The Effect of Dipeptidyl Peptidase 4 Inhibition on Growth Hormone Secretion in Women with Polycystic Ovarian Syndrome

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The Effect of Dipeptidyl Peptidase 4 Inhibition on Growth Hormone Secretion in Women with Polycystic Ovarian Syndrome

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Table of Contents:

1. Background and Previous Human Studies

Obesity poses a significant health burden and is associated with an increased risk of heart disease. More than one-third of adults in the United States are considered obese and obesity-related conditions-including heart disease, stroke and type 2 diabetes mellitus-remain some of the leading causes of morbidity and mortality in this country. Obesity exacerbates cardio-metabolic risk by contributing to inflammation, glucose intolerance, impaired fibrinolysis and hypertension. In particular, visceral adiposity is a consistent and strong predictor of cardiovascular disease and insulin resistance while subcutaneous adiposity appears to exert a protective effect.(1)

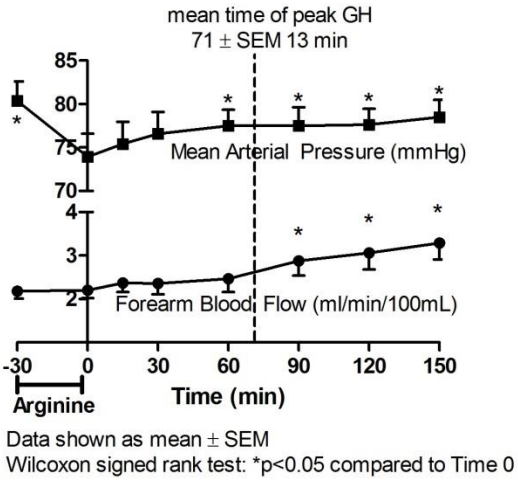
Growth hormone (GH) secretion is low in patients with obesity, insulin resistance, hyperlipidemia, the elderly, and those infected with HIV.(2) Hypothalamic GH releasing hormone (GHRH) is the primary regulator of pulsatile GH secretion from the pituitary gland.(3) Spontaneous and stimulated GH secretion is negatively associated with visceral adipose tissue.(4) This is due to a decreased response to GHRH stimulation and enhanced GH clearance as well as excessive somatostatin tone.(5) Compounds which inhibit hypothalamic somatostatin release improve, but do not normalize, GH secretion in response to GHRH. Similarly, drugs which lower free fatty acids improve GH secretion. A functional GH deficiency characterized by reduced pulsatile GH secretion appears to exist in obesity, and this is somewhat normalized after significant weight loss.(6)

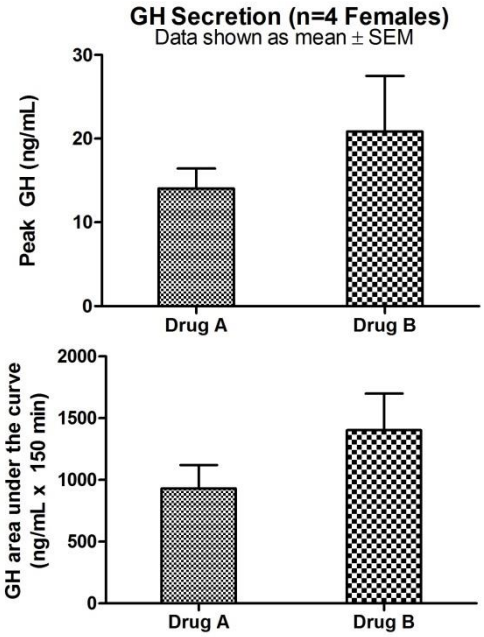
Individuals with hypopituitarism and untreated GH deficiency experience increased cardiovascular morbidity and increased prevalence of cardio-metabolic risk factors in particular visceral adiposity. Prospective studies in adults with hypopituitarism have demonstrated that GH replacement improves cardiac function as well as biochemical markers of cardiovascular and metabolic disease including fat distribution and inflammatory cytokines.(7-12) GH deficiency is associated with impaired endothelium-dependent vasodilation. Systemic and intra-arterial GH infusion increases forearm blood flow in healthy subjects, which is reversed by infusion of the nitric oxide synthase inhibitor, L-*N*-monomethylarginine (L-NMMA).(13;14) Despite these positive findings, exogenous GH therapy is not under physiologic negative feedback by IGF-1, does not restore pulsatile secretion, and is limited by the side effect of hyperglycemia.(15)

