Islet Hormone and Incretin Secretion in Cystic Fibrosis after Four Months of Ivacaftor Therapy

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Abstract

Rationale: Diabetes is associated with worse cystic fibrosis (CF) outcomes. The CFTR potentiator ivacaftor is suggested to improve glucose homeostasis in individuals with CF.

Objectives: To test the hypothesis that clinically indicated ivacaftor would be associated with improvements in glucose tolerance and insulin and incretin secretion.

Methods: Oral glucose tolerance tests, mixed-meal tolerance tests, and glucose-potentiated arginine tests were compared preivacaftor initiation and 16 weeks postivacaftor initiation in CF participants with at least one *CFTR* gating or conductance mutation. Meal-related 30-minute (early phase) and 180-minute incremental area under the curves were calculated as responses for glucose, insulin, C-peptide, and incretin hormones; glucagon-like peptide-1; and glucose-dependent insulintropic polypeptide. First-phase insulin secretion, glucose potentiation of arginine-induced insulin secretion, and disposition index were characterized by glucose-potentiated arginine stimulation tests.

Measurements and Main Results: Twelve subjects completed the study: six male/six female; seven normal/five abnormal glucose tolerance (oral glucose tolerance test 1-h glucose ≥ 155 and 2-h glucose < 200 mg/dl); of median (minimum–maximum) age (13.8 yr [6.0–42.0]), body mass index-Z of 0.66 (–2.4 to 1.9), and FEV₁% predicted of 102 (39–122). Glucose tolerance normalized in one abnormal glucose tolerance subject. Ivacaftor treatment did not alter meal responses except for an increase in early phase C-peptide ($P = 0.04$). First-phase ($P = 0.001$) and glucose potentiation of arginine-induced ($P = 0.027$) insulin secretion assessed by acute C-peptide responses improved after ivacaftor treatment. Consistent with an effect on β -cell function, the disposition index relating the amount of insulin secreted for insulin sensitivity also improved ($P = 0.04$).

Conclusions: Insulin secretion improved following 4 months of clinically indicated ivacaftor therapy in this relatively young group of patients with CF with normal to mildly impaired glucose tolerance, whereas incretin secretion remained unchanged.

Keywords: cystic fibrosis; insulin; diabetes; ivacaftor

With improved life expectancy for individuals with cystic fibrosis (CF), CF-related comorbidities that impose additional medical and psychological burden and that

may compromise pulmonary health and long-term survival are receiving increasing attention. CF-related diabetes (CFRD) is one such comorbidity: approximately 26%

of individuals age 10–20 years and 40–50% of adults with CF have CFRD (1, 2). CFRD is associated with worse clinical outcomes, including worse pulmonary function,

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At a Glance Commentary

Scientific Knowledge on the

Subject: Improved glucose tolerance has been reported in cystic fibrosis following initiation of ivacaftor therapy.

What This Study Adds to the

Field: Four months of ivacaftor therapy was associated with improved islet function, but meal-related incretin secretion seemed unchanged.

increased rates of pulmonary exacerbation, greater prevalence of important sputum pathogens, poorer nutritional status (body mass index [BMI]) (3), and higher mortality rates (4). Highlighting the importance of early derangements in glucose and insulin homeostasis, declines in BMI and lung function may begin up to 4 years before frank CFRD development (5).

CF arises from mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride and bicarbonate channel. A subset of CF-causing *CFTR* mutations cause defective CFTR function without causing CFTR mistrafficking, that is, CFTR is present at the plasma membrane but its anion transport function is impaired because of issues with conductance (restricted ion movement through channel) or gating (channel does not open appropriately). Ivacaftor, a novel therapy that potentiates CFTR channel function, directly addresses the underlying CFTR dysfunction, and its use in individuals with *CFTR* functional mutations results in improved pulmonary function and BMI (6).

Suggesting a potential role in glucose homeostasis, hypoglycemia was reported in a subject with CFRD randomized to ivacaftor in one of the seminal clinical trials (6). Moreover, improvements in insulin secretion during oral and intravenous glucose tolerance testing were found in five patients following ivacaftor treatment (7). Limited observational data are emerging that ivacaftor treatment is associated with CFRD resolution (8).

The mechanisms underlying the observed effects of ivacaftor on glucose homeostasis are not yet delineated. Some investigators have identified CFTR protein expression in both pancreatic β -cells (9, 10)

and α -cells (11) and have posited CFTR participates in the regulation of insulin (9, 10) and glucagon secretion (11). Ivacaftor may, thus, have a direct effect on β -cell and/or α -cell function. Other studies have 1) detected neither CFTR protein nor CFTR activity in β -cells and 2) suggested that CFTR does not play a central role in regulating insulin secretion (12). Alternatively, indirect β -cell effects could occur through improvements in incretin secretion by gastrointestinal neuroendocrine cells and decreased inflammation (12, 13). This study intended to determine the impact of 4 months of ivacaftor treatment on islet and incretin hormone secretion in response to nutrient ingestion and β -cell secretory capacity and sensitivity to glucose in ivacaftor-naïve patients with at least one *CFTR* conductance or gating mutation. Some of these data have been previously reported in the form of an abstract (14).

