Effects of GLP-1 and GIP on Islet Function in Glucose-Intolerant, Pancreatic-Insufficient Cystic Fibrosis

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Impaired insulin and incretin secretion underlie abnormal glucose tolerance (AGT) in pancreatic insufficient cystic fibrosis (PI-CF). Whether the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) can enhance pancreatic islet function in cystic fibrosis (CF) is not known. We studied 32 adults with PI-CF and AGT randomized to receive either GLP-1 (*n* = 16) or GIP (*n* = 16) during glucose-potentiated arginine (GPA) testing of islet function on two occasions, with either incretin or placebo infused, in a randomized, double-blind, cross-over fashion. Another four adults with PI-CF and normal glucose tolerance (NGT) and four matched control participants without CF underwent similar assessment with GIP. In PI-CF with AGT, GLP-1 substantially augmented second-phase insulin secretion but without effect on the acute insulin response to GPA or the proinsulin secretory ratio (PISR), while GIP infusion did not enhance second-phase or GPA-induced insulin secretion but increased the PISR. GIP also did not enhance second-phase insulin in PI-CF with NGT but did so markedly in control participants without CF controls. These data indicate that GLP-1, but not GIP, augments glucose-dependent insulin secretion in PI-CF, supporting the likelihood that GLP-1 agonists could have therapeutic benefit in this population. Understanding loss of GIP's insulinotropic action in PI-CF may lead to novel insights into diabetes pathogenesis.

Cystic fibrosis (CF) is caused by autosomal recessive inheritance of loss-of-function mutations affecting the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel important to bicarbonate and chloride transport across epithelial membranes. Loss of CFTR function results in multiorgan dysfunction that typically first manifests as exocrine pancreatic insufficiency and, later, impaired respiratory secretion clearance and pulmonary inflammation leading to sinopulmonary infections, deterioration in lung function, and ultimately, respiratory failure. Cystic fibrosis–related diabetes (CFRD) develops in ∼40% of individuals with CF by age 30 years and is associated with up to sixfold greater mortality (1). CFRD primarily develops in individuals with pancreatic-insufficient CF (PI-CF) (2) due to progressive loss of meal-related insulin secretion (3) and is associated with deterioration in nutritional status and pulmonary function that contribute to the increased mortality risk (4).

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Exocrine pancreatic insufficiency and related digestive abnormalities may diminish the actions of the enteroinsular axis, a key mechanism mediating prandial insulin secretion (5,6). Impaired nutrient digestion leads to reduced secretion of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) from intestinal L and K cells, respectively (5,6). GLP-1 and GIP both augment insulin production and glucose-dependent insulin secretion by pancreatic islet β-cells, with reciprocal effects on islet α-cells (i.e., GLP-1 inhibits and GIP stimulates glucagon secretion) (7). Optimization of pancreatic enzyme replacement improves GLP-1 and GIP responses to nutrient ingestion in CF and improves, but does not normalize, postprandial insulin secretion and glucose tolerance (8). However, pancreatic enzyme replacement does not mimic normal exocrine pancreatic physiology, and compliance with pancreatic enzyme replacement is unlikely optimal over a lifetime of multiple daily meal exposures and still does not normalize GIP secretion (8,9) that is more dependent than GLP-1 secretion on fat digestion.

Whether the insulinotropic actions of GLP-1 and GIP are also compromised in individuals with PI-CF is unknown. In type 2 diabetes, β-cell responsiveness to GLP-1 is preserved, but β-cell responsiveness to GIP is lost (10,11), presumably due to hyperglycemia-mediated downregulation of GIP receptor expression (12). Importantly, individuals without CF but with impaired glucose tolerance, although not overt diabetes, maintain β-cell responsiveness to GIP (13). Because functional CFTR is important for islet β-cell, and possibly α-cell, function (14), understanding whether loss of CFTR function may affect islet-cell responsiveness to incretin hormones in PI-CF is important to determine whether the incretin system may be an effective therapeutic target for CFRD prevention or treatment. This study was designed to investigate pancreatic islet β- and α-cell responsiveness to acute administration of either GLP-1 or GIP in PI-CF.

RESEARCH DESIGN AND METHODS

Participants

All participants were aged ≥18 years and had completed a 75-g oral glucose tolerance test (OGTT) within 6 months prior to enrollment. CF diagnosis was confirmed by *CFTR* mutation analysis or positive sweat test per the Cystic Fibrosis Foundation diagnostic criteria (15), and pancreatic insufficiency was confirmed by requirement for pancreatic enzyme supplementation. The initial group of participants studied had PI-CF with abnormal glucose tolerance (AGT) defined by an OGTT as either early glucose intolerance (1-h glucose ≥155 mg/dL, 2-h glucose <140 mg/dL), impaired glucose tolerance (2-h glucose ≥140 mg/dL and <200 mg/dL), or CFRD (2-h glucose ≥200mg/dL or previously confirmed diagnosis) without fasting hyperglycemia (fasting glucose <126 mg/dL) that we have previously associated with impaired β-cell function (3). Individuals having CFRD with fasting hyperglycemia were excluded to ensure participants maintained a sufficient β-cell secretory reserve that could respond to stimulation.

To control for possible effects of hyperglycemia affecting GIP action, additional participants with PI-CF and matched control participants without CF and with normal glucose tolerance (NGT), defined by 1-h glucose <155 mg/dL and 2-h glucose <140 mg/dL, were also studied. Those with PI-CF and NGT were further required to harbor at least one class I, II, or III mutation, and for class III mutations, participants were excluded if receiving they ivacaftor alone or as part of combination CFTR modulator treatment. Additional protocol details are available at ClinicalTrials.gov (identifier: NCT01851694).

The study was approved by the institutional review boards of the University of Pennsylvania and the Children's Hospital of Philadelphia and conducted under an investigational new drug application with the U.S. Food and Drug Administration (IND 117381). All participants provided written informed consent to participate.

Study Design

Eligible patients with PI-CF and AGT were randomly assigned to participate in either the GLP-1 or GIP group (Fig. 1*A*), with randomization stratified by glucose tolerance (early glucose intolerance, impaired glucose tolerance, or CFRD). All participants completed a mixed-meal tolerance test (16), as previously described (9), and, in a randomized, double-blind, cross-over fashion, underwent glucose-potentiated arginine (GPA) testing of islet β- and α-cell function on two occasions with either incretin or placebo infused. Those with PI-CF and NGT and the matched control participants without CF underwent randomized, double-blind, cross-over investigation with GIP only (Fig. 1*B*).

