



# Inhibition of Renal Sodium–Glucose Cotransport With Empagliflozin Lowers Fasting Plasma Glucose and Improves $\beta$ -Cell Function in Subjects With Impaired Fasting Glucose

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**The objective of this study was to examine the effect of renal sodium–glucose cotransporter inhibition with empagliflozin on the fasting plasma glucose (FPG) concentration and  $\beta$ -cell function in subjects with impaired fasting glucose (IFG). Eight subjects with normal fasting glucose (NFG) and eight subjects with IFG received empagliflozin (25 mg/day) for 2 weeks. FPG concentration and  $\beta$ -cell function was measured with a nine-step hyperglycemic clamp before and 48 h and 14 days after the start of empagliflozin. Empagliflozin caused  $50 \pm 4$  and  $45 \pm 4$  g glucosuria on day 2 in subjects with IFG and NFG, respectively, and the glucosuria was maintained for 2 weeks in both groups. The FPG concentration decreased only in subjects with IFG from  $110 \pm 2$  to  $103 \pm 3$  mg/dL ( $P < 0.01$ ) after 14 days. The FPG concentration remained unchanged ( $95 \pm 2$  to  $94 \pm 2$  mg/dL) in subjects with NFG. Empagliflozin enhanced  $\beta$ -cell function only in subjects with IFG. The incremental area under the plasma C-peptide concentration curve during the hyperglycemic clamp increased by  $22 \pm 4$  and  $23 \pm 4\%$  after 48 h and 14 days, respectively ( $P < 0.01$ ); the plasma C-peptide response remained unchanged in subjects with NFG. Insulin sensitivity during the hyperglycemic clamp was not affected by empagliflozin in either IFG or NFG. Thus,  $\beta$ -cell function measured with the insulin secretion/insulin sensitivity (disposition) index increased significantly in IFG, but not in subjects with normal glucose tolerance. Inhibition of renal sodium–glucose cotransport with empagliflozin in subjects with IFG and NFG produces comparable glucosuria but lowers the plasma glucose concentration and improves  $\beta$ -cell function only in subjects with IFG.**

Impaired fasting glucose (IFG) (fasting plasma glucose [FPG] 100–125 mg/dL) was introduced by the American Diabetes Association in 1997 to describe an intermediate state in the transition from normal glucose tolerance (NGT) to type 2 diabetes mellitus (T2DM) (1). Like impaired glucose tolerance (IGT), IFG describes a prediabetic state with a similar risk of developing T2DM to that in IGT (2). However, IFG and IGT have distinct pathophysiologic abnormalities (3). Subjects with IFG are characterized by hepatic insulin resistance and loss of first-phase insulin secretion, whereas subjects with IGT are characterized by muscle insulin resistance and loss of second-phase, as well as first-phase, insulin secretion (3,4).

Progressive  $\beta$ -cell failure is the principal factor responsible for the development of hyperglycemia and continuous rise in plasma glucose concentration in patients with T2DM (4–6). Interventions that halt or reverse the progressive  $\beta$ -cell failure have proven effective in preventing the conversion of prediabetes to T2DM (7,8) and produce a durable reduction in the HbA<sub>1c</sub> in patients with T2DM (reviewed in Ref. 6).

Subjects with IFG manifest a severe defect in first-phase insulin secretion (4,9,10), and studies with the intravenous glucose tolerance test (11) and hyperglycemic clamp (4,9,12) have demonstrated a progressive decline in first-phase insulin secretion with increasing FPG concentration. First-phase insulin secretion is essential for insulinizing the liver, resulting in the early and rapid suppression of hepatic glucose production (13). Excessive hepatic glucose production during the sleeping hours is the primary determinant

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of the FPG concentration (14). Chronic elevation in the plasma glucose concentration has been shown to impair  $\beta$ -cell function (i.e., glucotoxicity) (15). Thus, it is difficult to determine the cause-effect relationship between the increase in FPG concentration and impaired  $\beta$ -cell function in individuals with IFG. To address this question, we used the sodium-glucose cotransporter (SGLT) inhibitor empagliflozin, which lowers the plasma glucose concentration by promoting urinary glucose loss (16), and quantitated the effect of reduction in the FPG concentration on  $\beta$ -cell function with the stepped hyperglycemic clamp technique.

## RESEARCH DESIGN AND METHODS

### Subjects

Eight subjects with normal fasting glucose (NFG; FPG <100 mg/dL) and eight subjects with IFG (FPG 100–125 mg/dL) participated in the study. The subjects were part of a large study that was recently published (17). The patient characteristics are shown in Table 1. All subjects were in good general health as determined by medical history, physical exam, screening laboratory tests, and electrocardiogram. Body weight was stable ( $\pm 3$  pounds) in all subjects for 3 months prior to study, and no subject participated in any excessively heavy exercise program. No subject was taking any medication known to affect glucose metabolism.

