Official Study Title: Durability of Combination Therapy With Exenatide/Pioglitazone/Metformin vs. Conventional Therapy in New Onset T2DM

NCT number: NCT01107717

IRB Approval Date:12.04.2019

Unique Protocol ID: HSC20080456H

Click here for a complete set of instructions for this form.

If an item does not apply to your research project, simply indicate that the question is "**not applicable**."

For the following Sections: 1. "Purpose and Objectives"; 3. "Study Design"; and 4. "Study Population," and 5 – 12, you may copy and paste the relevant passages from the sponsor's full protocol or grant application (only citing the page number and section **will not** be acceptable).
For Section 2, "Background" you may cite the relevant passages (page number and section) from the sponsor's full protocol or grant application.

Click once on the highlighted entry in each box to provide your response. Click the item number/letter for detailed instructions for that question. If your response requires inserting a table, picture, etc, you will need to first delete the box that surrounds the answer and then insert your table or other special document.

1. Purpose and objectives.
List your purpose and objectives:

We hypothesize that initiation of combination therapy at the time of initial diagnosis of type 2 diabetes with antihyperglycemic drugs (pioglitazone, metformin, and exenatide) that specifically target known pathogenic defects (insulin resistance and beta cell dysfunction) responsible for impaired glucose homeostasis will achieve superior and more durable glycemic control and more effectively reduce cardiovascular risk factors compared to the stepwise approach currently recommended by the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD).
Specifically, We will test the effect of initiating therapy in newly diagnosed type 2 diabetic subjects with combination pioglitazone/metformin/exenatide therapy compared to the ADA recommended approach (start with metformin and add a second or third oral agent, and then proceed to insulin) on:

1) Achievement and maintenance of glycemic control (HbA_{1C} <6.5%)
2) Reduction in whole body (muscle) insulin resistance and improvement in beta cell function.
3) Improvement in cardiovascular risk factors
4) Decrease in carotid intima media thickness, a surrogate measure of atherosclerosis

2. Background.

Describe past experimental and/or clinical findings leading to the formulation of your study. For research involving investigational drugs, describe the previously conducted animal and human studies. For research that involves FDA approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol. Attach a copy of the approved labeling as a product package insert or from the Physician's Desk Reference. You may reference sponsor's full protocol or grant application (page number and section) or if none, ensure background includes references.

The prevalence of type 2 diabetes mellitus (T2DM) has increased progressively to epidemic proportions over the last 20 years. More than 150 million individuals worldwide were affected by the disease in the year 2000 and this number is expected to increase to more than 300 million by the year 2025 (1) The epidemic of type 2 diabetes is closely related to the epidemic of obesity (2) which, not only has resulted in a marked increase in the incidence of diabetes, but also in a decrease in the age of the disease onset. Type 2 diabetes, once a rare disease in youth, now frequently is encountered among adolescents and children (1-2). The decrease in the age of disease onset will result in a more prolong period of hyperglycemic exposure and eventuate in an increased risk for both micro- and macrovascular complications.
T2DM individuals experience increased morbidity and mortality secondary to vascular damage involving both the microvasculature (retinopathy, nephropathy, neuropathy) and macrovasculature (heart attack, stroke, amputation) (3). Diabetes is the leading cause of blindness and end stage renal disease in western countries (4), and T2DM individuals have a 2.5-4 fold increase in the risk of cardiovascular disease - heart attack and stroke (5). Moreover, clinically significant morbidity often is present long before the diagnosis of diabetes is made (6), and eventually, one-third to one-half of all people with diabetes have evidence for organ/tissue damage (7). Although not everyone with diabetes is destined to develop complications, a recent

epidemiological study (7) reported that two or more complications are apparent in almost one-fifth of people with diabetes.

The increased prevalence of T2DM, together with its associated morbidity and mortality, have placed a heavy burden on the health care system. In the US, the estimated annual cost for the treatment of diabetes exceeds $100 billion (8). Unfortunately, less than half of all T2DM patients in the US achieve the ADA's recommended goal ($HbA_{1C} \leq 7.0\%$) for glycemic control (9). Therefore, more effective therapeutic strategies with durable glycemic control will be required in order to reduce morbidity/mortality from vascular complications and decrease the cost of therapy for this devastating metabolic disease.

Glycemic Control and Diabetic Complications

Two landmark studies, the DCCT (10) and the UKPDS (11), as well as others (12-15) unequivocally have documented that improved control of the plasma glucose concentration in T2DM subjects reduces the risk of both microvascular and macrovascular complications, demonstrating that chronic exposure of tissues to hyperglycemia triggers pathologic processes that lead to organ damage. A 1% decrease in HbA_{1C} has been shown to cause an ~35% reduction in the incidence of microvascular complications (10,11,14) and maintaining the HbA_{1C} <6% has been reported to completely prevent the development of retinopathy in T2DM subjects (13). Further, in the DCCT continuation study (EDIC), subjects who initially were treated aggressively with intensive insulin therapy and achieved a HbA_{1C} <7% early in the course of their disease maintained a lower risk for microvascular complications many years later even though their HbA_{1C} increased to 8% compared to subjects who initially were less controlled (16). This observation emphasizes the importance of achieving good glycemic control at the time of diagnosis and of maintaining the level of glycemic control in order to reduce the risk of microvascular complications. The UKPDS (11), DCCT (15), and Kumamoto [14] studies also have shown that improved glycemic control reduces the risk of atherosclerotic cardiovascular complications. The results of these studies have prompted the professional organizations of health care providers (American Diabetes Association [17], American College of Physicians [18], American Association for Clinical Endocrinology (AACE) [19], and the European Association for the Study of Diabetes [20]) to recommend glycemic goals as close to normal as possible while avoiding hypoglycemia. The ADA's target goal for HbA_{1C} is ≤7.0%, while the AACE's and the EASD's goal is ≤ 6.5%.

ADA Recommendation for Therapy

The ADA recommends that, at the time of diagnosis, subjects with type 2 diabetes be started on metformin, in addition to lifestyle intervention (17). Metformin is safe and effective, reducing HbA_{1C} by ~1.5% (21). However, its efficacy wanes with time (22,23). Both the UKPDS (23) and ADOPT (22) studies have demonstrated that, despite a significant initial decrease in HbA_{1C} following initiation of metformin therapy, beta cell function continues to deteriorate progressively, resulting in subsequent treatment failure. The current ADA (and EASD) recommendation is to add a sulfonylurea (less commonly a thiazolidinedione) or basal insulin in stepwise fashion upon therapeutic failure, i.e. increase in HbA_{1C} to > 7.0% (17,20). Although this therapeutic approach will achieve adequate glycemic control in many T2DM subjects, diabetes is a progressive disease (22,23) and after 1-2 years the HbA_{1C} rises to values > 7.0%, requiring the addition of a second antidiabetic medication, usually a sulfonylurea. Moreover, this therapeutic approach is based upon reduction of the plasma glucose concentration rather than on correction of the underling defects which cause the hyperglycemia (beta cell failure and insulin resistance). Most importantly, we (24) and others (25) have shown that at the stage of impaired glucose tolerance (i.e., upper tertile of 2-hour plasma glucose during OGTT = 180-199 mg/dl) approximately 75-80% of beta cell function has been lost (24-26). Thus, if therapy is initiated with drugs, i.e., metformin or sulfonylureas that do not halt the progressive beta cell failure (22,23,27), eventually there will be a complete loss of beta cell function, rendering the diabetic patient dependent on insulin therapy for glycemic control (28). In most studies (28-30) insulin therapy is associated

with significant weight gain and an increased rate of hypoglycemia, which presents a major obstacle in achieving the desired level of glycemic control (28-30).

