Inhibition of Renal Sodium-Glucose Co-Transport with Empagliflozin Lowers Fasting Plasma Glucose and Improves Beta Cell Function in Subjects With Impaired Fasting Glucose

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ABSTRACT

To examine the effect of renal sodium glucose co-transporter inhibition with empagliflozin on the fasting plasma glucose concentration and beta cell function in subjects with impaired fasting glucose (IFG).

8 subjects with normal fasting glucose and 8 subjects with IFG received empagliflozin (25 mg/day) for 2 weeks. Fasting plasma glucose concentration and beta cell function was measured with a 9-step hyperglycemic clamp before and 48 hours and 14 days after the start of empagliflozin.

Empagliflozin caused 50±4 and 45±4 grams glucosuria on day 2 in IFG and NFG subjects, respectively, and the glucosuria was maintained for 2 weeks in both groups. The fasting plasma glucose (FPG) concentration decreased only in IFG subjects from 110±2 to 103±3 mg/dl ($p<0.01$) after 14 days. The FPG concentration remained unchanged (95±2 to 94±2 mg/dl) in NFG subjects. Empagliflozin enhanced beta cell function only in IFG subjects. The incremental area under the plasma C-peptide concentration curve during the hyperglycemic clamp increased by 22±4% and 23±4% after 48 hours and 14 days, respectively ($p<0.01$); the plasma C-peptide response remained unchanged in NFG subjects. 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 NGT subjects.

Inhibition of renal sodium-glucose co-transport with empagliflozin in IFG and NFG subjects produces comparable glucosuria but lowers the plasma glucose concentration and improves beta cell function only in IFG subjects.

INTRODUCTION

Impaired fasting glucose (IFG, FPG = 100-125mg/dl) was introduced by the ADA 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). IFG subjects are characterized by hepatic insulin resistance and loss of first phase insulin secretion, while IGT subjects are characterized by muscle insulin resistance and loss of second, 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 T2DM patients (4-6). Interventions which halt/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 HbA1c in T2DM patients (reviewed in reference #6).

IFG subjects 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 fasting plasma glucose 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 of the fasting plasma glucose 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 IFG

individuals. To address this question, we utilized the sodium-glucose cotransporter inhibitor, empagliflozin, which lowers the plasma glucose concentration by promoting urinary glucose loss (16), and quantitated the effect of reduction in the fasting plasma glucose concentration on beta cell function with the stepped hyperglycemic clamp technique.

RESEARCH DESIGN AND METHODS

Subjects. 8 subjects with normal fasting glucose (FPG <100 mg/dl) and 8 subjects with IFG (FPG=100-125 mg/dl) participated in the study. The subjects were part of a large study that recently was 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 lab tests, and EKG. Body weight was stable (±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 on the Clinical Research Center at the Texas Diabetes Institute following an overnight fast. On day -5 and day -4, a 24-hour urine was collected for measurement of urinary glucose excretion. On day -3 a nine-step hyperglycemic clamp was performed following a 10-hour overnight fast. Subjects reported to the Clinical Research Center at 6AM, 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 minutes. 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 ~100 to 140 mg/dl) for 40 minutes and urine was collected from 0-40 minutes for