Incretin Administration

Lypophilized GLP-1 (7–36 amide) or GIP (1–42 amide; Bachem AG, Bubendorf, Switzerland) was reconstituted in 0.9% saline containing 0.25% human serum albumin as a 1 μg/mL solution the evening before study. After a 12-h overnight fast, baseline blood samples were taken at 35 and 30 min before incretin administration (i.e., $t = -35$ min and $t = -30$ min, respectively). Then, incretin or matching placebo infusion was initiated, with the alternate condition performed at a subsequent visit between 1 week and 1 month apart. GLP-1 was infused at a rate of 1.5 pmol/kg/min, with a double infusion rate for the first 10 min, from $t = -30$ min until the completion of blood sampling at $t = 60$ min (Fig. 1*C*). This rate of administration has been demonstrated to produce supraphysiologic concentrations of GLP-1 that augment insulin responses in individuals without CF but with type 2 diabetes (17–19). GIP was infused at a rate of 4 pmol/kg/min, with a double infusion rate for the first 10 min, from $t = -30$ min until the completion of blood sampling at $t = 60$ min (Fig. 1*D*–*F*). This rate of administration has been demonstrated to produce supraphysiologic concentrations of GIP that augment

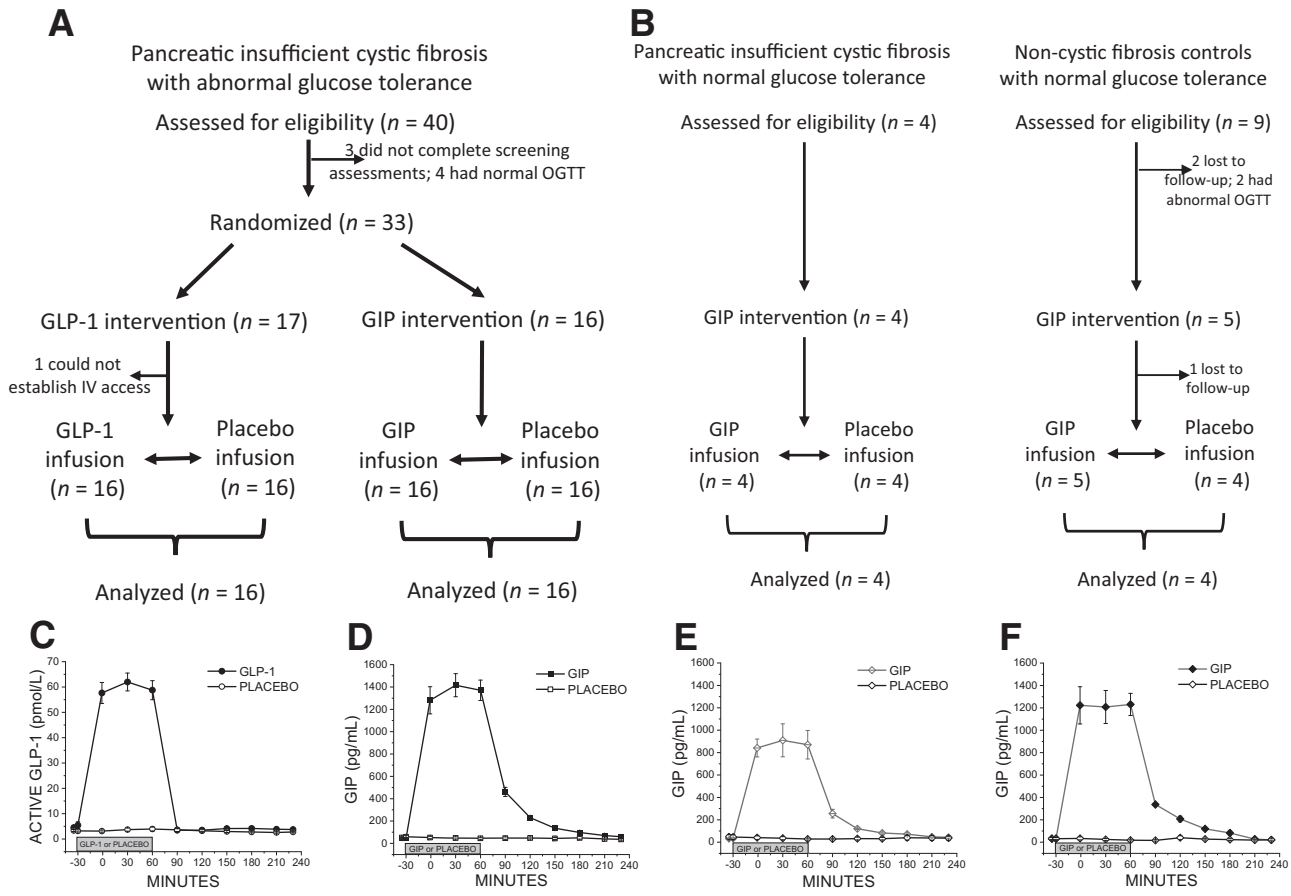

Figure 1—Study design and participant flow. *A*: Eligible participants with PI-CF and AGT were randomized to receive either intervention with either GLP-1 or GIP and underwent GPA testing of islet function on two occasions with either incretin or placebo infused in a randomized, double-blind, cross-over fashion. *B*: Eligible participants with PI-CF and NGT and matched control participants without CF received intervention with GIP and underwent GPA testing with either GIP or placebo infused in a randomized, double-blind, cross-over fashion. Plasma levels of active GLP-1 during infusion of GLP-1 or placebo in participants with PI-CF and AGT (*C*), and plasma levels of GIP during infusion of GIP or placebo in participants with PI-CF and AGT (*D*), in participants with PI-CF and NGT (*E*), and in control participants without CF (*F*). Data are reported as mean ± SE. IV, intravenous.

glucose-dependent insulin secretion in individuals without CF but with impaired glucose tolerance (13).

GPA Test

The GPA test avoids enteric stimulation and so was used during infusion of GLP-1 or GIP and placebo to independently assess the effect of each incretin hormone on glucose-dependent islet β- and α-cell function (20). Prestimulus blood samples were taken at 5 and 1 min before the injection of 5 g of 10% arginine over a 1-min period starting at t = 0. Additional blood samples were collected at 2, 3, 4, and 5 min after injection. Beginning at t = 10 min, a hyperglycemic clamp technique (21) using a variable rate infusion of 20% glucose was performed to achieve a plasma glucose concentration of ∼230 mg/dL. Blood samples were taken every 5 min, centrifuged, and measured in duplicate at bedside with an automated glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH) to adjust the glucose infusion rate and achieve the desired plasma glucose concentration. After 45 min of glucose infusion (at t = 55 min), a 5-g arginine pulse was injected again with identical blood sampling. Subsequently, the infusion of incretin or placebo was stopped. Following a 2-h period without glucose infusion to allow plasma glucose levels to return to baseline, a hyperglycemic clamp was performed to achieve a plasma glucose concentration of ∼340 mg/dL. After 45 min of glucose infusion, a 5-g arginine pulse was injected again with identical blood sampling.

Biochemical Analysis

Blood samples were collected into tubes on ice containing EDTA and a protease inhibitor cocktail, and for the mixed-meal tolerance test, DPP4 inhibitor (Sigma-Aldrich, St. Louis, MO). Samples were centrifuged at 4°C, separated, and frozen at −80°C for subsequent analysis of insulin, C-peptide, glucagon, proinsulin, active GLP-1, total GIP, and free fatty acids, as previously described (9).

Calculations

Second-phase glucose, insulin, and glucagon levels were assessed from the prestimulus levels between 40 and 45 min of the ∼230 mg/dL hyperglycemic clamp. Intravenous glucose tolerance was assessed from the glucose infusion rate (M) required during the ∼230 mg/dL hyperglycemic clamp. Acute insulin, C-peptide, proinsulin, and glucagon responses to arginine (AIR_{arg}, ACR_{arg}, APR_{arg}, and AGR_{arg}, respectively) were determined as the difference in mean of the 2-, 3-, 4-, and 5-min values and the mean of the prestimulus values (21). Glucose potentiation of arginine-induced insulin, C-peptide, and proinsulin (respectively, AIR_{pot}, ACR_{pot}, APR_{pot}) release, and glucose-inhibition of arginine-induced glucagon (AGR_{inh}) release were determined from the acute responses during the ∼230 mg/dL glucose clamp. Acute responses during the ∼340 mg/dL glucose clamp allowed for determination of the maximum arginine-induced insulin (AIR_{max}), C-peptide, and proinsulin release, and of the minimum arginine-induced glucagon release (22). The proinsulin secretory ratio (PISR) in response to each injection to arginine was calculated as the molar ratio of the acute proinsulin and C-peptide response to arginine (9).