Despite the irrefutable evidence for the beneficial effect of plasma glucose reduction in the prevention of microvascular complications, and the ADA's recommendation to maintain glycemic control as close to normal as possible, in current clinical practice many of type 2 diabetic subjects do not achieve the recommended treatment goals. In a recent survey from the NHANES (9), the mean HbA_{1C} in the US was ~7.2% and only ~55% of subjects achieved a $HbA_{1c} < 7\%$. In a survey of US academic medical centers, only 29% of subjects have achieved $HbA_{1c} < 7\%$ (31). Increased frequency of hypoglycemia, associated with improved glycemic control, and progressive beta cell failure are the principal obstacles which have prevented the attainment of recommended levels of HbA_{1C} (10,28,29).

From the above discussion, it is clear that, despite the availability of a number of therapeutic agents with various mechanisms of action, including insulin, the majority of type 2 diabetic individuals remain suboptimally controlled and, therefore, are at increased risk for both microvascular and macrovascular (see below) complications. In the present study I propose a new therapeutic strategy for achieving and maintaining target levels of glycemic control, $HbA_{1C} < 6.5\%$.

Diabetes and Cardiovascular Disease

Subjects with type 2 diabetes have a markedly increased risk for cardiovascular disease (heart attack and stroke) and a worse prognosis following any cardiovascular event (5,31,32). Poor glycemic control is an important risk factor for cardiovascular disease (11,15) but improved glycemic control only modestly reduces the increased risk of cardiovascular disease (11). The UKPDS has demonstrated that hyperglycemia per se has a less prominent role in the development of macrovascular complications compared to microvascular complications (11). Hypertension (34-36) and dyslipidemia (37) are major risk factors for coronary artery disease. Nonetheless, despite reduction of blood pressure (35,36) and plasma LDL cholesterol to target levels (37), the cardiovascular risk in subjects with type 2 diabetes remains greater than in nondiabetic subjects. Many recent studies (38-41) have demonstrated that insulin resistance is an additional risk factor for cardiovascular disease. Insulin resistance also has been shown to be associated with a cluster of metabolic-cardiovascular risk factors (central obesity, hypertriglyceridemia, hypertension, elevated procoagulant and inflammatory markers) which collectively have been referred to as the Metabolic Syndrome (42,43,43a). In the present study, we also will examine whether antidiabetic therapy, which corrects known pathophysiologic abnormalities responsible for type 2 diabetes (i.e., insulin resistance), improves cardiovascular risk factors compared to therapy which reduces plasma glucose without direct effects to improve insulin sensitivity. Some evidence in support of this concept has been presented. Thus, pioglitazone, which improves muscle insulin resistance in type 2 diabetic subjects, has been shown to reduce cardiovascular risk factors, (i.e., plasminogen activator inhibitor-1, inflammatory markers, TNF alpha, and NFkB) independent of its glucose lowering effect and these beneficial actions may, in part, contribute to the decrease in carotid intima medial thickness compared to other hypoglycemic therapies (27,44-47). In the PROACTIVE study, pioglitazone tended to decrease the composite primary cardiovascular endpoint and significantly reduced the principal secondary endpoint (heart attack, stroke, death) (48).

Pathophysiology of Type 2 Diabetes: Implications for Therapy

Subjects with type 2 diabetes manifest two major pathophysiologic defects (49-51): (i) impaired insulin secretion in response to glucose and other stimuli, i.e beta cell failure, and (ii) impaired insulin action in the liver and peripheral (muscle and adipose) tissues, i.e. insulin resistance. Both insulin resistance and beta cell failure are present long before the onset of overt diabetes (49-54). The earliest detectable abnormality in glucose metabolism is an increase in insulin resistance in liver, muscle, and other insulin target tissues. Both genetic and environmental factors, e.g. obesity and sedentary lifestyle, contribute to the development of

insulin resistance (50). In response to insulin resistance, the beta cell increases its secretion of insulin (both under fasting conditions and in response to nutrient stimuli) and this results in hyperinsulinemia. Longitudinal and cross sectional studies have demonstrated that, initially, the compensatory hyperinsulinemia is sufficient to offset the insulin resistance and maintain normal glucose tolerance (51,54,55). Most overweight and obese individuals are insulin resistant (2,42,51), but the majority maintain normal glucose tolerance throughout life due to the ability of the beta cell to fully compensate for the insulin resistance. However, when the beta cell fails to adequately compensate for the insulin resistance, glucose homeostasis deteriorates. Initially, this is manifest as impaired glucose tolerance and later as overt diabetes (51,54). By the time overt diabetes is diagnosed, ~80% of beta cell function has been lost (24,25). Furthermore, the deterioration in beta cell function continues after diabetes is diagnosed and is the major factor responsible for the progressive deterioration in glycemic control in subjects with type 2 diabetes despite an initial good response to "standard" antidiabetic therapies, i.e. metformin and sulfonylureas (22,23,27). Thus, only drugs which halt/slow the progressive beta cell failure can be expected to achieve durable glycemic control. Results from the UKPDS indicate that subjects who were started on monotherapy with metformin, sulfonylurea, or insulin continue to experience a deterioration in glycemic control and beta cell function at a rate which is identical to subjects treated with diet alone (23). These results suggest that none of the "standard" antidiabetic agents (i.e., metformin, sulfonylureas, insulin), when used alone in sequential therapy, prevent the progressive decline in beta cell function observed in subjects with type 2 diabetes.

Alternative Approach for More Effective and Durable Glycemic Control

Two novel classes of antihyperglycemic drugs have been developed in the past decade: (i) thiazolidinediones and (ii) the GLP-1 analogue, exanetide. Thiazolidinediones are peroxisome proliferator activated receptor-gamma (PPARγ) agonists and are potent insulin sensitizers in skeletal muscle, liver, and adipocytes (56-58). Two thiazolidinediones currently are approved for the treatment of T2DM: rosiglitazone and pioglitazone. In this study we propose to initiate therapy with pioglitazone at the time of diagnosis of T2DM. Pioglitazone has a much more favorable plasma lipid profile than rosiglitazone (58a) and reduced cardiovascular events (heart attack, stroke, death) in high risk T2DM patients in the PROactive Study (48). Clinical trials have demonstrated that the efficacy of pioglitazone in reducing the fasting plasma glucose concentration and HbA_{1C} is similar to that of metformin and sulfonylureas. In drug naïve subjects, pioglitazone decreases the HbA_{1C} by ~ 1.5% (59-61). Studies in humans with IGT and T2DM (62-65) and in experimental animals (66-68) have demonstrated that the thiazolidinedione class of drugs, in addition to their insulin sensitizing action, improve and preserve beta cell function. Consistent with its effect to preserve beta cell function, recent clinical trials have reported more durable glycemic control with thiazolidinediones compared to sulfonylureas (22,27). In the recently completed ACT NOW study, pioglitazone compared to placebo reduced the conversion rate of IGT to T2DM by 85% over a period of 3.75 years via its combined effects to improve beta cell function and augment insulin sensitivity (Ralph A. DeFronzo, PI, unpublished results). Pioglitazone also has been shown to reduce plasma FFA (69), adipocytokine/other inflammatory markers/procoagulant factors and increase plasma adiponectin concentration (44-47,70), all of which would be expected to provide cardiovascular benefit. Pioglitazone also decreases insulin resistance (by 35-40%) in skeletal muscle and liver (62) and, together with the decrease in plasma triglyceride and increase in HDL cholesterol concentrations (58a), would be expected to provide additional cardiovascular benefits, independent of the reduction in plasma glucose concentration (71-73). A recent study has demonstrated that pioglitazone slows the progression in carotid intima medial thickness in subjects with type 2 diabetes (27) and in a large clinical trial (PROactive study), the addition of pioglitazone as an adjunct to "standard" antihyperglycemic therapy resulted in significant 16% reduction in the combined endpoint of myocardial infarction, stroke and death compared to placebo

in subjects with type 2 diabetes and pre-existing cardiovascular disease (48). Collectively these results indicate that pioglitazone, in addition to lowering plasma glucose concentration, reduces insulin resistance, preserves beta cell function, and may have a direct action on the vasculature (74) to reduce the vascular complications of diabetes above and beyond its glucose lowering effect.