GH releasing hormone (GHRH) is a substrate of dipeptidyl peptidase 4 (DPP4) and thus inhibition of DPP4 may influence GH secretion. DPP4 is a ubiquitously expressed cell surface serine protease which cleaves a dipeptide containing a penultimate proline or alanine. *In vitro* DPP4 rapidly cleaves human GHRH(1-44)-NH_2 to the biologically inactive GHRH (3-44)-NH_2.(16;17) Conversion to GHRH (3-44)-NH_2 is 82% blocked *in vitro* by diprotin A, a DPP4 competitive inhibitor, or by amino acid substitution at either position 1 or 2.(18) *In vivo,* injection of exogenous GHRH(1-44)-NH_2 into healthy subjects produces GHRH (3-44)-NH_2 within 1 minute, demonstrating a half-life of GHRH (1-44) of only 6.7 minutes.(19) Others have exploited these findings to develop a DPP4-resistant GHRH analogue therapy that increases endogenous pulsatile GH secretion, specifically daily subcutaneous Tesamorelin which was approved in 2010 for the reduction of visceral adiposity in HIV-associated lipodystrophy.(20) Twelve months of Tesamorelin therapy in 60 obese subjects selectively reduced visceral adiposity and improved triglycerides, C-reactive protein and carotid intima-media thickness without affecting glucose homeostasis.(21) Two weeks of Tesamorelin given to 13 healthy males elicited a non-significant trend toward increased homeostasis model assessment insulin resistance index (HOMA-IR).(22) Tesamorelin is associated with a small increase in hemoglobin A1c in patients with HIV.(23) This side effect in high risk individuals is a drawback to Tesamorelin.

DPP4 inhibitors improve post-prandial hyperglycemia in patients with type 2 diabetes mellitus by decreasing the degradation of the incretin hormones, including glucagon like peptide-1 (GLP-1).(16;17) DPP4 inhibitor therapies, such as sitagliptin, are popular due to their once daily oral dosing, tolerability, and low incidence of hypoglycemia. Consistent with the ubiquitous expression of DPP4, other off-target effects of DPP4 inhibition are increasingly recognized. DPP4 is widely expressed in the cardiovascular system, including endothelial cells.(24) Sitagliptin therapy in patients with type 2 diabetes or the metabolic syndrome produces an anti-inflammatory effect, lowers post-prandial free fatty acids, and decreases platelet aggregation.(25-27) DPP4 inhibitor therapy has the potential to restore GH secretion by decreasing the degradation of the GHRH stimulus, while simultaneously addressing cardio-metabolic risk through a variety of mechanisms.

We are currently evaluating the effect of acute DPP4 inhibition on stimulated GH levels and forearm blood flow in 14 healthy adults. Our study biostatistician (CY) has thus far been un-blinded to the first 6 subjects (4 females, 2 males) who have completed this double-blinded, placebo-controlled, crossover study. On each study day, subjects are randomized to sitagliptin (200 mg p.o.) vs. matching placebo and receive a thirty minute intravenous infusion of Arginine 30 grams which stimulates GH secretion via a GHRH-dependent mechanism. GH level was positively associated with forearm blood flow (FBF) (r_s=0.442; p<0.001) and FBF significantly increased only after mean time of peak GH.*(left)* All subjects demonstrated sufficient responses to arginine (peak GH >3 ng/mL), with females demonstrating a higher peak GH (mean 17.5 ng/mL ± SEM 3.5 vs. 13.3 ± SEM 7.9 in males; p=NS) as has previously been reported.(28-30). Our preliminary data demonstrates a discrete effect of Drug A vs. Drug B on GH secretion which is consistent among 4 females, where the two drugs represent blinded sitagliptin vs. placebo.*(above)*