Statistical Analysis

Comparisons were made using the nonparametric Wilcoxon signed rank test for paired data and the Mann-Whitney *U* test for independent samples. The primary outcome was second-phase insulin concentration during the 230 mg/dL hyperglycemic clamp. Mixed-effects models were used to test the relative influence of either GLP-1 or GIP versus placebo infusions on the primary and multiple secondary outcomes in PI-CF with AGT groups using time-by-condition, group-by-condition, and time-by-condition-by-group interactions (23). A similar approach was used to assess the relative effect of GIP versus placebo infusion for the group of patients with PI-CF and NGT and control group without CF (24). Analyses were performed using Stata, version 15 (StataCorp LP, College Station, TX). Data are presented as mean ± SD or difference estimates from the mixed-effects models with 95% CIs. Significance was considered at $P < 0.05$ (two-tailed).

Data and Resource Availability

The datasets generated during this study are available from the corresponding authors upon reasonable request.

RESULTS

Participant Characteristics

A total of 33 patients with PI-CF and AGT were randomized with 17 enrolled in the GLP-1 group and 16 enrolled in the GIP group; 1 patient randomized to GLP-1 did not complete either GPA test because of insufficient intravenous access (Fig. 1*A*). The GLP-1 and GIP groups were comparable in sex, age, BMI, HbA_{1c}, pulmonary function, and glucose tolerance values (Table 1; Supplementary Fig. 1). Six patients randomized to the GLP-1 group were receiving CFTR modulator therapy, five were receiving ivacaftor, and one was receiving lumacaftor and ivacaftor; one patient randomized to the GIP group was receiving lumacaftor and ivacaftor.

Four patients with PI-CF and NGT and five in the non-CF control group were subsequently enrolled for GIP intervention; one participant in the non-CF control group moved from the region after completing just one GPA test (Fig. 1*B*). The PI-CF and non-CF groups with NGT were comparable in sex, age, BMI, HbA_{1c}, and glucose tolerance values (Table 1). All four patients with PI-CF and NGT were receiving combination CFTR modulator therapy, two were receiving tezacaftor and ivacaftor, one was receiving lumacaftor and ivacaftor, and one was receiving elexacaftor, tezacaftor, and ivacaftor.

Responses to GLP-1 and GIP in PI-CF With AGT

Active GLP-1 concentrations increased from 5.4 ± 1.3 to 62.0 ± 3.5 pmol/L during GLP-1 infusion and remained unchanged during placebo infusion (Fig. 1*C*). GIP concentrations increased from 46.6 ± 6.2 to 1,416.3 ± 103.6 pg/mL during GIP infusion and remained unchanged during placebo infusion (Fig. 1*D*).

Basal glucose concentrations decreased by more after 30 min of GLP-1 versus placebo infusion than after 30 min of GIP versus placebo infusion (−8.4 [95% CI −4.7 to −12.2] mg/dL; $P < 0.001$) (Fig. 2, *A* and *B*). During the ∼230 mg/dL hyperglycemic clamp, plasma glucose level was lower with GLP-1 versus placebo infusion (208 ± 16 vs. 225 ± 12 mg/dL; $P < 0.001$) despite M being greater with GLP-1 versus placebo infusion (11.4 ± 2.1 vs. 9.0 ± 1.5 mg/kg/min; $P < 0.001$), whereas the plasma glucose level was not different with GIP versus placebo infusion (219 ± 13 vs. 225 ± 9 mg/dL), with no difference in M (9.1 ± 1.5 vs. 8.8 ± 1.4 mg/kg/min). The increase in M during the ∼230 mg/dL hyperglycemic clamp with GLP-1 versus placebo treatment was greater than with GIP versus placebo (+2.2 [95% CI 1.1–3.2] mg/kg/min; $P < 0.001$) (Fig. 2, *A* and *B*).

Basal insulin tended to increase more after 30 min of GLP-1 versus placebo infusion than after 30 min of GIP versus placebo infusion (+2.8 [95% CI −0.08 to 5.7] μU/mL; $P = 0.057$) (Fig. 2, *C* and *D*). During the ∼230 mg/dL hyperglycemic clamp, the difference in second-phase insulin concentrations during GLP-1 versus placebo infusion was markedly greater than during GIP versus placebo infusion (+38.1 [95% CI 23.8–52.4] μU/mL; $P < 0.001$) (Fig. 2, *C* and *D*), with parallel findings for second-phase C-peptide (+2.66 [95% CI 1.56–3.76] ng/mL; $P < 0.001$). Similar results were obtained when excluding the seven participants ($n = 6$ in the GLP-1 group and 1 in the GIP group) receiving CFTR modulator therapy.

Basal glucagon concentrations decreased after 30 min of GLP-1 versus placebo infusion and increased after 30 min of GIP versus placebo infusion, which differed between the GLP-1 and GIP groups (−9.6 [95% CI −14.3 to

Table 1—Participant characteristics

	PI-CF with AGT			
	GLP-1 group	GIP group	PI-CF group with NGT	Non-CF group with NGT
n	16	16	4	4
Demographics				
Male/female	8/8	7/9	2/2	2/2
Age (years)	27 (19–43)	23 (18–40)	24 (20–28)	28 (19–30)
BMI (kg/m^2)	22 (19–34)	23 (19–32)	25 (18–27)	23 (21–27)
HbA_{1c} (%)	5.8 (4.8–6.2)	5.6 (5.1–6.1)	5.5 (5.4–5.6)	5.2 (5.2–5.3)
HbA1c (mmol/mol)	40 (29–44)	38 (32–43)	37 (36–38)	33 (33–34)
FEV_1 (% predicted)	80.5 (48–122)	89 (32–112)	92 (35–109)	ND
FVC (% predicted)	90 (65–121)	90 (51–123)	93 (64–110)	ND
OGTT profile				
Fasting glucose (mg/dL)	93 (73–110)	91 (77–104)	89 (88–94)	88 (85–90)
1-h glucose (mg/dL)	220 (171–260)	213 (163–246)	138 (124–147)	139 (121–141)
2-h glucose (mg/dL)	154 (34–270)	144 (53–309)	89 (84–130)	91 (87–105)

Data are medians and ranges (minimum–maximum). FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; ND, not done.

−4.8] pg/mL; $P < 0.001$) (Fig. 2, *E* and *F*). During the ∼230 mg/dL hyperglycemic clamp, glucagon concentrations were similarly suppressed during incretin and placebo infusions in both the GLP-1 and GIP groups.

Changes in acute insulin, C-peptide, glucagon, and proinsulin responses with incretin versus placebo infusion were not different between the GLP-1 and GIP groups (Table 2; Supplementary Fig. 2; Fig. 3*A–D*). The PISR under the ∼230 mg/dL hyperglycemic clamp condition was notably lower with GLP-1 versus placebo infusion when compared with GIP versus placebo intervention (−1.18% [95% CI −2.17 to −0.19%]; $P = 0.019$) (Table 2; Fig. 3, *E* and *F*). The GLP-1–induced change in second-phase insulin concentrations was related to the underlying β-cell secretory capacity (Supplementary Fig. 3), whether estimated by the AIR_{pot} ($r = 0.54$; $P = 0.034$) or AIR_{max} ($r = 0.50$; $P = 0.046$).

