# Comparative Effects of Antidiabetic Medications on Postprandial Hyperlipidemia, Free Fatty Acid Signaling, and Endothelial Dysfunction in Individuals with Prediabetes

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**Protocol Title:** Comparative Effects of Antidiabetic Medications on Postprandial Hyperlipidemia, Free Fatty Acid Signaling, and Endothelial Dysfunction in Individuals with Prediabetes

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**Population:** This study will require 40 obese prediabetic (otherwise generally healthy) subjects, males and female, aged 30-70 years old, from the Houston, TX area.

**Number of Sites:** Single site

**Study Duration:** 3 years

**Subject Duration:** 4 months (not including optional extension study). Optional extension study will take 6 additional weeks, and may be completed within one year of finishing required study procedures.

## General Information

It is a paradox that medical efforts to control blood glucose in type 2 diabetes mellitus have not decreased the risk of cardiovascular disease. Postprandial lipid concentrations are a strong predictor of cardiovascular risk, independent of traditional cardiovascular risk factors. The new classes of antidiabetic medications - GLP-1 agonists and DPP-IV inhibitors - affect lipid as well as glucose metabolism. This study will investigate the efficacy of these medications in reducing postprandial hyperlipidemia, disrupting the concurrent proinflammatory free fatty acid signaling, and ameliorating endothelial dysfunction in individuals with prediabetes. This will consist of a single center, randomized, crossover, placebo-controlled double-blinded prospective trial involving three study arms representing the aforementioned medications: exenatide (GLP-1 agonist), saxagliptin (DPP-IV inhibitor), and placebo (control arm). For each study arm, subjects will eat a standardized atherogenic high-fat test lunch. Venous blood draws and measurements of forearm blood flow will be done prior to the meal and periodically during a 6-hour period after the meal. Forearm blood flow measurements will assess for changes in endothelial function. The blood will be analyzed for multiple markers of hyperlipidemia and free fatty acid signaling. The results will provide new insights into the anti-inflammatory effects of multiple antidiabetic medications via the mechanisms of postprandial hyperlipidemia, free fatty acid signaling, and endothelial function in prediabetic individuals. At the request of the sponsor (CCTS) and due to updates in the medical literature, pioglitazone is no longer being studied. The pioglitazone study arm has been removed. Also at the request of the sponsor (CCTS), we have added an optional, nonrandomized extension study to address the issue of exenatide efficacy during longer treatment duration.

## Background Information

Coronary heart disease accounts for more than two-thirds of all deaths in patients with type 2 diabetes mellitus.[1] In spite of aggressive efforts to improve blood glucose control, these measures generally yield little to no positive impact on cardiovascular outcomes.[2-4] Recent large epidemiological studies suggest that postprandial lipid concentrations are a strong predictor of cardiovascular risk, independent of traditional cardiovascular risk factors.[5-8] As some of the newer antidiabetic medications affect lipid as well as glucose metabolism, this research will focus on the effects of these medications on the treatment of postprandial hyperlipidemia.

Exenatide is a glucagon-like receptor agonist (GLP-1 agonist) which lowers postprandial glucose and positively modifies several cardiovascular risk factors when given long-term. Recently, it was shown that a single dose of exenatide reduced postprandial elevations in lipids, lipoproteins, and free fatty acids in both impaired glucose tolerance and early type 2 diabetes mellitus.[9] Moreover, exenatide improved postprandial endothelial function.[10] Exenatide treatment also has a potent effect on free fatty acid signaling.[11]

Saxagliptin is a dipeptidyl peptidase-IV (DPP-IV) inhibitor which prevents the inactivation of native GLP-1 and glucose-dependent insulinotropic peptide (GIP). Saxagliptin also has potent effects on free fatty acid signaling.[12] There are no studies showing whether a single dose of saxagliptin would reduce postprandial elevations in lipids, lipoproteins, free fatty acids, and endothelial dysfunction in a manner similar to exenatide.