Methods

Participants

Individuals aged greater than or equal to 6 years with a confirmed diagnosis of CF and who were initiating ivacaftor for clinical indication of either a *CFTR* gating or conductance mutation were eligible to participate. Individuals were recruited from North America and Italy and studied at the Children's Hospital of Philadelphia (CHOP). Participants were categorized as pancreatic insufficient (PI), defined by clinical diagnosis and treatment with pancreatic enzyme replacement therapy, or pancreatic sufficient (PS). Individuals were excluded for prior lung or liver transplant, significant liver or kidney dysfunction, hemoglobin less than 10 g/dl, pregnancy or nursing, or CFRD with fasting hyperglycemia (fasting glucose ≥ 126 mg/dl). Participants were required to be pulmonary exacerbation-free (defined as requiring intravenous antibiotics or administration of oral or intravenous glucocorticoids) within the previous 4 weeks of procedures.

The CHOP institutional review board approved the study. Participants age greater than or equal to 18 years gave written informed consent. For participants age less than 18 years, both parents were required to consent, and assent was obtained (when age-appropriate) from the child to participate.

Study procedures were performed over 3 study days on two occasions: baseline (just before starting ivacaftor) and 14–16 weeks after ivacaftor therapy initiation. Metabolic tests were conducted at the CHOP Center for Human Phenomic Science after a 12-hour overnight fast.

Spirometry

FEV₁ was measured using standard techniques (15) in the Center for Human Phenomic Science Pulmonary Testing Core. Values are reported as percent of predicted (FEV₁% predicted) using National Health and Nutrition Examination Survey III prediction equations.

Oral Glucose Tolerance Test

Fasting glucose was assessed at $t = 0$ minutes before ingestion of Glucola (1.75 g/kg; maximum 75 g) over 5 minutes. Glucose tolerance was defined using glucose obtained at $t = 60$ and 120 minutes (16, 17):

- Normal (normal glucose tolerance [NGT]): 1-hour glucose <155 and 2-hour glucose <140 mg/dl (16)
- Early intolerance (early glucose intolerance [EGI]): 1-hour glucose ≥ 155 and 2-hour glucose <140 mg/dl (16)
- Impaired (impaired glucose tolerance [IGT]): 2-hour glucose ≥ 140 and <200 mg/dl (17)
- CFRD: 2-hour glucose ≥ 200 mg/dl (17)

Mixed-Meal Tolerance Test

Mixed-meal tolerance tests (MMTTs) have previously been used to differentiate insulin and incretin secretory responses (18). After an overnight fast, an intravenous catheter was placed and baseline blood samples were obtained 10 minutes and 1 minute before ingestion of breakfast equal to approximately one-third daily caloric requirement for age and weight (maximum 820 kcal) over 15 minutes starting at $t = 0$. Carbohydrate, fat, and protein composition were 47%, 40%, and 13% of total energy content (18). Subjects with PI took their usual prescribed pancreatic enzyme replacement with MMTT. Additional blood samples were obtained at $t = 10, 20, 30, 60, 90, 120, 150,$ and 180 minutes from meal start.

Glucose-potentiated Arginine Test

The glucose-potentiated arginine (GPA) test followed established methodology for evaluation of β -cell secretory capacity and

sensitivity to glucose (19–21). After an overnight fast, an intravenous catheter was placed for infusions and a second catheter was placed for blood sampling. Baseline blood samples were taken 5 minutes and 1 minute before injection of 10% arginine (0.07 g/kg, maximum 5 g) over 1 minute starting at $t = 0$. Additional blood samples were collected at $t = +2, 3, 4$, and 5 minutes. Beginning at $t = 10$ minutes, a hyperglycemic clamp technique (22) using a variable rate infusion of 20% dextrose was performed to achieve a plasma glucose concentration of approximately 230 mg/dl. Blood samples were taken every 5 minutes to adjust the infusion rate and achieve the desired plasma glucose concentration. After 45 minutes of glucose infusion (at $t = 55$ min), a second 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 (23). A subsequent 2-hour period without glucose infusion allowed plasma glucose to return to baseline. A second hyperglycemic clamp was then performed to achieve a plasma glucose concentration of approximately 340 mg/dl. After 45 minutes of glucose infusion, a third arginine pulse was injected with identical sampling.

Biochemical Analysis

Glucose was measured in duplicate by Nova StatStrip Glucose Hospital Meter System (Nova Biomedical), which is calibrated to provide plasma equivalent results to laboratory methods (24). Other samples were collected on ice into tubes containing ethylenediaminetetraacetic acid and protease inhibitor cocktail (and for MMTT samples, dipeptidyl peptidase-4 inhibitor; Sigma-Aldrich), centrifuged at 4°C, separated, and frozen at –80°C. Plasma insulin, C-peptide, proinsulin, and glucagon were measured in duplicate by double-antibody radioimmunoassays (Millipore). Active glucagon-like peptide-1 (GLP-1) and total glucose-dependent insulinotropic polypeptide (GIP) were measured in duplicate by enzyme-linked immunosorbent assays (Millipore).

Calculations

MMTT. Incremental areas under the curve (AUC) for glucose, insulin, C-peptide, glucagon, GLP-1, and GIP were calculated for 30 minutes (early phase) and

180 minutes with baseline values subtracted by trapezoidal method.