Responses to GIP in PI-CF and Matched Controls With NGT

GIP concentrations increased similarly with GIP versus placebo infusion in the PI-CF and non-CF groups (+852.6 ± 225.6 vs. +1,193.3 ± 253.3 pg/mL, respectively) (Fig. 1, *E* and *F*).

Basal glucose decreased by less after 30 min of GIP versus placebo infusion in the PI-CF group than in the non-CF group (+7.5 mg/dL [95% CI 1.9–13.2] mg/dL; $P = 0.009$) (Fig. 4, *A* and *B*). During the ∼230 mg/dL hyperglycemic clamp, plasma glucose concentrations were not different with GIP versus placebo infusion in patients with PI-CF (222 ± 12 vs. 228 ± 7 mg/dL, respectively) and those without CF (171 ± 24 vs. 206 ± 29 mg/dL, respectively). M was similar with GIP versus placebo infusion in the PI-CF group (9.7 ± 1.7 vs. 9.7 ± 1.4 mg/kg/min, respectively) (Fig. 4*A*) and was higher with GIP infusion than the placebo infusion in the non-CF group (respectively, 15.9 ± 3.2 vs. 9.7 ± 1.7 mg/kg/min and +6.19 [95% CI 7.6–11.9; $P < 0.001$) (Fig. 4*B*). The absent difference in M in the PI-CF group compared with the increase in M in the non-CF group translated into an M that was greater with GIP relative to placebo infusion in participants without CF versus those with PI-CF (+6.2 mg/kg/min [95% CI 2.6–9.7]; $P = 0.001$).

Basal insulin level remained unchanged after 30 min of GIP versus placebo infusion in both PI-CF and non-CF groups. During the ∼230 mg/dL hyperglycemic clamp, augmentation of second-phase insulin concentrations with GIP versus placebo infusion was markedly less in participants with PI-CF than in those without CF (−78.4 μU/mL [95%CI −146.9 to −9.9]; $P = 0.025$) (Fig. 4, *C* and *D*), with similar results for second-phase C-peptide (−6.23 [95% CI −8.1 to −4.37] ng/mL; $P < 0.001$).

The change in glucagon level after 30 min of GIP versus placebo was not different between the PI-CF and non-CF groups. During the ∼230 mg/dL hyperglycemic clamp, glucagon concentrations were similarly suppressed during GIP and placebo infusions in both PI-CF and non-CF groups (Fig. 4, *E* and *F*).

Neither AIR_{arg} nor ACR_{arg} was different during GIP versus placebo in patients with PI-CF (Table 3; Supplementary Fig. 4*A*; Fig. 5*A*) and both were lower during GIP versus placebo infusion in the non-CF group ($P \leq 0.02$ for both) (Table 3; Supplementary Fig. 4*B*; Fig. 5*B*), an effect explained by lower fasting glucose level and so a priming stimulus for arginine during GIP infusion in control participants. The change in AIR_{arg} between GIP and placebo infusion was less in the PI-CF group than the non-CF group (−2.1 ± 9.0 vs. −21.7 ± 8.8 μU/mL, respectively; $P = 0.02$) with similar results for ACR_{arg} (−0.24 ± 0.74 vs. −1.61 ± 0.78 ng/mL, respectively; $P = 0.04$). Neither AIR_{pot} nor ACR_{pot} was different with GIP versus placebo infusion in either the PI-CF group or non-CF group (Table 3; Supplementary Fig. 4 *A* and *B*; Fig. 5, *A* and *B*). AGR_{arg}

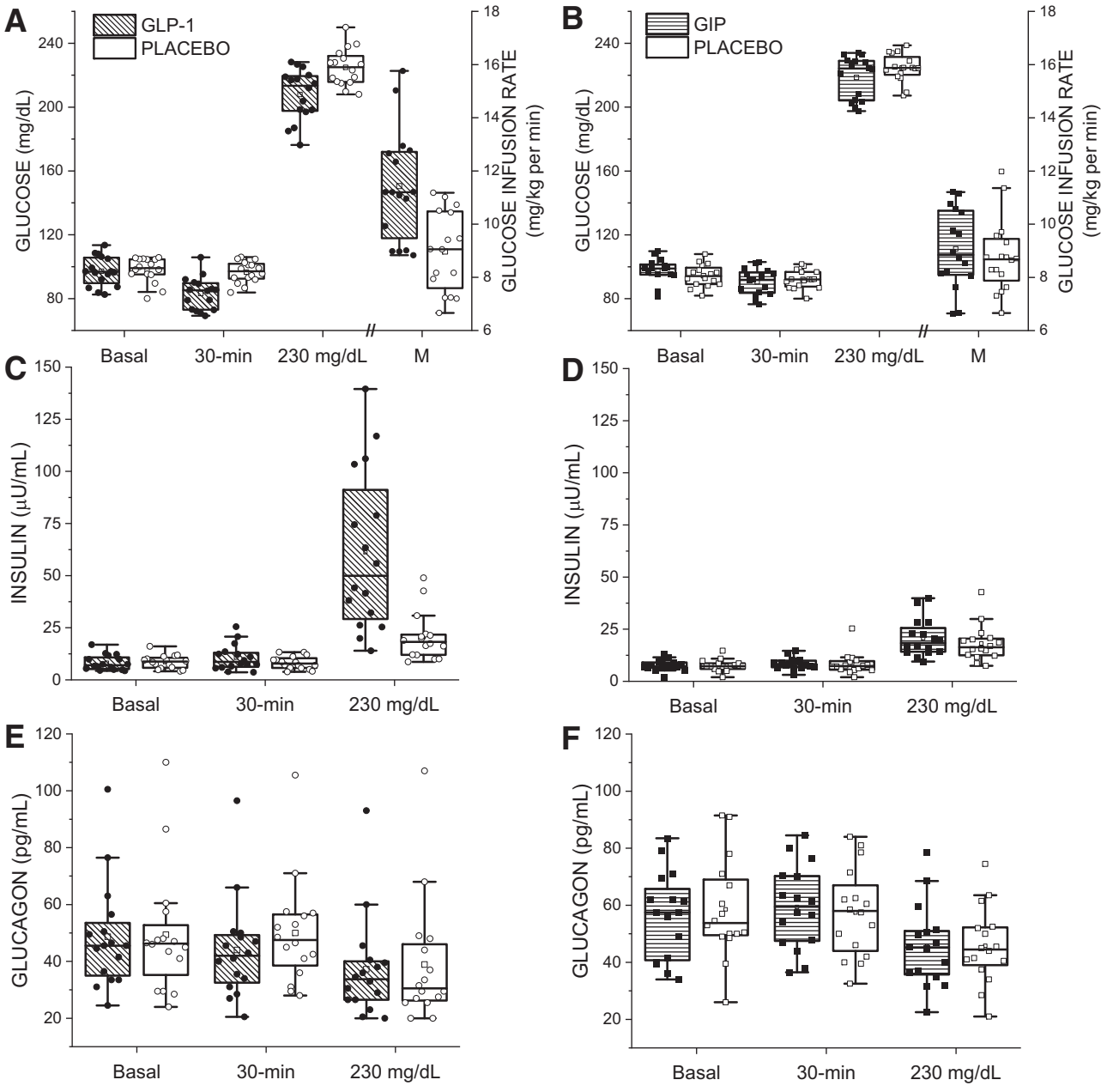

Figure 2— Glucose and islet hormone concentrations in response to incretin infusion. Plasma glucose (*A* and *B*), insulin (*C* and *D*), and glucagon (*E* and *F*) before and 30 min after infusion of incretin (lined box plots) or placebo (open box plots), and at the end of the ∼230 mg/dL hyperglycemic clamp in participants with PI-CF and AGT in the GLP-1 (left) and GIP (right) groups. On the opposite *y*-axis (*A* and *B*), M represents the requisite glucose infusion rate during the hyperglycemic clamp. Box plots give the median and interquartile range.