Exenatide is a GLP-1 mimetic drugs which binds to the GLP-1 receptor on the beta cell and markedly augments glucose-stimulated insulin secretion (75,76). Because its stimulatory effect on insulin secretion is glucose dependent, hypoglycemia is rarely encountered when exenatide is used as monotherapy or in combination with metformin or a thiazolidnedione (77-79). Exenatide also acts on the alpha cell to suppress glucagon secretion (78). The combination of increased insulin secretion and decreased glucagon secretion results in suppression of basal/post-meal hepatic glucose production (HGP) and enhanced peripheral tissue (muscle) glucose disposal (80). Clinical studies have demonstrated that exenatide causes a significant reduction in fasting (decreased HGP) and post-prandial (increased muscle glucose uptake) plasma glucose concentration, leading to a significant drop in HbA_{1c} (79-84), and is well tolerated when added to metformin (84) or to a thiazolidinedione (79). Exenatide also has extrapancreatic actions: it delays gastric emptying, thereby reducing postprandial hyperglycemia (85), and suppresses appetite, resulting in significant weight loss (86,87). The weight loss is associated with a decrease in plasma triglycerides, an increase in plasma HDL concentration, and a reduction in blood pressure (80-86). These latter actions of exenatide would be expected to have a favorable effect to reduce cardiovascular complications (88,89). Studies in humans have demonstrated that exenatide markedly enhances beta cell function in subjects with type 2 diabetes (90). In experimental animals exenatide stimulates beta cell neogenesis and replication and inhibits beta cell apoptosis (91). If these later effects were to extend to humans, additional beneficial effects on beta cell health would be expected.

No previous study has evaluated the efficacy and durability of combined pharmacological therapy at the time of diagnosis of diabetes with drugs that preserve beta cell function. Furthermore, no previous study has achieved the recommended goal of glycemic control (HbA_{1C} <6.5%) as its primary outcome. In this study we will initiate therapy in subjects with type 2 diabetes at the time of diagnosis with triple therapy (pioglitazone, metformin, exenatide). Pioglitazone and exenatide both exert effects to preserve beta cell function, while pioglitazone, and metformin to a lesser extent, are excellent sensitizers. All three drugs effectively reduce the HbA_{1C} and have beneficial cardiovascular actions. The primary outcome variable is the achievement and maintenance of the recommended target for glycemic control (HbA_{1c} <6.5%) in subjects with type 2 diabetes. We will compare the efficacy of two therapeutic regimens: (i) triple therapy (pioglitazone, metformin, exenatide) at the time of diagnosis of T2DM versus (ii) stepwise therapy starting with metformin and subsequent addition of sulfonylurea and basal insulin (i.e., the "standard" approach) in achieving this goal. The first intervention is based on the novel concept of initiating therapy at the time of diagnosis with combination therapy using agents that correct specific pathophysiologic defects that are characteristic of T2DM (insulin resistance and beta cell failure). The second intervention is based upon stepwise addition of antidiabetic agents to reduce the HbA_{1C} according to the current ADA therapeutic recommendation.

3. Study Design.

Describe the study design (e.g., single/double blind, parallel, crossover, etc.) Consider inserting a scheme to visually present the study design.

Research Design and Methods

Subjects:

720 newly diagnosed T2DM subjects (less than 2 years) in good general health as determined by physical exam, medical history, blood chemistries, CBC, TSH/free T4/total T4, lipid profile, urinanalysis, and EKG will take part in the study. Subjects must have a HbA_{1c} > 6.5% and must not have taken any oral or injectable antidiabetic medication for more than 2 days, except metformin, and must not be taking any medications known to affect glucose homeostasis. Only subjects whose body weight has been stable (± 3lbs) 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, albuminuria >300 mg/dl, symptomatic diabetic neuropathy, or history of cardiovascular disease (class III-IV CHF) will be excluded. Subjects with AST or ALT greater than 3.5-foldl above the upper limit of normal will be excluded. All subjects must be GAD antibody negative. Subjects meeting these criteria will meet with a dietician and be instructed in a weight maintaining diet containing 50% carbohydrate, 30% fat, and 20% protein. They also will receive instructions about a modest intensity exercise program (40 minutes of walking 3-4 times per week). After one week on the diet, all subjects will receive: (1) oral glucose tolerance test; (2) hyperglycemic clamp-will be performed in a subgroup of 50 subjects (25 in each group) to provide a measure of beta cell function; (3) DEXA for total body fat content/fat distribution; (4) carotid ultrasound-for measurement in carotid intima media thickness; (5) fundus examination; (6) 24-hour urine for albumin and creatinine; (7) ankle arm blood pressure measurement.
Following completion of these tests, subjects will be randomized (using a table of random numbers) to receive, in open label fashion, one of the following treatment regimens: (i) Group I will be started on pioglitazone (15 mg/day) plus metformin (1000 mg/day) with the supper meal and exenatide (5 mcg s.c. bid 30 min before breakfast and supper) and up-titrated to 45 pioglitazone plus 2000 metformin and 10 mcg s.c. bid exenatide to achieve HbA1c <6.0%; (II) Group II will be started on metformin, 1000 mg with breakfast and 1000 mg with supper, glyburide and basal insulin will be added and up titrated to achieve HbA1c <6.0% Subjects will be recruited during the first year and followed up for additional two years. After completing the repeat studies, subjects will be asked to continue in the study and with the study medications without any change. Follow up visits will occur every 3 months) until the last patient recruited into the study completes 2 –years of follow-up. At the Follow visit Fasting Glucose and HbA1C will be performed. At the annual visits blood work to check the following: fasting glucose, HbA1C, and other repeat labs. Every 2 years or if the subject decides to discontinue participation, we will repeat the annual labs, OGTT, eye photographs, and carotid ultrasound.

4. Study Population(s).
You will be drawing subjects from one or more populations. In medical research, for example, a population can be individuals with type 2 diabetes controlled with diet, or a population of healthy individuals. In social behavioral research, a population can be individuals attending an education program, etc.

a. How many different populations are you enrolling in this study?	1
b. For each different population, provide a short descriptive label: *(e.g., normal-healthy, diabetics, patients needing surgery, parents, child, etc.)*	720 Newly diagnosed type 2 diabetic subjects Will be enrolled to have 600 completers .

*For each **specific** population identified 4b, provide the following information in the table provided below.* ***(One table is provided, use copy and paste for additional tables if enrolling more than one population.)***

Population # [Type # given to population in 4b] XCheck here if enrolling only one population	**Population Descriptive Label:** [Insert short label from 4b] Enrollment of **[Insert #]** requested to obtain **[Insert #]** completers.
(1)	Identify the criteria for **inclusion**:

1) Subjects with type 2 diabetes diagnosed in the past 2 years
2) subjects must be on diet or metformin only
3) age 18-75 years
4) must be in general good health and without a major organ disease
5) must have creatinine < 1.5
6) must have LFT < twice the upper normal limit
7) subjects must have HbA1c > 6.5%

(2)	Identify the criteria for exclusion:

1) subjects with type 1 diabetes or positive for anti diabetic antibodies
2) subjects with type 2 diabetes who were diagnosed more than 2 years or who receive insulin or pharmacological therapy other than metformin
3) subjects with a cardiovascular event in the past year

(3)	Recruitment Describe plans how the population will be **identified** for the purpose of recruiting. (Consider HIPAA Waiver if needed to allow those without existing legitimate access to PHI to use it to identify potential subjects.) (e.g., database search, personal contacts, referrals, patients under the care research team, etc.)