### Research Design

All studies were performed in the Clinical Research Center at the Texas Diabetes Institute following an overnight fast. On days  $-5$  and  $-4$ , a 24-h urine was collected for measurement of urinary glucose excretion. On day  $-3$ , a nine-step hyperglycemic clamp was performed following a 10-h overnight fast. Subjects reported to the Clinical Research Center at 6 A.M., and a catheter was placed into an antecubital vein for glucose infusion. A second catheter was placed into a vein on the dorsum of the hand and the hand was placed in a thermo-regulated box heated to 60°C for arterialized blood draws. Blood was drawn at  $-30$ ,  $-15$ , and 0 min. At time zero, a stepped hyperglycemic clamp was performed (16). The plasma glucose concentration was acutely raised and maintained at 40 mg/dL above the fasting level (i.e., from  $\sim 100$  to 140 mg/dL) for 40 min and urine was collected from 0–40 min for measurement of

glucose excretion. From 40–80, 80–120, 120–160, 160–200, 200–240, 240–280, 280–320, and 320–360 min the plasma glucose concentration was acutely raised and maintained at 180, 220, 260, 300, 340, 380, 420, and 460 mg/dL, respectively, with the variable infusion of 20% dextrose solution. Plasma C-peptide concentration was measured at 2, 4, 6, 8, and 10 min and every 10 min thereafter until 360 min. To ensure sufficient urine volume to all spontaneous voiding during the study, subjects were asked to drink one quart of water upon awakening at home and received a water load (15 mL/kg) at the time of arrival at the Clinical Research Center. Each voided volume of urine was quantitatively replaced with drinking water during the hyperglycemic clamp to ensure spontaneous voiding. Urine was collected during each 40 min period for measurement of urinary glucose excretion. The mean urine volume during the 40 min collection periods was 504 mL. On days  $-2$  and  $-1$ , 24-h urines were collected for measurement of urinary glucose excretion.

On day zero, subjects were started on empagliflozin, 25 mg/day, which they took in the morning for 14 days (including on the morning of day 14). At 48 h and 14 days after the start of empagliflozin, the stepped hyperglycemic clamp was repeated as described above.

### Analytical Techniques

Plasma and urine glucose concentration was determined by glucose oxidation method (Analox; Analox Instruments, Lunenburg, MA). Plasma insulin, C-peptide, and glucagon concentrations were determined by radioimmunoassay (Linco Research, St. Louis, MO).

### Calculations and Statistical Analyses

Insulin secretion during each step of the hyperglycemic clamp was calculated as the incremental urea above baseline under the plasma C-peptide concentration during that step according to the trapezoid rule. First- and second-phase insulin secretion during the hyperglycemic clamp were calculated as the incremental area above baseline for the plasma C-peptide concentration curve between 0 and 10 ( $\Delta C\text{-Pep}_{[0-10]}$ ) and 10 and 360 ( $\Delta C\text{-Pep}_{[10-360]}$ ) minutes, respectively. Tissue glucose uptake (TGU) during each step was calculated as the glucose infusion rate during the last

**Table 1—Baseline patient characteristics**

	NFG	IFG	P value
Age (years)	58 $\pm$ 2	59 $\pm$ 3	NS
Sex (male/female)	6/2	5/3	NS
BMI (kg/m <sup>2</sup> )	27.0 $\pm$ 1.1	30.3 $\pm$ 0.8	0.04
FPG (mg/dL)	95 $\pm$ 2	110 $\pm$ 2	<0.0001
HbA <sub>1c</sub> (%)	5.6 $\pm$ 0.1	5.7 $\pm$ 0.1	NS
Plasma creatinine (mg/dL)	0.70 $\pm$ 0.05	0.79 $\pm$ 0.04	NS
eGFR (mL/min/1.73 m <sup>2</sup> )	120 $\pm$ 10	99 $\pm$ 6	NS
Fasting plasma FFA (mmol/L)	0.54 $\pm$ 0.07	0.46 $\pm$ 0.04	NS

eGFR, estimated glomerular filtration rate; FFA, free fatty acid.