measurement of glucose excretion. From 40-80, 80-120, 120-160, 160-200, 200-240, 240-280, 280-320, and 320-360 minutes 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 minutes and every 10 minutes thereafter until 360 minutes. 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 CRC. 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 minute period for measurement of urinary glucose excretion. The mean urine volume during the 40 minute collection periods was 504 ml. On day -2 and day -1, 24 hour 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 hours 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, Lumenburg, 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 0-10 [$(\Delta C\text{-}Pep)_{0\text{-}10}$] and 10-360 [$(\Delta C\text{-}Pep)_{10\text{-}360}$] minutes, respectively. Tissue glucose uptake during each step was calculated as the glucose infusion rate during the last 20 minutes 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 tissue glucose uptake (TGU) during the last 20 minutes of each step (glucose infusion rate minus urinary glucose excretion) divided by the mean plasma insulin (MRI) concentration during the same time period. Previous studies have demonstrated that this index of insulin sensitivity (TGU/mean plasma insulin) strongly correlates (86%) with total body insulin-mediated glucose disposal measured in the same subject with the euglycemic hyperinsulinemic clamp (16) and, therefore, was utilized to calculate beta cell function. Beta cell function was calculated as the insulin secretion/insulin resistance (disposition) index: [$\Delta C\text{-}Pep_{(0\text{-}360)}$] x [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 ± SEM. Insulin secretion, beta cell function, glucose infusion rate and beta cell glucose sensitivity on day 2 and day 14 after the start of empagliflozin were compared to baseline with paired t-test. To test for repeated measures for FPG and insulin secretion on day 2 and on day 14 versus baseline, two way ANOVA with post hoc analysis (Bonferroni) was utilized. To estimate the contribution of the change in FPG concentration on day 2 and on day 14 to the increase in insulin secretion and beta cell function, a linear regression

model was constructed with the change in ΔC-Pep$_{(10-360)}$ and insulin secretion/insulin resistance (disposition) index as the dependent variable and the change in FPG as the independent variable. FPG and other anthropomentric variables were included as covariates in the model. P value <0.05 (two tailed) was considered for statistical significant level. SPSS 22.0 statistical package was used for statistical analysis.

Study Approval. The study protocol was approved by the IRB of the University of Texas Health Science Center in San Antonio, Texas and all subjects gave their written informed voluntary consent prior to participation. The study is registered at clinical trials.gov NCT01867307.

RESULTS

NFG and IFG subjects were matched in age (58±2 and 59±3 years), gender (6 males/2 females and 5 males/3 females), and HbA1c (5.6±0.1 vs 5.7±0.1%) (Table1). IFG subjects had significantly higher BMI (30.3±0.8 vs 27.0±1.1 kg/m^2, p=0.04) and FPG concentration (110±2 vs 95±2 mg/dl, p<0.0001). Plasma creatinine concentration was similar in NFG and IFG subjects (0.70±0.05 vs 0.79±0.04 mg/dl). Empagliflozin caused 45±4 and 50±5 grams urinary glucose excretion per 24 hours in NFG and IFG subjects, respectively, (P=NS) on day 2; urinary glucose excretion remained unchanged on day 14 in NFG and IFG subjects (38±5 and 48±7 grams/24 hours, respectively; both p=NS versus day 2). Despite comparable urinary glucose loss, FPG decreased significantly only in IFG subjects on day 2 (from 110±2 to 103±3, p<0.01), while it remained unchanged in NGT subjects (95±2 versus 94±3 mg/dl) (Table 2). The decrease in FPG in IFG subjects was maintained at day 14 (Table 2). In two-way ANOVA, the decrease in

FPG concentration on day 2 and on day 14 in IFG subjects was significantly reduced compared to baseline after adjustment for the baseline FPG concentration. There was no significant change in FPG in NFG subjects. There was no significant difference in FPG between day 2 and day 14 in IFG subjects. Further, the decrement (change) in the FPG concentration following empagliflozin administration was strongly and inversely correlated with the baseline fasting plasma glucose concentration ($r=-0.61$, $p<0.01$, Figure 1). Consistent with previous studies (3,4,18) and compared to NFG subjects, IFG subjects had a significantly higher fasting plasma C-peptide concentration (Table 2).

Insulin Secretion in IFG and NFG subjects at baseline

At baseline, the fasting plasma C-peptide concentration and the plasma C-peptide concentration during the hyperglycemic clamp were significantly higher in IFG versus NGT subjects (Figure 2). However, the incremental area under the plasma C-peptide concentration curve from 0-10 minutes (ΔC-pep$_{(0-10)}$) and from 10 to 360 minutes (ΔC-pep$_{(10-360)}$) during the baseline hyperglycemic clamp was not significantly different between NFG and IFG individuals (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 NFG and IFG subjects (Table 2).