Advertising material will be posted in the medical center and near by hospitals or in primary care offices (advertising material is included for IRB approval). Subjects interested in participating in the study will be asked to contact one of the investigators.
We also have access to TDI patient database that can search and select patients that are newly diagnosed diabetic patients or have diabetes markers such as HbA1c . We are planning on identifying such potential subjects and then contacting their health care providers for referral. We will send out "dear Doctor" letter informing the providers about our research study and ask them to contact participants. The treating physician will be making the initial patient contact, not the researcher. If the patient is interested, then either the patient will contact the PI or, with the permission of the patient, the PI will be invited to talk with the patient about study participation.

(4)	Recruitment Describe how **initial contact** will be made with potential subjects. Describe how those making initial contact have a legitimate access to the subjects' identity and the subjects' information. (Consider HIPAA Waiver if needed to disclose PHI to individuals who would not have otherwise had legitimate access.)

Subjects who express their interest in participating in the study will be contacted via phone by Dr DeFronzo or one of the co investigators. During this initial telephone conversation the study aim and procedures are explained to the patient, he also will be questioned about the inclusion and exclusion criteria. During this conversation subjects will be encouraged to discuss their participation in the study with their doctors. Subjects who fulfill the inclusion criteria and express their interest in participating in the study will be invited to the clinical research center for visit I

(5)	Recruitment Describe the **setting** in which an individual will be initially approached. (e.g., private room, inpatient unit, waiting area, group setting, over internet, over phone, in public). Also, describe all interaction between the research staff and the potential subject between the time they contact the research team or vice versa and the time they sign a consent form (including pre-screening activities-see instructions for detailed guidance)

Subjects who will respond to the advertisement will be contacted via telephone and asked about possible exclusion criteria. Subjects who are interested in participating in the study and met the inclusion criteria will be invited to the GCRC for the first visit

(6)	Recruitment Specify if any advertising will be performed. xYes ☐ No ☐ Pending (e.g., submitting amendment after approval) If yes, please see Section 4, Form L for instructions on attaching copies of the information to be used in flyers or advertisements. Advertisements must be reviewed and approved by the IRB prior to use.

Adverising material is included in this submission (See form L)

(7)	Consent Process Describe the consent/assent **procedures** to be followed by the research team. (Be sure to cover how information is provided, how the consent interview is conducted, the person(s) who will conduct the consent interview and how the consent is signed (privacy, who signs the consent, etc.).) If consenting a single subject will involve more than one member of the research team, describe how this process will be coordinated from start to finish.
During visit 1 all subjects will be interviewed by Dr. DeFronzo or one of the co-investigators, who will describe in detail the purpose, nature and potential risks of the study. Each subject is then asked to read the consent form. Subjects then will be asked if they have any questions concerning any aspect of the study. All questions will be answered thoroughly and candidly by Dr. DeFronzo or one of the co-investigators. Immediately prior to the study, subjects are asked to sign the consent form and are given a photocopy to keep for themselves. The consent forms will be kept in room at TDI. The quality and content of the consent process will be monitored throughout the study period and any changes will be communicated to IRB and the GAC.	
(8)	Consent Process Describe the **timing** of obtaining informed consent, whether there is any waiting period between informing the prospective subject and obtaining consent. (e.g., take consent home, waiting period of X hours, after consulting with family members, etc.)
Subjects will be consented at the first visit, there will be no waiting period.	
(9)	Describe measures taken to **minimize** the possibility of coercion or undue influence during consent.
The study procedures will be explained for the subjects via the phone during the initial contact. They will be encouraged to consult their primary care doctors about their participation in the study. It will be explained to them that their participation in the study is voluntary and there is no penalty for not participating in the study.	
(10)	Will subjects from this population be assigned to different research groups? X Yes No
If yes, list the groups using a short descriptive label. E.g., experimental group A, B, etc., control group, etc. (this information is needed later in the Risk: Benefit Analysis section) [Group I: Combination therapy; Group II: Conventional therapy	

5. Informed Consent for Research Involving Non-English Speaking Subjects

A	Individuals who do not speak English will not be enrolled.	
	☐	There is **no** expected benefit for those participating. (Skip B, and (1) and (2) below this table and go to #6)

B. X	Individuals who do not speak English will be enrolled. The foreign language translation of the consent will be submitted to the IRB:		
	☐	Immediately following approval of the English consent document. (go to (1) and (2) below this table.)	
	X	Only after a potential non-English speaking participant is identified. Indicate why this method that will result in a delay in consenting non-English speaking subjects is acceptable by selecting on of the choices below:	
		☐	There is **no** expected benefit for those participating. (go to (1) and (2) below this table.)
		X	There is an expected benefit for those participating. The anticipated delay in enrolling non-English speaking subjects is acceptable because: [Describe the rationale for determining as acceptable, the potential delay in start of the research related treatment pending approval under this circumstance then go to (1) and (2):]

We rarely encounter non-English speaking individuals. However, if an individual who speaks Spanish is to be recruited we anticipate being able to develop the consent form and get it approved by the IRB within 2-3 weeks. Since during the time period between identification of subjects and the start of the intervention patient will continue their previous care for their diabetes, the delay will not have any significant adverse effect on the patient's diabetes condition and/or treatment ot increase their risk compared to English speaker participants.

(1) If you are recruiting non-English speaking subjects, the method by which consent is obtained should be in language in which the subject is proficient. Describe the process for obtaining informed consent from prospective subjects in their respective language (or the legally authorized representative's respective language).

If a Spanish speaking individual is to be recruited, a nurse or individual listed on the consent form who is fluent in Spanish will obtain the consent.

(2) In order to ensure that individuals are appropriately informed about the study when English is their second-language, describe a plan for evaluating the level of English comprehension, and the threshold for providing a translation, or explain why an evaluation would not be necessary.

Subjects will be asked if English is their first language. If not, they will be consent with a consent form that has been translated into Spanish.

6. Research Plan / Methods:

a. Description of Methods – Provide a comprehensive narrative describing the research methods. Provide the sequence of events for conducting the research and a description of the methods used to protect privacy during the study.

Specific Methods:

1) **OGTT**: A 75 gram OGTT will be performed at 0800h after a 10-12 hour overnight fast. Plasma glucose, insulin, C-peptide, and FFA concentrations are measured at baseline (15, -10 and 0 min) and every 15 min for 2 hours. Insulin sensitivity will be measured with the Matsuda index =

$$10{,}000/\sqrt{(FPG \times FPI) \bullet (\overline{PG} \times \overline{PI})}$$

This index agrees closely with insulin sensitivity measured with euglycemic insulin clamp technique (92). The following indices of insulin secretion will be measured: early insulin response ($\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$) and total insulin response ($\Delta I_{0\text{-}120}/\Delta G_{0\text{-}120}$ and $\Delta ISR_{0\text{-}120}/\Delta G_{0\text{-}120}$) (24,26,52,93), where ISR = insulin secretory rate calculated by deconvolution of the plasma C-peptide curve (93). The insulin secretion/insulin resistance (so called "disposition") also index will be calculated as $\Delta I/\Delta G$ x Matsuda index and $\Delta ISR/\Delta G$ x Matsuda index.
Total blood loss for OGTT is up to 88 ml. Total blood loss for screening tests is up to 40 ml.

2) **Adipocytokines** plasma(adiponectin, leptin, resistin, IL6, TNFα), urinary alpha-1 isoprostane (a measure of reactive oxygen species [94]), and highly sensitive C-reactive protein (hsCRP) will be measured as previously described (94a).
3) **Whole Body DEXA.** Total and regional fat and fat-free mass will be measured by a licensed radiology technician using a Discovery 010-1596 instrument from Hologic that is housed on the CRC at the TDI.
4) **Carotid Intima Media Thickness:** Carotid IMT will be measured by high resolution B-mode carotid artery ultrasound using an HDI 5000 Ultrasound System with a linear-array 7.5-MHz transducer (Philips Medical System, Wash). All scanning throughout the study will be performed by a single ultrasonographer at the same location using the same equipment. The mean difference between 2 readings amongst 20 examinations is 0.002 mm (SD 0.002). The HDI 5000 Ultrasound system is housed on the CRC of the TDI and a skilled ultrasonographer , will make the

measurements and read them blindly. Because the HDI 5000 is owned by the Diabetes Division, there is no cost for the procedure. Previous studies with thiazolidinediones have shown that significant reductions in carotid IMT are observed within 12-18 months or less (27,72,73).