20 min of each step minus urinary glucose excretion during the same time period. We previously have shown that under conditions of combined hyperglycemia plus hyperinsulinemia, hepatic glucose production is maximally/near maximally suppressed (18). Insulin sensitivity during each step of the hyperglycemic clamp was calculated as TGU during the last 20 min of each step (glucose infusion rate minus urinary glucose excretion) divided by the mean plasma insulin (MPI) concentration during the same period. Previous studies have demonstrated that this index of insulin sensitivity (TGU/MPI) strongly correlates (86%) with total-body insulin-mediated glucose disposal measured in the same subject with the euglycemic-hyperinsulinemic clamp (16) and, therefore, was used to calculate  $\beta$ -cell function.  $\beta$ -Cell function was calculated as the insulin secretion/insulin resistance (disposition; IS/IR) index:  $(\Delta C\text{-Pep}_{(10-360)}) \times$  (insulin sensitivity) (19).  $\beta$ -Cell glucose sensitivity was calculated as the slope of the line relating insulin secretion

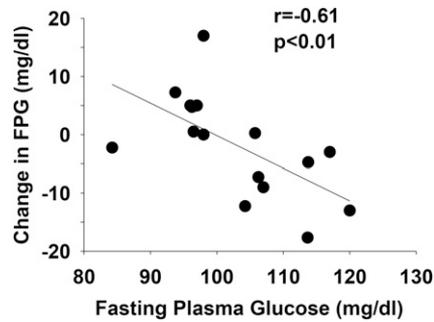
(measured as the incremental area under the plasma C-peptide concentration curve) and the plasma glucose concentration during each hyperglycemic clamp step.

Values are expressed as the mean  $\pm$  SEM. Insulin secretion,  $\beta$ -cell function, glucose infusion rate, and  $\beta$ -cell glucose sensitivity on days 2 and 14 after the start of empagliflozin were compared with baseline with paired *t* test. To test for repeated measures for FPG and insulin secretion on days 2 and 14 versus baseline, two-way ANOVA with post hoc analysis (Bonferroni) was used. To estimate the contribution of the change in FPG concentration on days 2 and 14 to the increase in insulin secretion and  $\beta$ -cell function, a linear regression model was constructed with the change in  $\Delta C\text{-Pep}_{(10-360)}$  and IS/IR index as the dependent variable and the change in FPG as the independent variable. FPG and other anthropometric variables were included as covariates in the model. A *P* value  $<0.05$  (two-tailed) was considered for statistically

**Table 2—Effect of empagliflozin on the FPG concentration and insulin secretion during the stepped hyperglycemic clamp**

	NFG	IFG	<i>P</i> value
FPG (mg/dL)			
Baseline	95 $\pm$ 2 (96)	110 $\pm$ 2 (113)	<0.0001
48 h	94 $\pm$ 3 (97)	103 $\pm$ 3* (102)	0.07
Day 14	100 $\pm$ 3 (97)	103 $\pm$ 3* (103)	0.06
FP C-peptide (ng/mL)			
Baseline	2.1 $\pm$ 0.3 (2.0)	4.0 $\pm$ 0.4 (4.1)	0.002
48 h	2.0 $\pm$ 0.3 (2.0)	3.7 $\pm$ 0.5 (3.4)	0.008
Day 14	2.5 $\pm$ 0.4 (2.9)	3.7 $\pm$ 0.5 (3.8)	0.02
Fasting FFA (mmol)			
Baseline	0.54 $\pm$ 0.07 (0.48)	0.46 $\pm$ 0.04 (0.44)	NS
48 h	0.65 $\pm$ 0.09 (0.57)	0.62 $\pm$ 0.09* (0.61)	NS
Day 14	0.42 $\pm$ 0.05 (0.43)	0.49 $\pm$ 0.04 (0.46)	NS
$\Delta C\text{-Pep}_{(0-10)}$ (ng/mL/min)			
Baseline	15 $\pm$ 4 (14)	26 $\pm$ 5 (18)	NS
48 h	14 $\pm$ 4 (13)	34 $\pm$ 4 (27)	0.05
Day 14	16 $\pm$ 4 (10)	32 $\pm$ 4 (30)	NS
$\Delta C\text{-Pep}_{(10-360)}$ (ng/mL/min)			
Baseline	98 $\pm$ 11 (81)	116 $\pm$ 10 (94)	NS
48 h	107 $\pm$ 11 (105)	141 $\pm$ 8* (141)	0.07
Day 14	96 $\pm$ 9 (91)	141 $\pm$ 8* (138)	<0.05
Slope (C-Pep/PG)			
Baseline	0.10 $\pm$ 0.01 (0.08)	0.10 $\pm$ 0.01 (0.11)	NS
48 h	0.10 $\pm$ 0.01 (0.09)	0.11 $\pm$ 0.01 (0.11)	NS
Day 14	0.09 $\pm$ 0.01 (0.10)	0.12 $\pm$ 0.01 (0.12)	NS
TGU (mg/kg/min)			
Baseline	15.7 $\pm$ 1.2 (15.3)	12.4 $\pm$ 0.8 (12.2)	0.2
48 h	16.3 $\pm$ 1.5 (17.3)	12.8 $\pm$ 0.5 (12.9)	0.01
Day 14	16.3 $\pm$ 1.2 (17.0)	12.9 $\pm$ 0.9 (12.7)	0.02
TGU/MPI			
Baseline	48 $\pm$ 10 (49)	17.2 $\pm$ 2 (16.3)	0.01
48 h	44 $\pm$ 7 (47)	21 $\pm$ 3 (20.1)	0.01
Day 14	50 $\pm$ 10 (48)	20 $\pm$ 3 (17.4)	0.01
IS/IR index			
Baseline	4,324 $\pm$ 1,004 (3,854)	1,784 $\pm$ 160 (1,879)	<0.05
48 h	4,410 $\pm$ 927 (4,138)	2,863 $\pm$ 497* (2,898)	NS
Day 14	4,322 $\pm$ 810 (3,575)	2,697 $\pm$ 391* (2,623)	NS