Effect of Empagliflozin on Insulin Secretion in NFG and IFG subjects

Empagliflozin had no significant effect on insulin secretion, measured as the incremental area under the plasma C-peptide concentration curve [ΔC-Pep$_{(0-10)}$ and ΔC-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 NFG subjects (Figure

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 (ΔC-Pep$_{(10-360)}$) after 48 hours and 14 days in IFG subjects (Figure 4, Table 2), and the decrease in ΔC-Pep$_{(10-360)}$ on day 2 and on day 14 remained significant after adjustment for FPG concentration (both 0<0.05). Empagliflozin caused a small, but non-significant, increase in ΔC-Pep$_{(0-10)}$ in IFG subjects. Beta cell glucose sensitivity during the hyperglycemic clamp was unaffected by empagliflozin in both NFG and IFG subjects.

Plasma Glucagon Concentration

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

Insulin Sensitivity

NFG subjects had a higher mean tissue glucose uptake and insulin sensitivity index during the hyperglycemic clamp compared to IFG subjects (15.7±1.2 versus 12.4±0.7 mg/kg.min, p<0.05, and 48±10 versus 17±2, p=0.01, respectively). Empagliflozin treatment did not significantly affect the insulin sensitivity index during the hyperglycemic clamp performed after 48 hours or 14 days in either group in either group (Table 2).

Effect of Empagliflozin on Beta Cell Function

In IFG subjects, beta cell function, measured with the insulin secretion/insulin resistance (disposition) index, increased by 60% and 51% (both $p<0.05$) after 48 hours and 14 days (from 1784±160 at baseline to 2863±497 and to 2697±391 after 48 hours and 14 days, respectively). In the NFG subjects the IS/IR after 48 hours (4410±927) and 14 days (4322±810) was not changed significantly from baseline (4324±1004). The change in IS/IR after 48 hours was strongly and inversely correlated with the change in fasting plasma glucose concentration after 48 hours (Figure 5).

Relationship Between the Increase in Insulin Secretion and Beta Cell Function and Decrease in FPG Concentration

The change in ΔC-Pep$_{(10\text{-}360)}$ during the hyperglycemic clamp after 48 hours and 14 days in IFG subjects 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. insulin secretion/insulin resistance [disposition] 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 tissue glucose uptake or insulin sensitivity during the hyperglycemic clamp after 48 hours and 14 days in IFG subjects.

In a linear regression model (Table 3), the decrease in FPG concentration significantly contributed to the increase in insulin secretion (ΔC-Pep$_{(10\text{-}360)}$) on day 14 and to the increase in the insulin secretion/insulin resistance (disposition) index on day 2 and on day 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 present study, these results should be confirmed in a large group of subjects.

DISCUSSION

The present study provides four novel findings: (i) first, in subjects with normal fasting glucose (NFG) concentration, SGLT2 inhibition has no significant effect on the fasting plasma glucose concentration despite the large increase in glucose excretion; (ii) second, in IFG subjects SGLT2 inhibition significantly reduces the FPG concentration; (iii) in IFG subjects the decrease in FPG concentration is associated with a significant increase in insulin secretion and beta cell function; (iv) in NFG and IFG subjects 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 type 2 diabetic patients, SGLT2 inhibitors produce 70-90 grams/day glucosuria and this results in a 20-40 mg/dl decrease in fasting plasma glucose concentration (20). However, in subjects with normal fasting plasma glucose concentration in the present study, despite 45 grams/day glucosuria, the fasting plasma glucose concentration remained unchanged. This only can be explained by a rapid increase in endogenous glucose production which 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 grams per day) (20). Although endogenous glucose production was not measured in the present study, we (21) previously have shown that, in T2DM individuals, two weeks of treatment

with dapagliflozin augments endogenous glucose production but reduces the FPG concentration. Thus, unlike the present study where the increase in EGP in NFG subjects closely matches the urinary glucose loss, the increase in urinary glucose excretion in T2DM subjects exceeded the increase in EGP and the FPG concentration declined. Of note, in the present study, and unlike T2DM patients in our previous study (21), the fasting plasma glucagon concentration in NFG subjects 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 present study argues for a more important role of the decrease in fasting plasma glucose concentration in T2DM patients in stimulating glucagon secretion following SGLT2 administration. These findings underscore the importance of the **reno-hepatic interaction** in the regulation of plasma glucose concentration in NGT individuals.

