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Official Study Title: Effect of Combined Incretin-Based Therapy Plus Canagliflozin on Glycemic Control and the Compensatory Rise in Hepatic Glucose Production in Type 2 Diabetic Patients

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Effect of Combined Incretin-Based Therapy Plus Canagliflozin on Glycemic Control and the Compensatory Rise in Hepatic Glucose Production in Type 2 Diabetic Patients

RATIONALE: Endogenous (primarily hepatic) glucose production (HGP) plays a central role in the maintenance of normal glucose homeostasis and impaired regulation of HGP plays a pivotal role in the development of fasting and postprandial hyperglycemia in type 2 diabetes (1-9).Because the liver is responsible for the majority (>80%) of HGP, it has become an important target for antidiabetic therapy (10).

HGP is under tight hormonal control (11-14). Insulin is the primary regulator of HGP and a small increase in plasma insulin concentration strongly suppresses HGP (13). Conversely, glucagon and other counter-regulatory hormones (catecholamines, cortisol and growth hormone) stimulate HGP (14). The plasma glucose concentration also is a strong regulator of HGP, and an increase in the plasma glucose concentration, independent of plasma insulin and glucagon concentrations, strongly inhibits HGP(14). Conversely, hypoglycemia is a powerful stimulator of HGP (15).

SGLT2 inhibitors represent a novel class of drugs which inhibit the renal sodium-glucose cotransporter 2 (SGLT2), produce glucosuria and decrease the plasma glucose concentration (16). The urinary glucose loss also promotes weight loss (16). Despite the large glucosuria produced by SGLT2 inhibitors and the accompanying weight loss, the decrease in HbA1c brought about SGLT2

inhibitors is less than expected. For example, canagliflozin an SGLT2 inhibitor approved as an adjunct to diet and exercise to improve glycemic control in adults with T2DM, promotes a urinary glucose loss of ~70-90 grams per day, but it only decreases HbA1c by ~0.6-0.8% (17). Therefore, we hypothesized that the glucosuria produced by SGLT2 inhibition stimulates compensatory mechanisms that act to raise the plasma glucose concentration and compensate for (oppose) the urinary glucose loss. To test this hypothesis, we examined the effect of glucosuria produced by dapagliflozin administration on the rate of HGP in T2DM individuals. Administration of dapagliflozin (10 mg/day) produced glucosuria (~3 gram/hour) and reduced the fasting plasma glucose (FPG) concentration by 37 mg/dl (see preliminary results). Because of the close relationship between the FPG and HGP, we anticipated that the decrease in FPG produced by the induction of glucosuria would be accompanied by a parallel decrease in HGP. However, to our surprise, following an initial decline in HGP, the decrease in FPG concentration was associated with a **paradoxical increase in HGP**. This suggests the presence of a "compensatory" mechanism in which loss of glucose in the urine and the accompanying decrease in plasma glucose concentration following dapagliflozin administration triggers an increase in HGP in an attempt to maintain the plasma glucose concentration at its fasting hyperglycemic level(18). The increase in HGP following dapagliflozin administration was associated with an increase in plasma glucagon concentration which was closely related to the increase in HGP. Because glucosuria results in decreased return of glucose from the glomerular filtrate to the renal vein, it results in widening the glucose A-V difference across the kidney and this could provide a signal to the CNS to stimulate (directly or indirectly via glucagon secretion) HGP to compensate for urinary glucose loss. Alternatively, the increase in glucagon secretion could represent the response to the acute decline in FPG concentration induced by dapagliflozin. Whatever the mechanism(s), our findings indicate

the presence of a "reno-hepatic" interaction that participates in the regulation of plasma glucose concentration. The primary aim of the present study is to examine whether the combination of incretin-based therapy, liraglutide, plus canagliflozin can block the compensatory increase in HGP, augment the decrease in plasma glucose concentration produced by canagliflozin, and enhance the decline in A1c.

Canagliflozin, like other SGLT2 inhibitors, is associated with weight loss and a reduction in systolic blood pressure (17,19). The weight loss amounts to ~2.5 kg during the first year and plateaus thereafter, without any attenuation of the drug's glucosuric effect. This suggests a resetting of the appetite regulation center in the hypothalamus, leading to an increase in caloric intake that negates the loss of glucose calories in the urine. GLP-1 receptor agonists bind to the GLP-1 receptor in the hypothalamus, causing appetite suppression and weight loss. We hypothesize that combined liraglutide-canagliflozin treatment will result in additive, even synergistic, decreases in body weight, hepatic fat content, and visceral fat content in type 2 diabetic patients.

Canagliflozin also reduces systolic (by 4-5 mmHg), and to a lesser extent diastolic (by 1-2 mmHg), blood pressure (17). The decline in blood pressure is evident within the initial 2-4 weeks of treatment and most likely is related to the mild intravascular volume depletion associated with the natriuretic action of canagliflozin. GLP-1receptor agonists also possess natriuretic properties (20), improve endothelial dysfunction by augmenting release of the potent vasodilator nitric oxide (21), and cause a modest reduction in blood pressure (21). Therefore, one might expect combination therapy with liraglutide plus canagliflozin to have an additive, even synergitic, effect to reduce systolic/diastolic blood pressure and in the present study we will examine the hypotensive action of combined therapy with canagliflozin plus liraglutide.

The **SPECIFIC AIMS** of the study are:

Specific Aim 1.**To examine whether the combination of liraglutide plus canagliflozin can prevent the increase in HGP following institution of canagliflozin therapy and produce an additive or even synergistic effect to lower the plasma glucose concentration and A1c.** We will examine this hypothesis by comparing the effect of administration of liraglutide alone, canagliflozin alone, and the combination of liraglutide plus canagliflozin on:(i) the rate of HGP; (ii) decrease in fasting plasma glucose concentration; (iii) counter-regulatory hormone response and (iv) A1c. We anticipate that the addition of liraglutide to canagliflozin will prevent the increase in plasma glucagon concentration, augment insulin secretion, and blunt/block the increase in HGP in response to canagliflozin, resulting in a greater decrease in fasting plasma glucose concentration and A1c than observed with each therapy alone.

Specific Aim 2: To examine whether combination therapy with liraglutide plus canagliflozin can produce an additive, or even synergistic, effect to promote weight loss and reduction in hepatic and visceral fat content.

Specific Aim 3. To examine whether combination therapy with liraglutide plus canagliflozin can produce an additive or even synergistic effect to reduce systolic/diastolic blood pressure and 24-hour integrated blood pressure.

Background:

Endogenous (primarily hepatic) glucose production (HGP) plays a central role in the maintenance of glucose homeostasis and impaired regulation of HGP plays a pivotal role in the development of both fasting and postprandial hyperglycemia in patients with type 2 diabetes mellitus (T2DM) (1-9).

The tissues/mechanisms responsible for the regulation of glucose metabolism during the fasting state, e.g. after an overnight fast, are very different than those responsible for the regulation of the plasma glucose concentration following glucose ingestion, e.g. after a mixed meal (1). After an overnight fast, tissue glucose uptake (~2 mg/kg.min) is closely matched by the rate of HGP (3,9). The rate of HGP is under tight hormonal control (11-14). Insulin is the primary determinant of basal HGP, and a small increase in the portal plasma insulin concentration markedly suppresses HGP (13).Conversely, glucagon is a strong stimulator of HGP and an increase in the portal glucagon concentration augments HGP (11,12,14,22).