was not different during GIP infusion than placebo infusion in the PI-CF group (Table 3; Supplementary Fig. 4*C*) and was greater during GIP than placebo infusion in the non-CF group (P = 0.006) (Table 3; Supplementary Fig. 4*D*), an effect explained by the decrease in fasting glucose levels in control participants that is known to augment α-cell function, particularly in the presence of GIP (25,26). The change in AGR_{arg} between GIP and placebo infusion was less in participants with PI-CF than those without -CF (+6.2 ± 16.5 vs. +32.3 ± 9.4 pg/mL, respectively; P = 0.03). AGR_{inh} with GIP versus placebo infusion was not different in either the PI-CF or non-CF groups (Table 3; Supplementary Fig. 4 *C* and *D*). Neither the changes in acute proinsulin responses (Table 3; Fig. 5, *C* and *D*) nor in PISRs (Table 3; Fig. 5, *E* and *F*) were different between the PI-CF and non-CF groups.

DISCUSSION

These results advance our understanding of the individual contributions of GLP-1 and GIP to incretin action in PI-CF. Individuals with PI-CF with AGT who received GLP-1 exhibited a marked augmentation in glucose-dependent

Table 2—Acute islet cell hormone responses during glucose-potentiated arginine testing in PI-CF with AGT

	GLP-1 group (n = 16)			GIP group (n = 16)		
	Incretin	Placebo	P value	Incretin	Placebo	P value
AIR, μU/mL						
AIR_{arg}	11.6 (7.6–15.6)	14.5 (9.2–23.1)	0.004	8.6 (6.9–12.0)	14.3 (11.1–18.1)	0.01
AIR_{pot}	44.7 (23.8–60.2)	43.4 (23.8–54.8)	0.61	26.0 (18.4–46.7)	32.1 (24.8–55.9)	0.001
AIR_{max}	49.3 (21.5–61.3)	48.6 (30.9–64.4)	0.61	38.7 (23.7–78.3)	41.3 (32.0–60.9)	0.02
AGR, pg/mL						
AGR_{arg}	29.0 (20.9–38.6)	31.3 (18.9–46.9)	0.74	40.0 (24.5–57.3)	29.0 (20.8–47.6)	0.21
AGR_{inh}	18.5 (9.4–30.6)	27.3 (16.3–35.1)	0.07	25.3 (15.8–43.3)	27.5 (18.0–42.9)	0.57
AGR_{min}	18.9 (12.0–26.4)	26.1 (12.8–33.9)	0.21	22.4 (16.4–28.8)	28.4 (15.5–39.8)	0.49
ACR, ng/mL						
ACR_{arg}	0.49 (0.36–0.68)	0.61 (0.46–0.90)	0.002	0.40 (0.33–0.64)	0.69 (0.61–0.80)	<0.001
ACR_{pot}	2.04 (1.30–2.85)	2.04 (1.19–2.74)	0.57	1.69 (0.99–2.15)	1.62 (1.37–2.13)	0.09
ACR_{max}	1.90 (0.96–2.57)	2.07 (1.32–2.64)	0.06	1.85 (1.23–2.99)	2.16 (1.60–2.58)	0.07
APR, pmol/L						
APR_{arg}	3.5 (1.3–5.0)	3.5 (2.2–6.0)	0.20	2.1 (1.1–2.9)	3.4 (1.7–4.6)	0.04
APR_{pot}	8.5 (5.5–11.8)	12.1 (6.1–14.6)	0.14	11.7 (7.4–14.3)	11.2 (6.7–14.6)	0.62
APR_{max}	10.8 (7.6–13.5)	11.5 (7.3–18.2)	0.44	11.5 (7.4–17.0)	11.9 (9.3–15.4)	0.53
PISR, %						
$PISR_{arg}$	2.06 (0.91–2.68)	1.68 (1.07–2.22)	0.61	1.37 (0.77–2.15)	1.56 (0.79–1.98)	0.80
$PISR_{pot}$	1.12 (0.62–2.61)	1.54 (1.02–2.25)	0.23	2.32 (1.94–3.04)	1.96 (1.57–2.43)	0.03
$PISR_{max}$	1.70 (1.12–2.10)	1.57 (1.07–2.40)	0.57	1.88 (1.23–2.04)	1.91 (1.52–2.32)	1.00

Data are medians and interquartile ranges. Changes in acute islet cell hormone responses with incretin versus placebo infusion were not different between the GLP-1 and GIP groups; the change in $PISR_{pot}$ with incretin versus placebo was different between the GLP-1 and GIP groups (P = 0.03). AIR, acute insulin response; AGR, acute glucagon response; ACR, acute C-peptide response; APR, acute proinsulin response; PISR, proinsulin secretory ratio; arg, arginine; pot, glucose-potentiated arginine; inh, glucose-inhibited arginine; max, maximum arginine; min, minimum arginine.

insulin secretion evident by increased second-phase insulin and C-peptide concentrations as well as intravenous glucose tolerance. There was no effect of GLP-1 on measures of maximal β-cell secretory capacity, indicating preservation of the reserve capacity for insulin secretion. In contrast, individuals with PI-CF with AGT who received GIP demonstrated no increase in second-phase insulin or C-peptide concentrations. Moreover, the differential responsiveness to GLP-1 and GIP in PI-CF with AGT does not appear to be the result of dysregulated glucose metabolism, because individuals with PI-CF with NGT also exhibited impaired second-phase insulin and C-peptide responses during GIP infusion. The attenuated insulinotropic action of GIP suggests loss of a key component of the incretin effect could contribute to diabetes development in CF.

The effect of GLP-1 to augment second-phase insulin secretion in PI-CF is like that reported in individuals with type 2 diabetes (17,27). Patients with type 2 diabetes have decreased β-cell sensitivity to GLP-1 compared with individuals without diabetes (19), but the former retain their response to supraphysiologic concentrations of GLP-1 and to pharmacologic agonists of the GLP-1 receptor. Inhibitors of dipeptidyl peptidase-4 (DPP-4) prevent inactivation of endogenous GLP-1 (and GIP) and can improve pancreatic islet function in type 2 diabetes. In individuals with PI-CF and AGT, our group has shown that 6 months' treatment with the DPP-4 inhibitor sitagliptin improved the rapidity and magnitude of postprandial insulin secretion and led to greater suppression of glucagon concentrations (28). However, these islet effects did not translate into an improvement in postprandial glucose tolerance with sitagliptin in PI-CF (28), a benefit of DPP-4 inhibition reported in most studies of patients with type 2 diabetes (29,30). The hyperglucagonemia that characterizes type 2 diabetes is not present in PI-CF, which features reduced islet α-cell as well as β-cell secretory capacity (9). Thus, more potent stimulation of insulin secretion rather than suppression of glucagon may be necessary to improve glucose tolerance in PI-CF. The results of this study support that the improved glucose-dependent regulation of islet function observed with the DPP-4 inhibitor sitagliptin in PI-CF (28) can be attributed to the increased levels of intact GLP-1, rather than GIP, and that use of GLP-1 agonists to further enhance GLP-1 effects may lead to enhanced insulin secretion and glucose tolerance.