While these medications are traditionally used to lower blood glucose in diabetic individuals, their additional effects in altering postprandial hyperlipidemia will provide new insights in the mechanisms of atherogenesis. Though glycemic control might have little impact on cardiovascular outcomes,[2-4] these therapies potentially show new promise in the future treatment of coronary heart disease.

The following Specific Aims will test the hypotheses that exenatide and saxagliptin have potent anti-inflammatory postprandial effects as evidenced by modulation of free fatty acid signaling, the reduction of hyperlipidemia, and amelioration of endothelial dysfunction in prediabetic subjects. At the request of sponsor and due to updates in the medical literature, pioglitazone is no longer being studied. The pioglitazone study arm and relevant specific aim have been removed.

**Objectives**

**Specific Aim #1: Determine How Incretin-Based Therapies Modulate the Mechanisms of Postprandial Hyperlipidemia in the Prediabetic State**

**Hypothesis:** A single dose of exenatide or saxagliptin will reduce serum free fatty acid (FFA) levels and subsequently downregulate FFA-induced cellular proinflammatory signaling.

**Hypothesis:** A single dose of exenatide or saxagliptin will attenuate postprandial excursions of proatherogenic lipids and lipoproteins.

**Hypothesis:** Six weeks of treatment with long-acting exenatide will further reduce serum free fatty acid (FFA) levels, downregulate FFA-induced cellular proinflammatory signaling, and attenuate postprandial excursions of proatherogenic lipids and lipoproteins.

**Specific Aim #2:** Identify the Effect of the Above Therapies on the Amelioration of Endothelial Dysfunction in Postprandial Hyperlipidemia

**Hypothesis:** Changes in endothelial dysfunction (measured by forearm blood blow) will correlate with changes in triglycerides and free fatty acids in the postprandial state.

**Study Design**

Potential study subjects in UT clinics will be identified via All Scripts. A study coordinator or investigator will go out to clinics with high volumes of potential subjects and inform patients of the study, given the approval of the clinic's attending physician. Furthermore, we will use CPHS-approved newspaper ads and flyers as means to recruit patients outside of the UT clinics. All interested patients (both in UT clinics and outside of UT clinics) will be given a phone number to call for the purpose of discussing study participation further.

Interested potential subjects will be prescreened for the above criteria via a telephone interview. If the subject meets criteria via telephone interview, he or she will be invited to participate in an hour-long outpatient screening visit. At this time, subjects will be consented for participation in the study. We will perform a complete history and physical exam. We will also draw the following baseline fasting labs (npo since midnight, except for water and chronic medications): complete metabolic panel, lipid panel, hemoglobin A1C, complete blood count, and urine pregnancy test (if applicable). Subjects will receive monetary compensation for this screening visit ($40) Results will be reviewed. If the subject meets the inclusion and exclusion criteria, then he or she will be invited to participate in the trial.

This will be a single center, randomized, crossover, placebo-controlled double-blinded prospective trial. Each subject will participate in three separate, daylong outpatient studies at the Clinical Research Unit (CRU). Each study will represent one of the three arms of the trial: Exenatide 10 mcg subcutaneous injection, saxagliptin 5 mg oral tablet, or placebo. The order of the studies will be randomized in a crossover design, and each study will take place at least ≥ 10 days apart to ensure sufficient washout of each medication. The three studies, however, must all be completed in period of no longer than four months. Subjects will receive monetary compensation for each study visit ($95 each).

Each study will begin with the patient presenting to Clinical Research Unit (CRU) at approximately 0800 after an overnight fast. Peripheral venous access will be obtained in a stable vein in an upper extremity. A blood pressure cuff and a strain gauge will be placed at the widest part of the opposite forearm (to measure forearm blood flow via venous occlusion plethysmography). Baseline blood draws (described below) and forearm venous occlusion plethysmography will be performed at 1045 (± 10 minutes). At this time, the study medication (exenatide, saxagliptin, or placebo) will be given. At 1100 (± 10 minutes), the participant will be fed an atherogenic high-fat test lunch equivalent to a McDonald's® Big Mac meal. The standardized meal will be prepared by the Memorial Hermann TMC cafeteria.