The plasma glucose concentration also is an important regulator of HGP (23-27). Studies with the pancreatic clamp technique have demonstrated that hyperglycemia strongly inhibits HGP in the absence of changes in plasma insulin and glucagon concentrations (26). Conversely, hypoglycemia strongly activates HGP (15). Thus, changes in the plasma glucose concentration can affect HGP both directly and indirectly via alterations in both insulin and glucagon secretion.

Suppression of HGP by hyperinsulinemia and hyperglycemia following glucose ingestion is a critical determinant of postprandial hyperglycemia (26,29,30. Thus, glucose balance across the liver (uptake versus production) is an important factor which determines the rise in postprandial plasma glucose concentration.

Subjects with T2DM manifest severe hepatic insulin resistance and an increased rate of basal HGP, leading to fasting hyperglycemia (1). Excessive glucagon secretion also contributes to accelerated rate of HGP in T2DM patients (1,11). Numerous clinical studies have demonstrated that the rise in basal HGP is the principal factor responsible for the increase in FPG concentration in T2DM subjects (1-3,5,6,).

Canagliflozin is a member of a new class of antidiabetic drugs and recently has been approved by the FDA for the treatment of T2DM. Canagliflozin lowers the plasma glucose concentration by inhibiting the SGLT2 transporter and producing glucosuria (17). Because SGLT2 inhibitors produce urinary glucose loss, they also promote weight loss (17). Clinical studies have demonstrated that canagliflozin promotes the excretion of ~70-90 grams of glucose per day and results in a weight loss of 2.5-3 kg during the first 6-12 months of therapy (31,32). Despite the significant weight loss and large glucosuria produced by canagliflozin, the decrease in HbA1c caused by the drug is modest. Clinical studies have reported that a maximal dose of canagliflozin and other SGLT2 inhibitors cause only a 0.6-0.8% decrease in HbA1c in drug naïve T2DM diabetic subjects and in diabetic patients treated with metformin, sulfonylureas or pioglitazone (31-42)(reviewed in ref #43). Thus, we hypothesize that the glucosuria produced by canagliflozin stimulates a compensatory response in glucose homeostasis that offsets the urinary glucose loss in an attempt to maintain the plasma glucose concentration constant at its fasting level.

Results from our lab(see below) demonstrate that the SGLT2 inhibitor dapagliflozin (10 mg) produces significant glucosuria and decreases the fasting plasma glucose concentration. Because of the strong correlation between the basal rate of HGP and the fasting plasma glucose concentration (4),we anticipated that the decrease in FPG concentration brought about dapagliflozin in T2DM patients would be accompanied by a decline in HGP. In contrast, the decrease in FPG produced by dapagliflozin was accompanied by a **paradoxical increase** in the rate of HGP. The increase in HGP in response to glucosuria suggests the presence of a "reno-hepatic" interaction in the regulation of glucose metabolism. In normal glucose tolerant (NGT) individuals, a "reno-hepatic axis" that leads to a compensatory increase in HGP in response to urinary glucose loss and maintains the plasma glucose concentration can be viewed as beneficial

because it prevents the development of hypoglycemia. However, this "compensatory" increase in HGP in T2DM individuals is paradoxical because it occurs while the plasma glucose concentration is well within the hyperglycemic range, and quantitatively offsets the reduction in plasma glucose concentration in response to the glucosuric effect of the SGLT2 inhibitor. Delineation of the mechanisms responsible for the "paradoxical" increase in HGP produced by glucosuria (i) will improve our understanding of the regulation of glucose homeostasis, and (ii) has important clinical implications. Thus, if the rise in HGP in response to glucosuria can be blocked, the decrease in plasma glucose concentration produced by SGLT2 inhibitors can be enhanced. We hypothesize that the combination of an SGLT2 inhibitor with incretin-based therapy, which stimulates insulin and inhibits glucagon secretion (43,44), will block the paradoxical rise in HGP and augment the ability of the SGLT2 inhibitor to lower the plasma glucose concentration and HbA1c.Although the DPP4 inhibitors augment insulin and inhibit glucose secretion, their effect on the beta cell is weak and it is likely that they will not inhibit the paradoxical increase in HGP observed following the initiation of canagliflozin therapy (44). In contrast, the GLP-1 receptor agonists have a potent effect on the beta cell to augment insulin secretion and inhibit glucagon (45),thus causing a greater more durable reduction in A1c (46). Further, the DPP4 inhibitors are weight neutral and do not reduce the blood pressure, whereas the GLP-1 receptor agonists promote weight loss and decrease the blood pressure. Although canagliflozin is approved for use with the GP-1 receptor agonists, it is unclear whether they produce an additive, or even synergistic, effect to reduce body weight and blood pressure. Therefore, in the present study we have chosen to examine combination therapy with canagliflozin and liraglutide.

Preliminary Results: To examine the effect of lowering the plasma glucose concentration with a SGLT2 inhibitor on the rate of endogenous (primarily reflects hepatic) glucose production,

we treated T2DM individuals with dapagliflozin, which lowers the plasma glucose concentration by inhibiting renal glucose reabsorption and producing glucosuria. 15 T2DM subjects (age=54±4, BMI=32.7±2.7 kg/m^2, HbA1c= 8.3±0.2%) treated with metformin alone or in combination with a sulfonylurea participated in the study. Subjects received dapagliflozin (10 mg/day, n=12) or placebo (n=6) for 2 days and HGP was measured before and after dapagliflozin administration. Subjects were admitted to the Clinical Research Center (CRC) for 3 days. On day 1, basal HGP was measured with 3-^{3}H-glucose. Measurement of HGP was performed at 6 AM following a 10hour overnight fast. At 6 AM, subjects received a prime (25 μCi X FPG/100) -continuous (0.25 μCi/min) infusion of 3-^{3}H-glucose. Arterialized blood (heated hand technique) was drawn from a catheter placed retrogradely in a vein in the dorsum of the hand. After a 3 hour tracer equilibration period, 4 blood samples (-20, -10, -5, and 0 minutes) were obtained and plasma insulin, glucagon, and glucose concentrations and 3-^{3}H-glucose specific activity were measured. On day 2, subjects received a 3-^{3}H-glucose infusion as on day 1 and the tritiated glucose infusion was continued until 1 PM. After a 3 hour tracer equilibration period, subjects received dapagliflozin or placebo (at 9 AM) and blood samples were drawn every 20 minutes for 4 hours after drug administration. Plasma glucose, insulin, and glucagon concentrations and 3-^{3}H-glucose specific activity were measured. On day 3, subjects received a second dose of dapagliflozin or placebo and HGP was measured as described for day 1. 24-hour urinary collections, to quantitate urinary glucose excretion, were obtained on days 1, 2 and 3. Dapagliflozin caused a significant increase in urinary glucose excretion (78±18 and 91±23 grams/day on days 2 and 3after dapagliflozin, respectively)compared to 2±1 grams/day on day 1 (before dapagliflozin) ($p<0.0001$). Figure 1 depicts the fasting plasma glucose, insulin and glucagon concentrations and the rate of HGP measured on day 2 (before and immediately after the start of dapagliflozin).

