{I}$ during the MMTT) in our NGT individuals with PI-CF did not reveal insulin sensitivity that was different than controls. As all subjects were free of a recent pulmonary exacerbation and not receiving glucocorticoids, decreased insulin sensitivity is unlikely to have confounded our insulin secretion measures. Nevertheless, periods of previous illness and glucocorticoid exposure may have contributed to increased β-cell demand prior to the time of study that may have affected the β-cell secretory capacity. Curiously, the PS-CF group had increased β-cell secretory responses under fasting conditions and higher post-prandial glucose and insulin secretion than healthy controls of comparable age, sex, and BMI; these findings are suggestive of impaired insulin sensitivity with compensatory increased insulin secretion. Exclusion of the one obese subject with a BMI of 33 kg/m$^2$ attenuated the difference in acute insulin and C-peptide responses between the PS-CF group and controls, but did not affect the differences between the PS-CF and PI-CF groups. While insulin sensitivity estimated from the oral minimal model was lower in the PS-CF participants, a previous study utilizing the more sensitive hyperinsulinemic euglycemic clamp did not identify insulin resistance in PS-CF (46).

In conclusion, patients with PI-CF who demonstrate strictly normal glucose tolerance defined by standard OGTT criteria manifest decreased insulin and incretin secretion, disproportionately increased proinsulin secretion during hyperglycemia, and higher post-prandial
glucose excursions than PS-CF and otherwise healthy individuals without CF. Given these defects in glucose regulation and in insulin and incretin secretion, introduction of interventions that enhance incretin effects or reduce β-cell demand early in the development of glucose abnormalities, for example when the 1-hour glucose during an OGTT becomes “elevated,” may be beneficial in the prevention of or delay in progression to CFRD.
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**Author Contributions.** S.S. and L.G. participated in the conduct of the study, researched and analyzed data, and wrote the manuscript, D.D.D. and D.H. contributed to the design, researched data, and revised the manuscript critically for important intellectual content, C.K., N.K.R., S.N., A.P., and S.M. participated in the conduct of the study, researched data, and reviewed/edited the manuscript, D.S. researched and analyzed data, and reviewed/edited the manuscript, M.C. researched data and reviewed/edited the manuscript, R.C.R., A.K., and M.R.R. designed and
conducted the study, researched and analyzed data, and wrote the manuscript. A.K. and M.R.R. are the guarantors of this work and, as such, take full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.
References

1. Moran, A., Dunitz, J., Nathan, B., Saeed, A., Holme, B., and Thomas, W. (2009) Cystic fibrosis-related diabetes: current trends in prevalence, incidence, and mortality. *Diabetes Care* **32**, 1626-1631

2. Moran, A., Becker, D., Casella, S. J., Gottlieb, P. A., Kirkman, M. S., Marshall, B. C., Slovis, B., and Committee, C. C. C. (2010) Epidemiology, pathophysiology, and prognostic implications of cystic fibrosis-related diabetes: a technical review. *Diabetes Care* **33**, 2677-2683

3. Marshall, B. C., Butler, S. M., Stoddard, M., Moran, A. M., Liou, T. G., and Morgan, W. J. (2005) Epidemiology of cystic fibrosis-related diabetes. *J Pediatr* **146**, 681-687

4. Kuo, P., Stevens, J. E., Russo, A., Maddox, A., Wishart, J. M., Jones, K. L., Greville, H., Hetzel, D., Chapman, I., Horowitz, M., and Rayner, C. K. (2011) Gastric emptying, incretin hormone secretion, and postprandial glycemia in cystic fibrosis--effects of pancreatic enzyme supplementation. *J Clin Endocrinol Metab* **96**, E851-855