Peripheral venous blood will be drawn for analysis before the meal and every 2 hours (± 10 minutes for each blood draw) during the 6 hours after eating the meal (see below). Forearm venous occlusion plethysmography will be measured every 3 hours (± 10 minutes for each measurement) during the 6 hours after eating the meal.

After completing the three study visits, subjects will be invited to participate in an optional, nonrandomized extension study. For the extension study, subjects will take exenatide ER (extended-release exenatide) weekly for total of six weeks. This will start with a brief CRU visit, where subjects will be provided with exenatide ER (brand name "Bydureon") pens and necessary education on dosing and administration. Vital signs will be taken. A urine pregnancy test will be done if applicable. Subjects with prior history of pancreatitis, medullary thyroid cancer, or multiple endocrine neoplasia type 2 (MEN 2) will be excluded from this extension study. Subjects will receive monetary compensation for this screening visit ($25)

Approximately one week after completing the sixth and final dose of exenatide ER, the patient will be brought back to CRU after an overnight fast at approximately 0900. Similar to prior visits, peripheral venous access will be obtained in a stable vein in an upper extremity. A blood pressure cuff and a strain gauge will be placed at the widest part of the opposite forearm (to measure forearm blood flow via venous occlusion plethysmography). Baseline blood draws (described below) and forearm venous occlusion plethysmography will be performed at 1045 (± 10 minutes). No study medication will be given, as the exenatide ER has a long half-life (2 weeks). At 1100 (± 10 minutes), the participant will be fed an atherogenic high-fat test lunch equivalent to a McDonald's® Big Mac meal. The standardized meal will be prepared by the Memorial Hermann TMC cafeteria. Peripheral venous blood will be drawn for analysis before the meal and at the 2 hour timepoint (± 10 minutes for each blood draw). Forearm venous occlusion plethysmography will be measured before the meal and at 3 hours (± 10 minutes for each measurement). After all measurements are completed (at 3 hours after the meal), the extension study is completed. Subjects will receive monetary compensation for this visit ($75).

The research aims will be addressed as follows:

**Specific Aim #1A: The effect of a single dose of exenatide or saxagliptin on serum free fatty acid (FFA) levels and FFA-induced cellular proinflammatory signaling.** On the exenatide and saxagliptin studies, plasma levels of free fatty acids, high-sensitivity C-reactive protein, adiponectin, insulin, and glucose will be drawn at baseline and at every two hours (± 10 minutes) for up to 6 hours after ingestion of the meal.

Blood will also be drawn at baseline and every 2 hours (± 10 minutes) after ingestion of meal for monocyte isolation. This blood will be processed by the CRU and in my lab at UTHSC. The processed samples will be analyzed by Quest Laboratories, my laboratory at UTHSC, and Dr. Taegtmeyer's laboratory at UTHSC. All samples not analyzed by Quest will be stored in a -80°C freezer for later analyses described in Study Procedures. Also, a drop of blood will be analyzed at baseline and 2 hours for blood cells counts and oxygen levels via a co-oximeter. The results will be analyzed by my laboratory and Dr. Qingchun Tong's laboratory.

**Specific Aim #1B: The effect of a single dose of exenatide or saxagliptin on postprandial excursions of proatherogenic lipids and lipoproteins**. On the exenatide and saxagliptin studies, plasma levels of triglycerides (TG), total cholesterol, apolipoprotein B-48, apolipoprotein C-III, remnant lipoprotein TG, and remnant lipoprotein cholesterol will be drawn at baseline and then at every two hours after ingestion of the meal. This blood will be processed at the CRU. Some of the blood will be analyzed by Quest Laboratories. The rest of the blood will be stored in a -80°C freezer; it will later be analyzed by my laboratory at UTHSC and Dr. Taegtmeyer's laboratory at UTHSC.

**Specific Aim #2: The Effect of the Above Therapies on the Amelioration of Endothelial Dysfunction in Postprandial Hyperlipidemia.** On all studies, endothelial dysfunction will be assessed via non-invasive venous occlusion strain gauge plethysmography (for the measurement of forearm blood flow), per the method described by Skilton *et al.*[13] These measurements will be done at baseline and every three hours after the meal.