Unlike subjects with NFG, IFG subjects 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. Since tracers were not employed in the present study, we only can speculate on the mechanism(s) responsible for the different effect of empagliflozin on the FPG in IFG vs NFG subjects despite similar urinary glucose loss in both groups. We (21) previously have shown that basal glucose clearance is decreased in IFG subjects compared to subjects with NGT. Although not measured in the present 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 fasting plasma glucose concentration in IFG but not in NGT individuals.

The third major finding of the present study is that a small decrease in the FPG concentration in IFG subjects 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 IFG subjects is secondary to the increase in FPG concentration, i.e. glucotoxicity. Of note, previous studies have demonstrated that, compared to NGT subjects, IFG subjects manifest a decrease in first phase insulin secretion while second phase insulin secretion is comparable to that in NGT individuals (9-12). However, in the present study, the increase in first phase insulin secretion caused by empagliflozin did not reach statistical significance in IFG subjects. Rather, a more robust increase in insulin secretion during the hyperglycemic clamp in IFG subjects was observed between 200-360 minutes. It is possible that the small magnitude (+40 mg/dl) of the first hyperglycemic step employed in the present 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 IFG subjects, though comparable to NGT individuals in magnitude, is affected by the deleterious effect of a small rise in the fasting plasma glucose concentration in IFG individuals, 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 type 2 diabetic patients in which both empagliflozin (22) 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 T2DM patients. In the

present study, the glucose infusion rate and insulin sensitivity index during the hyperglycemic clamp was not enhanced by empagliflozin. Since endogenous glucose production was not measured in the present 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 IFG subjects could obscure a significant increase in whole body (muscle) insulin sensitivity following empagliflozin therapy. Alternatively, since 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 IFG subjects, although significant, was modest. Thus, it should not be surprising that, although lowering the FPG with empagliflozin in IFG subjects 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 (4,24,25) have shown that whole body insulin sensitivity in IFG individuals, measured with the euglycemic insulin clamp, is comparable to that in NGT subjects. Thus, it is possible that a small increase in the FPG in IFG subjects exerts a detrimental effect on beta cell function without worsening insulin sensitivity. This would explain the normal/near normal insulin sensitivity in IFG subjects and the lack of effect of plasma glucose reduction on the glucose infusion rate in IFG subjects.

There is an inverse dynamic relationship between insulin secretion and insulin sensitivity (26). Because lowering the FPG concentration with empagliflozin in IFG subjects 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 due to change in insulin sensitivity. Although SGLT2 transporters have not been reported to be present in the beta cell, we (21) and others (27) 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 IFG subjects, 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 IFG subjects.

Because of the intensive nature of the experimental procedures utilized in the present study, a relatively small number of subjects were studied. Nonetheless, the effect of empagliflozin to reduce the FPG concentration and enhance beta cell function in IFG subjects was highly significant. It should be noted that the hyperglycemic clamp is highly reproducible with less than 5% variation when the same subject is studied on two occasions. Another limitation of the present 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 NFG subjects, making it unlikely that the metabolic effects of empagliflozin on FPG and especially insulin secretion in IFG subjects were due to day-to-day variation in the FPG or change in life habits. Further, similar variations would have been expected to occur NFG subjects and none were observed. Most importantly, as mentioned above, the hyperglycemic clamp technique is highly reproducible.

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

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All authors contributed to performing the study. Drs. Abdul-Ghani and DeFronzo wrote the initial draft of the manuscript which then was reviewed by all authors. Dr. DeFronzo is the guarantor of the data.