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 mean of 2-, 3-, 4-, and 5-minute values minus mean of baseline values (22). Acute responses during the 230 mg/dl 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_{230}) release. Acute responses during the 340 mg/dl clamp allow for determination of maximum arginine-induced insulin (AIR_{max}), C-peptide (ACR_{max}), and proinsulin (APR_{max}) release (i.e., β -cell secretory capacity), and minimum arginine-induced glucagon (AGR_{340}) release (19).

The fasting proinsulin-to-C-peptide ratio was calculated as the molar concentration of proinsulin divided by the molar concentration of C-peptide $\times 100$ (25). Estimation of proinsulin-to-C-peptide ratio within β -cell secretory granules is most reliable after acute stimulation of secretion (26); therefore, we examined the proinsulin secretory ratio in response to each injection of arginine from respective acute proinsulin/C-peptide responses (20).

PG_{50} , plasma glucose at which half-maximal insulin secretion is achieved, was calculated to assess β -cell sensitivity to glucose (19–21). Insulin sensitivity (M/I) was determined by dividing mean glucose infusion rate required during the 230 mg/dl glucose clamp (M) by mean prestimulus insulin level (I) between 40 and 45 minutes of glucose infusion; disposition index, a measure of β -cell function that relates insulin secretion to insulin sensitivity, was then calculated as $\text{AIR}_{\text{arg}} \times \text{M/I}$ (20).

Statistical analyses. Graphs were first generated to permit visual inspection of data and included differentiation by potentially important patient characteristics (age, *CFTR* mutation, PI/PS status) and plots examining relationships among various GPA responses (e.g., ACR_{pot} and AGR_{230} ; ACR_{pot} and MMTT outcomes). Paired comparisons between baseline and 4-month follow-up data were performed using paired Student's *t* test or Wilcoxon matched-pairs signed rank test. Potential relationships among various GPA responses were sought using Spearman

correlation. Subsequently, generalized estimating equations with an autoregressive correlation structure were performed to 1) assess for changes in acute insulin and C-peptide responses adjusted for potential covariates (prestimulus glucose, PI status, age), 2) test relationships of C-peptide and glucagon secretion during GPA, and 3) assess the relationship of C-peptide secretion and glucose tolerance during MMTT. Analyses were performed using STATA 15 (StataCorp LP).

Results

Participants

A total of 12 participants (six male) were studied at baseline (visit 1; median, minimum–maximum, age: 13.5 yr, 6–42; BMI-Z: 0.71, –2.39 to 1.97; FEV₁% predicted: 102, 39–122) and 4 months after ivacaftor initiation (Table 1). One additional participant was unable to complete follow-up studies (hospitalization and ivacaftor discontinuation by primary CF team for increased liver enzymes); the data from this subject were not included in analyses. One study participant was unable to complete the MMTT because intravenous access could not be secured. As expected, most participants (9/12) were PI, and overall increases in FEV₁% predicted and BMI-Z were observed after 4 months of ivacaftor therapy (Table 1).

Oral Glucose Tolerance Test

Fasting glucose (median [minimum–maximum]) was not different between baseline and follow-up (93 [53–104] vs. 88.5 [76–105] mg/dl; $P > 0.5$; Table 1). At baseline, 7/12 had NGT, 4 subjects had EGI, and 1 had IGT. Four of these five individuals with abnormal glucose tolerance remained with either EGI or IGT, whereas one subject with EGI had normalization of glucose tolerance. The 1-hour and 2-hour glucose were similar at baseline and follow-up (all $P > 0.5$). Hemoglobin A_{1c} did not change with 4 months of ivacaftor ($P = 0.44$), including in those subjects who vacillated between abnormal glucose tolerance categories (Table 1).

MMTT

Glucose, insulin, and incretin secretion during MMTT. Fasting glucose, insulin, C-peptide, free fatty acids, active GLP-1, and

Table 1. Study Participant Characteristics (n = 12) at Baseline and after 4 Months of Ivacaftor

Subject	Ivacaftor-Responsive CFTR Mutation	Second CFTR Mutation	Visit	Age (yr)	PS/PI	BMI-Z	FEV ₁ % Predicted	HbA _{1c}	Oral Glucose Tolerance	Fasting Glucose (mg/dl)	1-Hour OGTT Glucose (mg/dl)	2-Hour OGTT Glucose (mg/dl)
1	R117H	F508del	1	14.0	PS	-0.21	112	5.02	NGT	98	91	102
			2	14.3		-0.44	104	5.04	NGT	88	96	79
2	G551D	P67L	1	6.8	PS*	0.66	122	5.18	NGT	89	121	121
			2	7.1		0.89	124	5.09	NGT	76	Not done	85
3	S549R	F508del	1	16.9	PS	-0.70	39	5.92	NGT	99	114	112
			2	17.3		-0.27	85	5.56	NGT	89	122	93
4	G551D	F508del	1	6.3	PI	-2.36	87	5.4	NGT	98	104	108
			2	6.6		-1.54	88	5.28	NGT	93	113	109
5	G551D	F508del	1	6.0	PI	-1.21	62	5.15	NGT	97	138	135
			2	6.4		-0.25	90	5.24	NGT	97	146	120
6	R117H	F508del	1	8.3	PI	1.90	108	5.11	IGT	99	237	183
			2	8.7		1.79	109	5.23	EGI	97	165	138
7	S549R	711 + 3A->G	1	17.5	PI†	0.25	113	4.71	NGT	82	96	78
			2	17.8		0.13	110	4.89	NGT	91	106	83
8	G1244E	F508del	1	42.3	PI	0.73	51	5.13	EGI	104	182	132
			2	42.6		0.86	58	5.2	EGI	105	168	133
9	G1244E	T3381	1	19.1	PI†	0.66	102	5.01	NGT	64	118	103
			2	19.4		0.89	107	4.74	NGT	78	131	116
10‡	G1244E	N1303K	1	13.5	PI	1.22	92	5.6	EGI	73	168	78
			2	14.0		1.24	113	5.67	IGT	79	154	160
11‡	G1244E	N1303K	1	11.1	PI	1.15	103	5.35	EGI	53	168	71
			2	11.6		1.56	109	5.29	IGT	79	233	159
12	S549N	Q98X	1	15.3	PI	1.13	105	5.9	EGI	88	204	94
			2	15.6		1.33	111	5.8	NGT	85	102	85
P value for paired pre vs. post data										0.97	0.57	0.69