All above procedures will be repeated in the placebo study.

The entire trial will be completed in about three years. 40 subjects will need to complete the study (see Data Analysis Plan). To accomplish this, about 50 subjects will be consented. A 20% drop out rate may be expected for personal reasons alone.

**Efficacy Assessment:**

The primary efficacy objective is to determine whether a single dose of exenatide or saxagliptin compared with placebo will reduce levels of serum free fatty acids and disrupt FFA-induced proinflammatory signaling in monocytes during the postprandial period in patient with prediabetes.

The secondary efficacy objective is to determine whether a single dose of exenatide or saxagliptin compared with placebo will reduce levels of serum triglycerides during the postprandial period in patient with prediabetes.

Other efficacy objectives are (1) to determine whether a single dose of exenatide or saxagliptin compared with placebo will reduce lipoprotein levels, and improve endothelial function during the postprandial period in patient with prediabetes, and (2) to determine if six weeks of exenatide ER treatment reduce levels of serum free fatty acids, disrupt FFA-induced proinflammatory signaling in monocytes, and will reduce levels of serum triglycerides during the postprandial period in patient with prediabetes.

**Safety Assessment:**

Study drug toxicities will be assessed throughout the study. Adverse events will be evaluated from the first dose of study medication while the patient is in the study, until 30 days after the last dose of study medication. Patients will be followed until all treatment-related adverse events have recovered to baseline or are deemed irreversible by the principal investigator. Patients will be monitored for the following adverse reactions related to each study drug:

Exenatide (single dose): nausea, vomiting, hypoglycemia, diarrhea, and hypersensitivity-related reactions including skin rash (rare). Other risks listed in package insert would relate only to long-term use of this medication and are not expected for this study.

Saxagliptin (single dose): nausea, vomiting, hypoglycemia, headache, minor upper respiratory infections, and hypersensitivity-related reactions including skin rash (rare). Other risks listed in package insert would relate only to long-term use of this medication and are not expected for this study.

Exenatide ER (Six doses): nausea, vomiting, hypoglycemia, diarrhea, hypersensitivity-related reactions including skin rash (rare). A rare adverse event related to prolonged use of exenatide is pancreatitis.

**Study Population**

Inclusion Criteria:

1. Men and women, ages 30 to 70 years of age inclusive
2. Diagnosis of Prediabetes - defined as either impaired fasting glucose (fasting glucose of 100-125 mg/dL), impaired glucose tolerance (2-hour postprandial blood glucose of 140-199 mg/dL after 75 gram oral glucose challenge), and/or a hemoglobin A1C ranging from 5.7% to 6.4%
3. Subjects are allowed, but not required, to be on statins, ACE-inhibitors, beta-blockers, angiotensin-receptor blockers, thiazide diuretics, and/or loop diuretics at doses that have been stable for at least the last 3 months

4. BMI between 30-35 kg/m2 (±1 kg/m2)
5. Body weight has been stable (±4-5 pounds) over the prior three months.
6. Women of childbearing age must agree to use an acceptable method of pregnancy prevention (barrier methods, abstinence, or surgical sterilization) for the duration of the study
7. Patients must have the following laboratory values: Hematocrit ≥ 34 vol% S. creatinine < 1.5 mg/dl in men and 1.4 mg/dl in women AST (SGOT) < 2.5 times ULN, ALT (SGPT) < 2.5 times ULN, alkaline phosphatase< 2.5 times ULN

Exclusion Criteria:

1. History of Type 1 or Type 2 diabetes mellitus
2. History of diabetic ketoacidosis or hyperosmolar nonketotic coma
3. Pregnant or breastfeeding women
4. Patients must not be receiving lipid-lowering medications other than statins within the last 3 months
5. Patient must not be receiving metformin, DPP-IV inhibitors, GLP-1 agonists, thiazolidinediones, insulin, sulfonylureas, acarbose, SGLT-2 inhibitors, corticosteroids, or immunosuppressive therapy within the last 3 months and cannot take them for the duration of the study. Patient must not be receiving NSAIDS or antioxidant vitamins within the last 1 week, and cannot take them for the duration of the study.
6. Patients must not be on hormone replacement therapy.
7. Patients with diabetic gastroparesis
8. Patients with current tobacco use
9. Patients with active malignancy
10. Patients with history of urinary bladder cancer
11. Patients with dietary restrictions precluding a high-fat meal
12. Patients with a history of clinically significant heart disease (NYHA III or IV; more than non-specific ST-T wave changes on the EKG), peripheral vascular disease (history of claudication), or pulmonary disease (dyspnea on exertion of one flight or less; abnormal breath sounds on auscultation) will not be studied
13. Subjects with a history of any serious hypersensitivity reaction to the study medications
14. Prisoners or subjects who are involuntarily incarcerated
15. Subjects who are compulsorily detained for treatment of either a psychiatric or physical (e.g., infectious disease) illness
16. Subjects with known allergic reactions to the study medications or test meal
17. Subjects unwilling or unable to provide informed consent
18. Subjects determined by the investigator(s) to not be appropriate candidates for the study
19. Subjects with prior history of pancreatitis, medullary thyroid cancer, or multiple endocrine neoplasia type 2 (MEN 2) will be excluded from the extension study only.

For Recruitment

- Allscripts will be scanned for subjects meeting entry criteria. These subjects will then be contacted during their next clinic visit, with the approval of their physicians. The encounter is strictly voluntary on the patient's behalf.
- CPHS approved newspaper advertisements will be used
- CPHS approved flyers will be posted at UT Health Sciences Center



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## Study Procedures

**Table 1: Time and Event Schedule for Required Protocol Procedures**

| Procedure | Visit 1 (Screening) | Visit 2 | Visit 3 | Visit 4 |
|---|---|---|---|---|
| Obtain Informed Consent | X | | | |
| Confirm Eligibility | X | | | |
| Medical History | X | X | X | X |
| Concomitant Medications | X | X | X | X |
| Physical Examination | X | X | X | X |
| Vital Signs | X | X | X | X |
| EKG | X | | | |
| Pregnancy Test (if applicable) | X | X | X | X |
| CMP – including FPG, LFTs, serum creatinine | X | | | |
| Lipid panel (including serum TG and total cholesterol) | X | X | X | X |
| HbA1C | X | | | |
| CBC | X | | | |
| FFA | | X | X | X |
| hsCRP | | X | X | X |
| Adiponectin | | X | X | X |
| Glucose, Insulin | | X | X | X |
| Monocyte isolation (for NfKB, TLR2, TLR4) | | X | X | X |
| Apolipoproteins B-48, C-III | | X | X | X |
| Remnant lipoproteins (TG, cholesterol) | | X | X | X |
| Blood cell counts, oxygen levels | | X | X | X |
| Venous Occlusion Plethysmography | | X | X | X |
| Assess for Adverse Events | | X | X | X |
| Administration of study drug/placebo | | X | X | X |

There are 4 total required visits which will involve the procedures listed above. There are two optional visits (5 and 6) which are discussed later. Visit 1 (screening visit) will take one hour. Visits 2, 3, and 4 are the study visits. Each study visit (Visits 2, 3, and 4) will begin at about 0800 and end at about 1700. Each study visit will take place ≥ 10 days apart to ensure sufficient washout of each study medication. The three visits, however, must all be completed in period of no longer than four months. Weight will be measured at each study visit.

In visit 1, a total of 20 cc of blood will be drawn for screening labs. The blood drawn at these visits will be analyzed by Quest Laboratories.