5) **Measurement of Ankle-Arm Blood Pressure** will be performed and ankle brachial index (ABI) will be determined as previously described (95). The ABI measurement is independently and inversely related to carotid and femoral arterial intima-media thickness and to the extent of coronary artery disease and enables early detection of atherosclerosis (95). ABI is computed by dividing the ankle systolic blood pressure by the arm systolic blood pressure. In normal individuals ankle pressures are similar to (or somewhat higher than) arm pressures when the patient is supine. When peripheral arterial occlusive disease is present, a low ABI (less than 0.9 is considered to represent presence of PAD) is found.
6) **Hyperglycemic Clamp:** (96) After a 10-12h overnight fast, subjects will report to the CRC at the TDI at 0600 h and catheters will be placed into an anteculital vein (for glucose infusion) and retrogradely into a vein on the dorsum of the hand (for blood withdrawal). Low dose insulin (4-10 mU/m^2 • min) will be started to reduce plasma glucose concentration to ~100 mg/dl. 30-60 minutes after plasma glucose concentration is stabilized at ~100 mg/dl, the hand will be inserted into a heated box (70^0C) to ensure arterialization of the venous blood. Following the collection of three baseline blood samples (-20, -10, and 0 minutes), the plasma glucose concentration will be acutely raised and maintained at 125 mg/dl above baseline (i.e., from about 100 mg/dl to 225 mg/dl) for 90 minutes. Plasma glucose, insulin, and C-peptide concentrations are measured every 2 minutes during the first 10 minutes and every 15 minutes until 90 minutes. At 90 minutes, the plasma glucose concentration is raised and maintained at 400 mg/dl for an additional 90 minutes. Plasma glucose, insulin, and, C-peptide are measured every 2 minutes from 90 to 105 minutes and every 10-15 minutes from 105 to 180 minutes. This will provide a maximum hyperglycemic stimulus for insulin secretion, with an early phase of insulin release during the first 10 minutes of each hyperglycemic clamp step followed by a gradually increasing phase of insulin release over the subsequent 80 minutes. At 180 minutes, an intravenous bolus of 5 grams of arginine will be given and the plasma glucose, insulin, and C-peptide levels will be measured every 2 minutes for 10 minutes and every 5 minutes thereafter for a total of 30 minutes following the arginine injection. The combination of hyperglycemia plus hyperargininemia provides a maximal stimulus for insulin secretion (97) and the insulin secretory rate (quantitated by deconvolution of the plasma C-peptide concentration) provides the most sensitive recognized measure of beta cell health (55). Under steady state conditions of physiologic (+125 mg/dl) hyperglycemia (60-90 minute time period of the hyperglycemic clamp), the glucose infusion rate divided by the mean plasma insulin concentration provides a measure of insulin sensitivity that agrees closely (r=0.92) with the euglycemic insulin clamp (95). Total blood loss for the procedure is up to 255 ml.
7) **7) EUGLYCEMIC INSULIN CLAMP** On a separate day, subjects will be admitted to the CRC 6:45 and 8 AM for a four-hour, two-step hyperinsulinemic euglycemic insulin (20 and 80 mU/m^2•min) clamp with a prime-continuous infusion of 3-^{3}H-glucose. The 3-^{3}H glucose prime will be 25 μCi x (fasting plasma glucose/100); the continuous infusion will be given at the rate of 0.40 μCi/min for 240 minutes Plasma samples for tritiated glucose will be obtained at 5-15 minute intervals during the 30 minutes before and throughout the insulin clamp, to quantitate rates of whole body glucose disposal and suppression of endogenous (primarily hepatic) glucose production. Continuous indirect calorimetry (Deltatrac, Sensormedics, Anaheim, CA) will be performed for 30 minutes prior to the start of the insulin clamp, and during the 210-240 period of

the insulin clamp to calculate rates of glucose and lipid oxidation Total blood loss for the procedure is up to 160 ml.

8) **Fundus Examination:** All diabetic subjects seen at the Texas Diabetes Institute have an annual ophthalmologic examination as part of their routine care. The fundus photographs will be read blindly by University of Wisconsin-Madison, Department of Ophthalmology and Visual Sciences.

Following completion of these tests, subjects will be randomized (using a table of random numbers) to receive, in open label fashion, one of the following treatment regimens:

GROUP I: Group I will be started on pioglitazone (15 mg/day) plus metformin (1000 mg/day) with the supper meal and exenatide (5 mcg s.c. bid 30 min before breakfast and supper).

If, after month one, the FPG ≥ 110 mg/dl or HbA_{1C} ≥6.0%, the exenatide dose will be increased to 10 mcg bid, pioglitazone will be increased to 30 mg/day, and metformin to 1000 mg bid.

If, after month two, the FPG ≥ 110 mg/dl or HbA_{1C} ≥ 6.0%, pioglitazone will be increased to 45 mg/day. Subjects who will not tolerate 5 mg bid exenatide because of GI side effects will be started on lower dose (1 mg s.c bid 30 min before breakfast and supper): and the dose will be titrated up to the maximal tolerated dose.

If, at any time after achieving a HbA1c ≤ 6.0%, the HbA1c rises above 6.0% and the subject is on submaximal doses of metformin or pioglitazone or exenatide, the medication(s) will be titrated up to the maximally effective dose and the subject will not be considered a treatment failure.

If, after month six, the HbA_{1C} ≥ 6.0% and the subject is on maximum doses of all triple therapy medications, the subject will be considered a treatment failure. If, after month 6, the HbA1c rises to > 6.5%, bedtime glargine insulin (10-20 units) will be started and titrated to a maximum of 60 units to achieve a FPG < 110 mg/dl and HbA1c < 6.0%. If more than 60 units of glargine insulin is required to reduce the HbA1c < 6.5%, regular insulin will be added to each meal and adjusted based upon the pre- and post-meal blood glucose levels. These "treatment failure" subjects will continue with all follow up visits/procedures. Throughout the titration process, downward adjustments will be allowed for the development of hypoglycemia, as defined by a blood glucose less than 60 mg/dl with (symptomatic hypoglycemia) or without (asymptomatic hypoglycemia) symptoms. Serious hypoglycemia is defined as any hypoglycemic reaction requiring assistance from a third party for recovery.

GROUP II: Group II will be started on metformin, 1000 mg with breakfast and 1000 mg with supper.

If, after month one, the FPG ≥ 110 mg/dl or HbA_{1C} ≥6.0%, glipizide (5 mg) will be added: 1-2 tablets with breakfast depending on the FPG, HbA_{1C}, and discretion of the investigator in order to avoid hypoglycemia.

If, after month two, the FPG ≥ 110 mg/dl or HbA_{1C} ≥6.0%, the glipizide dose will be increased to 4 tablets (20 mg/dl).

If, after month three, the FPG > 110 mg/dl or HbA_{1C} > 6.0%, bedtime basal insulin (10-20 units) will be started and titrated up to achieve a FPG <110 mg/dl or HbA_{1c} <6.0%

If, at any time after achieving a HbA1c < 6.0%, the HbA1c rises above 6.0% and the subject is on submaximal doses of metformin or glipizide or glargine insulin, the medication(s) can be titrated up to the maximally effective dose and the subject will not be considered a treatment failure. If, after month six, the HbA_{1C} > 6.0%, and the subject is on maximum doses of metformin, glipizide, and glargine insulin, the subject will be considered a treatment failure. Regular insulin will be added to each meal when the HbA1c rises to > 6.5% and adjusted based upon the pre- and post-meal blood glucose levels. These "treatment failure" subjects will continue with all follow up visits/procedures.