Definition of abbreviations: BMI = body mass index; CFTR = cystic fibrosis transmembrane conductance regulator; EGI = early glucose intolerance; HbA_{1c} = hemoglobin A_{1c}; IGT = impaired glucose tolerance; NGT = normal glucose tolerance; OGTT = oral glucose tolerance test; PI = pancreatic insufficient; PS = pancreatic sufficient.
*Pancreatic enzyme replacement therapy at visit 1 but not visit 2.
†PI but not taking enzymes.
‡Siblings.

GIP were not different from baseline after 4 months of ivacaftor. Similarly, maximum glucose during MMTT was not different: 137 (102–179) versus 140 (102–173) mg/dl. In addition, glucose, insulin, C-peptide, active GLP-1, and GIP AUC during MMTT were not different except for an increase in early phase C-peptide response calculated over the first 30 minutes ($P = 0.04$) (Table 2).

Glucose-potentiated Arginine Test

Glucose, insulin, C-peptide, and glucagon during GPA test.

Prestimulus glucose (mg/dl) under fasting conditions was slightly lower at baseline (92 [74–99]) than follow-up (96 [85–115]; $P = 0.04$). Prestimulus glucose during the 230 mg/dl (224 [180–241] vs. 224 [190–242]; $P = 0.56$) and 340 mg/dl clamps (329 [290–377] vs. 330 [294–378]; $P = 0.95$) were not different between baseline and follow-up.

Fasting islet cell hormone concentrations were not different between baseline and follow-up (Table 3). For insulin and C-peptide responses (Figure 1A, Table 3), under fasting conditions both AIR_{arg} and ACR_{arg} increased (both $P \leq 0.005$). Under 230 mg/dl glucose-potentiated conditions, ACR_{pot} was greater ($P = 0.027$) (Figure 1B and Table 3), a difference that persisted with adjustment

for prestimulus glucose, age, and PS status ($P = 0.005$). With adjustment for prestimulus glucose, age, and PI status, AIR_{pot} was on average 22.4 U/ml (95% confidence interval [CI], -1.7 to 46.6) greater at follow-up, but this difference did not reach statistical significance ($P = 0.069$). AIR_{max} and ACR_{max} were not different after 4 months of ivacaftor therapy even after adjustment for prestimulus glucose, age, PI status (both $P > 0.5$). As expected, AIR_{arg} and AIR_{pot} (baseline: $\rho = 0.65$; $P = 0.032$) (follow-up: $\rho = 0.77$; $P = 0.003$) and ACR_{arg} and ACR_{pot} (baseline: $\rho = 0.64$; $P = 0.024$) (follow-up: $\rho = 0.87$; $P = 0.0003$) were positively correlated at each visit.

With respect to glucagon, AGR at fasting, 230 mg/dl, and 340 mg/dl were not different between baseline and follow-up. Adjustment for prestimulus glucose, PI status, and age did not alter these results. However, because visual inspection suggested the relationship between ACR_{pot} and AGR_{230} was different between baseline and follow-up, longitudinal analyses were pursued. Indeed, the 230 mg/dl AGR_{230} was positively associated with ACR_{pot} ($\beta = 7.5$; 95% CI, 2 – 12.9 ; $P = 0.007$), but, after 4 months of ivacaftor, the relationship of AGR_{230} with ACR_{pot} was dampened with increasing ACR_{pot} (visit $\times ACR_{pot}$

interaction: $\beta = -4.42$; 95% CI, -6.68 to -2.16 ; $P < 0.0001$) (Figure 2) even after adjustment for prestimulus glucose ($\beta = -0.5$; 95% CI, -0.8 to -0.2 ; $P = 0.001$).

Proinsulin and proinsulin secretory ratios. For proinsulin responses, APR_{arg} was greater ($P = 0.03$), but APR_{pot} and APR_{max} were not different following 4 months of ivacaftor therapy (Table 3). The fasting proinsulin-to-C-peptide ratio and proinsulin secretory ratio under fasting, 230 mg/dl, and 340 mg/dl conditions were not different between baseline and follow-up visits (Table 3). Adjustment for prestimulus glucose, PI status, and age did not alter these results.