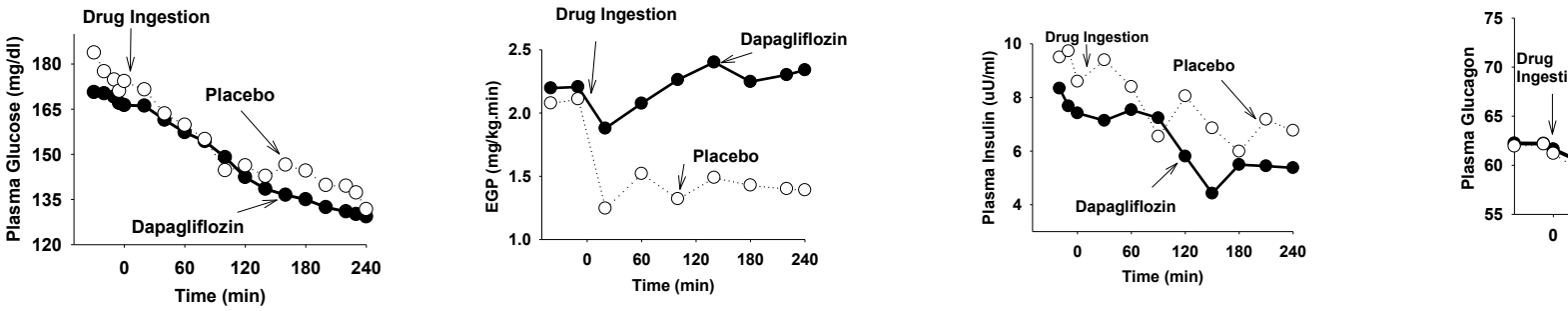

Figure 1: Plasma glucose (A), insulin (C), and glucagon (D) concentration and the rate of HGP (B) before and immediately after dapagliflozin and placebo administration at time zero.

The plasma glucose concentration decreased similarly in subjects receiving dapagliflozin and placebo (by 37±18 and 38 ± 13 mg/dl, respectively, at 240 minutes). The decrease in the plasma glucose concentration in subjects receiving placebo can be explained by the prolonged fasting period (~20 hours). Consistent with this, the rate of HGP decreased significantly in subjects receiving placebo from 2.10±0.24 at baseline to 1.40±0.16 mg/kg.min at the end of the study ($p<0.001$).In marked contrast, during the first 30 minutes following dapagliflozin administration, HGP initially decreased slightly and then increased progressively (Figure 1B) such that the rate of HGP during the last 40 minutes of the study was above baseline (2.32 ± 0.42 versus 2.20 ±0.28 mg/kg.min, P=0.12). The mean difference in HGP between dapagliflozin-treated and placebo-treated subjects (0.70±0.34 mg/kg.min, $p<0.001$) virtually is identical to the amount of glucose excreted in the urine in dapagliflozin-treated subjects (~15 grams). Thus, the decrease in FPG in placebo-treated subjects was entirely accounted for by a decline in HGP, while the decline in FPG in dapagliflozin-treated subjects was entirely accounted for by renal glucose excretion. On day 3, the fasting plasma glucose concentration was reduced by 23 mg/dl in dapagliflozin-treated subjects, while the FPG increased by 5 mg/dl in placebo-treated subjects. The blunted decline in FPG concentration in dapagliflozin-treated subjects was explained by a 16% increase in HGP

(from 2.20 ±0.25 to 2.56 ± 0.52, P<0.05). There was no significant change in HGP in subjects who received placebo. On day 3, HGP remained elevated in dapagliflozin-treated subjects (0.36 mg/kg.min) and resulted in the addition of ~47 grams glucose per day to the circulation. This amount of glucose (~47 grams), produced as a result of the increase in HGP, was equal to approximately one half (52%) of the amount of glucose excreted in the urine secondary to SGLT2 inhibition by dapagliflozin. Thus, the increase in HGP in response to glucosuria offsets, by at least half, the amount of glucosuria produced by inhibiting SGLT2. If the increase in HGP had been prevented, the decrease in the FPG caused by dapagliflozin would have been at least double that achieved with the drug.

The plasma insulin concentration decreased progressively in both placebo-treated and dapagliflozin-treated groups on day 2. On day 3, the plasma insulin concentration in both groups was unchanged compared to day 1, while the plasma glucagon concentration progressively increased in subjects receiving dapagliflozin on day 2 (Figure 1D) and did not change in placebo-treated subjects. On day 3, the plasma glucagon concentration was 32% higher compared to day 1 ($p<0.05$) in dapagliflozin-treated subjects, while there was no significant change in plasma glucagon concentration in placebo-treated subjects. The progressive decline in plasma insulin concentration (despite the rebound increase in HGP and plasma glucose concentration) and the paradoxical rise in plasma glucagon concentration facilitate the increase in HGP and plasma glucose concentration following dapagliflozin administration.

RESEARCH HYPOTHESIS:

We hypothesize that the glucosuria produced by the inhibition of SGLT2 triggers an increase in the rate of HGP to compensate for the urinary glucose loss in an attempt to maintain

the plasma glucose concentration constant at the fasting level. Based upon our preliminary data, we hypothesize that the rise in plasma glucagon concentration, in concert with the decline in plasma insulin concentration, is the critical trigger that mediates the compensatory rise in HGP. The compensatory increase in HGP offsets by ~50% the amount of glucose lost in the urine. This "paradoxical" increase in HGP attenuates the decrease in plasma glucose concentration and A1cand reduces the clinical efficacy of the SGLT2 inhibitor. Further, we hypothesize that combination therapy with an incretin-based agent, e.g. liraglutide, plus an SGLT2 inhibitor will, by inhibiting glucagon and stimulating insulin secretion, prevent the compensatory rise in HGP and amplify the decrease in plasma glucose concentration and A1c caused by the SGLT2 inhibitor. We also hypothesize that combination therapy with canagliflozin/liraglutide will cause an additive, or even synergistic, decrease in body weight/hepatic fat content/visceral fat content and systolic/diastolic blood pressure.

Significance: Increased basal rate of HGP and impaired suppression of HGP following a meal are major causes of fasting and postprandial hyperglycemia, respectively, in T2DM individuals (1-9). Based on our preliminary data, we hypothesize that the increase in HGP in response to glucosuria indicates the presence of an interaction between the kidney and liver, i.e. "a reno-hepatic axis" that plays a pivotal role in the regulation of glucose homeostasis. The present study will provide novel insights about the regulation of glucose homeostasis, and has important clinical implications. Although SGLT2 inhibitors produce large glucosuria and promote 2-3 kg weight loss, the decline inA1c (0.6-0.8%) is less than expected (11). In NGT individuals, the presence of a "reno-hepatic axis" that leads to a compensatory increase in HGP in response to glucosuria and maintains the FPG within the normal range represents a protective response against the development of hypoglycemia. On the other hand, the "compensatory" increase in HGP in

T2DM individuals is paradoxical in that it occurs while the plasma glucose concentration is well within the hyperglycemic range and opposes by ~50% the urinary glucose loss produced by SGLT2 inhibitors. This markedly attenuates the glucose lowering effect of the SGLT2 inhibitors. The present study will help to elucidate the mechanisms that mediate the rise in HGP in response to glucosuria and allow the development of strategies to prevent this compensatory rise in HGP and increase the efficacy of SGLT2 inhibitors. Our preliminary data suggest an important role for increased glucagon secretion in mediating the "reno-haptic" interaction. In this study, we will examine the ability of liraglutide to block the increase in plasma glucagon concentration and subsequent stimulation of HGP in response to canagliflozin -induced glucosuria. Liraglutide stimulates insulin and inhibits glucagon secretion. Therefore, it has the potential to prevent the compensatory rise in HGP following canagliflozin administration and provide an additive, even synergistic, decrease in the plasma glucose concentration and HbA1c. Further, we anticipate that combination therapy with canagliflozin plus liraglutide will produce an additive, even synergistic, effect to reduce body fat content and blood pressure.