Throughout the titration process, downward adjustments will be allowed for the development of hypoglycemia, as defined by a blood glucose less than 60 mg/dl with (symptomatic hypoglycemia) or without (asymptomatic hypoglycemia) symptoms. Serious hypoglycemia is defined as any hypoglycemic reaction requiring assistance from a third party for recovery.

Following their enrollment into the study, all T2DM participants will return to the Clinical Research Center (CRC) at the TDI for follow up visits every 3 months. Telephone calls also will be made to each participant every 6 weeks. During the telephone/CRC visits, subjects will be asked questions about interim medical history, home glucose monitoring results, and compliance with the medical regimen.

During each 3-month follow-up visit to the CRC, body weight, waist circumference (Gulick III tape measure), and blood pressure (Dynamop) will be measured. Blood will be drawn for FPG and HbA_{1C}, and pharmacological therapy will be adjusted based on the HbA_{1c}, FPG, and HBGM results (see prior algorithm). Plasma lipids and 24-hour urine albumin excretion will be measured annually or more frequently as indicated by test results. In addition to glycemic control, all subjects will receive therapy for any abnormal cardiovascular risk factors: hypercholesterolemia (goal LDL <100 mg/dl), hypertriglyceridemia (goal <150 mg/dl), hypertension (goal <130/80 monthly), albumin excretion (goal < 30 mg/day). At the end of three years, the OGTT, DEXA, fundus photography, 24 hour urine for albumin, hyperglycemic clamp, ankle-arm blood pressure, and carotid IMT will be repeated.

Primary End Point: The primary end point of the study is the difference in HbA_{1c} between Group I (triple combination therapy at time of initial diagnosis) and Group II (stepwise therapy).
Secondary End Points: The following parameters will be measured as secondary endpoints;

1) Number of subjects in each group with $HbA_{1C} < 6.0\%$
2) Number of treatment failures in each group as defined by $HbA_{1C} > 6.0\%$and requiring the addition of rapid acting insulin with meals
3) Change in beta cell function
4) Change in whole body insulin resistance
5) Incidence of hypoglycemia (asymptomatic, symptomatic, serious)
6) Change in body weight, total body fat, and waist circumference
7) Change in carotid intima media thickness
8) Change in ankle-arm blood pressure
9) Change in stage of diabetic retinopathy

Change in GFR and urinary albumin secretion rate

Subjects who will complete 3-year follow-up and the repeat studies will continue to receive therapy as described above and continue in the follow-up visit every 3 months as previously described until the last subjects recruited to the study has completed 2-year follow-up. During this follow up period we will repeat the OGTT, eye photographs, DEXA and carotid ultrasound every other year (Year 3 and 6).

Washout Phase

At 6 years, all baseline studies will be repeated as stated above (OGTT, annual labs, eye photographs, ankle-arm blood pressure and carotid ultrasound). After the completion of the repeat studies, hepatic fat content and liver fibrosis will be measured with fibroscan and MRI Spectroscopy.
MRI Spectroscopy is the gold standard method to quantitate hepatic fat content. Pharmacologic therapy will be stopped if participants HbA1C is less than 7.5%. Participants with HbA1C greater than 7.5% and in the triple therapy arm, dapagliflozin (10mg/day) will be added. Participants with HbA1C greater than 7.5% in the conventional therapy arm will be switched to triple therapy. Patients will be seen every 3 months for a follow-up visit. Medical history, physical exam, body weight, blood pressure, Fasting blood sugar and HBA1c will be measured at each follow-up visit. The OGTT will be repeated at 3 months and every 6 months thereafter. Liver fat content and liver fibrosis will be measured with fibroscan annually. The 3-month follow-up visits will continue for additional 5 years, to obtain information about the long-term durability of triple therapy. Liver fat content and liver fibrosis will be measured with fibroscan and MRI annually, and the OGTT will be repeated every 2 years, and all baseline measurements will be repeated at 5 years.

Measurement of Hepatic fat content and Liver fibrosis: this measurement is made with fibroscan, which uses ultrasound wave sent through the liver from a probe placed on the skin above the liver and connected to an ultrasound machine. From the speed by which the ultrasound wave reflected back to the probe and the amplitude of the reflected wave, the machine calculates a score that reflects hepatic fat content and the degree of fibrosis in the liver. The measurement is non-invasive, lasts 10-15 minutes, does not use radiation, and there are no adverse events associated with it.

b. List of Research Procedures or Components (Components can be two or more procedures) (Click here for example)
(add rows or delete unused rows) (Consider as one component, multiple procedures that may lend themselves to having identical risks and benefits described in a single table in c, although exceptions exist to this guidance.)

Note that: A + B + C +D

#	Research components	Local Standard Practice required by the research plan	Research Only (Indicate # performed solely for research (research only) or indicate # performed outside frequency or timing for acceptable local practice)	Total Number of Research Related Procedures for 36 month study	Follow up Visits after completing 36 month study	Washout Phase (Patients who have completed 6years and HbA1C is less than 7.5%)
1	**OGTT**		Research only	**2**	***OGTT will be performed at years 3 and 6 after completing 36 months**	**OGTT will be performed at 6 year point and then after 2 years into the follow-up phase**
2	**DEXA**		Research only	**2**	**At last study visit**	
3	**Carotid intima media thickness**		Research only	**2**	**Carotid will be performed years 3 and 6 after completing 36 months**	**1**

4	**Ankle-arm blood pressure**		Research only	**2**	**Ankle-arm blood pressure will be performed at years 3 and 6 after completing 36 months**	**1**
5	**Hyperglycemic clamp**		Research only	**2**	**0**	
6	**Euglycemic clamp**		Research only	**2**	**0**	
7	**Fundus examination**	Standard practice		**2**	**Fundus examination will be performed at years 3 and 6 after completing 36 months**	**1**
8	**Initiation of pharmacological therapy**	Standard practice				**Maintain therapy if HbA1C is greater than 7.5% (with the addition of dapagliflozin)**
9	**Follow-up**		Research only	**14**	**Every 3 months after completing 36 months in years 3-6**	**Every 3 Months there will be a follow up for 5 years**
10	**FibroScan & MRI Spectroscopy (without contrast)**		Research only			**Measured annually**

c. Risk:Benefit Analysis

For each research procedure identified in section 6b above, complete a risk:benefit analysis table.
(Two tables are provided, copy and paste additional tables as needed)

Procedure or component 1 OGTT	
List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
Group I	None
Group II	None

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• None	• None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	• none	None
		Serious
Rare These risks are expected to occur in less than 5 [or insert different #] subjects out of 100	Local reaction at iv site Total blood loss for the procedure is up to 88 ml.	• none

Are all groups exposed to the risks listed above? X Yes ☐ No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

Procedure or component 2 DEXA	
List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
GROUP I	NONE
GROUP II	NONE

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• Radiation exposure (6 mrems). Although we cannot be sure that nothing will happen from such a small amount of radiation, we know that any risk is very small.	None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	None	• none
		Serious
Rare These risks are expected to occur in less than 5 [or insert different #] subjects out of 100	**none**	none

Are all groups exposed to the risks listed above? X Yes ☐ No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

Procedure or component 3 Carotid intima media thickness	
List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
GROUP I	NONE
GROUP II	NONE

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• none	None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	None	• none
		Serious
Rare These risks are expected to occur in less than 5 [or insert different #] subjects out of 100	**none**	none

Are all groups exposed to the risks listed above? ☐ Yes ☐No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

Procedure or component 4 Measurement of Ankle-Arm blood pressure	
List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
GROUP I	NONE
GROUP II	NONE

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• none	None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	None	• none
		Serious
Rare These risks are expected to occur in less than 5 [or insert different #] subjects out of 100	**none**	none