β -Cell sensitivity to glucose and insulin sensitivity. PG_{50} was not different after 4 months of ivacaftor therapy ($P = 0.86$) (Table 3). Insulin sensitivity, as measured by M/I, was not different over the 4 months ($P = 0.16$) (Table 3). However, disposition index (amount of insulin secreted for degree of insulin resistance) improved after 4 months ($P = 0.04$) (Table 3).

Relationship of GPA and MMTT parameters.

We next tested the relationships of ACR_{arg} , ACR_{pot} , and ACR_{max} measured during GPA as indices of insulin secretion with 1) MMTT early phase insulin secretion during the first 30 minutes, 2) MMTT peak glucose, 3) MMTT 180-minute insulin AUC, 4) MMTT 180-minute glucose AUC, and

Table 2. MMTT Responses during the 180 Minutes after Ingestion, Median (Minimum to Maximum)

	Baseline	Follow-up	P Value
Fasting			
Glucose, mg/dl	85 (70 to 91)	90 (76 to 97)	0.06
Insulin, μ U/ml	7.4 (4.2 to 24.4)	8.9 (5.1 to 22.7)	0.40
C-peptide, ng/ml	0.99 (0.5 to 2.2)	1.05 (0.7 to 2.25)	0.37
FFA, mmol/L	0.48 (0.23 to 0.92)	0.45 (0.24 to 0.85)	0.81
GIP, pg/ml	37.9 (13.7 to 141.7)	38.7 (10.3 to 93.7)	0.73
Active GLP-1, pmol/L	4.01 (1.99 to 14.77)	3.09 (1.9 to 14.4)	0.77
Peak glucose	137 (102 to 179)	140 (102 to 173)	0.99
30-min AUC			
Glucose, mg $\cdot$ min/dl	205 (–30 to 745)	440 (70 to 840)	0.13
Insulin, μ U $\cdot$ min/ml	210 (3.5 to 2,271)	586 (136 to 1,701)	0.25
C-peptide, ng $\cdot$ min/ml	12.9 (1.35 to 75)	23.3 (6.35 to 123)	0.04
GIP, pg $\cdot$ min/ml	1,049 (–63.5 to 4,414)	2,290 (529 to 5,096)	0.24
Active GLP-1, pmol $\cdot$ min/L	41.9 (0 to 201)	64.3 (3.8 to 230)	0.7
180-min AUC			
Glucose, mg $\cdot$ min/dl	4,428 (1,325 to 9,675)	4,815 (–170 to 10,190)	0.86
Insulin, μ U $\cdot$ min/ml	3,519 (543 to 15,781)	5,644 (1,380 to 28,255)	0.13
C-peptide, ng $\cdot$ min/ml	378 (66 to 915)	396 (194 to 1,786)	0.48
GIP, pg $\cdot$ min/ml	30,565 (8,206 to 144,410)	33,447 (23,086 to 57,899)	0.09
Active GLP-1, pmol $\cdot$ min/L	370 (36 to 1682)	327 (154 to 856)	0.72
Insulin AUC/glucose AUC, (μ U/ml)/(mg/dl)	0.79 (0.34 to 5.36)	0.86 (–23.5 to 15.6)	0.86

Definition of abbreviations: AUC = area under the curve; FFA = free fatty acids; GIP = glucose-dependent insulinotropic polypeptide; GLP-1 = glucagon-like peptide-1; MMTT = mixed-meal tolerance test.

Table 3. Glucose-potentiated Arginine Test, Median (Minimum to Maximum)