CONFLICT OF INTEREST

MA-G, HAJ, GD, JA, have no conflicts. EC is on the speakers bureaus of Janssen, Lilly-Boehringer Ingelheim, Astra Zeneca, and Sanofi and receives research support from Astra Zeneca and Jansssen, CT is on the speakers bureaus of Boehringer-Ingelheim, Astra Zeneca, and Janssen. RAD is on the advisory boards of AstraZeneca, Novo Nordisk, Janssen, Intarcia, Elcelyx, and Boehringer-Ingelheim; receives research support from Boehringer-Ingelheim, Takeda, Janssen, and AstraZeneca; and is on the speaker's bureaus of Novo Nordisk and AstraZeneca.

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FIGURE LEGENDS

Figure 1: Relationship between the change in plasma glucose concentration 48 hours after following empagliflozin (25 mg/day) administration and the fasting plasma glucose concentration at baseline in NFG and IFG subjects.

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

Figure 3: Increment above fasting in plasma C-peptide concentration during the hyperglycemic clamp in NFG [A] and IFG [B] subjects at baseline and at 48 hours after starting empagliflozin treatment. Figure 3C depicts first phase insulin secretion in IFG subjects before and at 48 hours after starting empagliflozin.

Figure 4: Relationship between the change in ΔC-Pep$_{10\text{-}360}$ 48 hours after the start of empagliflozin relative to baseline and the change in the FPG concentration after 48 hours of empagliflozin treatment.

Figure 5. Correlation between the change in beta cell function (IS/IR index) and the change in FPG concentration after 48 hours of empagliflozin treatment.

Table 1: Baseline patient characteristics

	NFG	IFG	P
Age (years)	58±2	59±3	NS
Gender (m/f)	6/2	5/3	NS
BMI (kg/m^2)	27.0±1.1	30.3±0.8	0.04
FPG (mg/dl)	95±2	110±2	<0.0001
HbA1c (%)	5.6±0.1	5.7±0.1	NS
Plasma Creatinine (mg/dl)	0.70±0.05	0.79±0.04	NS
eGFR (ml/min.1.73 m^2)	120±10	99±6	NS
Fasting plasma FFA (mmol/L)	0.54±0.07	0.46±0.04	NS

Table 2: Effect of empagliflozin on the FPG concentration and insulin secretion during the stepped hyperglycemic clamp. FPG= fasting plasma glucose; TGU = tissue glucose uptake; MRI = mean plasma insulin concentration; IS/IR Index=Insulin secretion/insulin resistance (disposition) index. * p<0.05 versus baseline. Values are mean±SEM (Median)

	NFG	IFG	P
FPG (mg/dl)			
Baseline	95±2(96)	110±2(113)	<0.0001
48 hours	94±3(97)	103±3*(102)	0.07
Day 14	100±3(97)	103±3*(103)	0.06
FP C-Peptide (ng/ml)			
Baseline	2.1±0.3(2.0)	4.0±0.4(4.1)	0.002
48 hours	2.0±0.3(2.0)	3.7±0.5(3.4)	0.008
Day 14	2.5±0.4(2.9)	3.7±0.5(3.8)	0.02
Fasting FFA (mM)			
Baseline	0.54±0.07(0.48)	0.46±0.04(0.44)	NS
48 hours	0.65±0.09(0.57)	0.62±0.09*(0.61)	NS
Day 14	0.42±0.05(0.43)	0.49±0.04(0.46)	NS
ΔC-Pep$_{(0\text{-}10)}$ ng/ml.min			
Baseline	15±4(14)	26±5(18)	NS
48 hours	14±4(13)	34±4(27)	0.05
Day 14	16±4(10)	32±4(30)	NS
ΔC-Pep$_{(10\text{-}360)}$ ng/ml.min			
Baseline	98±11(81)	116±10(94)	NS
48 hours	107±11(105)	141±8*(141)	0.07
Day 14	96±9(91)	141±8*(138)	<0.05
Slope (C-Pep/PG)			
Baseline	0.10±0.01(0.08)	0.10±0.01(0.11)	NS
48 hours	0.10±0.01(0.09)	0.11±0.01(0.11)	NS
Day 14	0.09±0.01(0.10)	0.12±0.01(0.12)	NS
TGU (mg/kg.min)			
Baseline	15.7±1.2(15.3)	12.4±0.8(12.2)	0.2
48 hours	16.3±1.5(17.3)	12.8±0.5(12.9)	0.01
Day 14	16.3±1.2(17.0)	12.9±0.9(12.7)	0.02
TGU/MPI			
Baseline	48±10 (49)	17.2±2(16.3)	0.01
48 hours	44±7 (47)	21±3(20.1)	0.01
Day 14	50±10 (48)	20±3(17.4)	0.01
IS/IR Index			
Baseline	4324±1004(3854)	1784±160(0.11)	<0.05
48 hours	4410±927(4138)	(1879)	NS
Day 14	4322±810(3575)	2863±497*(2898)	NS
		2697±391*(2623)	