Data are mean  $\pm$  SEM (median) unless otherwise indicated. FFA, free fatty acid; FP, fasting plasma. \**P* < 0.05 versus baseline.



**Figure 1**—Relationship between the change in plasma glucose concentration 48 h after empagliflozin (25 mg/day) administration and the FPG concentration at baseline in subjects with NFG and IFG.

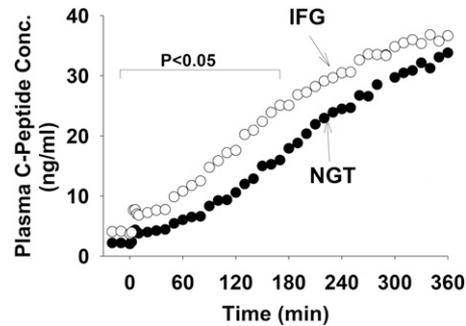
significant level. SPSS 22.0 statistical package (SPSS Inc.) was used for statistical analysis.

#### Study Approval

The study protocol was approved by the institutional review board of The University of Texas Health Science Center at San Antonio in San Antonio, TX, and all subjects gave their written, informed, voluntary consent prior to participation. The study is registered at ClinicalTrials.gov under NCT01867307.

#### RESULTS

Subjects with NFG and IFG were matched in age ( $58 \pm 2$  and  $59 \pm 3$  years), sex (six males and two females; and five males and three females, respectively), and  $HbA_{1c}$  ( $5.6 \pm 0.1$  vs.  $5.7 \pm 0.1\%$ ) (Table 1). Subjects with IFG had significantly higher BMI ( $30.3 \pm 0.8$  vs.  $27.0 \pm 1.1$  kg/m<sup>2</sup>;  $P = 0.04$ ) and FPG concentration ( $110 \pm 2$  vs.  $95 \pm 2$  mg/dL;  $P < 0.0001$ ). Plasma creatinine concentration was similar in subjects with NFG and IFG ( $0.70 \pm 0.05$  vs.  $0.79 \pm 0.04$  mg/dL). Empagliflozin caused  $45 \pm 4$  and  $50 \pm 5$  g urinary glucose excretion per 24 h in subjects with NFG and IFG, respectively ( $P$  was not significant) on day 2; urinary glucose excretion remained unchanged on day 14 in subjects with NFG and IFG ( $38 \pm 5$  and  $48 \pm 7$  g/24 h, respectively; both  $P$  values were not significant vs. day 2). Despite comparable urinary glucose loss, FPG decreased significantly only in subjects with IFG on day 2 (from  $110 \pm 2$  to  $103 \pm 3$ ;  $P < 0.01$ ), whereas it remained unchanged in subjects with NGT ( $95 \pm 2$  vs.  $94 \pm 3$  mg/dL) (Table 2). The decrease in FPG in subjects with IFG was maintained at day 14 (Table 2). In two-way ANOVA, the decrease in FPG concentration on days 2 and 14 in subjects with IFG was significantly reduced compared with baseline after adjustment for the baseline FPG concentration. There was no significant change in FPG in subjects with NFG. There was no significant difference in FPG between days 2 and 14 in subjects with IFG. Further, the decrement (change) in the FPG concentration following empagliflozin administration was strongly and inversely correlated with the