Basal glucagon secretion in individuals with PI-CF and AGT was inhibited during GLP-1 infusion and stimulated during GIP infusion—the expected effects for each incretin hormone on pancreatic α-cell function. GLP-1 likely inhibits glucagon secretion indirectly through the paracrine effects of insulin and somatostatin; in contrast, GIP appears to directly stimulate glucagon secretion through GIP receptors expressed on α-cells (7,25,26). However, AGRs, which are lower in individuals with PI-CF than in

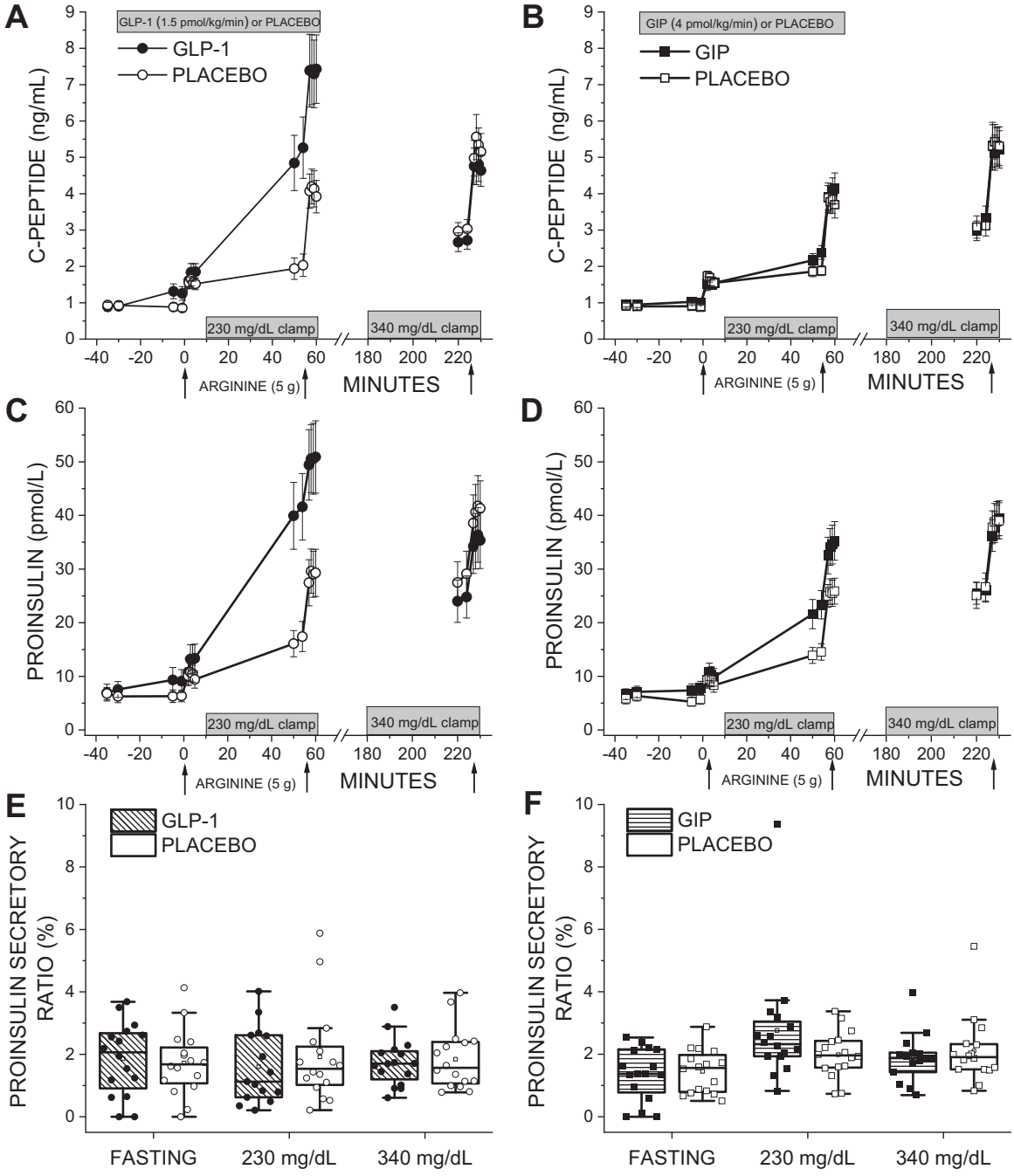

Figure 3—C-peptide, proinsulin, and proinsulin secretory ratios in response to GPA testing. Islet β-cell release of processed C-peptide (*A* and *B*), unprocessed proinsulin (*C* and *D*), and PISRs (*E* and *F*) of the acute proinsulin and C-peptide responses to arginine under fasting and ∼230 mg/dL hyperglycemic clamp conditions during incretin or placebo infusion, and again under ∼340 mg/dL hyperglycemic clamp conditions without incretin or placebo infusion in participants with PI-CF and AGT in the GLP-1 (left) and GIP (right) groups. Data are reported as mean ± SE or in box plots giving the median and interquartile range.

those without CF (9), were not increased during GIP infusion, as was observed for AGR_{arg} in our non-CF control group. Thus, individuals with PI-CF may exhibit impairment of both islet β- and α-cell responsiveness to GIP.

In type 2 diabetes, the insulinotropic effect of GIP is markedly impaired with loss of GIP-induced augmentation of second-phase insulin secretion (10,11,31,32). Both GLP-1 and GIP signal through specific G-protein coupled receptors that lead to stimulation of adenylate cyclase and activation of protein kinase A and appear to potentiate insulin secretion using the same downstream intracellular machinery (33). Work in Zucker diabetic rats suggested that hyperglycemia leads to decreased islet β-cell GIP receptor expression and function as a mechanism for