	Baseline	Follow-up	P Value*	Visit 2 vs. Visit 1 β-Coefficient† (95% CI)	P Value‡
Insulin, μU/ml					
Fasting (n = 12)	8.7 (5.4 to 24.8)	9.5 (6.0 to 23.7)	0.47	0.76 (−1.9 to 3.4)	0.57
AI _{Rarg} (n = 11)	17.0 (5.6 to 91.7)	34.4 (16 to 93.4)	0.005	16.7 (7.4 to 25.9)	<0.0001
AI _{Rpot} (n = 12)	79.3 (18.5 to 392)	117 (27.6 to 400)	0.17	22.4 (−1.7 to 46.6)	0.069
AI _{Rmax} (n = 10)	125 (33.7 to 410)	132 (59 to 303)	0.69	−18.1 (−53.2 to 17)	0.31
C-peptide, ng/ml					
Fasting (n = 12)	1.08 (0.45 to 2.24)	1.11 (0.69 to 3.22)	0.12	0.11 (−0.18 to 0.39)	0.45
AC _{Rarg} (n = 12)	0.98 (0.19 to 3.3)	1.20 (0.07 to 4.82)	0.001	0.5 (0.2 to 0.8)	0.001
AC _{Rpot} (n = 12)	3.74 (0.16 to 7.66)	4.10 (0.45 to 8.64)	0.027	0.73 (0.23 to 1.22)	0.004
AC _{Rmax} (n = 11)	3.76 (0.003 to 9.51)	4.28 (0.53 to 7.66)	0.85	0.03 (−0.76 to 0.81)	0.95
Glucagon, pg/ml					
Fasting (n = 10)	57.8 (39.5 to 93)	70.3 (32.5 to 83)	0.69	1.0 (−7.3 to 9.3)	0.86
AG _{Rarg} (n = 9)	45.5 (17.5 to 131.5)	60.9 (19.3 to 108)	0.71	4.4 (−11.3 to 20.1)	0.58
AG _{R230} (n = 10)	25.3 (4.3 to 105)	34.8 (0.5 to 76.3)	0.75	−0.4 (−8.5 to 7.8)	0.93
AG _{R340} (n = 9)	32.5 (9 to 116)	31.0 (20.3 to 63.3)	0.50	−7.5 (−25 to 10.4)	0.41
Proinsulin, pmol/L					
Fasting (n = 11)	9.34 (1.99 to 22.3)	10.8 (1.99 to 31.8)	0.11	2.05 (−0.34 to 4.45)	0.093
AP _{Rarg} (n = 10)	5.30 (0 to 11.9)	8.00 (0 to 14.2)	0.03	2.1 (0.2 to 3.9)	0.03
AP _{Rpot} (n = 12)	19.8 (5.0 to 31.9)	18.8 (6.5 to 31.7)	0.58	2.5 (−3.1 to 8.1)	0.39
AP _{Rmax} (n = 11)	11.6 (2.8 to 22.8)	17.2 (5.1 to 25.1)	0.57	0.7 (−3.9 to 5.3)	0.76
Proinsulin/C-peptide					
Fasting proinsulin/C-peptide ratio, % (n = 11)	1.90 (0.66 to 5.2)	2.60 (0.7 to 3.9)	0.83	0.13 (−0.32 to 0.59)	0.57
PIS _{Rarg} , % (n = 10)	1.44 (0 to 2.5)	1.44 (0 to 2.8)	0.71	−0.14 (−0.57 to 0.3)	0.54
PIS _{Rpot} , % (n = 11)	1.39 (0.59 to 2.83)	1.39 (0.65 to 2.46)	0.8	−0.09 (−0.36 to 0.18)	0.5
PIS _{Rmax} , % (n = 10)	1.01 (0.14 to 2.0)	1.20 (0.20 to 4.5)	0.36	0.18 (−0.5 to 0.86)	0.64
β-Cell sensitivity to glucose					
PG ₅₀ , mg/dl	136 (119 to 185)	136 (92 to 200)	0.86		
Insulin sensitivity					
M/I, (mg/kg/min)/(μU/ml)	0.51 (0.10 to 7.1)	0.30 (0.13 to 7.5)	0.16		
DI, mg/kg/min	9.3 (3.2 to 101)	11.6 (4.2 to 139)	0.04		

Definition of abbreviations: ACR = acute C-peptide response; AGR = acute glucagon response; AI_R = acute insulin response; AP_R = acute proinsulin response; arg = arginine; DI = disposition index calculated as AI_{Rarg} × M/I; M/I = insulin sensitivity calculated as the mean glucose infusion rate during 230 mg/dl glucose clamp (M) divided by the mean prestimulus insulin level (I); PIS_R = proinsulin secretory ratio; PG₅₀ = plasma glucose at half-maximal insulin secretion; pot = potentiated.

*Sign rank test for paired data.

†Adjusted for prestimulus glucose, age, and pancreatic insufficiency status (PS vs. PI).

‡Longitudinal model.

5) whether these relationships differed between preivacaftor and postivacaftor visits. ACR_{arg}, ACR_{pot}, and ACR_{max} were all positively associated with early phase insulin secretion (Table 4). Greater ACR_{arg}, ACR_{pot}, and ACR_{max} were all associated with lower MMTT peak glucose and glucose AUC. These relationships, however, were not further improved after 4 months of ivacaftor (Figure 3, Table 4).

Discussion

This study evaluated the impact of 4 months of clinically indicated ivacaftor therapy on islet hormone and incretin secretion in

a cohort of young individuals with CF and normal to mild glucose intolerance. Alterations in neither meal-related glucose tolerance nor incretin secretion were evident. However, islet function seemed improved; insulin secretion, as quantitated by GPA, increased overall and in relation to insulin sensitivity. Improved suppression of glucagon secretion, seen with glucose-inhibition of the glucagon response to arginine, was found. These findings suggest that reported improvements in glucose tolerance following ivacaftor therapy (7) may be conferred through improved β-cell and α-cell function.

Highlighting clinical implications of GPA parameters, greater first-phase insulin secretion and GPA-induced insulin

secretion were associated with better MMTT outcomes: 1) lower peak glucose, 2) lower glucose AUC, and 3) higher C-peptide during the first 30 minutes. Islet function improvements over 4 months of ivacaftor, however, did not translate readily into lower meal-related glucose excursion, which may reflect the relatively NGT of our young cohort. Importantly, in patients without diabetes with CF, a reduction in “early” phase insulin secretion is well-documented and considered an initial, clinically measurable marker of defective insulin secretion (16). Thus, although glucose excursion was not altered, finding improved early phase insulin secretion and improved disposition index during GPA

Figure 1. Acute C-peptide responses to arginine (0.07 g/kg, maximum dose 5 g, intravenous over 1 min) during (A) fasting (ACR_{arg}) and (B) 230 mg/dl clamp (ACR_{pot}) before and after 4 months of clinically indicated ivacaftor therapy in individuals with cystic fibrosis caused by at least one *CFTR* gating or conductance mutation. Individuals described clinically as “pancreatic-sufficient” are shown in dashed lines; participant numbers correlating with data in Table 1 are also shown. In age-, pancreatic sufficiency status-, and prestimulus glucose-adjusted models, ACR_{arg} ($P = 0.001$) and ACR_{pot} ($P = 0.004$) improved after 4 months of ivacaftor therapy. *CFTR* = cystic fibrosis transmembrane conductance regulator.