Obese females with polycystic ovary syndrome (PCOS) have increased visceral adiposity and diminished GH secretion-independent of weight or degree of insulin resistance**.** Females with PCOS exhibit an impaired GH response to stimuli including GHRH and a greater than 50% reduction in 24 hour mean GH levels secondary to diminished GH pulse amplitude.(31-34) Ten percent of reproductive-aged women are affected by PCOS; these women are often obese and typically at higher risk for the future development of cardiovascular disease and a ten-times higher risk for the onset of type 2 diabetes mellitus.(35) Similar to adults with GH deficiency, young women with PCOS demonstrate impaired glucose metabolism with a normal fasting blood glucose, increased visceral fat and altered endothelial function.(36) Management options are limited to lifestyle intervention with weight loss or the use of metformin. These therapies can be challenging to implement or poorly tolerated. Furthermore, metformin therapy is associated with a modest reduction in visceral adiposity and an undesirable loss of protective subcutaneous fat.(37)

In conclusion, patients with obesity and insulin resistance, including young females with PCOS, have impaired pulsatile GH secretion. Sitagliptin and other DPP4 inhibitor therapies are a currently approved and well-tolerated oral medication for patients with type 2 diabetes mellitus. DPP4 inhibition has the potential to restore physiologic GH secretion in at-risk populations through a variety of mechanisms that affect GHRH-stimulated GH secretion. GH replacement therapy improves visceral adiposity and markers of cardiovascular risk; its administration, however, is limited by undesirable metabolic effects. The DPP4-resistant GHRH analog, Tesamorelin, is limited by cost and adherence issues given its administration by daily subcutaneous injection, in addition to its lack of a beneficial effect on glucose homeostasis. We hypothesize that DPP4 inhibition is able to pharmacologically manipulate the GH axis and restore GH secretion in a more physiologic fashion while improving glucose homeostasis. The translational application of currently available DPP4 inhibitor therapies to patients with impaired GH secretion at high cardio-metabolic risk represents a cost-effective, high impact strategy which may rapidly be put into effect to address our nation's obesity epidemic.

2. Rationale and Specific Aims

Forty percent of women affected by PCOS develop glucose intolerance or diabetes by age 40 and diabetes in this population is associated with a BMI >30 kg/m^2. Women with PCOS are at increased risk for the development of impaired fibrinolysis and vascular function as well as cardiovascular disease.(36) Overnight and pharmacologically stimulated GH secretion is reduced in women with PCOS syndrome. (31;33;34) Flow-mediated vasodilation, and specifically nitric oxide-mediated vasodilation, is impaired in obese women with PCOS.(38;39) We hypothesize that chronic therapy with the DPP4 inhibitor, sitagliptin, will increase mean overnight GH secretion and improve endothelium-dependent vasodilation in obese women with PCOS.

We will secondarily evaluate the effect of chronic DPP4 inhibition on glucose tolerance, as well as glucose-suppressed GH and post-glucose rebound GH release. In healthy individuals, GH secretion initially declines to a nadir within 90 minutes of glucose ingestion and then increases above basal secretion, peaking 3-5 hours after glucose ingestion.(40-42) This "rebound" increase in GH secretion following oral glucose is mediated by GHRH.(2) In obese subjects, the early inhibitory effect on GH secretion is lost and the "rebound" response is blunted.(41) DPP4 inhibition lowers post-prandial blood glucoses and free fatty acids by decreasing degradation of the incretin hormones. Both free fatty acids and hyperglycemia negatively regulate GH secretion; DPP4 inhibition may thus indirectly enhance GH secretion by decreasing post-prandial hyperglycemia and free fatty acids.(2;25) We also hypothesize that chronic DPP4 inhibition will improve glucose tolerance, and restore early GH inhibition and "rebound" GH release after glucose ingestion.

3. Inclusion/Exclusion Criteria

Thirty-four obese (BMI ≥ 30 kg/m^2) females (18-40 years old) with PCOS will participate in this randomized, double-blind, placebo-controlled crossover study. The use of oral contraceptives or metformin will be discontinued at least 8 weeks prior. In females experiencing monthly cycles, the timing of the inpatient visit will be standardized and will take place during the early follicular phase.