EXPERIMENTAL DESIGN AND METHODS

Subjects: 45subjects with T2DM according to the ADA criteria will participate in the study. Subjects must be between 18-70 years of age, drug naïve or on a stable dose (more than 3 months) of metformin or on metformin and a sulfonylurea and have HbA1c >7.0% and <10.0%. Subjects taking drugs known to affect glucose metabolism (other than metformin) will be excluded. Other than diabetes, subjects must be in good general health as determined by physical exam, medical history, blood chemistries, CBC, TSH, T4, and urinalysis. Only subjects whose body weight has been stable (± 3 lbs) over the preceding three months and who do not participate in an excessively heavy exercise program will be included. Individuals with evidence of

proliferative diabetic retinopathy or plasma creatinine >1.4 females or >1.5 males or eGFR< 60 ml/min.172m^2 will be excluded.

Study design: Each subject will receive the following: (i) quantitation of basal hepatic glucose production with 3-^{3}H-glucose; (ii) OGTT with measurement of plasma glucose, insulin, C-peptide, and glucagon; (iii) determination of total body fat content with DEXA; (iv) measurement of hepatic, visceral, and abdominal subcutaneous fat content with MRS/MRI; (v) 24-hour continuous blood pressure monitoring; (vi) A1c x 2 and then started on one of the following therapies for 4 months: (1) canagliflozin (film-coated tablet), 100 mg/day, increased to 300 mg/day after week two if tolerated without side effects; (2) liraglutide, 1.2 mg/day, increased to 1.8 mg/day after week two if tolerated without side effects; (3) canagliflozin, 100 mg/day, plus liraglutide, 1.2 mg/day, increased to 300 mg/day and 1.8 mg/day, respectively at week two, if tolerated without side effects.

Visit 1: Screening. After a 10 hour overnight fast, medical history will be obtained and physical exam is performed. Blood will be drawn for FPG, routine blood chemistries (including renal function tests), lipid profile, A1c, and thyroid function. An ECG, urinalysis and pregnancy test will be performed. DEXA and OGTT will be performed and 24-hour blood pressure monitoring will be initiated. Preventative foot exam will be performed (see description in follow-up section).

Visit 2: Hepatic, visceral, and subcutaneous abdominal fat content will be measured by MRI/MRS after an overnight fast.

Visit 3: The rates of endogenous (primarily hepatic) glucose production and whole body glucose disposal will be measured with 3-^{3}H-glucose. Subjects will report to the CRC at 6 AM

after a 10 hour overnight fast. At 6 AM a catheter will be placed into an antecubital vein and a prime (40 uCi x FPG/100)-continuous (0.4uCi) infusion of [3-^{3}H]-glucose will be started and continued until 3 PM. At 8 AM, a second catheter will be placed retrogradely in a vein on the dorsum of the hand, which is placed in a heated box (70°C)for sampling of arterialized blood. After 3 hours of tracer equilibration, blood samples will be drawn from the catheter on the dorsum of the hand at -30,-20,-15,-10,-5 and 0 (time zero is drug ingestion time) minutes. At 9 AM, blood samples will be obtained from the retrograde catheter on the dorsum of the hand every 20 minutes from 9 AM to 3 PM. Plasma glucose, insulin, C-peptide, glucagon, cortisol, growth hormone, and catecholamine concentrations, and [3-^{3}H]-glucose specific activity will be measured. Urine will be collected from 6 to 9 AM and from 9 AM to 3 PM. Urinary volume and urinary glucose concentration will be measured to obtain urinary glucose excretion. The study will end at 3 PM and subjects will be allowed to return home.

Visit 4: After completing visit 3, subjects will return to the CRC within 7-14 days for a repeat measurement of HGP with tritiated glucose as per visit 3, with the following exception: at time zero (at 9 AM), using a table of random numbers, subjects will be randomized to receive one of the following:(i) canagliflozin (100 mg orally) n=15; (ii) liraglutide (1.2 mg sc); n=15; or (iii) canagliflozin 100 mg, plus liraglutide, 1.2 sc; n=15. We will also draw blood to monitor kidney function. Prior to initiation of therapy, subjects have a one hour session with a dietician who will discuss a nutritionally balanced, weight maintaining diet.

After completion of the above studies, subjects will continue to take canagliflozin, liraglutide, or canagliflozin plus liraglutide for 4 months. Subjects will return for follow up visits at weeks 2, 4, 6, 8, 12, 15 and 16. During each follow-up visit we will draw blood to monitor kidney function. We will also draw some blood to measure A1c, lipid profile and FPG (fasting

plasma glucose) and weight and blood pressure will be measured. Measurements for A1c and lipid profile will be taken during visits 15 or 16, not on both. In addition, preventative foot exams will be done on a monthly basis (see description in follow-up section). During weeks 15-16 measurement of total body fat (DEXA), hepatic/visceral/abdominal subcutaneous fat content (MRS/MRI), OGTT, 24-hour blood pressure monitoring, and HGP (as described in visits 4) will be repeated.

Schedule of events for Visits 1-4

Visit 1:

- Screening procedures (including tests to monitor renal function and preventative foot exam)– about 60-90 minutes
- Test 1: Oral Glucose Tolerance Test (OGTT) – about 2 hours
- Test 2: DEXA scan – about 10 minutes
- Test 3: 24-hour blood pressure monitoring – carried out over a 24 hour period during normal daily life

Visit 2:

- MRI or an MRS – about 2 hours

Visit 3:

- HGP (hepatic glucose production) measurement
  - Catheter placed at 6AM (elbow); small amount of radioactive glucose given
    - Infusion continued until 3PM
  - 2nd catheter placed at 8AM (hand); hand placed in heated box
  - From 8:30AM to 9AM blood samples will be drawn from hand at 6 time points within 30 minutes (-30, -20, -15, -10, -5 and 0)
  - At 9AM blood samples will be drawn from the hand every 20 minutes from 9AM to 3PM.
  - 6AM – 9AM there will be one urine collection
  - 9AM -3PM there will be one urine collection
  - Procedure ends at 3PM

Visit 4: within 7-14 days of visit 3

- Repeat HGP as in Visit 3
  - Difference: at 9AM (time 0) the subject will be randomly assigned one of the following: (i) canagliflozin (100 mg orally) alone, (ii) liraglutide (1.2 mg

subcutaneously) alone, or (iii) both canagliflozin 100 mg and lirgaglutide 1.2 mg sc
- Renal function tests

** Return home with instructions on taking medication assigned during visit 4 for 4 months.

Follow-up visits

- At weeks 2, 4, 6, 8, 12, 15 and 16 blood will be drawn for A1c, lipid profile, renal function tests (BMP on visits 4-16) and, FPG (fasting plasma glucose), and weight and blood pressure will be measured.
- 3 visits during weeks 15 and 16 for repeat testing
  - 1: DEXA scan (as in visit 1, test 2) ~ 10 min
  - 1: OGTT (as in visit 1, test 1) ~ 3 hours
  - 1: 24-hour blood pressure monitoring (as in visit 1, test 3)
  - 2: MRI/MRS (as in visit 2)- ~2 hours
  - 3: HGP (as in visit 3) ~ 9 hours

- Preventative foot exams will be done on a monthly bases during the study. These exams include the following:

    - Visual inspection of both feet
    - Monofilament sensory tests for both feet
    - Pulse exams for both legs and feet

Data Analysis and Statistical Methods:

The primary end point of the study is the change in the rate of HGP during the last hour of the study (i.e. from 300-360 minutes) following drug administration compared to the basal rate of HGP (before drug administration). This change in HGP will be compared among the 3 different treatments (canagliflozin, liraglutide, canagliflozin plus liraglutide) with ANOVA. Post hoc analysis will be performed with paired t-test.