5. Moran, A., Brunzell, C., Cohen, R. C., Katz, M., Marshall, B. C., Onady, G., Robinson, K. A., Sabadosa, K. A., Scencko, A., Slovis, B., and Committee, C. G. (2010) Clinical care guidelines for cystic fibrosis-related diabetes: a position statement of the American Diabetes Association and a clinical practice guideline of the Cystic Fibrosis Foundation, endorsed by the Pediatric Endocrine Society. *Diabetes Care* **33**, 2697-2708

6. Dobson, L., Sheldon, C. D., and Hattersley, A. T. (2004) Conventional measures underestimate glycaemia in cystic fibrosis patients. *Diabet Med* **21**, 691-696

7. Farrell, P. M., Rosenstein, B. J., White, T. B., Accurso, F. J., Castellani, C., Cutting, G. R., Durie, P. R., Legrys, V. A., Massie, J., Parand, R. B., Rock, M. J., Campbell, P. W., 3rd, and Cystic Fibrosis, F. (2008) Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr* **153**, S4-S14

8. Loser, C., Mollgaard, A., and Folsch, U. R. (1996) Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. *Gut* **39**, 580-586

9. Rickels, M. R., Goeser, E. S., Fuller, C., Lord, C., Bowler, A. M., Doliba, N. M., Hegele, R. A., and Cuchel, M. (2015) Loss-of-function mutations in ABCA1 and enhanced beta-cell secretory capacity in young adults. *Diabetes* **64**, 193-199

10. Seaquist, E. R., and Robertson, R. P. (1992) Effects of hemipancreatectomy on pancreatic alpha and beta cell function in healthy human donors. *J Clin Invest* **89**, 1761-1766

11. Guldstrand, M., Ahren, B., and Adamson, U. (2003) Improved beta-cell function after standardized weight reduction in severely obese subjects. *Am J Physiol Endocrinol Metab* **284**, E557-565

12. Gudipaty, L., Rosenfeld, N. K., Fuller, C. S., Gallop, R., Schutta, M. H., and Rickels, M. R. (2014) Effect of exenatide, sitagliptin, or gliimipiride on beta-cell secretory capacity in early type 2 diabetes. *Diabetes Care* **37**, 2451-2458

13. Ward, W. K., Halter, J. B., Beard, J. C., and Porte, D., Jr. (1984) Adaptation of B and A cell function during prolonged glucose infusion in human subjects. *Am J Physiol* **246**, E405-411
15. Larsson, H., and Ahren, B. (1998) Glucose-dependent arginine stimulation test for characterization of islet function: studies on reproducibility and priming effect of arginine. *Diabetologia* **41**, 772-777

16. Vollmer, K., Holst, J. J., Baller, B., Ellrichmann, M., Nauck, M. A., Schmidt, W. E., and Meier, J. J. (2008) Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. *Diabetes* **57**, 678-687

17. Dobson, L., Sheldon, C. D., and Hattersley, A. T. (2003) Validation of interstitial fluid continuous glucose monitoring in cystic fibrosis. *Diabetes Care* **26**, 1940-1941

18. Loopstra-Masters, R. C., Haffner, S. M., Lorenzo, C., Wagenknecht, L. E., and Hanley, A. J. (2011) Proinsulin-to-C-peptide ratio versus proinsulin-to-insulin ratio in the prediction of incident diabetes: the Insulin Resistance Atherosclerosis Study (IRAS). *Diabetologia* **54**, 3047-3054

19. Horwitz, D. L., Starr, J. I., Mako, M. E., Blackard, W. G., and Rubenstein, A. H. (1975) Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest* **55**, 1278-1283

20. Polonsky, K. S., Given, B. D., Hirsch, L., Shapiro, E. T., Tillil, H., Beebe, C., Galloway, J. A., Frank, B. H., Karrison, T., and Van Cauter, E. (1988) Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* **81**, 435-441

21. Dalla Man, C., Caumo, A., Basu, R., Rizza, R., Toffolo, G., and Cobelli, C. (2004) Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method. *Am J Physiol Endocrinol Metab* **287**, E637-643

