Project Title: Effects of intervention with the GLP-1 analog liraglutide plus metformin versus metformin monotherapy in overweight/obese women with metabolic defects and recent history of gestational diabetes mellitus (GDM)

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Project Overview/Summary

The objective of the present proposal is to compare the clinical, endocrine and metabolic effects of therapy with combination liraglutide and metformin to metformin monotherapy in overweight women with a recent history of GDM. This study will serve as a pilot investigation to open perspectives for future investigations combining insulin-sensitizing drugs with different mechanisms of action in former GDM women especially overweight women for whom standard treatment with metformin is less effective

Primary Objective:

- To determine if the addition of liraglutide to metformin therapy is more effective than monotherapy with metformin in improving pancreatic beta cell compensation (ß-cell glucose sensitivity and rate sensitivity) in overweight/obese women with prior GDM who are at high risk of developing Type 2 diabetes (DM2).

Secondary Objective(s)

- To determine if the addition of liraglutide to metformin therapy is more effective than monotherapy with metformin in improving insulin sensitivity in overweight/obese women with prior GDM who are at high risk of developing Type 2 diabetes (DM2).
- To determine the impact of liraglutide in combination with metformin compared to metformin monotherapy on anthropometric measures in overweight/obese women with prior GDM who are at risk of developing DM2.
- To determine if the addition of liraglutide to metformin therapy is more effective than metformin alone in improving cardiometabolic markers (lipid profile and blood pressure) in overweight/obese women with prior GDM who are at risk of developing DM2.

Study Background and Rationale

Gestational diabetes mellitus (GDM) is defined as "any degree of glucose intolerance with onset or first recognition during pregnancy."[1] This simple definition belies the complexity of a condition that spans a spectrum of glycemia, pathophysiology, and clinical effects and for

which there is a wide diversity of opinion regarding detection and clinical management. GDM is one of the most frequent metabolic disorders occurring during pregnancy that complicates both, the course of pregnancy and the delivery and also has significant implications for the future health of the mother. Approximately 7% of all pregnancies in the United States are complicated by gestational diabetes resulting in more than 200,000 cases annually,[2] but the prevalence ranges from 1% to 14% of all pregnancies.[3] depending on the population studied and the diagnostic tests employed. Although the precise definition of gestational diabetes, its clinical significance and its treatment protocols remain controversial, it is clear that GDM increases the risk of pregnancy complications and strongly predicts the subsequent development of diabetes in the mother and her offspring.[4-6]

Insulin resistance and beta cell dysfunction are the main pathophysiological factors of GDM just as they are for diabetes outside of pregnancy.[7-8] Insulin resistance is a significant factor for many women who eventually develop GDM. It is possible that recurrent episodes of heightened insulin resistance, such as with recurrent GDM, place high demands on the pancreas and contribute to an eventual decline in beta cell function that leads to type 2 diabetes (DM2) in high-risk individuals. Peters and coworkers[9] studied 666 Latino women with a history of GDM. Among the 87 (13%) who completed an additional pregnancy, the rate ratio of DM2 increased significantly in comparison to women without an additional pregnancy. There may be a finite level of pancreatic "beta cell reserve" that is further depleted with recurring GDM. Although the longevity of beta cell function may be genetically determined, some individuals may respond to repeated demands on pancreatic insulin secretion (as seen in normal pregnancy, obesity, and polycystic ovarian disease) in a way that eventually exhausts beta cell reserve leading to the development of frank diabetes mellitus. After an index pregnancy with GDM, gestational diabetes recurs in 30-84% of subsequent pregnancies.[10,11] Interestingly, for the nonpregnant population, decreasing insulin resistance through diet and exercise or medications such as metformin or rosiglitazone has been shown to reduce the risk of subsequent type 2 diabetes or delay its onset.[12-14] Gestational diabetes is also a strong risk factor for the development of diabetes mellitus at a later stage of life in previous GDM woman. Among all the risk factors of DM2, the experience of GDM is the strongest one. The incidence of various forms of diabetes in this group balances from 10 to 60% over a period from 2 to 10 years. Russell et al[15] found that 36% of GDM women had some degree of persistent abnormal glucose tolerance when tested at

any time postpartum. Recently, Nelson et al[16] found that 8.9% of patients tested immediately postpartum had diabetes, and another 31.3% had other glucose metabolism abnormalities; only 55.8% had normal 2-hour glucose tolerance tests. Thus, women with previous GDM constitute an ideal group for primary diabetes prevention.

Gestational diabetes is a common medical problem that results from an increased severity of insulin resistance as well as an impairment of the compensatory increase in insulin secretion. A diagnosis of GDM has significant implications for the future health of the mother. Approximately 40% to 60% of women with a diagnosis GDM will exhibit further deterioration of carbohydrate metabolism. GDM is often the culmination of years of unrecognized and unmodified diabetes risk factors that lead to overt and occult clinical manifestations during pregnancy. Pregnancy, in essence, serves as a metabolic stress test and uncovers underlying insulin resistance and ß-cell dysfunction. Postpartum management of women with GDM is critical because of their markedly increased risk of DM2 in the future. Women with prior GDM have substantial rates of impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and DM2 after pregnancy. Women with a history of GDM are also at increased risk of other cardiovascular risk factors, such as obesity, hypertension, dyslipidemia, and the metabolic syndrome as well as subclinical atherosclerosis. Taken together, these findings suggest that GDM identifies a population of young women at increased risk for cardiovascular disease (CVD). Presently, in the literature, there are described new, more efficient methods of diabetes prevention in groups with a high risk of this disorder, which involve both, lifestyle modification and pharmacological therapies. In the 2.8 years of Diabetes Prevention Program (DPP) randomized clinical trial, diabetes incidence in a nonpregnant population with impaired glucose tolerance was reduced by 58% with intensive lifestyle intervention and 31% with metformin compared with placebo.[17] . Treatment of insulin resistance with the thiazolidinedione drug, troglitazone, improved insulin sensitivity and reduced the incidence of type 2 diabetes for nearly 5 years in Hispanic women with prior gestational diabetes[17]. During a median follow-up of 30 months on blinded medication, average annual diabetes incidence rates in the 236 women who returned for at least one follow-up visit were 12.1 and 5.4% in women assigned to placebo and troglitazone, respectively. Clinical studies using rosiglitazone or pioglitazone for a period of 3 years have demonstrated a beneficial effect of both agents in improving insulin sensitivity and recovery or improvement of ß-cell function, which are sustained in some individuals over