Inclusion Criteria

- Females, age 18-45 years
- BMI ≥ 25 kg/m^2
- Diagnosis of polycystic ovary syndrome defined by 2003 Rotterdam criteria as meeting two out of the three below criteria :
    - Oligomenorrhea (menstrual cycles occurring at intervals >35 days, or only 4-9 cycles per year) or secondary amenorrhea (no cycle in 3 months if previously regular OR no cycle in 9 months if previous oligomenorrhea)
    - clinical or biochemical evidence of hyperandrogenism (hirsutism and/or documented upper normal or elevated serum testosterone)
    - documented history of polycystic ovaries on ultrasound examination
    - exclusion of other endocrine disorders must be documented in the medical record or obtained at the time of the screening visit (normal TSH, prolactin, 17 OH progesterone)

Exclusion Criteria

- Smoking
- Type 1 or Type 2 diabetes mellitus, as defined by a fasting glucose of 126 mg/dL or greater at the time of screening visit or the use of anti-diabetic medication (Metformin use is allowed, but must be presently at a stable dose and continued at the same dose throughout the study)
- Hypertension, as defined by an untreated seated SBP greater than 150 mmHg and/or an untreated DBP greater than 95 mmHg at the time of screening visit or the use of anti-hypertensive medication (with the exception of spironolactone, which may be discontinued 30 days prior to initiation of study drug)
- History of reported or recorded hypoglycemia (plasma glucose < 70 mg/dL)
- Pregnancy and/or breast-feeding (negative serum pregnancy test will be confirmed prior to the initiation of study medication and at each inpatient study visit.)
- Surgical menopause, defined as s/p total hysterectomy including bilateral salpingo-oophorectomy
- Use of oral contraceptive therapy. The use of these contraceptives must be discontinued at least 8 weeks prior to study initiation.
- Anemia defined as hematocrit <35% at screening visit
- Cardiovascular or cerebrovascular disease, including history of myocardial infarction, history of congestive heart failure, history of stroke, or abnormal ECG at screening visit
- Pulmonary hypertension
- Impaired renal function, defined as eGFR <60 mL/min/1.73M^2
- Impaired hepatic function (AST or ALT > 2 X upper limit of normal range)
- Treatment with an investigational drug in the 1 month preceding the study
- Regular NSAID use including, but not limited to, naproxen, ibuprofen, and aspirin
- Allergy to any of the medications used in this protocol
- Regular work of a night-shift or unusual schedule which may disrupt circadian rhythm.
- Personal or family history (defined as first-degree relative) of pancreatic cancer
- Personal history of pancreatitis or known pancreatic lesions
- Coagulopathy as defined by history
- Mental conditions rendering the subject unable to understand the nature, scope, and possible consequences of the study

- Inability to comply with the protocol, e.g., uncooperative attitude, inability to return for follow-up visits, and unlikelihood of completing the study
- Any underlying or acute disease requiring regular medication that could possibly pose a threat to the subject or make implementation of the protocol or interpretation of the study results difficult