22. Abdul-Ghani, M. A., Abdul-Ghani, T., Ali, N., and Defronzo, R. A. (2008) One-hour plasma glucose concentration and the metabolic syndrome identify subjects at high risk for future type 2 diabetes. *Diabetes Care* **31**, 1650-1655

23. Succurro, E., Marini, M. A., Arturi, F., Grembiale, A., Lugara, M., Andreozzi, F., Sciacqua, A., Lauro, R., Hribal, M. L., Perticone, F., and Sesti, G. (2009) Elevated one-hour post-load plasma glucose levels identifies subjects with normal glucose tolerance but early carotid atherosclerosis. *Atherosclerosis* **207**, 245-249

24. Sheikh, S., Putt, M. E., Forde, K. A., Rubenstein, R. C., and Kelly, A. (2015) Elevation of one hour plasma glucose during oral glucose tolerance testing. *Pediatr Pulmonol* **50**, 963-969

25. Moran, A., Milla, C., Ducret, R., and Nair, K. S. (2001) Protein metabolism in clinically stable adult cystic fibrosis patients with abnormal glucose tolerance. *Diabetes* **50**, 1336-1343

26. Coriati, A., Ziai, S., Lavoie, A., Berthiaume, Y., and Rabasa-Lhoret, R. (2016) The 1-h oral glucose tolerance test glucose and insulin values are associated with markers of clinical deterioration in cystic fibrosis. *Acta Diabetol* **53**, 359-366

27. Robertson, R. P., Bogachus, L. D., Oseid, E., Parazzoli, S., Patti, M. E., Rickels, M. R., Schuetz, C., Dunn, T., Pruett, T., Balamurugan, A. N., Sutherland, D. E., Beilman, G., and Bellin, M. D. (2015) Assessment of beta-cell mass and alpha- and beta-cell survival and function by arginine stimulation in human autologous islet recipients. *Diabetes* **64**, 565-572

28. Mohan, V., Alagappan, V., Snehalatha, C., Ramachandran, A., Thiruvengadam, K. V., and Viswanathan, M. (1985) Insulin and C-peptide responses to glucose load in cystic fibrosis. *Diabet Med* **11**, 376-379
29. Moran, A., Diem, P., Klein, D. J., Levitt, M. D., and Robertson, R. P. (1991) Pancreatic endocrine function in cystic fibrosis. *J Pediatr* **118**, 715-723

30. Lanng, S., Thorsteinsson, B., Roder, M. E., Orskov, C., Holst, J. J., Nerup, J., and Koch, C. (1993) Pancreas and gut hormone responses to oral glucose and intravenous glucagon in cystic fibrosis patients with normal, impaired, and diabetic glucose tolerance. *Acta Endocrinol (Copenh)* **128**, 207-214

31. Elder, D. A., Wooldridge, J. L., Dolan, L. M., and D’Alessio, D. A. (2007) Glucose tolerance, insulin secretion, and insulin sensitivity in children and adolescents with cystic fibrosis and no prior history of diabetes. *J Pediatr* **151**, 653-658

32. Anzeneder, L., Kircher, F., Feghelm, N., Fischer, R., and Seissler, J. (2011) Kinetics of insulin secretion and glucose intolerance in adult patients with cystic fibrosis. *Horm Metab Res* **43**, 355-360

33. Iannucci, A., Mukai, K., Johnson, D., and Burke, B. (1984) Endocrine pancreas in cystic fibrosis: an immunohistochemical study. *Hum Pathol* **15**, 278-284

34. Abdul-Karim, F. W., Dahms, B. B., Velasco, M. E., and Rodman, H. M. (1986) Islets of Langerhans in adolescents and adults with cystic fibrosis. A quantitative study. *Arch Pathol Lab Med* **110**, 602-606

35. Soejima, K., and Landing, B. H. (1986) Pancreatic islets in older patients with cystic fibrosis with and without diabetes mellitus: morphometric and immunocytologic studies. *Pediatr Pathol* **6**, 25-46