**Figure 2**—Plasma C-peptide concentration during the baseline hyperglycemic clamp in subjects with IFG and NGT.

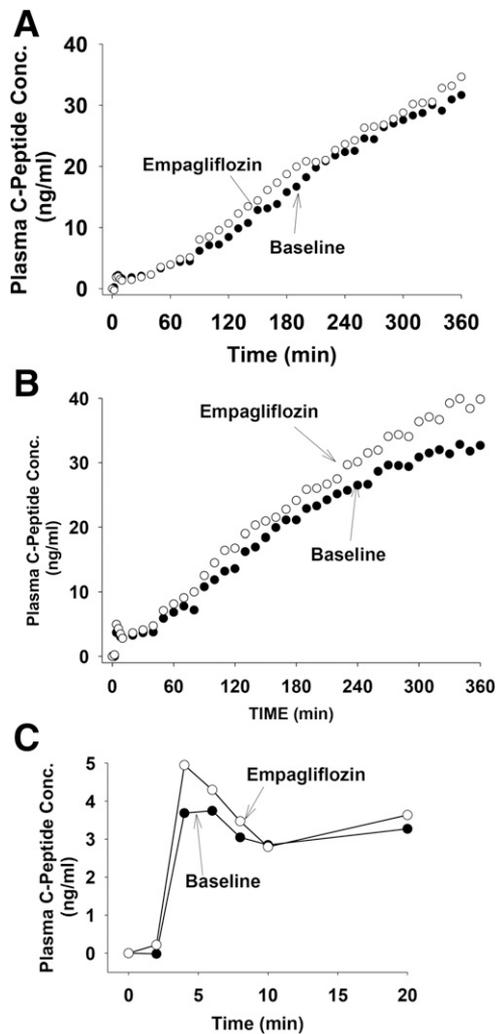
baseline FPG concentration ( $r = -0.61$ ;  $P < 0.01$ ) (Fig. 1). Consistent with previous studies (3,4,18) and compared with subjects with NFG, subjects with IFG had a significantly higher fasting plasma C-peptide concentration (Table 2).

#### Insulin Secretion in Subjects With IFG and NFG at Baseline

At baseline, the fasting plasma C-peptide concentration and plasma C-peptide concentration during the hyperglycemic clamp were significantly higher in IFG versus subjects with NGT (Fig. 2). However, the incremental area under the plasma C-peptide concentration curve from 0 to 10 min ( $\Delta C\text{-Pep}_{[0-10]}$ ) and from 10 to 360 min ( $\Delta C\text{-Pep}_{[10-360]}$ ) during the baseline hyperglycemic clamp was not significantly different between individuals with NFG and IFG (Table 2).  $\beta$ -Cell glucose sensitivity, measured as the slope of the line relating the plasma C-peptide concentration and the plasma glucose concentration at each step of the hyperglycemic clamp, was comparable in subjects with NFG and IFG (Table 2).

#### Effect of Empagliflozin on Insulin Secretion in Subjects With NFG and IFG

Empagliflozin had no significant effect on insulin secretion, measured as the incremental area under the plasma C-peptide concentration curve ( $\Delta C\text{-Pep}_{[0-10]}$  and  $\Delta C\text{-Pep}_{[10-360]}$ ), or on  $\beta$ -cell glucose sensitivity (measured as the slope of the line relating insulin secretion and plasma glucose concentration at each step of the hyperglycemic clamp) in subjects with NFG (Fig. 3 and Table 2). In contrast, empagliflozin caused a significant increase in insulin secretion, measured as the incremental area under the plasma C-peptide concentration curve ( $\Delta C\text{-Pep}_{[10-360]}$ ) after 48 h and 14 days in subjects with IFG (Fig. 4 and Table 2), and the decrease in  $\Delta C\text{-Pep}_{[10-360]}$  on days 2 and 14 remained significant after adjustment for FPG concentration (both  $P < 0.05$ ). Empagliflozin caused a small, but nonsignificant, increase in  $\Delta C\text{-Pep}_{[0-10]}$  in IFG subjects.  $\beta$ -Cell glucose sensitivity during the hyperglycemic clamp was unaffected by empagliflozin in subjects with both NFG and IFG.



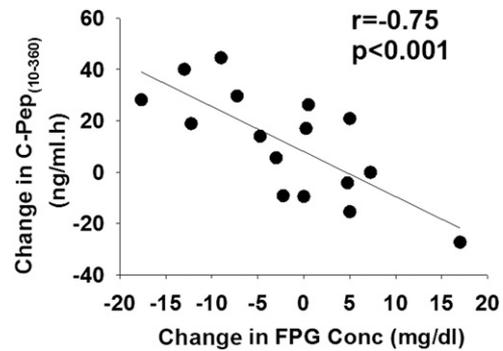
**Figure 3**—Increment above fasting in plasma C-peptide concentration during the hyperglycemic clamp in subjects with NFG (A) and IFG (B) at baseline and at 48 h after starting empagliflozin treatment. C: First-phase insulin secretion in subjects with IFG before and 48 h after starting empagliflozin.