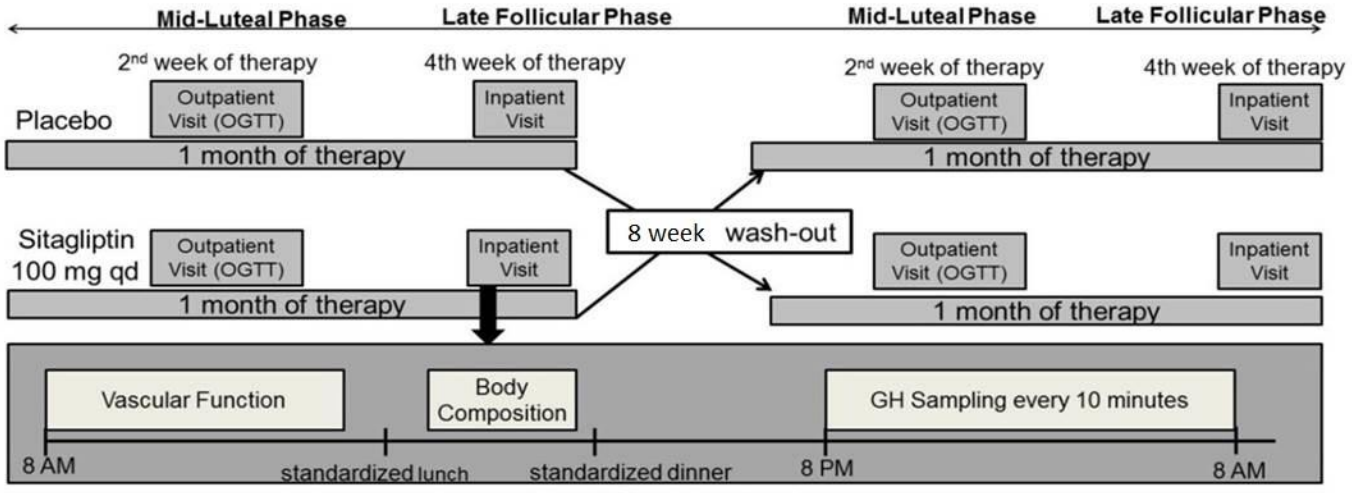
4. Enrollment/Randomization

Following their **initial screening visit**, subjects who qualify for the study will be randomized to treatment order (sitagliptin 100 mg daily or placebo) using a block randomization algorithm with a block size of two. The dose of sitagliptin was chosen as it is currently the FDA-recommended dose of sitagliptin for type 2 diabetic patients with unimpaired renal function. Visits will be standardized to the menstrual cycle when possible. Subjects will return to the CRC for a **medication-dispensing visit** which will consist of a pregnancy test and dispensing of double-blinded oral study medication (placebo or sitagliptin). They will take their first dose of study medication in the CRC. Subjects will take each therapy for one month; a minimum 8-week wash-out will separate study treatments. Side effects and compliance with study medication will be assessed at each visit in the CRC. Prior to initiation of the second drug, subjects will return to the CRC for an **interim screening visit** to update their medical history and have a blood count checked. If they still meet criteria for inclusion, they will then return to the CRC for a **medication-dispensing visit** which will again consist of a pregnancy test and dispensing of the double-blinded oral study medication.

5. Study Procedures

Protocol:

Each subject will undergo one **outpatient visit** (day 15 ± 4 days of treatment) and **one inpatient visit** (day 30 ± 4 days of treatment) during each treatment. *(above Figure)* On each study day, subjects will report fasting to the CRC in the morning having had no caffeine or alcohol for 24



Aim 2 Study Protocol

hours and having abstained from exercise that morning. They will have a baseline blood pressure and pulse obtained in triplicate. Height and weight will be obtained. On each study day, subjects will receive an intravenous catheter. Subjects will undergo an oral glucose tolerance test (OGTT) during the outpatient study visit, which will start one hour after their dose of study drug. During the inpatient

study visit, baseline venous samples will obtained 1 hour after the last dose of study drug. Vasodilation at baseline, during reactive hyperemia, and following nitroglycerin will be assessed, as detailed below, using high-resolution vascular ultrasound. Standardized meals will be provided at lunch and dinner. Body composition will be determined in the afternoon. At 8 PM overnight frequent sampling for venous GH will begin every 10 minutes for 12 hours to determine overnight GH secretion. Subjects will also complete a health-related quality-of-life questionnaire specific for women with PCOS (PCOSQ)(43;44) at the initiation of the study and at each inpatient visit; they will complete a CTSA Health Risk Assessment at the beginning of the study.

Assessments:

OGTT will be performed using 75 grams of glucose solution ingested within 10 minutes. Baseline venous blood samples will be obtained immediately prior to ingestion of oral glucose solution and 1 hour following ingestion of treatment drug that morning. Venous samples will be obtained at the following timepoints post ingestion: 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, and 270 minutes post-glucose load, given that the late rise in GH occurs three to five hours following glucose ingestion.(42)

Flow-mediated Dilation. Patients will be studied in a quiet, temperature-controlled room (22-23C). After 15 min of rest in a supine position, the right brachial artery will be imaged with a high ultrasound resolution machine for 1 minute. The artery will be scanned over a longitudinal section 3-5 cm above the elbow. The focus zone will be set to the depth of the anterior vessel wall. Depth and gain settings will be optimized to identify the lumen vessel wall interface. The diameter of the brachial artery on the dominant arm will be measured continuously at rest, during reactive hyperemia and after 15 minutes recovery period. A pneumatic tourniquet will be placed on the arm and will be inflated to a pressure 200 mm Hg for 5 minutes. Reactive hyperemia will be induced by sudden cuff deflation. The brachial artery will be continuously imaged for about 4 minutes after cuff release. After the recovery period, patients will receive 0.4 mg nitroglycerin by mouth (held if SBP<110 mmHg or pulse <60 bpm). The brachial artery will then be imaged again for an additional 4 minutes. Brachial artery diameter will be analyzed using continuous edge detection and wall tracking software (Brachial analyzer 5.0, Medical Imaging Applications LLC, Iowa city, Iowa). We will determine peak diameter using an automated mathematical algorithm, previously published (Black MA. et al. Hypertension.2008;51:203-210). FMD will be reported as absolute values at baseline, peak hyperemia, and peak following nitroglycerin. FMD will also be expressed as % change from baseline and will also be analyzed using allometric modelling (Atkinson G and AM Batterham Vascular Medicine. 2013; 18:354-365).