36. Adler, A. I., Shine, B. S., Chamnan, P., Haworth, C. S., and Bilton, D. (2008) Genetic determinants and epidemiology of cystic fibrosis-related diabetes: results from a British cohort of children and adults. *Diabetes Care* **31**, 1789-1794

37. Olivier, A. K., Yi, Y., Sun, X., Sui, H., Liang, B., Hu, S., Xie, W., Fisher, J. T., Keiser, N. W., Lei, D., Zhou, W., Yan, Z., Li, G., Evans, T. I., Meyerholz, D. K., Wang, K., Stewart, Z. A., Norris, A. W., and Engelhardt, J. F. (2012) Abnormal endocrine pancreas function at birth in cystic fibrosis ferrets. *J Clin Invest* **122**, 3755-3768

38. Uc, A., Olivier, A. K., Griffin, M. A., Meyerholz, D. K., Yao, J., Abu-El-Haija, M., Buchanan, K. M., Vanegas Calderon, O. G., Abu-El-Haija, M., Pezzulo, A. A., Reznikov, L. R., Hoegger, M. J., Rector, M. V., Ostedgaard, L. S., Taft, P. J., Gansemer, N. D., Ludwig, P. S., Hornick, E. E., Stoltz, D. A., Ode, K. L., Welsh, M. J., Engelhardt, J. F., and Norris, A. W. (2015) Glycaemic regulation and insulin secretion are abnormal in cystic fibrosis pigs despite sparing of islet cell mass. *Clin Sci (Lond)* **128**, 131-142

39. Guo, J. H., Chen, H., Ruan, Y. C., Zhang, X. L., Zhang, X. H., Fok, K. L., Tsang, L. L., Yu, M. K., Huang, W. Q., Sun, X., Chung, Y. W., Jiang, X., Sohma, Y., and Chan, H. C. (2014) Glucose-induced electrical activities and insulin secretion in pancreatic islet beta-cells are modulated by CFTR. *Nat Commun* **5**, 4420

40. Stalvey, M. S., Muller, C., Schatz, D. A., Wasserfall, C. H., Campbell-Thompson, M. L., Theriaque, D. W., Flotte, T. R., and Atkinson, M. A. (2006) Cystic fibrosis transmembrane conductance regulator deficiency exacerbates islet cell dysfunction after beta-cell injury. *Diabetes* **55**, 1939-1945

41. Bellin, M. D., Laguna, T., Leschyshyn, J., Regelmann, W., Dunitz, J., Billings, J., and Moran, A. (2013) Insulin secretion improves in cystic fibrosis following ivacaftor correction of CFTR: a small pilot study. *Pediatr Diabetes* **14**, 417-421

42. Hayes, D., Jr., McCoy, K. S., and Sheikh, S. I. (2014) Resolution of cystic fibrosis-related diabetes with ivacaftor therapy. *Am J Respir Crit Care Med* **190**, 590-591
43. Ross, S. A., Morrison, D., and McArthur, R. G. (1981) Hypersecretion of gastric inhibitory polypeptide in nondiabetic children with cystic fibrosis. *Pediatrics* **67**, 252-254

44. Perano, S. J., Couper, J. J., Horowitz, M., Martin, A. J., Kritas, S., Sullivan, T., and Rayner, C. K. (2014) Pancreatic enzyme supplementation improves the incretin hormone response and attenuates postprandial glycemia in adolescents with cystic fibrosis: a randomized crossover trial. *J Clin Endocrinol Metab* **99**, 2486-2493

45. Mohan, K., Miller, H., Dyce, P., Grainger, R., Hughes, R., Vora, J., Ledson, M., and Walshaw, M. (2009) Mechanisms of glucose intolerance in cystic fibrosis. *Diabet Med* **26**, 582-588