### Plasma Glucagon Concentration

The fasting plasma glucagon concentration was modestly but not significantly increased in IFG compared with subjects with NGT ( $82 \pm 5$  vs.  $68 \pm 7$ ), and it was similarly suppressed during the hyperglycemic clamp in both groups (by  $49 \pm 3$  and  $58 \pm 4\%$ , respectively;  $P$  was not significant). Empagliflozin caused a small nonsignificant increase in the fasting plasma glucagon concentration in subjects with IFG and no change in subjects with NGT ( $92 \pm 8$  and  $70 \pm 5$ , respectively); there was no significant change in the suppression of plasma glucagon concentration during the hyperglycemic clamp in subjects with either IFG ( $61 \pm 5\%$ ) or NGT ( $52 \pm 5\%$ ).

### Insulin Sensitivity

Subjects with NFG had a higher mean tissue glucose uptake and insulin sensitivity index during the hyperglycemic clamp compared with subjects with IFG ( $15.7 \pm 1.2$  vs.



**Figure 4**—Relationship between the change in  $\Delta C\text{-Pep}_{(10-360)}$  48 h after the start of empagliflozin relative to baseline and the change in the FPG concentration after 48 h of empagliflozin treatment.

$12.4 \pm 0.7$  mg/kg/min,  $P < 0.05$ ; and  $48 \pm 10$  vs.  $17 \pm 2$ ,  $P = 0.01$ , respectively). Empagliflozin treatment did not significantly affect the insulin sensitivity index during the hyperglycemic clamp performed after 48 h or 14 days in either group (Table 2).

### Effect of Empagliflozin on $\beta$ -Cell Function

In subjects with IFG,  $\beta$ -cell function, measured with the IS/IR index, increased by 60 and 51% (both  $P < 0.05$ ) after 48 h and 14 days (from  $1,784 \pm 160$  at baseline to  $2,863 \pm 497$  and to  $2,697 \pm 391$  after 48 h and 14 days, respectively). In the subjects with NFG, the IS/IR after 48 h ( $4,410 \pm 927$ ) and 14 days ( $4,322 \pm 810$ ) was not changed significantly from baseline ( $4,324 \pm 1,004$ ). The change in IS/IR after 48 h was strongly and inversely correlated with the change in FPG concentration after 48 h (Fig. 5).

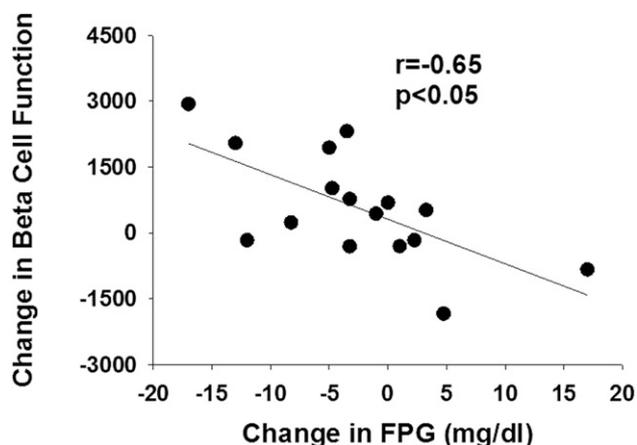
### Relationship Between the Increase in Insulin Secretion and $\beta$ -Cell Function and Decrease in FPG Concentration

The change in  $\Delta C\text{-Pep}_{(10-360)}$  during the hyperglycemic clamp after 48 h and 14 days in subjects with IFG strongly and inversely correlated with the decrease in FPG concentration ( $r = -0.74$  and  $-0.75$ , respectively; both  $P < 0.001$ ). Similarly, the increase in  $\beta$ -cell function (i.e., IS/IR index) caused by empagliflozin strongly and inversely correlated with the decrease in the FPG concentration ( $r = -0.65$ ;  $P < 0.05$ ). No significant relationship was observed between the change in FPG concentration and change in TGU or insulin sensitivity during the hyperglycemic clamp after 48 h and 14 days in subjects with IFG.

In a linear regression model (Table 3), the decrease in FPG concentration significantly contributed to the increase in insulin secretion ( $\Delta C\text{-Pep}_{(10-360)}$ ) on day 14 and to the increase in the IS/IR index on days 2 and 14. Approximately 20% of the increase in  $\beta$ -cell function could be accounted for by the decrease in FPG concentration. Because of the relatively small number of individuals in the current study, these results should be confirmed in a large group of subjects.