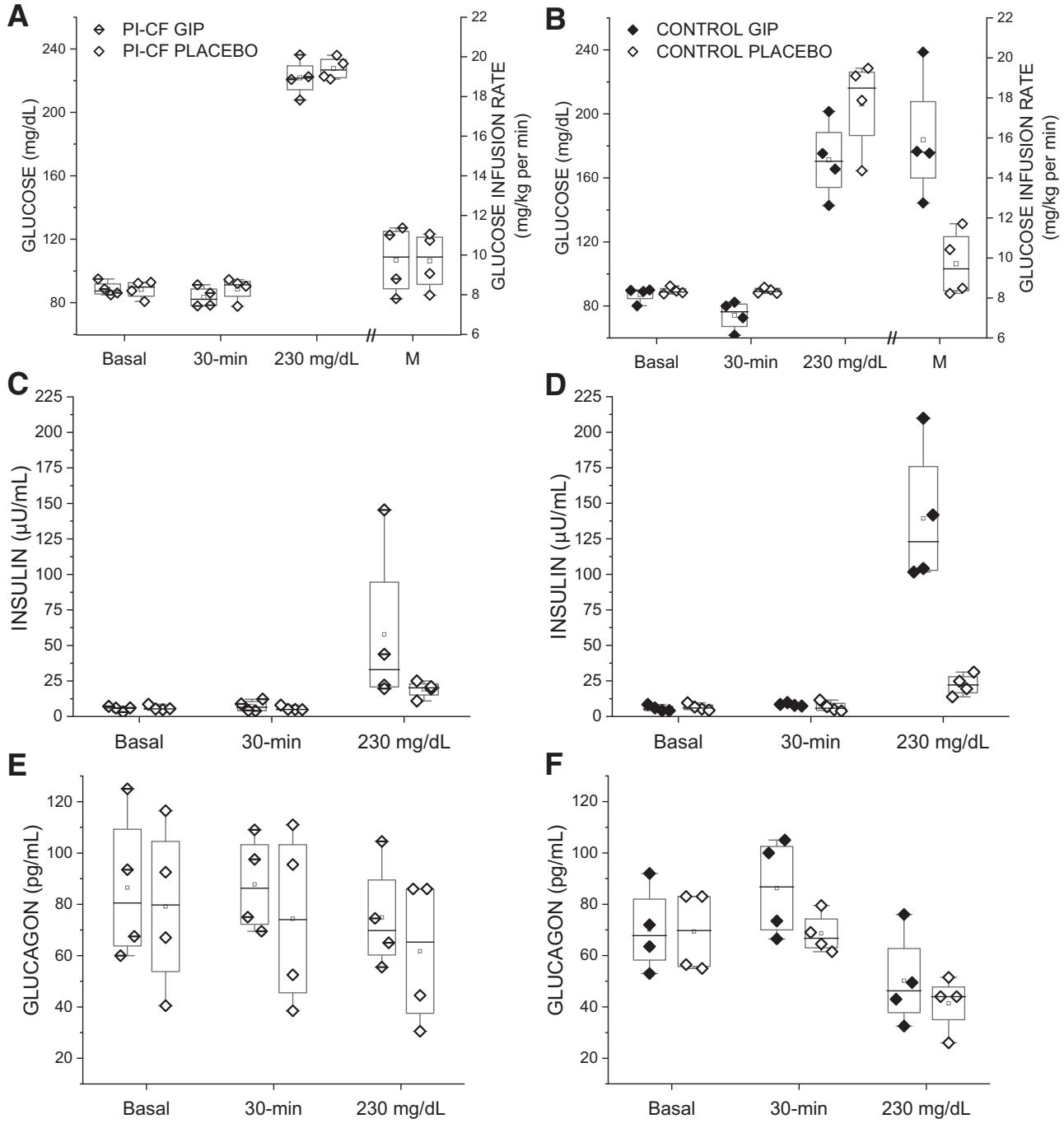

Figure 4—Glucose and islet hormone levels in response to GIP infusion in NGT patients with PI-CF and control participants without CF. Plasma glucose (*A* and *B*), insulin (*C* and *D*), and glucagon (*E* and *F*) levels before and 30 min after infusion of GIP (marked/filled diamonds) or placebo (open diamonds), and at the end of the ~230 mg/dL hyperglycemic clamp in participants with PI-CF and NGT (left) and in control participants without CF (right). On the opposite *y*-axis (*A* and *B*), M represents the requisite glucose infusion rate during the hyperglycemic clamp. Box plots give the median and interquartile range.

loss of GIP augmentation of glucose-dependent insulin secretion (12). Moreover, 4 weeks of near normalization of glycemia in type 2 diabetes modestly improved second-phase insulin and C-peptide levels during GIP infusion (34), suggesting that this effect of hyperglycemia is reversible.

In contrast, our study in PI-CF demonstrates impaired GIP action to augment insulin secretion even in individuals with NGT. This impaired GIP action in PI-CF is unlikely a consequence of pancreatic exocrine disease, because a previous study in individuals with chronic pancreatitis and exocrine pancreatic insufficiency demonstrated intact augmentation

Table 3—Acute islet-cell hormone responses during glucose-potentiated arginine testing in patients with PI-CF with NGT and in control participants

	PI-CF group (n = 4)			Non-CF control group (n = 4)		
	GIP	Placebo	P value	GIP	Placebo	P value
AIR, μU/mL						
AIR_{arg}	14.2 (13.1–15.7)	15.3 (8.9–24.0)	0.68	17.5 (12.3–21.5)	35.7 (33.1–44.1)	0.02
AIR_{pot}	82.1 (37.9–223.4)	38.3 (23.6–66.4)	0.29	143.0 (113.0–191.2)	97.7 (80.2–249.0)	0.82
AGR, pg/mL						
AGR_{arg}	39.4 (28.0–54.0)	33.8 (16.8–52.9)	0.51	69.9 (45.4–121)	44.0 (8.1–93.6)	0.006
AGR_{inh}	28.0 (19.9–46.9)	29.3 (8.5–49.8)	0.40	30.5 (14.1–53.0)	36.1 (18.0–53.4)	0.80
ACR, ng/mL						
ACR_{arg}	0.95 (0.84–1.11)	0.95 (0.64–1.78)	0.56	0.79 (0.46–1.37)	2.25 (2.17–2.87)	0.03
ACR_{pot}	3.34 (2.49–5.53)	1.97 (1.83–3.90)	0.05	4.48 (4.14–6.74)	5.03 (4.74–9.63)	0.24
APR, pmol/L						
APR_{arg}	2.9 (2.3–3.6)	2.9 (1.6–4.6)	0.84	3.1 (2.3–4.8)	5.9 (2.9–9.8)	0.34
APR_{pot}	14.7 (11.8–19.0)	9.7 (7.7–13.9)	0.02	21.0 (13.5–45.2)	17.5 (10.0–29.3)	0.50
PISR, %						
$PISR_{arg}$	0.90 (0.72–1.09)	0.75 (0.70–0.89)	0.32	1.15 (1.02–1.49)	0.60 (0.39–1.11)	0.05
$PISR_{pot}$	1.22 (1.07–1.44)	1.26 (1.02–1.49)	0.94	1.08 (0.66–3.17)	0.78 (0.61–1.12)	0.31

Data are reported as medians and interquartile ranges. AIR, acute insulin response; AGR, acute glucagon response; ACR, acute C-peptide response; APR, acute proinsulin response; PISR, proinsulin secretory ratio; arg, arginine; pot, glucose-potentiated arginine; inh, glucose-inhibited arginine.

of second-phase insulin and C-peptide responses during infusion of GIP in those with normal to impaired glucose tolerance (35). CFTR function has been proposed to be important for islet β-cell, and possibly α-cell, function (14), although a direct role of CFTR in insulin secretion via expression in a subpopulation of β-cells or an indirect role via expression in pancreatic ductal cells neighboring the islet (36,37) is debated. Nevertheless, loss of CFTR function appears to affect the amplification pathway for insulin secretion (38), with our study providing in vivo evidence that loss of CFTR function may affect nutrient-stimulated insulin secretion at least in part through impaired GIP action.

Both reduced β-cell secretory capacity and hyperglycemia can lead to disproportionately increased proinsulin secretion (39,40) with increased demand on a compromised functional β-cell mass for insulin secretion resulting in β-cell recruitment of immature secretory granules containing an abundance of incompletely processed proinsulin (41,42). Loss of CFTR function may impair proinsulin processing through effects on chloride ion flux important for insulin granule acidification and activation of the prohormone convertase enzymes that generate insulin and C-peptide (43). We have previously demonstrated that individuals with PI-CF have elevated proinsulin secretory ratios compared with individuals with pancreatic-sufficient CF and non-CF control individuals (9), and that the disproportionate increase in proinsulin secretion is related to the extent of impaired glucose tolerance (3). Unlike a previous study of GLP-1 infusion in which proinsulin secretory ratios increased under conditions of glucose-potentiated arginine stimulation (20), GLP-1 augmented second-phase insulin secretion without disproportionately increasing proinsulin release in this present study of PI-CF. Importantly, both studies demonstrate a relationship between the GLP-1–induced increase in second-phase insulin concentrations and the underlying β-cell secretory capacity. Interestingly, in participants with PI-CF and AGT (but not those with NGT), infusion of GIP led to disproportionately increased proinsulin secretion such that GLP-1 induced an improvement relative to worsening proinsulin secretory ratio with GIP. These results further suggest convergence of incretin signaling pathways and CFTR conductance on insulin processing and secretion.