46. Moran, A., Pyzdrowski, K. L., Weinreb, J., Kahn, B. B., Smith, S. A., Adams, K. S., and Seaquist, E. R. (1994) Insulin sensitivity in cystic fibrosis. *Diabetes* **43**, 1020-1026
Figure 1— Islet cell hormone levels in response to bolus administration of arginine (arrows) under fasting, ~230 mg/dL, and ~340 mg/dL hyperglycemic clamp conditions in pancreatic sufficient cystic fibrosis (PS-CF, closed circles), pancreatic insufficient cystic fibrosis (PI-CF, open circles) and healthy control subjects (normal range given as the 95% CI and shown as grey shaded area). CF subject data are represented at mean ± SE. * denotes $P < 0.05$; ** denotes $P < 0.01$.

Figure 2— A, Acute insulin responses (AIRs) to arginine as a function of the pre-stimulus plasma glucose concentration in pancreatic sufficient cystic fibrosis (PS-CF, closed circles), pancreatic insufficient cystic fibrosis (PI-CF, open circles), and healthy controls (normal, open triangles). Data are given as mean ± SE. The glucose-potentiation slope (GPS), calculated as the difference in the AIR at fasted and ~230 mg/dL glucose levels divided by the difference in plasma glucose, is impaired in PI-CF vs. both PS-CF and normal (0.3±0.2 vs. 1.0±0.6 & 0.7±0.4, $P = 0.002$ denoted with **). β-cell sensitivity to glucose is determined as the $PG_{50}$, the plasma glucose level at which half maximal insulin release was achieved, using the y-intercept (b) of the GPS to solve the equation $AIR_{max}/2 = GPS \times PG_{50} + b$, and was not different across groups. B, Box plot of insulin sensitivity (M/I) by study group, given as median and interquartile range (box), and mean (open squares) and range (error bars), is similar across PS-CF, PI-CF, and normal control subjects.

Figure 3— Glucose, insulin secretory rate (ISR), GLP-1 and GIP levels during the 4-hour mixed-meal tolerance test in pancreatic sufficient cystic fibrosis (PS-CF, closed circles), pancreatic insufficient cystic fibrosis (PI-CF, open circles), and healthy control subjects (normal range given as the 95% CI and shown as grey shaded area). CF subject data are given as mean ± SE.
|                          | GPA controls (n=11) | MMTT controls (n=10) | PS-CF (n=9) | PI-CF (n=11) | Overall P-value* |
|--------------------------|---------------------|----------------------|-------------|-------------|-----------------|
|                          | Controls vs. PI-CF  | Controls vs. PS-CF   | PI-CF vs. PS-CF |             |                 |
| Demographics             |                     |                      |              |             |                 |
| Sex (female)             | 5 (45)              | 5 (50)               | 6 (67)       | 5 (45)      | 0.80            |
| Age (years)              | 25 (21-38)          | 25 (20-41)           | 31 (16-56)   | 19 (16-50)  | 0.13            |
| BMI (kg/m²)              | 24 (21-29)          | 24 (18-27)           | 24 (19-33)   | 22 (17-31)  | 0.64            |
| HbA₁c (%)                | N/D                 | 5.0 (4.7-5.5)        | 5.2 (3.8-6.0)| 5.5 (5.3-6.2)| 0.0006 | 0.34 | 0.06 |
| OGTT Profile             |                     |                      |              |             |                 |
| Fasting glucose (mg/dL)  | 82 (74-107)         | 85 (68-94)           | 87 (74-102) | 92 (73-98)  | 0.18            |
| 1-hour glucose (mg/dL)   | 122 (70-199)        | 114 (60-157)         | 118 (67-165) | 162 (127-193)| 0.001³ | 0.01 | 0.009 |
| 2-hour glucose (mg/dL)   | 95 (60-128)         | 94 (74-111)          | 104 (46-115) | 97 (66-134) | 0.90            |

Data are medians and ranges (min-max) for continuous variables and number and (percentage) for categorical variables.