suggests ivacaftor is impacting β -cell function. Following pancreatic insulin secretion, approximately 50% of insulin is extracted by the liver (27); this hepatic

insulin extraction may be increased in the setting of CF (28). C-peptide bypasses the liver, and mathematical models that rely on C-peptide can be used to quantify insulin

Figure 2. Relationship between inhibition of glucagon (AGR_{230}) and the acute C-peptide response during the 230 mg/dl clamp (ACR_{pot}), before and after 4 months of clinically indicated ivacaftor therapy in individuals with cystic fibrosis caused by at least one *CFTR* gating or conductance mutation. Preivacaftor responses and fitted line are shown in blue, and postivacaftor initiation responses and fitted line are shown in red. With increasing C-peptide secretion under 230 mg/dl clamp conditions glucagon secretion is also greater, presumably reflecting overall better islet function. However, this relationship between higher C-peptide secretion and higher glucagon secretion is dampened after 4 months of ivacaftor therapy (visit $\times$ ACR_{pot} interaction: $\beta = -4.42$; 95% confidence interval, -6.68 to -2.16 ; $P < 0.0001$), suggesting the glucagon-secreting α -cell is more sensitive to inhibitory paracrine effects of insulin. CI = confidence interval.

secretory rates (29, 30). Unfortunately, meal-related insulin secretory rates could not be determined for our study participants using these published models (29, 30). These models make assumptions about C-peptide clearance rates derived from adults and gastrointestinal glucose absorption that may not be generalizable to CF in which delayed gastric emptying, bacterial overgrowth, and PI affect nutrient digestion and absorption rates.

By extension, we cannot exclude that improved nutrient digestion and absorption directly enhanced insulin secretion and therefore contributed not only to failure to observe improved meal-related glucose tolerance but also the higher C-peptide during the first 30 minutes of the meal. Indeed, ivacaftor therapy is associated with an improved intestinal pH profile (31) that may enhance pancreatic enzyme-replacement therapy efficiency and nutrient absorption. However, incretin responses were not different following 4 months of ivacaftor and suggest that any possible improvement in nutrient digestion and absorption did not translate into improved incretin secretion. This study was not designed to test for possible effects of ivacaftor on β -cell and α -cell sensitivity to incretins. Nevertheless, the improved acute C-peptide responses during fasting and glucose potentiation (conditions that excludes any contribution of gastrointestinal glucose absorption) are further evidence an ivacaftor effect on β -cell function.

Perhaps a more sensitive marker of improved islet function is the functional interaction of β -cells and α -cells. First, important to note are that 1) hyperglycemia suppresses glucagon secretion, 2) insulin secretion directly suppresses glucagon secretion through a paracrine effect, 3) hyperglucagonemia promotes hyperglycemia in the insulin deficiency of diabetes, 4) collateral damage of pancreatic islets in CF involves β -cells and α -cells, and 5) reduced glucagon secretion occurs in PI-CF (32). Longitudinal models permit insight into α -cell function. Insulin secretion and glucagon secretion were positively associated; at follow-up, greater insulin secretion under hyperglycemic clamp conditions (ACR_{pot}) was associated with more robust suppression of glucagon secretion (AGR_{230}). Whether these changes reflect improved α -cell sensitivity to glucose, α -cell sensitivity to insulin, or greater local insulin secretion is not known. The last is a particularly important

Table 4. Relationship of Insulin and Glucose Responses during MMTT and GPA-defined Acute C-Peptide Responses

	Insulin 30-Minute AUC ($\mu\text{U} \cdot \text{min}/\text{ml}$)	180-Minute Peak Glucose (mg/dl)	Glucose 180-Minute AUC ($\text{mg} \cdot \text{min}/\text{dl}$)
ACR _{arg}	$\beta = 281$ (47.7 to 514; $P = 0.018$)	$\beta = -9.3$ (-17.1 to -1.4 ; $P = 0.022$)	$\beta = -860$ ($-1,877$ to 155 ; $P = 0.097$)
ACR _{pot}	$\beta = 114$ (0.81 to 227; $P = 0.048$)	$\beta = -5.9$ (-8.7 to -3.1 ; $-P < 0.0001$)	$\beta = -690$ ($-1,023$ to -357 ; $P < 0.0001$)
ACR _{max}	$\beta = 172$ (99 to 245; $P < 0.001$)	$\beta = -5.2$ (-8.5 to -2.0 ; $P < 0.0001$)	$\beta = -501$ (-912 to -91 ; $P = 0.023$)

Definition of abbreviations: ACR = acute C-peptide response; arg = arginine; AUC = area under the curve; BMI = body mass index; GPA = glucose-potentiated arginine; MMTT = mixed-meal tolerance test; pot = potentiated.
Shown are β -coefficients* (95% confidence interval; P value) for acute C-peptide response during GPA[†].
*Models adjusted for BMI-Z.
[†]The relationship between ACR and MMTT outcomes did not vary between visits (i.e., no ACR $\times$ visit interactions).

consideration, because samples obtained during GPA and MMTT reflect systemic concentrations that follow hepatic extraction of islet insulin and glucagon secretion into the portal circulation. Direct local α -cell functional regulation by insulin measurement from sampling portal circulation would likely represent a more sensitive (but impractical) measure of islet cell function changes.