The rate of HGP will be calculated as previously described (18). Under steady-state post absorptive conditions, the basal rate of endogenous (primarily reflects hepatic) glucose appearance (Ra) equals the 3-^{3}H-glucose infusion rate divided by the steady state plasma tritiated glucose

specific activity. After drug administration, non-steady conditions for 3-^{3}H-glucose specific activity prevail and the total rate of glucose appearance (Ra) is calculated from Steele's equation. Ra will be calculated for each time point after the drug administration and compared to Ra in the placebo study.

Secondary end points will be: (i) the decrease in the fasting plasma glucose concentration and A1c at the end of the study compared to baseline, (ii) improvement in oral glucose tolerance and insulin secretion during OGTT; (iii) improvement in Matsuda index of insulin sensitivity during OGTT; (iv) change in plasma glucagon concentration at the end of the study compared to baseline; (v) change in plasma insulin concentration at the end of the study compared to baseline; (vi) change in total body, hepatic/visceral/abdominal subcutaneous fat content at study end compared to baseline; (vii) change in 24-hour blood pressure at study end compared to baseline.

Values will be presented as the mean $\pm$ SD. The difference in HGP and all secondary endpoints at study end versus baseline will be calculated and compared between each active treatment group with ANOVA.

We plan to perform additional analysis on plasma samples that were obtained during the study. We plan to carry out both lipidomic and metabolomic analyses on samples previously collected at baseline, week 2 and 16 for all 45 completers. These analyses utilize GCMS to identify specific lipids and metabolites in plasma that are increased or decreased. Using pathway analysis, this can provide insight into specific intracellular defects that are present in individuals with type 2 diabetes.

Anticipated Results and Data Interpretation

Consistent with our preliminary data with dapagliflozin we anticipate that, after an initial decline, HGP progressively will increase following canagliflozin administration compared to the

baseline study. We anticipate that plasma glucagon concentration will increase following canagliflozin administration and plasma insulin concentration will decline. Based upon previous studies (42), we anticipate that a single administration of liraglutide will cause an increase in plasma insulin concentration and a decrease in plasma glucagon concentration compared to the baseline study. The increase in plasma insulin and decrease in plasma glucagon concentration will result in greater decline in both HGP and fasting plasma glucose concentration compared to the baseline study. We also anticipate that the increase in plasma glucagon concentration and rise in HGP following canagliflozin administration will be prevented when canagliflozin is co-administered with liraglutide. Therefore, we anticipate an additive, and even synergistic, decrease in the plasma glucose concentration following the co-administration of canagliflozin plus liraglutide compared to either drug alone.

After 4 months of treatment, we anticipate that T2DM patients treated with canagliflozin plus liraglutide will have a greater decline in A1c, FPG, 24-hour blood pressure, total body fat, hepatic fat, and visceral and abdominal subcutaneous fat content when compared to either canagliflozin alone or liraglutide alone. We also expect to observe a greater improvement in beta cell function (insulin secretion/insulin resistance index) and insulin sensitivity (Matsuda index) during the OGTT in the canagliflozin/liraglutide group compared to the other two groups.

Sample Size Calculation: In preliminary results, the difference in the rate of basal HGP during the last hour between dapagliflozin-treated and placebo-treated individuals was 0.70±0.34 mg/kg.min (mean±SD). From this, we computed that 15 individuals per group would provide 95% power to detect a similar difference in HGP between (i)canagliflozin-treated versus canagliflozin plus liraglutide-treated individuals and (ii) between liraglutide-treated versus canagliflozin plus

liraglutide-treated individuals at alpha =0.05. To ensure 15 completers per group, we have set the sample size at 90.

SAFETY

All drugs can cause unwanted effects called side effects. As of 28 September 2015, approximately 13173 subjects have received canagliflozin in completed or ongoing studies of 12 weeks or longer.

Side effects found in these studies that are more likely to occur with canagliflozin include those below. The subject should speak with the doctor about any changes to his or her diet or other medications they are taking:

- **Dizziness or lightheadedness upon standing** - These adverse events, from a decrease in blood pressure, occur soon after starting canagliflozin and are more likely to occur in people on medicines to lower blood pressure including diuretics (water pills such as Lasix/furosemide), on a low salt diet, older patients or those who have reduced kidney function. While subject is taking canagliflozin, the subject should try to avoid becoming dehydrated and should speak to the doctor about any changes in his or her diet or other medications. This side effect may occur in up to 1 in 20 people on canagliflozin, or slightly more frequently in those at risk as described above.

- **Hypoglycemia in patients taking other medications associated with hypoglycemia**

  . If subject takes canagliflozin with another medicine that can cause low blood sugar, such as a sulfonylurea or insulin, the subject's risk of getting low blood sugar is higher. The dose of the subject's sulfonylurea medicine or insulin may need to be lowered while the subject takes canagliflozin and the subject should speak to the doctor about any changes in other medications. This side effect is very common and may occur in more than 1 in 10 people on canagliflozin.

Signs and symptoms of low blood sugar may include:

  - headache
  - drowsiness
  - weakness
  - dizziness

- confusion
- irritability
- hunger
- fast heart-beat
- sweating
- shaking or feeling jitter

- **Increased urination and thirst** – Symptoms might include feeling thirsty , having a dry tongue, urinating more frequently or in larger amounts, an urgent need to urinate or more frequent urination at night. These side effects may occur in up to 1 in 20 people taking canagliflozin.
- **Urinary tract infections** – Symptoms of urinary tract infections may include burning with urination, discomfort in passing urine, or fever. This side effect may occur in up to 1 in 20 people on canagliflozin.
- **Allergic reaction including rash or hives** – These events can occur shortly after starting canagliflozin, are generally not serious or associated with other serious symptoms, such as breathing problems. This side effect may occur in up to 1 in 20 people on canagliflozin.
- **Constipation** – The side effect of constipation may occur in slightly more than 1 in 50 people on canagliflozin.
- **Nausea** - There is a slightly higher rate of nausea (stomach sickness or queasy sensation) with canagliflozin. . The side effect of nausea may occur in slightly more than 1 in 50 people on canagliflozin.

- **Laboratory changes that have been observed in clinical studies with canagliflozin**
  - Blood test might show an increase in the LDL, or "bad", cholesterol.

- Blood test might show an increase in serum potassium, phosphate and/or hemoglobin; decreases in serum urate can also occur. These changes are generally not serious and not associated with serious symptoms.
- A change in lab tests associated with kidney function might occur. These changes have generally been temporary and may relate to hydration status. During the study, the doctor regularly monitors kidney function, and may discuss with subject any necessary changes in subject's diet or other medications.
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- Bone fractures may occur in up to 1 in 50 people per year on canagliflozin.

- Amputation of the toes (and to a lesser extent the foot or leg) could occur in up to 1 in 150 people per year on canagliflozin. This risk is greater in those with a prior history of amputation, disease of the circulation involving the legs or in those with nerve damage due to diabetes.

Side effects in patients not involved in clinical studies who have been prescribed canagliflozin to treat their diabetes include those below. It is difficult to know specifically how often these side effects occur or always be certain if they are more likely to occur as a result of canagliflozin because these were not reported in the manner similar to data collection in a clinical study.