PS-CF, pancreatic sufficient cystic fibrosis; PI-CF, pancreatic insufficient cystic fibrosis; GPA, glucose-potentiated arginine; MMTT, mixed-meal tolerance test; N/D, not done; OGTT, oral glucose tolerance test.

*Between group comparisons performed when overall P-value was significant at \( P \leq 0.05 \)

³To convert to mmol/mol, multiply by 10.93 and subtract 23.50.

³³Significant differences between PI-CF and MMTT controls.
| CGMS Measure               | Controls (n=6) | PS-CF (n=7)   | PI-CF (n=10) | Overall P-value* |
|----------------------------|----------------|---------------|--------------|-----------------|
|                            |                |               |              | Controls vs. PI-CF | Controls vs. PS-CF | PS-CF vs. PI-CF |
| Mean Glucose (mg/dL)       | 92 (88-96)     | 112 (96-122)  | 111 (104-121)| 0.009           |
|                            |                |               |              | 0.002 | 0.03 | 0.70 |
| Glucose SD (mg/dL)         | 14 (13-15)     | 14 (11-21)    | 22 (18-28)   | 0.001           |
|                            |                |               |              | 0.004 | 0.7  | 0.06 |
| Glucose CV                 | 0.14 (0.13-0.17)| 0.13 (0.11-0.19)| 0.19 (0.17-0.21)| 0.06           |
| % time > 140 mg/dL         | 0 (0-0)        | 5 (0-15)      | 10 (7-13)    | 0.006           |
|                            |                |               |              | 0.001 | 0.09 | 0.22 |
| % time > 180 mg/dL         | 0 (0-0)        | 0 (0-1)       | 2 (0-4)      | 0.06            |

Data are medians and interquartile ranges (IQR). PS-CF, pancreatic sufficient cystic fibrosis; PI-CF, pancreatic insufficient cystic fibrosis; CGMS, continuous glucose monitoring system; SD, standard deviation; CV, coefficient of variation.

*Between group comparisons performed when overall P-value was significant at $P \leq 0.05$
Table 3— Fasting islet cell hormones, acute responses, proinsulin to C-peptide and proinsulin secretory ratios (PISRs) during the glucose-potentiated arginine (GPA) test.