This study is limited by the exclusion of children, because our investigational new drug application required study in adults aged ≥18 years. Thus, whether the β-cell response to GIP may be preserved earlier in PI-CF, particularly prior to significant reduction in β-cell secretory capacity, remains uncertain. Additionally, because progressive β-cell dysfunction is so common in PI-CF, identifying adults with PI-CF and NGT according to our strict criteria of 1-h OGTT glucose <155 mg/dL was challenging, resulting in a small sample size for this cohort. Additionally, the four individuals with PI-CF and NGT were all receiving CFTR modulator therapy. Although CFTR modulator therapy with lumacaftor and ivacaftor has not been associated with improved insulin secretion or glucose tolerance in F508del homozygous individuals (44), and none of our participants had a class III mutation associated with improved insulin secretion after initiation of ivacaftor (45), we cannot exclude that partial correction of CFTR function may have been present in these individuals. In fact, one F508del homozygous individual with PI-CF and NGT receiving tezacaftor and ivacaftor exhibited a GIP-induced increase in second-phase insulin levels. Our study did not include

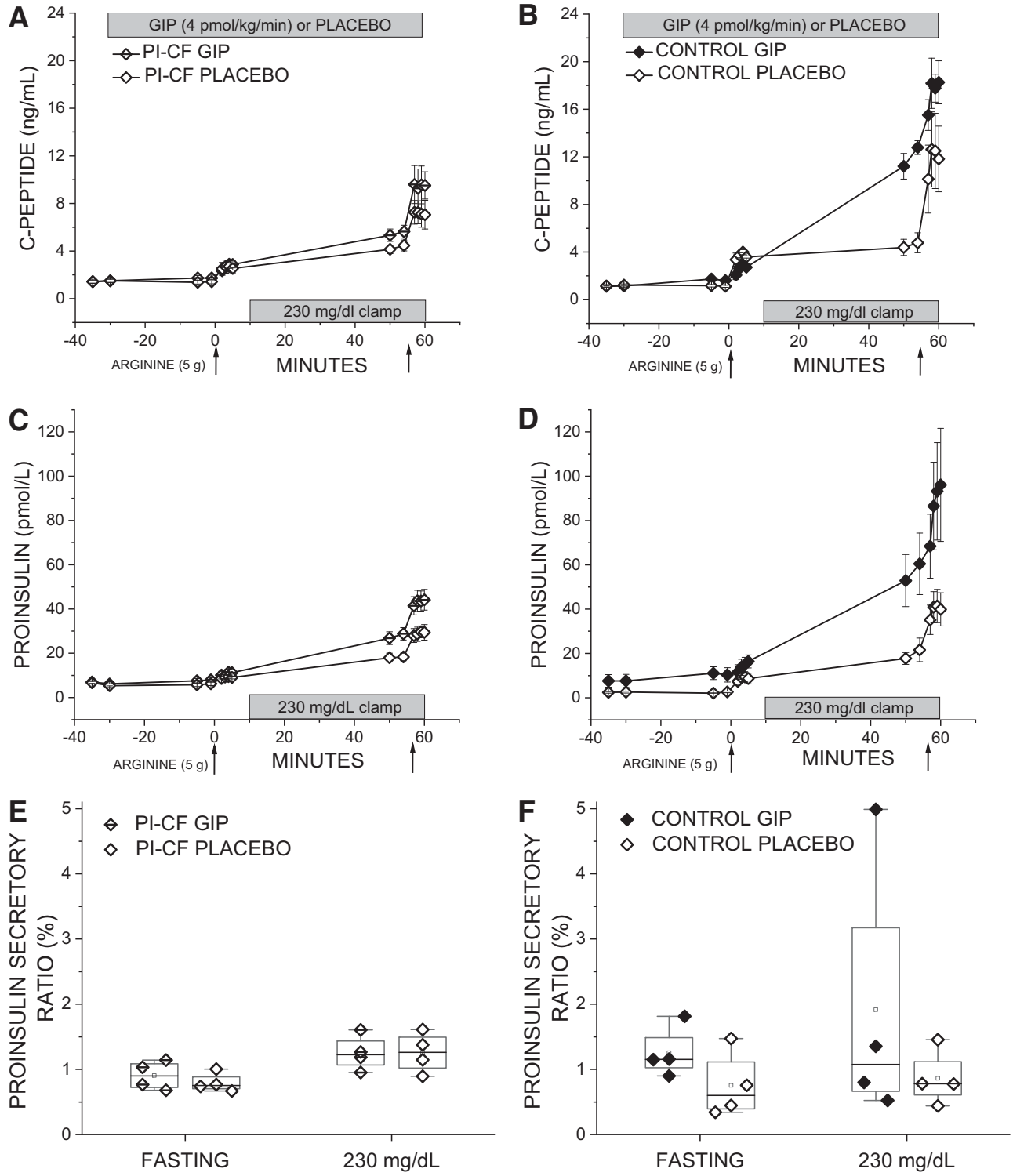

Figure 5—C-peptide, proinsulin, and proinsulin secretory ratios in response to GPA testing with GIP or placebo infusion in patients with PI-CF and control participants without CF. Islet β-cell release of processed C-peptide (*A* and *B*), unprocessed proinsulin (*C* and *D*), and PISRs (*E* and *F*) of the acute proinsulin and C-peptide responses to arginine under fasting and ∼230 mg/dL hyperglycemic clamp conditions during GIP or placebo infusion in participants with PI-CF and NGT (left) and in control participants without CF (right). Data are reported as mean ± SE or box plots giving the median and interquartile range.

individuals with pancreatic-sufficient CF who retain partial CFTR function and should be considered for inclusion as control participants in future studies needed to determine whether correction of CFTR function with highly effective CFTR channel modulation may restore more favorable GIP action on β-cell secretion.

In conclusion, the incretin hormone GLP-1 improves glucose-dependent β-cell insulin secretion in PI-CF without depleting the availability of mature β-cell secretory granules, whereas islet responsiveness to GIP is impaired independently of hyperglycemia in PI-CF. Although there is concern for the potential of GLP-1 receptor agonists to exacerbate underlying gastroparesis and dysmotility in CF, tolerability of low-dose, chronic administration has been reported in at least one individual (46). Future studies should determine whether peptide and/or nonpeptidic GLP-1 receptor agonists (47) may both be tolerated and preserve or even restore islet β-cell function long term in PI-CF to delay progression of AGT and/or treat CFRD.

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Author Contributions. S.C.N. conducted experiments, acquired and analyzed data, and wrote the manuscript; A.J.P., J.N.E., and K.G. conducted experiments, acquired data, and reviewed and edited the manuscript; A.D., A.T., and A.J.F. acquired and analyzed data, and reviewed and edited the manuscript; D.D.D. and D.H. contributed to the study design and study oversight, and reviewed and edited the manuscript; S.S., D.S., and R.G. analyzed data, and reviewed and edited the manuscript; D.A.D. contributed to the study design, provided reagents, and reviewed and edited the manuscript; R.C.R. contributed to the study design and study oversight, analyzed data, and reviewed and edited the manuscript; A.K. designed the study, provided study oversight, analyzed data, and reviewed and edited the manuscript; M.R.R. designed the study, conducted experiments, acquired and analyzed data, and wrote the manuscript. A.K. and M.R.R. are the guarantors of this work, and as such, had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

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