The extent to which increases in insulin secretion arise from β -cell-specific ivacaftor effects remains unclear. Although controversial, CFTR mRNA and protein expression in human and murine β -cells has been documented by some investigators (9, 10, 33), and a direct functional role for CFTR in regulation of cAMP-mediated

(as occurs with GLP-1) and of glucose-stimulated insulin secretion has been demonstrated in islets and isolated β -cells (9, 20). In contrast, others suggest more limited CFTR mRNA expression (pancreatic ductal cells but not islets) (13) and have demonstrated reductions in insulin secretion do not seem to rely on β -cell CFTR expression (13).

Reduced inflammation is an alternative mechanism for improved islet function. For instance, IL-1 β directly inhibits β -cell function in type 1 diabetes (34) and during high glucose (35). Furthermore, IL-1 β inhibition associates with improved β -cell secretory function in type 2 diabetes (36). In these situations, the islet is the inflammatory site. Supporting such a role

for islet inflammation in CF, increased IL-1 β immunoreactivity was recently reported in pancreatic tissue obtained at the time of autopsy in in patients with and without diabetes with CF (37). In addition, systemic inflammation, also seen with type 2 diabetes, is generally associated with insulin resistance (38–40), but how systemic inflammation may directly impact islet function is uncertain. CFTR knockout ferret islets display increased IL-6 secretion, whereas IL-6 treatment of wild-type ferret islets mimicked the CF phenotype of reduced islet insulin content but increased percentage of insulin secretion in low glucose (13). Because CF is well recognized as an inflammatory process, ivacaftor therapy may decrease inflammation as evidenced by a reduction in hospitalization rates, decreased sputum *Pseudomonas aeruginosa* isolation (31), and decreased sputum inflammatory markers (41). This study cannot differentiate the impact of improved inflammation versus direct islet cell effects of ivacaftor. Insulin resistance is present in patients with IGT and CFRD during pulmonary exacerbations (42, 43) but is largely not responsible for hyperglycemia in CF (16). In our cohort, insulin sensitivity remained essentially unchanged, an expected finding given participants were in the well-state and not diabetic.

Because enrollment required ivacaftor-naivety, participants were generally young with no or only mild glucose intolerance. Improvements in glucose tolerance were, thus, challenging to detect. In addition, insulin secretion in the youth was lower than what is reported in healthy, lean adults (32). Interpreting their insulin secretion changes further is challenging without a healthy pediatric reference.

In addition, this observational study was limited by not including a comparison of glucose excursion and islet hormone secretion

Figure 3. Relationship between glucose area under the curve during the mixed-meal tolerance test and the acute C-peptide response during the 230 mg/dl clamp (ACR_{pot}), before and after 4 months of clinically indicated ivacaftor therapy in individuals with CF caused by at least one CFTR gating or conductance mutation. Preivacaftor responses are shown in blue, and postivacaftor initiation responses are shown in red. With increasing ACR_{pot}, glucose area under the curve is lower ($\beta = -690$ mg $\cdot$ min/dl; 95% confidence interval, $-1,023$ to -357 ; $P < 0.0001$); this relationship persisted following 4 months of ivacaftor therapy. AUC = area under the curve; CI = confidence interval; MMTT = mixed-meal tolerance test.

in untreated CF. Variability of glucose excursion within individuals with CF is well documented, particularly with CF exacerbations. Moreover, evolution of GPA-derived measures in otherwise healthy growing children has not been documented. Indeed, one might anticipate enhanced insulin secretion with pubertal progression. Our pediatric subjects were either prepubertal or postpubertal, and large changes over just 4 months in pubertal children are not anticipated. Despite these limitations, on average within-subject insulin secretion improved over 4 months. Whether insulin secretion would further improve with longer duration of ivacaftor therapy is not known.

Improvements in insulin secretion did not seem related to PS status. This study relied on clinical diagnosis, and misclassification of PI status cannot be excluded. Misclassification of two “PS” participants was suspected based on abdominal symptoms.

Comparisons of insulin and C-peptide secretion during GPA and insulin, C-peptide, incretin, and glucose excursion during a meal were the primary aims of the study

protocol. Although these findings are internally consistent, the testing of multiple hypotheses within this dataset without adjusting for multiple comparisons should be acknowledged.

Finally, the extent to which these ivacaftor effects are “short-term” or enduring cannot be gleaned from this study. Reduced β -cell mass arising from collateral damage extending from pancreatic exocrine tissue may be irreversible, and with aging further decline in β -cell function and mass is anticipated. Obesity-related insulin resistance and high saturated fat and high glycemic index diets may also contribute to β -cell toxicity (44–48). Thus, ivacaftor may conceivably delay but not prevent CFRD.

In conclusion, we demonstrated improved insulin secretion and glucagon secretion regulation after 4 months of ivacaftor in the setting of fairly NGT. The long-term implications in preventing or delaying CFRD development with early CFTR modulator initiation and the specific mechanisms underlying these islet effects require additional investigation. ■

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