|                  | Controls (n=11) | PS-CF (n=9) | PI-CF (n=11) | Overall P-value* |
|------------------|----------------|-------------|--------------|-----------------|
| **Fasting insulin** (µU/mL) | 7.4 (6.8-10.4) | 8.8 (6.6-9.1) | 9.1 (6.2-10.4) | 0.85            |
| **AIR$_{arg}$** (µU/mL) | 19.2 (13.7-46.3) | 41.3 (36.4-49.8) | 19.6 (13.9-31.3) | 0.01            |
| **AIR$_{pot}$** (µU/mL) | 99.8 (82.6-128.7) | 162.3 (125.2-227.2) | 56.1 (42.9-94.7) | 0.002           |
| **AIR$_{max}$** (µU/mL) | 120.4 (91.7-190.2) | 116.7 (83.7-233.8) | 75.6 (52.4-143.0) | 0.13            |
| **Fasting C-peptide** (ng/mL) | 1.3 (1.00-1.8) | 1.6 (1.0-1.8) | 1.2 (0.8-1.2) | 0.70            |
| **ACR$_{arg}$** (ng/mL) | 1.2 (0.9-2.2) | 2.0 (1.6-2.6) | 0.8 (0.5-1.2) | 0.004           |
| **ACR$_{pot}$** (ng/mL) | 4.9 (4.3-5.5) | 6.7 (4.4-7.2) | 2.2 (1.5-3.4) | 0.003           |
| **ACR$_{max}$** (ng/mL) | 6.0 (3.8-6.9) | 5.5 (3.6-7.0) | 3.2 (1.5-4.4) | 0.03            |
| **Fasting glucagon** (pg/mL) | 62 (53-74) | 47 (43-60) | 48 (25-62) | 0.09            |
| **AGR$_{arg}$** (pg/mL) | 73 (39-94) | 62 (51-74) | 29 (17-38) | 0.008           |
| **AGR$_{inh}$** (pg/mL) | 42 (33-53) | 33 (28-51) | 16 (15-30) | 0.015           |
| **AGR$_{min}$** (pg/mL) | 33 (27-51) | 37 (19-43) | 20 (8-29) | 0.03            |
| **Fasting proinsulin** (pmol/L) | 8.6 (2.4-22.8) | 10.8 (2.0-13.1) | 11.7 (8.6-21.6) | 0.18            |
| **APR$_{arg}$** (pmol/L) | 5.3 (3.2-9.6) | 7.1 (6.0-11.9) | 2.7 (0.8-6.4) | 0.07            |
| **APR$_{pot}$** (pmol/L) | 23.1 (18.2-30.9) | 29.9 (17.7-33.5) | 18.7 (17.6-22.4) | 0.15            |
| **APR$_{max}$** (pmol/L) | 22.2 (15.2-26.7) | 19.0 (13.1-19.5) | 16.8 (13.1-19.5) | 0.20            |
| **Fasting proinsulin: C-peptide ratio (%)** | 2.3 (1.2-2.5) | 1.9 (1.7-2.4) | 3.4 (2.9-4.0) | 0.04            |
| **PISR, fasting (%)** | 1.3 (1.0-1.6) | 1.1 (1.0-1.6) | 1.1 (0.3-1.7) | 0.90            |
| **PISR, 230 mg/dL (%)** | 1.3 (1.2-1.6) | 1.4 (0.8-1.6) | 2.5 (1.3-3.4) | 0.04            |
| **PISR, 340 mg/dL (%)** | 1.2 (1.0-1.3) | 0.85 (0.8-1.3) | 1.4 (1.3-3.0) | 0.09            |

Data are medians and interquartile ranges (IQR). PS-CF, pancreatic sufficient cystic fibrosis; PI-CF, pancreatic insufficient cystic fibrosis.

*Between group comparisons performed when overall P-value was significant at $P \leq 0.05$.
Table 4— Mixed-meal tolerance test (MMTT) responses during the first 30 and 180 minutes post-ingestion

|                      | MMTT Controls (n=10) | PS-CF (n=8) | PI-CF (n=11) | Overall P-value* |
|----------------------|----------------------|-------------|--------------|------------------|
|                      |                      |             |              | Controls vs. PI-CF | Controls vs. PS-CF | PS-CF vs. PI-CF |
| 30 minutes           |                      |             |              |                  |                   |                |
| AUC<sub>glu</sub> (mg·min/dL) | 540 (276-729) | 447 (272-521) | 462 (268-509) | 0.60             |                   |                |
| AUC<sub>ISR</sub> (pmol/l) | 3041 (1459-4721) | 2857 (1950-4201) | 1033 (200-1677) | 0.03 | 0.03 | 0.02 |
| AUC<sub>ISR</sub>/AUC<sub>glu</sub> (mU·min/mg) | 0.09 (0.07-0.12) | 0.16 (0.10-0.17) | 0.05 (0.03-0.05) | 0.60 | 0.007 | 0.007 |
| AUC<sub>GLP-1</sub> (pmol·min/L) | 32 (21-81) | 91 (52-121) | 16 (10-71) | 0.32 | 0.05 | 0.02 |
| AUC<sub>GIP</sub> (pg·min/mL) | 1367 (734-1838) | 1900 (1048-2354) | 747 (355-1195) | 0.11 | 0.34 | 0.02 |
| 180 minutes           |                      |             |              |                  |                   |                |
| AUC<sub>glu</sub> (mg·min/dL) | 506 (-145-2958) | 3501 (2707-5123) | 5075 (2836-7989) | 0.004 | 0.02 | 0.46 |
| AUC<sub>ISR</sub> (pmol/l) | 25917 (23659-28693) | 53375 (47607-58495) | 26156 (23176-49651) | 0.60 | 0.0004 | 0.08 |
| AUC<sub>ISR</sub>/AUC<sub>glu</sub> (mU·min/mg) | 0.13 (-0.93-0.19) | 0.26 (0.15-0.35) | 0.13 (0.08-0.16) | 0.13 |                   |                |
| AUC<sub>GLP-1</sub> (pmol·min/L) | 395 (278-589) | 577 (230-738) | 216 (103-494) | 0.27 |                   |                |
| AUC<sub>GIP</sub> (pg·min/mL) | 34702 (28526-40934) | 30586 (23471-45227) | 18721 (11502-28675) | 0.006 | 0.42 | 0.03 |