Are all groups exposed to the risks listed above? ☐ Yes ☐No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

Procedure or component 5 Hyperglycemic clamp	
List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
GROUP I	NONE
GROUP II	NONE

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• none	None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	None	• none
		Serious
Rare These risks are expected to occur in less than 5 [or insert different #] subjects out of 100	**Local reaction at IV sites** Total blood loss for the procedure is up to 255 ml.	none

Are all groups exposed to the risks listed above? ☐ Yes ☐No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

Procedure or component 6 Euglycemic clamp	
List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
GROUP I	NONE
GROUP II	NONE

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• none	None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	None	• none
		Serious
Rare These risks are expected to occur in less than 5 [or insert different #] subjects out of 100	**Hypoglycemia** **Local reaction at iv sites** Total blood loss for the procedure is up to 160 ml.	none

Are all groups exposed to the risks listed above? ☐ Yes ☐No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

Procedure or component 7 Fundus examination	
List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
GROUP I	NONE
GROUP II	NONE

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• none	None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	None	• none
		Serious
Rare These risks are expected to occur in less than 5 [or insert different #] subjects out of 100	**none**	none

Are all groups exposed to the risks listed above? ☐ Yes ☐No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

Procedure or component 8
Pharmacological Therapy with metformin, and pioglitazone plus exenatide

List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
GROUP I	This drug combination is likely to reduce your blood sugar

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• Nausea (exenatide) • Weight gain (pioglitazone) • Anemia (pioglitazone) Itching where Exenatide once weekly (Bydureon) was injected • Feeling tired (lack of energy or tiredness) • Injection site events (for example: lumps, hardening at the site of injection, bruising, redness of skin, itching, rash, and/or pain)	None
	Not serious	**Serious**

Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	• Abdominal pain (metformin) • Headache (pioglitazone) • Flatulence (pioglitazone) • Vomiting (exenatide metformin) Hypoglycemia (glyburide) with a sulfonylurea) Hypoglycemia (with metformin and a sulfonylurea • Diarrhea (mertformin, exenatide) At least 1, but fewer than 10 patients out of 100 patients reported: • Injection site events (for example: bruising, bleeding, pain, itching, redness of skin, swelling and/or small lump where Exenatide was injected) Dyspepsia Gastroesophageal reflux disease Nasopharyngitis Asthenia Feeling Jittery Decreased appetite Dizziness Hyperhidrosis Fewer than 1 out of 100 patients reported: • Stomach swelling • Stomach pain • Constipation • Gas • Burping • Abnormal taste sensation	• none
		Serious

Rare These risks are expected to occur in less than 1 subjects out of 100	Hypoglycemia Fewer than 1 out of 1000 patients reported: • Sleepiness • Itching • Hives • Spotty/patchy or swollen rash • Rapid swelling of the tissues around the neck, face, mouth, and/or throat indicating an allergic reaction • Hair loss • Dehydration (the loss of too much water from the body), because of nausea, vomiting, and/or diarrhea • Worsening kidney function • Inflammation of the pancreas Fewer than 1 out of 10,000 patients reported: • Hives/itching with difficulty breathing and/or low blood pressure, indicating a severe allergic (anaphylactic) reaction • Thrombocytopenia- a condition in which you have a low blood platelet count.	Heart failure (pioglitazone) Bone fracture (pioglitazone)

Are all groups exposed to the risks listed above? ☐ Yes ☐No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

Procedure or component 9 Pharmacological therapy, metformin, glyburide/Glipizide and insulin	
List each group exposed to this procedure on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this procedure (either the procedure or a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
GROUP II	This drug combination is likely to reduce your blood sugar

For this procedure or component, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 [or insert different #] out of 100 subjects.	• Hypoglycemia • Weight gain	None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5 - 20 [or insert different range] subjects or less out of 100 subjects.	• Abdominal pain • vomiting	• none
		Serious
Rare These risks are expected to occur in less than 5 [or insert different #] subjects out of 100	**none**	none

Are all groups exposed to the risks listed above? ☐ Yes ☐No ☐ N/A (There is only one group)
If No, Describe: [Describe here]

7. Safety Precautions. (The study should at a minimum describe safeguards to address the serious risks listed above.)

(1) Describe the procedures for protecting against or minimizing any potential risks for each of the research procedures listed above.

PROTECTION OF SUBJECTS. The PI will review all results on a weekly basis to ensure that the timeliness and conformity with established clinical monitoring of the subjects is followed.

1. Any subject with clinically significant cardiac, pulmonary, gastrointestinal, or endocrine (other than diabetes) disease will be excluded from study.
2. Prior to study, a venous hematocrit will be obtained. Volunteers with a hematocrit less than 34 vol % will be excluded from the study.
3. Any serious adverse event will be reported to the IRB within 24 hours. All non-serious adverse events will be reported by the investigator to the IRB within 72 hours. An annual report summarizing all adverse events will be submitted to the IRB.
4. The timeliness and accuracy of all information documented in any form and/or records pertaining to the study will be maintained.
5. blood sugar level will be measured every 5 minutes during the clamp to prevent the risk for hypoglycemia

6. following the initiation of therapy subjects will contacted via phone calls every two weeks to ensure that no serious side effects have happened.
7. Subjects will be examined during the follow-up (every 1-3 months) for potential side effects and for the quality of their blood sugar control. If severe side effects will appear, drug dose will be adjusted accordingly. If blood glucose will not be adequately controlled, therapy will be adjusted accordingly. Subjects who will receive full dose therapy in either study arm and their A1c remains > 8% will be considered treatment failure and will be returned to their doctor for their standard care.
8. During the euglycemic clamp blood sugar will be tested every 5 minutes and glucose infusion will be adjusted accordingly to maintain blood sugar at target and prevent hypoglycemia.

(2) Where appropriate, discuss provisions for ensuring necessary medical or professional intervention in the event of adverse events, or unanticipated problems involving subjects.

If hypoglycemia during the clamp occurs, the study will be stopped and 20% glucose infusion will be started until plasma glucose concentration is stabilized

(3) Will the safeguards be different between/among groups? ☐ Yes xNo

[If yes, describe here]

8. Available Alternative.

(1) Describe alternative treatments and procedures that might be advantageous to the subjects, should they choose not to participate in the study. This should include a discussion of the current standard of care treatment(s). In some cases the only alternative may be not to participate in the study. This section should match the alternative section of the CF (though not written in second person like the ICF).

although all drugs used in this study are approved by the FDA for lowering blood sugar and group I will be treated according to the ADA recommendations, the participation of patients in this study is completely voluntary. Subjects can choose not to participate in the study and be treated with other combination of currently approved drugs or other drugs not used in this study (acarbose, DPP 1V inhibitors).

9. Confidentiality.

(1) Specify where the data and/or specimens will be stored and how the researcher will protect both the data and/or specimens with respect to confidentiality.

Everything we learn about the subjects will be confidential. If we publish the results of the study in a scientific journal or book, we will not identify them in any way. The Food and Drug Administration of the U.S. Government may also want to see the records. We will not identify any subject by name in any publication. The consent forms will be stored in a locked cabinet in Room at the TDI. Subjects will be assigned a study number for identification purposes and the identification code will be stored in a locked cabinet in the PI's office (Room 3.380s, UTHSCSA).
Every subject will be assigned a code number, and Data will be stored only on the UTHSCSA computer system which is protected with passwords.

(2) Provide a time table for destroying the data/specimens and identify how they will be destroyed, (this is not necessarily the same answer as used in a HIPAA Waiver as that waiver may only apply to some of the information collected during the study) or provide rationale for perpetual maintenance.

Data sheets will be saved in un identified manner

(3) Specify who will access the identified data/specimens, why they need access and whether they will be authorized to remove PHI from the institution.

PI and co investigators in the study

10. Payment.

(1) Describe the incentives (e.g., inducements) being offered to subjects for their time during participation in the research study.