- Serious allergic reactions, including those with the symptoms of swelling of the face, throat, and/or tongue or breathing problems.

- Related to changes in lab tests associated with kidney function, severe cases of decreases in kidney function have been reported more commonly in patients who were dehydrated. **Acute Kidney Injury**: Sudden kidney injury (acute kidney injury) has happened to people taking canagliflozin. Talk to your doctor right away if you:
  - reduce the amount of food or liquid you drink for example, if you are sick or cannot eat or
  - you start to lose liquids from your body for example, from vomiting, diarrhea or being in the sun too long. Seek medical attention immediately if you experience signs and symptoms while taking these medicines such as:
    - Decreased urine
    - Swelling in your legs or feet

- Infections of the urinary tract that can spread to the kidneys or into the bloodstream. Symptoms may include high fever, increased heart rate and breathing, low blood pressure, low urine output and lower back pain. Call your doctor if you experience these symptoms.

- **Diabetic ketoacidosis** - your blood may show increased levels of blood acids called ketones. Sometimes this can occur even if your blood sugar levels are not very high (e.g., less than 250 mg/dL [13.9 mmol/L]). The risk of developing diabetic ketoacidosis may be increased with prolonged fasting, excessive alcohol consumption, dehydration, sudden reductions in insulin dose, or a higher need of insulin due to major surgery or serious illness. Symptoms may include difficulty breathing, nausea, vomiting, excessive thirst, rapid weight loss, abdominal pain, confusion, fruity-smelling breath, a sweet or metallic taste in your mouth, a different odor to your urine or sweat, and unusual fatigue or sleepiness. This side effect may occur in up 1 in 1000 people with Type 2 diabetes. Call your doctor if you experience these symptoms. Do not stop or change study drug or diabetes medicines without first discussing with your doctor.There may be side effects with the use of canagliflozin that are not yet known. Sometimes the University of Texas Health Science Center at San Antonio may learn new facts about the study drugs/treatments. It is possible that this information might make the subject change their mind about being in the study. If new information is discovered, the study doctor will inform the subject of such new information in a timely manner.

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For Women:

- **Vaginal yeast infections and vaginal itching** - Symptoms such as vaginal itching, burning, irritation, odor or discharge. This side effect may occur in slightly more than 1 in 10 women on canagliflozin.

For Men:

Yeast infection at the head of the penis - Symptoms such as penile itching, irritation, burning, swelling, foul smelling discharge or pain. In 0.3% (1 in 300) of men who are not circumcised, this could lead to swelling of the foreskin, and require circumcision. The side effect of yeast infection may occur in up to 1 in 20 men on canagliflozin.

PREGNANCY AND BIRTH CONTROL DURING THE STUDY

Canagliflozin has been tested for its ability to cause harm to the fetus during pregnancy or cause birth defects. These studies have been done in animals. The studies that have been completed do not indicate that canagliflozin is associated with birth defects.

Nonetheless, women who are pregnant, lactating or intend to become pregnant during the study will not be allowed to participate in the study. Women who could possibly become pregnant must have a negative pregnancy test prior to starting on the study drug and report immediately to the study site if they suspect they are pregnant during the study.

Women who are able to have children *and are heterosexually active*, must use birth control (contraception) during the study. Birth control methods that can be used while in this study include: avoiding sex, birth control pills, birth control injections or patch, intrauterine device, barrier method (for example, condoms or diaphragm) combined with spermicide (foam, cream, or gel), or the male partner is sterile (e.g. sperm tubes are cut or blocked). The type of birth control used must be discussed with the study doctor before the start of the study. The study doctor must approve the method the subject uses before the subject can enter the study.

If the subject becomes pregnant during the study, he or she must tell the doctor immediately. The subject will have to stop taking the study drug. The doctor will advise the subject about medical care and will ask the subject to allow him/her to collect information about the pregnancy and the health of the subject's baby.

For male subjects, if his partner becomes pregnant, the subject must tell the study doctor immediately.

The following are the risks associated with **Liraglutide**:

Likely, not serious

In 100 people, approximately 20 or more may have:

- Nausea

Less likely, not serious

In 100 people, approximately 20 or less may have:

- Back pain
- Nasopharyngitis

- Sinusitis
- Dizziness
- UTI
- Influenza
- Headache
- Upper respiratory tract infection
- Constipation
- Vomiting
- Diarrhea

Rare and Serious

In 100 people, approximately 5 or less may have:

- Thyroid C-cell Tumors – There have been 4 reported cases of thyroid C-cell hyperplasia among Victoza-treated patients and 1 case in a comparator-treated patient (1.3 vs 0.6 cases per 1000 patient-years).
- Pancreatitis – There were 7 cases of pancreatitis among Victoza-treated patients and 1 case among comparator-treated patients (2.2 vs. 0.6 cases per 1000 patient-years). Five cases with Victoza were reported as acute pancreatitis and two cases with Victoza were reported as chronic pancreatitis.
- Hypoglycemia may occur in patients taking other antidiabetic medications such as a sulfonylurea or insulin but is uncommon in patients taking only metformin. Liraglutide taken with another medicine that can cause low blood sugar, such as a sulfonylurea, the risk of getting low blood sugar is increased. The dose of sulfonylurea medicine may need to be lowered while taking

liraglutide and a physician should be contacted about any changes in other medications. This side effect may occur in more than 1 in 10 people on liraglutide.

Adverse Events and Serious Adverse Events

I. Management of Safety Data

This Study has been designated as an interventional study. Janssen requirements for IIS interventional studies are all adverse events regardless of causality and special situations excluding those from subjects not exposed to a Janssen Medicinal Product and product quality complaints with or without an adverse event will be reported, once the subject has signed and dated an Informed Consent Form is obtained until the subject has completed participation in the study and for 30 days after the last dose of study drug.

II. Definitions

a. Adverse Event (AE)

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non- investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH]).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

b. Adverse Events of Special Interest

Events that Janssen Scientific Affairs is actively monitoring as a result of a previously identified signal (even if non-serious). These adverse events are:

Diabetic ketoacidosis (DKA), Amputation

The INSTITUTION and the PRINCIPAL INVESTIGATOR) will transmit all AEs related to Diabetic ketoacidosis following exposure to a Janssen product under study in a DKA form provided by the COMPANY in accordance with Section 10,Transmission Methods, in English. If also an SAE, please submit Janssen **within 24-hours of becoming aware of the event(s).**

c. Individual Case Safety Report (ICSR)

A valid ICSR must contain the four minimum criteria required to meet regulatory reporting requirements.

- an identifiable subject (not disclosing the subject's name and address)
- an identifiable reporter (investigational site)
- a Janssen medicinal product
- an adverse event, outcome, or certain special situation

The minimum information required is:

- suspected Janssen product (doses, indication)
- date of therapy (start and end date, if available)
- batch or lot number, if available
- subject details (subject ID and country)
- gender
- age at AE onset
- reporter ID
- adverse event detail (AE verbatim in English), onset date, relatedness, causality, action taken, outcome, (if available)
- Janssen protocol ID

d. Product Quality Complaint (PQC)

A product quality compliant is related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a subject. Lot and batch numbers are of high significance and need to be collected whenever available.