Data are medians and interquartile ranges (IQR). PS-CF, pancreatic sufficient cystic fibrosis; PI-CF, pancreatic insufficient cystic fibrosis; AUC, incremental area under the curve for glucose (glu), insulin secretory rates (ISR), glucagon (gln), free fatty acids (FFA), glucagon like peptide-1 (GLP), and gastric inhibitory peptide (GIP).

<sup>a</sup>n=9 for AUC<sub>ISR</sub> and AUC<sub>glu</sub>/AUC<sub>ISR</sub>.

<sup>*</sup>Between group comparisons performed when overall P-value was significant at P ≤ 0.05.
**FIGURE 2**

**A**

- **ACUTE INSULIN RESPONSE**
  - PANCREATIC SUFFICIENT
  - PANCREATIC INSUFFICIENT
  - NORMAL

**PLASMA GLUCOSE (mg/dL)**

**INSULIN SENSITIVITY**

- **INSULIN SENSITIVITY**
  - (mg·kg⁻¹·min⁻¹/µU/mL)

**B**

- **PS**
- **PI**
- **NORMAL**
FIGURE 3
**Supplementary Figure**— Acute islet hormone responses to bolus administration of arginine (5 g) under fasting, ~230 mg/dL, and ~340 mg/dL hyperglycemic clamp conditions for individual subjects by study group, pancreatic sufficient cystic fibrosis (PS-CF), pancreatic insufficient cystic fibrosis (PI-CF), and healthy control subjects. CFTR mutations are classified based on F508del status; F508del homozygous (grey triangle), F508del heterozygous (black triangle), other CFTR mutations (open grey circle), and other CFTR mutations on CFTR modulator, ivacaftor (black circles, diagonal pattern).
Supplementary Table — Cystic fibrosis transmembrane (CFTR) mutations in study subjects

| Subject | PS-CF Allele 1 | PS-CF Allele 2 | PI-CF Allele 1 | PI-CF Allele 2 |
|---------|----------------|----------------|----------------|----------------|
| 1       | F508del        | L206W          | 1              | F508del        |
| 2*      | G551D          | unknown        | 2              | F508del        |
| 3       | R709X          | P102IT         | 3*             | G551D          |
| 4       | F508del        | D1152H         | 4              | F508del        |
| 5       | W1282X         | 3849+10kbC->T  | 5              | F508del        |
| 6       | F508del        | 3849+10kbC->T  | 6              | F508del        |
| 7*      | R117H          | W1282X         | 7              | F508del        |
| 8       | F508del        | S549N          | 8              | F508del        |
| 9       | F508del        | unknown        | 9              | F508del        |
| 10      | F508del        | unknown        | 10             | F508del        |
| 11      | F508del        | F508del        |                |                |

PS-CF, pancreatic sufficient cystic fibrosis; PI-CF, pancreatic insufficient cystic fibrosis
*Denotes subjects on CFTR modulator, ivacaftor