For Participants who will have clamps: Participants will receive during the 36 month portion of the study the following: Qualification Study Visit: $20, Study -OGTT & DXA @ $40 each (Before treatment and end of treatment) total of $80, INTIMA MEDIA THICKNESS @ $30 each (Before treatment and end of treatment) total of $60, INSULIN SECRETION TEST @ $100 each (Before treatment and end of treatment) for a total of $200, INSULIN SENSITIVITY TEST @ $100 each (Before treatment and end of treatment) for a total of $200, FOLLOW UP VISITS @ $20 each for total of $280 for all visits, After Treatment phase FOLLOW UP VISITS (@ $20 per visit for a max of 8) for a total of $160, Year 2 After Treatment Phase FOLLOW UP VISIT OGTT, DXA, & INTIMA MEDIA THICKNESS for a total of $70. Total amount of compensation participant could receive if all procedures and visits are completed is $1070.

For Participants who will not have a clamp: Participants will receive during the 36 month portion of the study and for the follow-up portions are as follows: Qualification Study Visit: $20, Study -OGTT & DXA @ $40 each (Before treatment and end of treatment) total of $80, INTIMA MEDIA THICKNESS @ $30 each (Before treatment and end of treatment) total of $60, FOLLOW UP VISITS @ $20 each for total of $280 for all visits, After Treatment phase FOLLOW UP VISITS (@ $20 per visit for a max of 8) for a total of $160, Year 2 After Treatment Phase FOLLOW UP VISIT OGTT, DXA, & INTIMA MEDIA THICKNESS for a total of $70. Total amount of compensation you could receive if completed all procedures and visits is $670.

For all participants the compensation during the Follow Up Visits after completing the 36 month treatment phase and Washout Phase are as follows: $20 for each follow up visit, $40 for each OGTT, $30 for intima media thickness measurement and $30 for the MRI.

Reimbursement for unexpected or unusual expenses related to travel or other circumstances related to study participation, but this may not exceed $100.

(2) If monetary compensation is offered, indicate how much the subjects will be paid and describe the terms and schedule of payment. (It is IRB policy that provision should be made for providing partial payment to subjects who withdraw before the completion of the research. Monetary payments should be prorated or paid in full.) The Veterans Health Administration prohibits payment to human subjects participating in research when the research is integrated with the patient's medical care and when the research makes no special demands on the patient beyond those of standard medical care. Payment may be permitted, with IRB approval, under certain circumstances. Refer to the Veterans Affairs Research and the UTHSCSA IRB web page to review circumstances under which payment is permitted for VA research.

Payments will be made in checks that will be mailed to their home address within 6 weeks of the study date

11. Costs to Subjects.

(1) Describe any costs for care associated with research (including a breakdown of standard of care procedures versus research procedures), costs of test drugs or devices, and research procedure costs that are the subject's responsibility as a consequence of participating in the research.

The cost of all tests performed and medication used in this study will be covered by the investigator. Participants will not be asked to cover any cost associated with the procedures or medications of the study

(2) Describe any offer for reimbursement of costs by the sponsor for research related injury care. (Attach a copy of the section of the clinical trial agreement or contract describing research related injury care).

Any injury related to the study will be treated either in the VA hospital (for patients studied in the GCRC of the VA hospital) or at the Texas Diabetes Institute.

12. PI-Sponsored FDA-Regulated Research.

(1) If the PI is the IND/IDE holder, the PI is also considered a sponsor. When the PI assumes a sponsor function, the PI must meet the requirements for the sponsor and the investigator. Describe your (the PI's) experience/knowledge/training (if any) in serving as a sponsor.

N/A

(2) Explain what measures you, the PI, will take/have taken to ensure understanding of your responsibilities as both PI and sponsor, and how you will ensure compliance with FDA regulations (e.g., detail plans for attending the pre-study visit & review of FDA regulations; describe procedures for informing the research team of their responsibilities and a plan for the monitoring function).

I have drafted the study protocol, which is consistent with the FDA regulation for treating diabetes. All study procedures and time table will be reviewed with the study team prior to the start of the study. A monthly meeting will be held with all study team to update on the study progress and to ensure that all procedures are done in compliance with the study protocol.

IRB policy requires additional CITI training (GCP) for all investigators who are also FDA-regulated sponsors. If the protocol receives IRB approval, the investigator will be required to complete this mandatory training. Information on the training will be included with the IRB approval materials.

Abstract / Project Summary

Provide a succinct and accurate description of the proposed research. State the purpose/aims. Describe concisely the research design and methods for achieving the stated goals. This section should be understandable to all members of the IRB, scientific and non-scientific. This summary will also be needed in future IRB Progress Reports.

DO NOT EXCEED THE SPACE PROVIDED.

Abstract

Type 2 diabetes mellitus (T2DM) is a common metabolic disease that affects 21 million Americans and is associated with significant morbidity and mortality secondary to micro- and macrovascular complications. Subjects with T2DM manifest insulin resistance in skeletal muscle, liver and adipocytes and impaired insulin secretion. Although many therapeutic agents have been developed to target the pathogenic abnormalities in subjects with T2DM, the current ADA recommendation for pharmacological therapy in T2DM remains based on monotherapy, with dose escalation, followed by sequential addition of oral antidiabetic agents and eventually insulin to control blood glucose levels. Unfortunately, the majority of type 2 diabetic individuals in the US fail to achieve the recommended level of glycemic control (HbA_{1c} <6.0%) using this approach. In this study, I propose to explore the efficacy of a novel therapeutic approach to attain glycemic control in T2DM subjects. This novel approach is based upon the initiation of triple combination therapy at the time of diagnosis with agents that target specific pathogenic abnormalities present in T2DM. Two groups of newly diagnosed, drug naive T2DM subjects will be studied: (i) Group I will receive triple combination therapy with metformin, pioglitazone, and exenatide as initial therapy; (ii) Group II will be started on metformin monotherapy followed by sequential addition of a sulfonylurea and then glargine insulin. The goal of therapy in both groups is to reduce the HbA_{1C} < 6.0%. Subjects will be recruited over one year and followed for 2 years (total study duration =3 years). The primary outcome will be the difference in HbA_{1c} between Groups I and II. Secondary outcomes will include failure to achieve the glycemic goal (HbA_{1C}<6.0%), improvements in insulin resistance, beta cell function, markers of inflammation, markers of oxidative stress, and carotid intima media thickness. I hypothesize that subjects started on combination therapy at the time of diagnosis of T2DM will have better and more durable glycemic control, a lower rate of therapeutic failure, enhanced insulin sensitivity, and improved/preservation of beta cell function compared to subjects receiving the "conventional" ADA-recommended stepped-therapeutic approach with "standard" medications. I also hypothesize that subjects started on initial combination therapy will have: (i) lower levels of inflammatory markers and (ii) reduced carotid IMT. On a long-term basis, one would expect the superior metabolic control in T2DM who receive aggressive combination therapy from onset to have a lower rate of diabetic complications.

The results of this study will provide a novel, more progressive therapeutic approach to the treatment of T2DM, based upon correction of known pathogenic abnormalities responsible for the disease.

Data Analysis and Statistical Methods

Values are presented as mean 6 SEM. Two-sided t test was used to compare mean differences between treatment arms, and the x2 test was used to test the significance of discrete variables. The Cox proportional hazards model was used to estimate the influence of therapy on failure to maintain the treatment goal. The model was adjusted for other confounders (namely, age, sex, BMI, disease dura-on, and baseline HbA1c level).

Sample size calculation:

The study was powered to detect a 0.5% (60.95 SD) HbA1c difference between the two treatment arms based on the HbA1c decrease in the PROactive study (22).We calculated that 76 pa-ents who completed the study would be required in each arm to detect significant difference between the two groups at a , 0.05. A detailed description of the sample size

calculation is given in the Supplementary Material.