In visits 2 through 4, there will be four blood draws per visit. The first two blood draws require 40 cc of of blood each and the last two blood draws require 20 cc of blood. Hence, for each study visit (Visit 2-4) there will be 120 cc of blood will be drawn. About 20 cc of urine will be collected for urine pregnancy test in women of childbearing potential, at every visit (Visits 1-4). For these visits, the free fatty acids (FFA), hsCRP, insulin, glucose, and urine pregnancy will be analyzed by Quest Laboratories. The lipid panels after the approval of this protocol revision will also be analyzed by Quest Laboratories. The lipid panels (prior to the approval of this protocol revision), adiponectin, apolipoproteins B48 and C-III, remnant lipoproteins, and monocytes (NfKB), TLR2, and TLR4) will be analyzed by both Dr. Heinrich Taegtmeyer's laboratory at UTHSC and my laboratory at UTHSC. Blood cell counts and oxygen levels will be analyzed by my laboratory and Dr. Qingchun Tong's laboratory. These changes were made due to the heavy time burden and costs in analyzing these labs.\

We will gather the information listed in the above table.

Visit 5 is the first optional visit for subjects who choose to participate in the extension study. This consists of a brief CRU visit, where subjects will be provided with exenatide ER (brand name "Bydureon") pens and necessary education on dosing and administration. Vital signs will be taken. About 20 cc of urine will be collected for urine pregnancy test in women of childbearing potential. Subjects with prior history of pancreatitis, medullary thyroid cancer, or multiple endocrine neoplasia type 2 (MEN 2) will not participate in this this extension study.

Visit 6 is the second optional visit for subjects who choose to participate in the extension study. This visit will begin at about 0900 and end at about 1300. There will be two blood draws, each requiring 40 cc of blood. About 20 cc of urine will be collected for urine pregnancy test in women of childbearing potential. For this visit, the free fatty acids (FFA), hsCRP, insulin, glucose, and urine pregnancy will be analyzed by Quest Laboratories. The lipid panels after the approval of this protocol revision will also be analyzed by Quest Laboratories. The lipid panels (prior to the approval of this protocol revision), adiponectin, apolipoproteins B48 and C-III, remnant lipoproteins, and monocytes (NfKB), TLR2, and TLR4) will be analyzed by both Dr. Heinrich Taegtmeyer's laboratory at UTHSC and my laboratory at UTHSC. Blood cell counts and oxygen levels will be analyzed by my laboratory and Dr. Qingchun Tong's laboratory.

All data will be recorded in file folders and binders, which will be stored in the Clinical Research Unit in locked, secure cabinets which will only be accessible to the PI and his team.

Plasma free fatty acid (FFA) concentration will be determined by an enzymatic calorimetric quantification method. High-sensitivity CRP (hsCRP) will be determined by an immunoluminometric method. Plasma adiponectin concentration will be measured by ELISA. Plasma insulin is measured by radioimmunoassay. Plasma glucose is measured by an automated glucose analyzer. Plasma triglycerides and cholesterol are determined using a colorimetric method. Serum concentration of apolipoprotein B-48 (ApoB48) will be measured by ELISA. Plasma apolipoprotein C-III (ApoCIII) will be measured on an automated colorimetric immunoassay. Remnant –like particles of cholesterol and triglyceride will be isolated from plasma using an immunoaffinity assay, then measured using aforementioned methods for plasma cholesterol and triglycerides. Blood cell counts and oxygen levels will be analyzed via co-oximetry.

For mononuclear cells (monocytes), there is a specific isolation technique. Blood samples will be collected in EDTA tubes and layered on a density gradient cell separation medium. Samples will centrifuged serially to yield a pellet of mononuclear cells. For Western blotting, mononuclear cell lysates will be prepared, electrophoresed, and immunoblotted. Mononuclear antibodies against NfKb (p65), TLR2, TLR4 and actin will be used and all values will be corrected for loading to actin.

Venous occlusion plethysmography will be performed per the method described by Skilton *et al.*[13]

Serum and plasma specimens will be stored in a -80 degrees C freezer for no more than 6 years. The samples will be stored while waiting processing. Any unused sample may be used for future studies if the subject consents to this. The freezer will be locked an accessible only to the PI and his team.

**Data and Safety**
**Monitoring Adverse Event**

All adverse events, including those that are serious, will be graded by the investigator as follows:

- Mild (Grade 1): awareness of event but easily tolerated
- Moderate (Grade 2): discomfort enough to cause some interference with usual activity
- Severe (Grade 3): inability to carry out usual activity
- Very Severe (Grade 4): debilitating; significantly incapacitates subject despite symptomatic therapy.
-

The following categories and definitions of causal relationship to a study medication as determined by a physician should be used:

- Related: There is a reasonable causal relationship to study medication administration and the adverse event.
- Not Related: There is not a reasonable causal relationship to study medication administration and the adverse event.