### DISCUSSION

The current study provides four novel findings: 1) first, in subjects with NFG concentration, SGLT2 inhibition has no



**Figure 5**—Correlation between the change in  $\beta$ -cell function (IS/IR index) and the change in FPG concentration after 48 h of empagliflozin treatment.

significant effect on the FPG concentration despite the large increase in glucose excretion; 2) second, in subjects with IFG, SGLT2 inhibition significantly reduces the FPG concentration; 3) in subjects with IFG, the decrease in FPG concentration is associated with a significant increase in insulin secretion and  $\beta$ -cell function; 4) in subjects with NFG and IFG, empagliflozin had no effect on the plasma glucagon concentration.

SGLT2 inhibitors lower the plasma glucose concentration by inhibiting renal sodium–glucose reabsorption, resulting in urinary glucose loss. In patients with T2DM, SGLT2 inhibitors produce 70–90 g/day glucosuria, and this results in a 20–40-mg/dL decrease in FPG concentration (20). However, in subjects with normal FPG concentration in the current study, despite 45 g/day glucosuria, the FPG concentration remained unchanged. This can only be explained by a rapid increase in endogenous glucose production (EGP) that closely approximated the urinary glucose loss and maintained the FPG concentration at the fasting level despite the marked glucosuria. These findings are reminiscent of the situation in subjects with familial renal glucosuria who maintain a normal FPG concentration despite the excretion of large amount of glucose in the urine (up to 100 g/day) (20). Although EGP was not measured in the current study, we (21) previously have shown that, in individuals with T2DM, 2 weeks of treatment with dapagliflozin augments EGP but reduces the FPG concentration. Thus, unlike the current study in which the increase in EGP in

subjects with NFG closely matches the urinary glucose loss, the increase in urinary glucose excretion in subjects with T2DM exceeded the increase in EGP, and the FPG concentration declined. Of note, in the current study, and unlike patients with T2DM in our previous study (21), the fasting plasma glucagon concentration in subjects with NFG was not affected by empagliflozin treatment. Although SGLT2 inhibitors have been shown to stimulate glucagon secretion by direct action on the  $\alpha$ -cell (22), the lack of significant change in plasma glucagon concentration in NGT and IFG in the current study argues for a more important role of the decrease in FPG concentration in patients with T2DM in stimulating glucagon secretion following SGLT2 administration. These findings underscore the importance of the renohepatic interaction in the regulation of plasma glucose concentration in individuals with NGT.

Unlike subjects with NFG, subjects with IFG experienced a small (7 mg/dL) but significant decrease in FPG concentration following the administration of empagliflozin, and this decrease in FPG was maintained for the 2-week treatment period. Because tracers were not used in the current study, we only can speculate on the mechanism(s) responsible for the different effect of empagliflozin on the FPG in subjects with IFG versus NFG despite similar urinary glucose loss in both groups. We (21) previously have shown that basal glucose clearance is decreased in subjects with IFG compared with subjects with NGT. Although not measured in the current study, it is possible that SGLT2 inhibition enhanced basal glucose clearance, as it has been shown to enhance insulin-mediated glucose disposal (21). Such an effect of empagliflozin would lower the FPG concentration in individuals with IFG but not NGT.

The third major finding of the current study is that a small decrease in the FPG concentration in subjects with IFG results in a significant increase in insulin secretion, measured with the gold standard, the hyperglycemic clamp method. This result indicates that, at least in part, the impairment in  $\beta$ -cell function in subjects with IFG is secondary to the increase in FPG concentration (i.e., glucotoxicity). Of note, previous studies have demonstrated that, compared with subjects with NGT, subjects with IFG manifest a decrease in first-phase insulin secretion, whereas second-phase insulin secretion is comparable to that in individuals with NGT (9–12). However, in the current study, the increase in first-phase insulin secretion caused by empagliflozin did not reach statistical significance in subjects

**Table 3**—Linear regression analysis with the increase in insulin secretion ( $\Delta$ C-Pep<sub>(10–360)</sub>) and increase in IS/IR index on days 2 and 14 as the dependent variable and the decrease in FPG concentration as the independent variable

Independent variable	Dependent variable	Covariates	Standardized $\beta$	P value	R <sup>2</sup>
$\Delta$ FPG <sub>(BL→day2)</sub>	$\Delta$ C-Pep <sub>(10–360)</sub>	Age, sex, BMI, FPG	0.26	NS	0.17
$\Delta$ FPG <sub>(BL→day2)</sub>	IS/IS index	Age, sex, BMI, FPG	0.52	0.03	0.28
$\Delta$ FPG <sub>(BL→day14)</sub>	$\Delta$ C-Pep <sub>(10–360)</sub>	Age, sex, BMI, FPG	0.59	0.01	0.13
$\Delta$ FPG <sub>(BL→day14)</sub>	IS/IS index	Age, sex, BMI, FPG	0.43	0.05	0.21