Body composition will be assessed by dual-energy x-ray absorptiometry (DXA) to measure body composition with the inclusion of a visceral fat measurement. Abdominal visceral fat negatively predicts GH secretion following glucose load. (40) GH replacement decreases abdominal fat in GH deficient adults. (46)

Overnight spontaneous GH secretion will be assessed using deconvolution analysis from venous samples obtained from an indwelling catheter overnight. AutoDecon, a deconvolution algorithm validated for analysis of endogenous GH pulsatility, will be used to assess parameters of GH secretion including mean overnight GH secretion, GH half-life, GH peak and nadir, number of GH peaks, and GH peak area.(47-49) Intravenous catheter patency will be maintained during sampling by infusion of intravenous fluids via infusion pump at 20 mL/hour.

6. Risks

1. Repeated blood sampling from venous catheters may cause bleeding or infection.
2. Frequent blood draws can cause anemia.
3. Insertion of catheters may cause bleeding, bruising, or infection.
4. Stopping metformin or oral contraceptives may increase symptoms of high testosterone, including acne, hirsutism, and may make menstrual cycles more irregular.
5. Stopping spironolactone may increase symptoms of acne and hirsutism, and cause an increase in blood pressure and fluid retention.
6. Sitagliptin - Drugs like sitagliptin can cause lowered blood sugar (common) in individuals with high blood sugar (diabetes). This can result in dizziness, nausea, shaking, sweating, fast heartbeat, vision changes, headache, anxiety, tiredness, or confusion. It can rarely cause fainting, seizures, or coma. Other side effects are pancreatitis (damage to the pancreas), kidney damage (which can be life threatening), allergic reaction (which can be life threatening), rash, and liver damage (which can be life threatening).
7. Oral Glucose (75 grams) Solution-The oral glucose solution is a commercially available liquid containing a certain amount of glucose, 75 grams in this case. This solution will be swallowed during the outpatient study visit, after the subject has abstained from eating and drinking anything but water for at least 8 hours. Side effects may include nausea, sweatiness, light-headedness, short of breath or faint after drinking the glucose solution. Symptoms are generally limited to a few hours after the test and resolve with food intake.
8. Nitroglycerin - Nitroglycerin may lower blood pressure, increase or decrease heart rate, or cause the patient to feel dizzy, faint, lightheaded, weak, hot, nauseated or have a headache. If the blood pressure or pulse is too low, the study physician may not perform this part of the study.

7. Reporting of Adverse Events or Unanticipated Problems Involving Risk to Participants or Others

All adverse events and instances of non-compliance with the protocol, subject withdrawals, and subject complaints will be formally summarized and reviewed by the Data and Safety Monitoring Board (DSMB) bi-annually. The DSMB will receive these in written format and review the study for appropriate and timely subject accrual, adherence to the protocol, and data accuracy and completeness. The DSMB will provide the PI (Dr. Devin) with written confirmation of receipt of this bi-

annual summary as well as a recommendation with accompanying rationale for study termination or continuation. This written confirmation will be provided to the IRB and include results of the review and concerns, if any, regarding subject safety or study drug tolerability. The DSMB will ensure that all adverse events reporting to the IRB is in accordance with current policies. The members of the DSMB will be available for discussion of any questions regarding protocol adherence, consent issues, or adverse events. The DSMB will review final results of the study.

The DSMB is comprised of [redacted] is a clinical endocrinologist and translational researcher who has experience with the use of sitagliptin in both the clinical and research setting. [redacted] is a translational researcher who has experience with the administration of vasoactive substances. [redacted] is a clinical endocrinologist who has extensive experience in the management of patients with polycystic ovarian syndrome. [redacted] will serve as chair of the DSMB.