The expression “reasonable causal relationship” is meant to convey in general that there are facts (e.g., evidence such as de challenge/re challenge) or other arguments to suggest a positive causal relationship.

**Collection and Reporting**

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. To prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the event was administered, it should be recorded in the medical record. The investigator must supply the IRB with any additional information requested, notably for reported deaths of subjects.

**Serious Adverse Events**

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur during the screening period and within 30 days of discontinuation of dosing. If applicable, SAEs must be collected that relate to any later protocol-specified procedure. The investigator should report any SAE occurring after these time periods that is believed to be related to study drug or protocol-specified procedure.

An SAE report should be completed for any event where doubt exists regarding its status of seriousness. If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

All SAEs, whether related or unrelated to study medications, and all pregnancies must be reported to the University of Texas (UT) Health Sciences Center at Houston IRB within 24 hours of study personnel becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.
All SAEs should be reported to the UT Health Sciences Center at Houston IRB.

**Safety Monitoring**

The PI and his team will monitor the study at each visit. All treatment-emergent AEs will be recorded on source documents (i.e. original documents, data, and records). AEs include those reported spontaneously by the subject and those noted incidentally or as observed by the investigator or study personnel. All clinically significant abnormalities noted upon physical examination, or other diagnostic test results should be reported as an AE, except for baseline measurements that may be considered part of the medical history. In addition, all clinically significant AEs that continue at Study Termination will be followed up by the investigator and evaluated with additional tests if necessary, until the underlying cause is diagnosed or resolution occurs. All AEs will be evaluated for intensity and causal relationship with use of the study medication and/or study procedures by the investigator and reported to the UT Health Sciences Center at Houston IRB. All SAEs will be reported to the UT Health Sciences Center at Houston IRB and the sponsor within 24 hours. In addition, a safety report will be submitted to the IRB annually. Any new information regarding exenatide or saxagliptin will be submitted the IRB.

**Pregnancy**

Women of childbearing potential (WOCBP) must use an acceptable method of birth control (as described above) during the course of the study, in such a manner that the risk of failure is minimized.

All WOCBP MUST have a negative urine pregnancy test before receiving any study medications. A urine pregnancy test will be done at the beginning of each study day. If the urine pregnancy test is positive, the subject must not receive the study medication or any study procedures that day. The subject must see her primary care physician or OB/GYN physician for further evaluation. If pregnancy is confirmed by this medical provider, the subject will be discontinued from the study. If the medical provider determines that the subject is not pregnant, the subject is allowed to resume the study on a later date.

In addition, all WOCBP must be instructed to contact the investigator and/or other study personnel immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

If, following initiation of the study medication, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of study medication exposure, including at least 6 half-lives after medication administration, the subject will be discontinued from the study. The subject must establish or continue care with a qualified primary care or OB/GYN physician. A single dose of any of the study medications is not expected to have a significant impact on a pregnancy, but the pregnancy should be monitored by the above medical provider.

The investigator must immediately notify the UTHSC CPHS in accordance with CHPS reporting procedures.

**Statistics**

For univariate analysis, we will conduct Student's t-tests/ANOVA, or their nonparametric equivalents, as appropriate for the comparison among the groups. Variables with highly skewed distribution will be transformed such as natural log scale before the analysis. The models will be developed to evaluate the effect of medications for each of three Specific Aims. The response of metabolic markers and endothelial function (as measured by forearm blood flow) to different medications (exenatide, sitagliptin, or placebo), over time, will be analyzed using mixed effects model or generalized estimating equation (GEE) model that accounts for correlation of repeated measures over time in crossover design (please see paragraph below for a description of primary analyses and main time points of interest). A secondary consideration will be interaction effects, which will be also examined during regression model construction. All analyses will perform using SAS 9.3 (SAS Institute, Cary, NC, USA) assuming statistical significance at $P<0.05$.