Table 3: Linear regression analysis with the increase in insulin secretion (ΔC-Pep$_{(10\text{-}360)}$) and increase in IS/IR Index on day 2 and day 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^2
ΔFPG$_{(BL\rightarrow day2)}$	ΔC-Pep$_{(10\text{-}360)}$	Age, sex, BMI, FPG	0.26	NS	0.17
ΔFPG$_{(BL\rightarrow day2)}$	IS/IS Index	Age, sex, BMI, FPG	0.52	0.03	0.28
ΔFPG$_{(BL\rightarrow day14)}$	ΔC-Pep$_{(10\text{-}360)}$	Age, sex, BMI, FPG	0.59	0.01	0.13
ΔFPG$_{(BL\rightarrow day14)}$	IS/IS Index	Age, sex, BMI, FPG	0.43	0.05	0.21

Figure 1

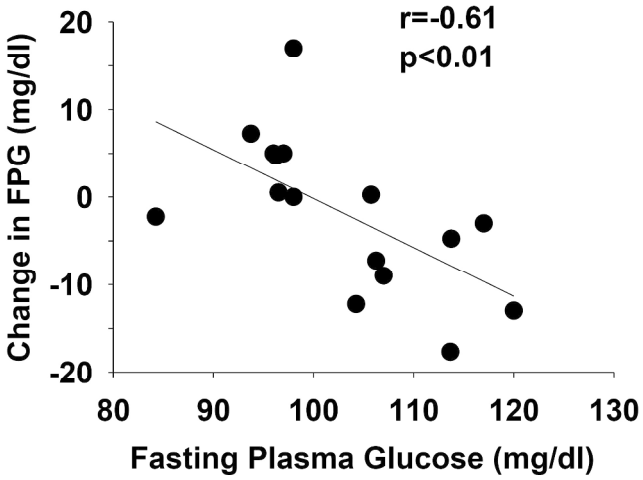

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Figure 2

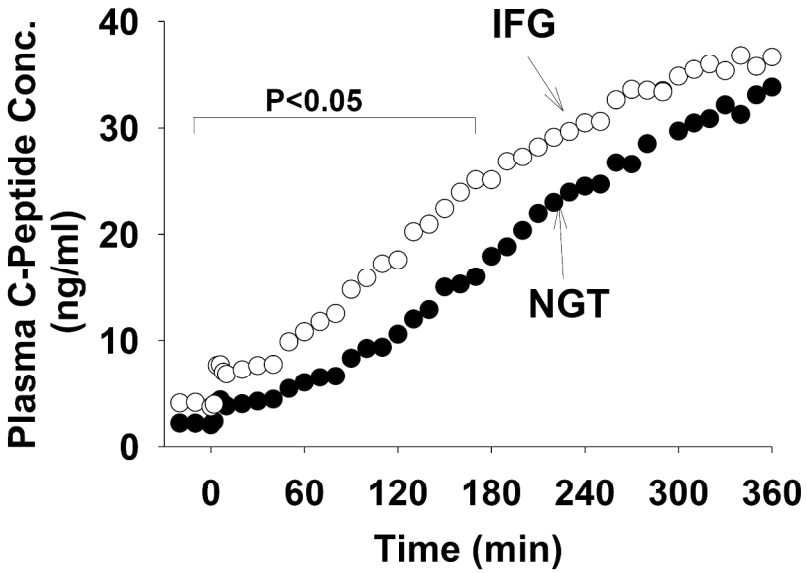

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Figure 3A

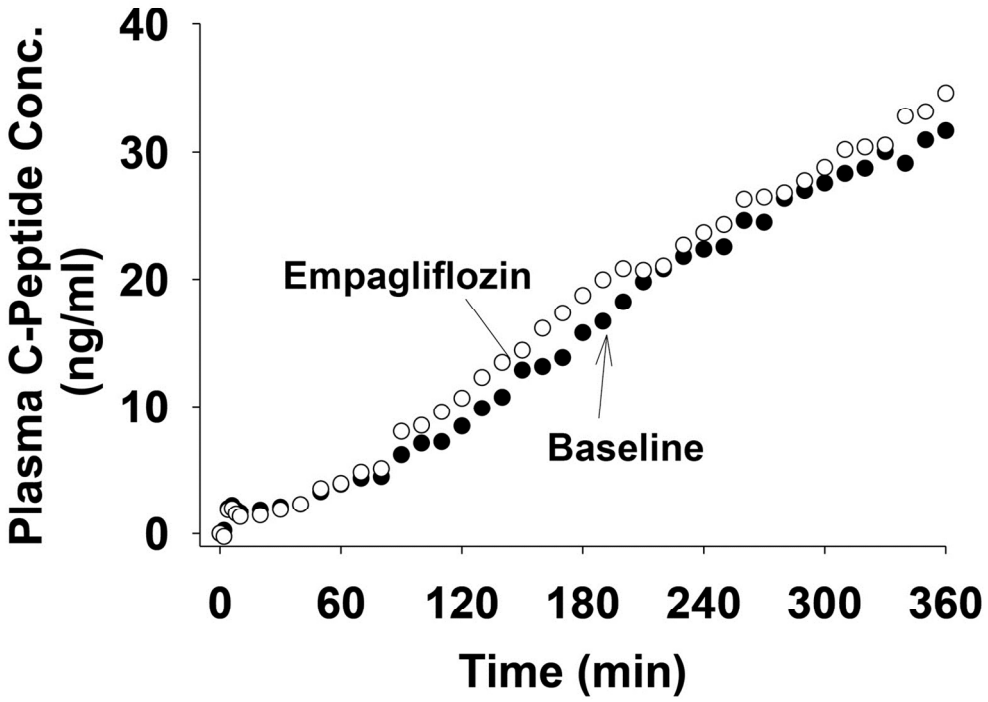