BL, baseline.

with IFG. Rather, a more robust increase in insulin secretion during the hyperglycemic clamp in subjects with IFG was observed between 200 and 360 min. It is possible that the small magnitude (40 mg/dL) of the first hyperglycemic step used in the current study was not sufficient to elicit a sufficiently robust first-phase insulin secretory response and, therefore, only resulted in a modest improvement in first-phase insulin secretion during empagliflozin treatment. Nonetheless, these results demonstrate that second-phase insulin secretion in subjects with IFG, though comparable in magnitude to individuals with NGT, is affected by the deleterious effect of a small rise in the FPG concentration in individuals with IFG (i.e., glucotoxicity).

Fourth, in subjects with NFG and IFG, empagliflozin had no effect on the fasting plasma glucagon concentration. This is in contrast to previous studies in patients with T2DM in whom both empagliflozin (23) and dapagliflozin (21) significantly increased the fasting glucagon level.

We (21) previously have shown that SGLT2 inhibition with dapagliflozin causes a significant increase in total-body insulin-mediated glucose disposal in patients with T2DM. In the current study, the glucose infusion rate and insulin sensitivity index during the hyperglycemic clamp were not enhanced by empagliflozin. Because EGP was not measured in the current study, it is possible that suppression of EGP was less complete during empagliflozin treatment. Failure to account for an increased rate of residual EGP in subjects with IFG could obscure a significant increase in whole-body (muscle) insulin sensitivity following empagliflozin therapy. Alternatively, because hepatic, not muscle, insulin resistance is the primary defect in IFG, it is not surprising that the glucose infusion rate was not increased following empagliflozin. Further, the decrease in the FPG concentration in subjects with IFG, although significant, was modest. Thus, it should not be surprising that, although lowering the FPG with empagliflozin in subjects with IFG caused a significant increase in insulin secretion, it failed to affect the glucose infusion rate or insulin sensitivity index during the stepped hyperglycemic clamp. As discussed above, we (19) and others (24–26) have shown that whole-body insulin sensitivity in individuals with IFG, measured with the euglycemic insulin clamp, is comparable to that in subjects with NGT. Thus, it is possible that a small increase in the FPG in subjects with IFG exerts a detrimental effect on  $\beta$ -cell function without worsening insulin sensitivity. This would explain the normal/near normal insulin sensitivity in subjects with IFG and the lack of effect of plasma glucose reduction on the glucose infusion rate in subjects with IFG.

There is an inverse dynamic relationship between insulin secretion and insulin sensitivity (27). Because lowering the FPG concentration with empagliflozin in subjects with IFG improved insulin secretion without affecting insulin sensitivity, this suggests that the increase in insulin secretion caused by empagliflozin is a primary effect on the  $\beta$ -cell and not a secondary effect because of change in insulin sensitivity. Although SGLT2 transporters have not been reported to be present in the  $\beta$ -cell, we (21) and others (22) have

shown that SGLT2 inhibitors increase the rate of whole-body fat oxidation. We speculate that an increased rate of fat oxidation in the  $\beta$ -cell, which potentially can reduce  $\beta$ -cell fat content in subjects with IFG, could contribute to the increase in insulin secretion caused by empagliflozin. Alternatively, removal of glucotoxicity on the  $\beta$ -cell following the decrease in the FPG concentration by empagliflozin could contribute to the increase in insulin secretion in subjects with IFG.

Because of the intensive nature of the experimental procedures used in the current study, a relatively small number of subjects was studied. Nonetheless, the effect of empagliflozin to reduce the FPG concentration and enhance  $\beta$ -cell function in subjects with IFG was highly significant. It should be noted that the hyperglycemic clamp is highly reproducible, with <5% variation when the same subject is studied on two occasions. Another limitation of the current study is lack of placebo-treated IFG group. Thus, a time-related change in the FPG and insulin secretion over the 14-day study period cannot be excluded. However, empagliflozin had no effect on FPG or insulin secretion in subjects with NFG, making it unlikely that the metabolic effects of empagliflozin on FPG and especially insulin secretion in subjects with IFG were because of day-to-day variation in the FPG or change in life habits. Further, similar variations would have been expected to occur in subjects with NFG, and none was observed. Most importantly, as mentioned above, the hyperglycemic clamp technique is highly reproducible.

In summary, inhibition of SGLT2 reduces the FPG concentration in subjects with IFG, but not in subjects with NFG, and is associated with a significant increase in insulin secretion and  $\beta$ -cell function.

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