For Specific Aim #1 – the primary outcome measure is free fatty acid (FFA) level, and the main time points of interest are baseline and 6 hours. A key secondary outcome measure for the same specific aims is triglyceride level, of which the main time points of interest are also baseline and 6 hours. Specific Aim #2 reflects largely exploratory work; all pertinent data (measurements of endothelial function) will be considered secondary outcomes. At this time, there is no reported effect size between venous occlusion plethysmography regarding placebo and exenatide, at baseline and 6 hours. The goal for Specific Aim #2 is to determine the effect size (between baseline and 6 hours) between groups – which will be ascertained after testing the first 20 subjects. Furthermore, we will have two nurses assessing identical measurements, in order to assess inter-rater reliability.

The sample size calculation is based on free fatty acid (FFA) levels being the primary outcome measure for this study, using the time points of baseline and 6 hours. Based on the reported effect sizes for fatty acids (in regard to both placebo and exenatide, prior to the meal and at the end of the post meal period) from Schwartz,[9] 40 subjects will provide >90% power to detect an effect size of 2.5 SDs. Regarding a key secondary outcome - triglyceride levels at baseline and 6 hours – Koska's data (10; Supplemental Table 2) shows that if we assume a 1.1 change for placebo and a 0.3 change for exenatide and assuming an SD=0.9 at each time point (prior to the meal and at the end of the post meal period), 40 subjects will provide 80% power to detect this difference (effect size=0.8/1.1=0.7). As stated above, measurements of endothelial function (at baseline and 6 hours) reflect secondary outcomes, and an effect size will be ascertained after testing the first 20 subjects.

**Ethics**

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonization (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50).

The study will be conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent will receive University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects (IRB) approval/favorable opinion before initiation of the study.

All potential serious breaches must be reported to the IRB immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure; debarment).

**Institutional Review Board**

Before study initiation, the investigator will have written and dated approval/favorable opinion from the IRB for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects.

**Informed Consent**

The investigators will ensure that subjects are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate. Freely given written informed consent must be obtained from every subject before clinical study participation, including informed consent for any screening procedures conducted to establish subject eligibility for the study.
Investigators must:

**1)** Provide a copy of the consent form and written information about the study in the language in which the subject is most proficient prior to clinical study participation. The language must be non-technical and easily understood.
**2)** Allow time necessary for subject to inquire about the details of the study.

**3)** Obtain an informed consent signed and personally dated by the subject and by the person who conducted the informed consent discussion.

**4)** Obtain the IRB's written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects, prior to the beginning of the study, and after any revisions are completed for new information.

**5)** Revise the informed consent whenever important new information becomes available that is relevant to the subject's consent. The investigator, or a person designated by the investigator, should fully inform the subject of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented.

The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

**Data handling and record keeping**

The PI will retain, in a confidential manner, all data pertinent to the study for all subjects. All data will be recorded in file folders and binders, which will be stored in the Clinical Research Unit in locked, secure cabinets which will only be accessible to the PI and his team. The PI and his team will retain source documents and accurate case histories that record all observations and other data pertinent to the investigation (e.g., the medical record) for the maximum period required by applicable regulations and guidelines or following institutional procedures. The PI will have direct access to subjects' identity; however, other team members will know the encoded identifying information. Data will be linked to subjects via a study identification code.

A record of the disposition of study drugs is will be maintained at the Memorial Hermann Central Pharmacy, where the study drugs will be stored. This will also be the site of blinding of medications.

**Quality control and assurance**

An annual report will be delivered to the Center for Clinical and Translational Sciences to assure that this clinical trial is progressing appropriately. A statistician will work with us to assure that assure that the data collected are accurate, consistent, complete and reliable. There are no plans for third-party monitoring.

**Publication Plan**

This research will result in publication of two papers by the end of the third year of the study. Data at screening is available to research subjects immediately after screening. All other data will not be available to subjects until after completion of the study. This takes into account the blinded nature of the protocol.

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