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Figure 3B

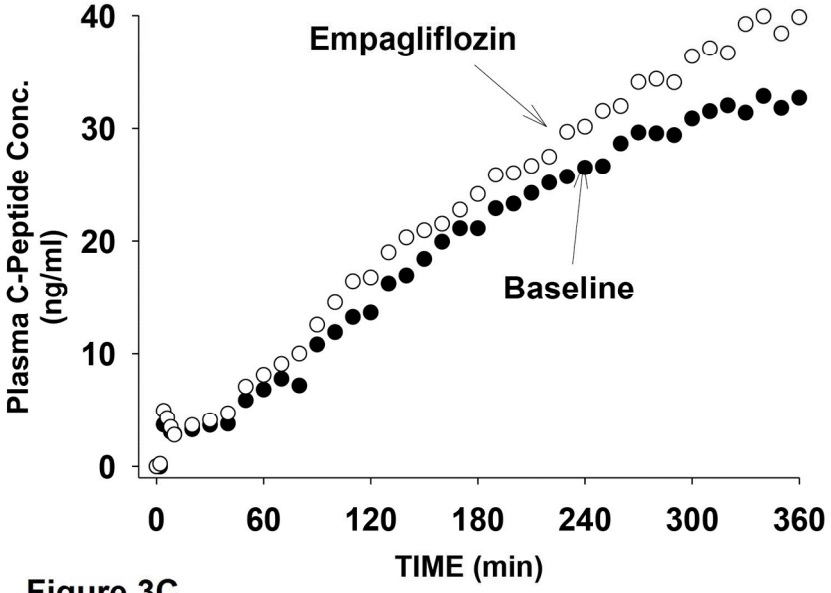

Figure 3C

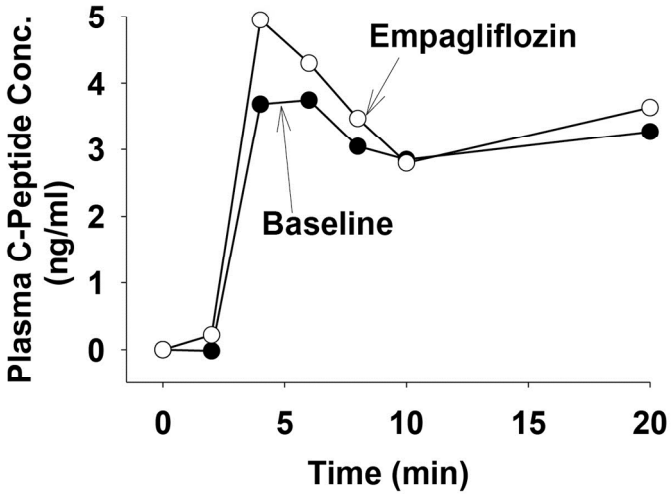

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Figure 4

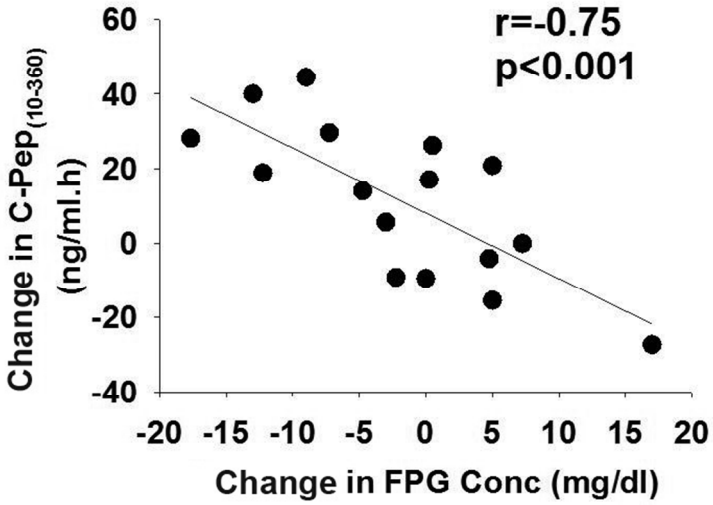

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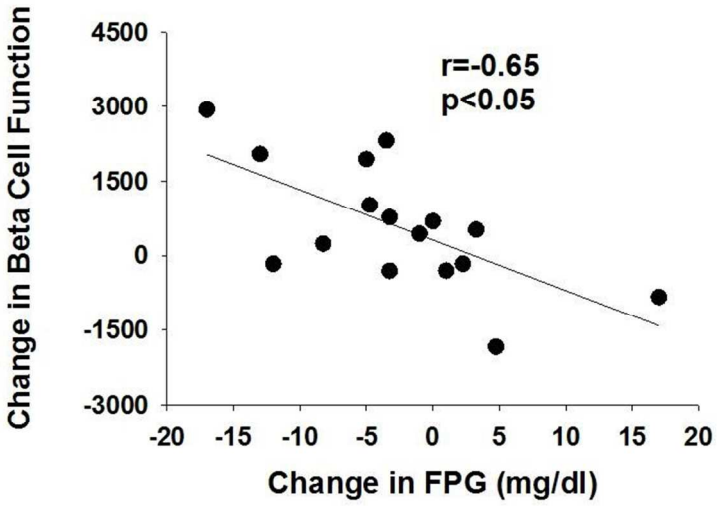
Figure 5



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