Examples of PQC include but not limited to:

- Functional Problem: e.g., altered delivery rate in a controlled release product
- Physical Defect: e.g. abnormal odor, broken or crushed tablets/capsules
- Potential Dosing Device Malfunction: e.g., autoinjector button not working, needle detaching from syringe
- Suspected Contamination
- Suspected Counterfeit.

e. Serious Adverse Event (SAE)

A serious adverse event based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)

- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is medically important*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

NOTE: DEATH FOR ANY REASON SHOULD BE REPORTED AS A SERIOUS ADVERSE EVENT.

For reports of hospitalization, it is the sign, symptom or diagnosis which led to the hospitalization that is the serious event for which details must be provided.

Any event requiring in-patient hospital admission (or the prolongation of hospitalization) must be reported as an SAE. Events that do not meet the criteria for SAE reporting are:

- Reasons described in the Protocol, e.g. drug administration, Protocol-required testing
- Social reasons, e.g. overnight stay because of distance between home and hospital
- Surgery or procedure planned and documented prior to entry into the Study.

Any event requiring hospitalization or prolongation of hospitalization that occurs during the study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study. [Note: Hospitalizations that were planned before the start of data collection and where the underlying condition for which the hospitalization was planned has not worsened will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.]

The cause of death of a subject in a study within 30 days of the last dose of study drug, whether or not the event is expected or associated with the study drug, is considered a serious adverse event.

f. Unlisted (Unexpected) Adverse Event/Reference Safety Information

An adverse event is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For a medicinal product(s) with a marketing authorization, the expectedness of an adverse event will be determined by whether or not it is listed in the applicable product information.

III. Special Situations

Safety events of interest for a Janssen medicinal product that require expediting reporting and/or safety evaluation include, but are not limited to:

- Drug exposure during pregnancy (maternal and paternal)
- Overdose of a Janssen medicinal product
- Exposure to a Janssen medicinal product from breastfeeding
- Suspected abuse/misuse of a Janssen medicinal product
- Inadvertent or accidental exposure to a Janssen medicinal product
- Any failure of expected pharmacological action (i.e., lack of effect) of a Janssen medicinal product
- Medication error involving a Janssen medicinal product (with or without patient exposure to the Janssen medicinal product, e.g., name confusion)
- Suspected transmission of any infectious agent via a medicinal
- Unexpected therapeutic or clinical benefit from use of a Janssen medicinal product

These safety events may not meet the definition of an adverse event; however, they are treated in the same manner as adverse events. Special situations should be recorded on the Adverse Event page of the CRF.

Any special situation that meets the criteria of a serious adverse event should be recorded on a Serious Adverse Event Report Form and be reported to Janssen Scientific Affairs **within 24 hours of becoming aware of the event.**

IV. Pregnancy

All initial reports of pregnancy must be reported to Janssen Scientific Affairs by the Sponsor Investigator **within 24 hours of their awareness of the event** using the Serious Adverse Event Form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported using the Serious Adverse Event Form.

Any subject who becomes pregnant during the study must be promptly withdrawn from the study and discontinue further study treatment.

Because the effect of the Janssen medicinal product on sperm is unknown, pregnancies in partners of male subjects exposed to a Janssen medicinal product will be reported by the Sponsor Investigator **within 24 hours of their awareness of the event** using the Serious Adverse Event Form. Depending on local legislation this may require prior consent of the partner.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

V. Reporting Procedures for Adverse Events and Pregnancies [and/or Pregnancies in Partners]

All adverse events, whether serious or non-serious, related or not related, special situations, pregnancy exposures and/or pregnancies in partners following exposure to a Janssen medicinal product are to be documented by the investigator and recorded in the CRF and in the subject's source records. Investigators must record in the CRF their opinion concerning the relationship of the adverse event to a Janssen medicinal product.

All (serious and non-serious) adverse events reported for a Janssen medicinal product should be followed-up in accordance with clinical practice.

All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

All serious adverse events, pregnancy exposures and/or pregnancies in partners for Janssen medicinal products under study should be reported directly by the Sponsor Investigator, **within 24 hours of becoming aware,** to Janssen Scientific Affairs using the Janssen Scientific Affairs Serious Adverse Event Report Form. In the event the study is blinded, the Sponsor Investigator will submit an unblinded SAE or pregnancy exposure report to Janssen Scientific Affairs.

All follow-up information for serious adverse events that are not resolved at the end of the study or by the time of patient withdrawal must be reported directly by the Sponsor Investigator, **within 24 hours becoming aware**, to Janssen Scientific Affairs using the Janssen Scientific Affair's Serious Adverse Event Report Form.

VI. Product Quality Complaints for Janssen Medicinal Products

A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of patients, investigators, and Janssen Scientific Affairs, and are mandated by regulatory agencies worldwide. Janssen Scientific Affairs has established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information. Lot and/or Batch #s shall be collected or any reports of failure of expected pharmacological action (i.e., lack of effect).

All initial PQCs involving a Janssen product under study must be reported to Janssen Scientific Affairs by the Sponsor Investigator **within 24 hours after being made aware of the event.**

If the defect for a Janssen product under study is combined with either a serious adverse event or non-serious adverse event, the Sponsor Investigator must report the PQC to Janssen Scientific Affairs according to the serious adverse event reporting timelines. A sample of the suspected product should be maintained for further investigation if requested by Janssen Scientific Affairs.

VII. Maintenance of Safety Information

All safety data should be maintained in a clinical database in a retrievable format. The Institution and Sponsor Investigator shall provide all adverse events, both serious and non-serious, in report format. However, in certain circumstances more frequent provision of safety data may be necessary, e.g. to fulfill a regulatory request, and as such the data shall be made available within a reasonable timeframe at Janssen Scientific Affair's request.

VIII. Transmission Methods:

The following methods are acceptable for transmission of safety information to Janssen Scientific Affairs:

- Electronically via Janssen SECURE Email service (preferred)
- For business continuity purposes, if SECURE Email is non-functional:
    - Facsimile (fax), receipt of which is evidenced in a successful fax transmission report
- Telephone (if fax is non-functional).

Please use the contact information and process information provided by Janssen Scientific Affairs.

IX. Procedures for Reporting Adverse Events (AE), Serious Adverse Events (SAE), Pregnancy, and Product Quality Complaints (PQC) to Janssen Scientific Affairs

A. AEs, SAEs, Special Situations and Pregnancy Reporting.

The Institution and the Sponsor Investigator will transmit SAEs and Special Situations in a form provided by Janssen Scientific Affairs in accordance with Section VIII Transmission methods, in English **within 24-hours** of becoming aware of the event(s).

All available clinical information relevant to the evaluation of a related SAE or Special Situation is required.

- The Institution and/or Sponsor Investigator are responsible for ensuring that these cases are complete and if not are promptly followed-up. A safety report is not considered complete until all clinical details needed to interpret the case are received. Reporting of follow-up information should follow the same timeline as initial reports.

- Copies of any and all relevant correspondences with regulatory authorities and ethics committees regarding any and all serious adverse events, irrespective of association with the Janssen Product under study, are to be provided to Janssen Scientific Affairs using a transmission method in Section VIII **within 24 hours of such report or correspondence being sent to applicable health authorities.**

B. PQC Reporting

The Institution and the Sponsor Investigator will report any suspected PQC to the Janssen contact within 24 hours of becoming aware of the complaint. The product should be quarantined immediately and if possible, take a picture.

X. Reconciliation of SAEs

At a minimum, on a quarterly basis and at the end of the Study, Janssen Scientific Affairs will provide to the Institution and/or Sponsor Investigator, a listing of all SAEs reported to Janssen Scientific Affairs. The Sponsor Investigator will review this listing and provide any discrepancies to Janssen Scientific Affairs.