All protocols will be reviewed and approved by the [redacted] before any subject is enrolled. Dr. Devin, her mentor and her co-investigators will closely oversee the protocol. Any serious adverse events or toxicities will be reported to the [redacted] as per policy (currently no later than within 10 working days of the investigator's knowledge) and will immediately be reviewed with the DSMB. Any noncompliance with the IRB-approved protocol that increases risk or affects participants' rights, safety or welfare will be similarly reported to the IRB within 10 working days of the investigators' knowledge and immediately reviewed by the DSMB.

Any untoward medical event will be classified as an adverse event, regardless of its causal relationship with the study. An adverse event will be classified as serious if it a) results in death, b) if life-threatening, c) requires inpatient hospitalization or prolongation of existing hospitalization, d) results in persistent or significant disability or incapacity, e) is a congenital anomaly or birth defect. Non-serious adverse events will be reported to the IRB at the time of continuing review, as is currently IRB policy.

Dr. Devin will review enrollment progress, all adverse events, protocol adherence data, and data quality entry at a minimum of every two weeks with her mentor. Dr. Devin will review with her Mentor each enrolled subject's chart every two weeks for side effects and tolerability of the investigational drug. These findings will be included in the DSMB bi-annual report unless previously dictated otherwise.

8. Study Withdrawal/Discontinuation

If at any time during the study, a subject develops any symptoms related to study participation that subject will be withdrawn from the study. If, in the opinion of the investigator, a subject is non-compliant, that subject will be withdrawn from the study. Subjects who are withdrawn will be followed until symptoms have resolved.

9. Statistical Considerations

Anticipated Results and Data Analysis Plan

We anticipate that chronic DPP4 inhibition will increase mean overnight GH secretion, enhance endothelium-dependent vasodilation, and normalize post-prandial GH secretion. The primary endpoints are mean overnight GH secretion and GH levels measured at various time points during oral GTT. Secondary endpoint of FMD will be measured at baseline, during reactive hyperemia, and following nitroglycerin. FMD will also be expressed as % change from baseline and will also be analyzed using allometric modelling (Atkinson G and AM Batterham Vascular Medicine. 2013; 18:354-365). Paired t-tests will be used to analyze the data obtained during placebo with sitagliptin. Mixed-effect models will be used to analyze the data, with the fixed effect being sitagliptin vs. placebo for endpoints such as mean overnight GH. For the post-glucose load GH secretion assessed after each of the two treatment periods (sitagliptin vs. placebo), time as a fixed effect and time by treatment interaction will be added into the mixed effect model. This will capture the time trend and how sitagliptin impacts the time trend. For each endpoint, a pre-specified set of covariates that would impact the endpoint will be adjusted in the model.

Sample Size and Power Calculation

Prior data indicates that a difference in mean overnight GH of the following is statistically significant ($p<0.005$): mean baseline overnight GH 0.7 ± SEM 0.2 vs mean post-therapy overnight GH 1.2 ± SEM 0.3 µg/L in 13 subjects.(22) Conservatively assuming a correlation coefficient of 0.6 for the two measures on the same subject, the SD for the within subject difference of GH would be 0.87. Assuming sitagliptin will increase GH from 0.7 (placebo) to 1.2 µg/L, a sample size of 26, 30, and 34 completed subjects would have 80, 85, or 90% power, respectively, to detect this clinically meaningful difference. Sample size was calculated with PS Software using the design for a paired t-test.(54) Selecting 85% power and assuming a 10% drop-out based upon previous experience with studies of this nature, we will enroll 34 patients.

10. Privacy/Confidentiality Issues

Clinical data, including clinical laboratory, will be entered by a member of the Key Study Personnel in a protected source database (RedCap). A unique identification case number will be used to protect the confidentiality of the study participants. The case numbers and participants' names will be included in the protected source database, accessible only by Key Study Personnel, but only case numbers will be included in any spreadsheet used for the statistical analysis.

11. Follow-up and Record Retention

The total duration of enrollment will be 2 years, and we anticipate completion of a manuscript within 3 years of initiation. All records will be retained for 7 years following publication of the data. After that time, records may be archived for an additional 5 years and then shredded.

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