Upon request, Institution and/or Sponsor Investigator shall provide Janssen Scientific Affairs with a summary list of all SAEs, and AEs of Special Interest and Special Reporting Situation reports to date, for reconciliation purposes.

XI. Dissemination of Safety Information from Janssen Scientific Affairs to Institution/Sponsor Investigator

Sponsor Investigator will be responsible for submitting IND safety reports for the Study Product to Institution's IRB in accordance with Federal regulations 21 CFR 312.66.

Janssen Scientific Affairs agrees to provide to the Sponsor Investigator IND safety reports for the Study Product as they become available until all subjects in the Protocol have completed their last Study visit according to the Protocol (i.e. Last Subject Last Visit has occurred).

All observed or volunteered AEs or suspected causal relationship to the study drugs or procedures will be reported. For all AEs, the investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets the criteria for classification as an SAE requiring immediate notification to Janssen Scientific Affairs, LLC and to UTHSCSA IRB (per UPIRSO policy). For all AEs, sufficient information should be obtained by the investigator to determine causality of the AE. For AEs with a causal relationship to the investigational product, follow-up by the investigator is required until the event is resolved or stabilized at a level acceptable to the investigator, and IRB.

As part of ongoing safety reviews, any non-serious adverse event that is determined by the investigator to be serious will be reported as an SAE. To assist in the determination of case seriousness, further information may be requested from the investigator to provide clarity and understanding of the event in the context of the clinical study.

All AEs related to protocol procedures will be reported to the IRB, Janssen Scientific Affairs, LLC and to FDA (by the study sponsor). Subjects will self-report AEs occurring at all other times. (iii) Any clinically significant findings from ECGs, labs, vital sign measurements, other procedures, etc. that result in a diagnosis will be reported to the IRB and subjects physician for a follow up outside of the research study. Any conditions that may put subjects to risk will be grounds for withdrawing the subject from the study. Investigators will report to the IRB their assessment of the potential relatedness of each AE to study procedure, disease, or study drug via Unanticipated Problems Involving Risk to Subjects or Others (UPIRSO) or AE Report.

The Collection of UPIRSO begins after the subject has signed informed consent and has started the study procedures. If a subject experiences an UPIRSO after signing informed consent, the

event will NOT be collected unless the investigator feels that the event may have been caused by a protocol procedure/study medication. UPIRSOs will be reported to the IRB in according to the IRB policy. Study site personnel will alert the IRB (within 24 hours) of becoming aware of any life threatening or fatal events via approved method. Alerts issued via telephone are to be immediately followed with official notification. An SAE is any AE from this study that results in 1 of the following outcomes:

- death
- initial or prolonged in subject hospitalization
- a life-threatening experience (that is, immediate risk of dying)
- persistent or significant disability/incapacity
- considered significant by the investigator for any other reason.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse drug events when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. All other AEs will be reported to the IRB once a year in the Continuing Review Report. As the PI/Sub-PI evaluates SAEs/AEs, actions will be taken to eliminate any hazards and/or changes that should be submitted to the IRB, if necessary, will be considered.

Risks will be minimized by a careful screening process including medical history, physical exam, and review of complete blood count, coagulation tests, chemistry and electrocardiogram. Experienced study research personnel during the visits and procedures and of at least one physician at all times (or on call during the 4-day in-patient visit) further minimizes any potential risks.

Severity Assessment

If required, when documenting the AE, the investigator will use the adjectives MILD, MODERATE or SEVERE to describe the maximum intensity of the AE. These intensity grades are defined as follows:

MILD Does not interfere with subject's usual function

MODERATE Interferes to some extent with subject's usual function

SEVERE Interferes significantly with subject's usual function

The terms "serious" and "severe" are not synonymous. The term "severe" is often used to describe the intensity (severity) of a specific event. The event itself, however, may be of relatively minor medical significance. This is not the same as "serious," which is based on subject/event outcome or action criteria. Accordingly, a severe event is not necessarily a serious event.

Causality Assessment

The investigator's assessment of causality must be provided for all AEs. The investigator must record the causal relationship in the CRF, as appropriate, and report such an assessment in accordance with the serious adverse event reporting requirements, if applicable. An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an AE. If the investigator

does not know whether or not the investigational product caused the event, then the event will be handled as "related to investigational product" for reporting purposes. If the investigator's causality assessment is "unknown but not related to investigational product", this should be clearly documented on study records. In addition, if the investigator determines an SAE to be associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with SAE reporting requirements.

Withdrawal Due to Adverse Events

Withdrawal due to AE should be distinguished from withdrawal due to insufficient response, and recorded on the appropriate AE CRF. When a subject withdraws due to an SAE, the SAE must be reported in accordance with the reporting requirements.

Eliciting Adverse Event Information and Reporting

The investigator is to report all directly observed AEs and all AEs spontaneously reported by the study subject. Each study subject will be questioned about AEs. Each AE is to be assessed to determine if it meets the criteria for SAEs. If an SAE occurs, expedited reporting will follow appropriate regulations.

Serious Adverse Event Reporting Requirements

If an SAE is determined as UPISRO, it will be reported to IRB in accordance with UPIRSO policy.

In the rare event that the investigator does not become aware of the occurrence of an SAE immediately (e.g., an outpatient study subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hr after learning such event and document the time of first awareness of the AE.

Non-Serious Adverse Event Reporting Requirements

All AEs will be reported on the AE page(s) log. AEs should be reported using concise medical terminology on the CRFs as well as on the form for collection of the SAE information.

Sponsor Reporting Requirements to Regulatory Authorities

AE reporting, including suspected serious unexpected adverse reactions, will be carried out in accordance with applicable regulations. Expected AEs or AEs that are not related to the study will be reported on the annual progress report to the IRB.

The investigator also must notify the IRB of the occurrence of the SAE, in writing, as soon as is practicable and in accordance with local law

Subject Monitoring

Subjects will be monitored for AEs throughout the study by the study unit staff. Safety parameters, including laboratory results and ECGs, will be assessed by the principal investigator or his delegate using the site's criteria for clinical laboratory and ECG acceptance ranges as suggested guidelines in making the medical assessment.

Scheduled safety measurements will be repeated according to appropriate SOPs or upon request from a physician. Any abnormal repeated measurement will be evaluated by a physician and repeated if judged necessary. Further action may be taken on the physician's request.

Subjects will be advised to notify their health care professionals (e.g., physician, dentist, and/or pharmacist) that they are participating in a clinical research study of a drug called synthetic glucagon for injection before taking any medicines or undergoing any medical procedure.

Data Integrity

The data collected during the study will be reviewed in a timely fashion by the PI or Sub-Investigator to ensure that the studies are performed as approved by the IRB and the study medication is well tolerated, and that plasma glucose concentration during the study meets the targets of the study. In the case that the levels of plasma glucose concentrations do not meet the targets, the PI will decide which measures are required to achieve that. The PI will convert the data in the sheet to electronic file and will be responsible for the data analysis. The PI will review the patient records with the nurse coordinator every month to ensure the safety of all study participants and that any encountered AE are within the expected frequency and severity. The Research Compliance Manager and/or UTHSCSA Compliance monitors may conduct periodic monitoring of the research protocol/procedure and participant records for compliance and adherence to the study protocol. The monitoring tool used by the Research Compliance Manager will indicate the date of the review.

Data will be discussed at the monthly Clinical Management Team with the PI or Sub-Investigator. Safety data will also be assed at the submission of annual progress reports to the IRB.

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