time.[19,20] Treatment of Hispanic women with a history of GDM with pioglitazone improved β-cell compensation for insulin resistance; 3 years of pioglitazone treatment given to Hispanic women with prior gestational diabetes was associated with stable pancreatic ß-cell function and a relatively low rate of diabetes (4.6% per year vs. 13.1% on placebo).[19] The use of rosiglitazone for 3 years in subjects with prediabetes resulted in a 60% reduction of the diabetes incidence rate.[20]

Incretin mimetics are a new class of pharmacological agents with multiple antihyperglycemic actions that mimic some effects of endogenous incretin hormones, including glucose-dependent enhancement of insulin secretion. Glucose lowering induced by glucagon-like peptide-1 (GLP-1) seems to be mediated by a potent insulin-releasing action as well as a range of other effects, including inhibition of glucagon secretion and gastric emptying, increased satiety, stimulation of glucose-uptake, and gluconeogenesis. GLP-1 also seems to exert trophic effects on the βcells, stimulating growth and differentiation and inhibiting cytokine- and free fatty acid- cytokine- and free fatty acid beta-cell-induced apoptosis.[21] Given the pivotal role of β-cell dysfunction in the progressive nature of GDM to DM2, the fact that certain GLP-1 analogs promote β-cell replication/neogenesis/mass in animal models is promising from the standpoint of modifying the pathophysiology of this condition. Liraglutide is a long-acting GLP-1 derivative, designed for once daily administration.[22-25] Liraglutide has 97% homology with GLP-1 and resists DPP-IV degradation by FA acylation and albumin binding.[26] Single-dose kinetic studies in DM2 subjects revealed a half-life of 10.0 ± 3.5 h, allowing for single daily-dose administration[27], whereas native GLP-1 with a very short half-life of 1.5 ± 0.35 min has limited clinical value.[28] The mechanisms of liraglutide action appear to be analogous to those exerted by endogenous incretins, thus liraglutide, can control diabetes by multiple actions through increasing insulin and lowering glucagon, yet it has a rapid and sustained glycemic effect and is associated with weight reduction.[29, 30] All actions are predictably strictly glucose-dependent, with low hypoglycemia risk and counterregulatory response to hypoglycemia not being impaired. Liraglutide has been shown to enhance several ß-cell function parameters, and the enhancement was correlated with the improvement in glycemic control.[31] Liraglutide may potentially delay disease progression in GDM considering the ß-cell function improvement in DM2 and ß-cell mass shown to increase in animal models.[32, 33]

Rationale

Women with previous GDM comprise a target group for future intervention trials with the aim to prevent or delay development of DM2. While a large number of studies have demonstrated reductions between 25 and 67% in the incidence of diabetes over 2.5 to 6-year intervention periods in subjects with prediabetes (IFG, IGT or both), most participants remain in a pre-diabetic state. Less often presented were the number of participants who did not progress to diabetes but in fact had a reduction in insulin resistance and normalization of pancreatic beta cell function. Postpartum follow up of women with a history of GDM is inadequate. The risk level for women with GDM suggests that these women may benefit from both preventive interventions and regular screening.

We evaluated 110 women having GDM in their index pregnancies between 6-12 weeks after delivery. A 75-g oral glucose tolerance test (OGTT) was performed with insulin and glucose measured. From the oral glucose tolerance (OGTT) data, it is possible to derive reliable indexes of insulin sensitivity and pancreatic beta-cell (ß-cell) function. [35-45] We quantified insulin sensitivity using the OGTT insulin sensitivity index of Matsuda and DeFronzo (SI_{OGTT}) because of its simplicity and good correlation with measurements generated by the clamp technique (the gold standard).[37] Fasting insulin resistance index was calculated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR).[36]. ß-cell secretory capacity was determined by dividing the insulinogenic index (IGI= Ins30-0/Glu30-0) by HOMA-IR (IGI/HOMA-IR) and by calculating the insulin secretion-sensitivity index (IS-SI).[40-45] The IS-SI expresses the overall ability of the beta cell to increase its release rate relative to insulin resistance in response to a glucose stimulus and reveals the progressive ß-cell dysfunction originally demonstrated using the disposition index. Our research was designed to evaluate the insulin secretory responses to oral glucose in women with former gestational diabetes (fGDM), and to determine if the insulin secretory response present in these subjects is appropriate for the degree of insulin resistance.

Insulin sensitivity and ß-cell secretory capacity derived from fasting values (HOMA-IR) and glucose-stimulated measures (SI_{OGTT} and IGI/HOMA) were calculated for all 110 former gestational diabetic (fGDM) women. Our findings demonstrated that measurement of insulin status using the 2 h-OGTT can actually identify distinct groups of fGDM women with differences in insulin sensitivity, β-cell function, and glycemia in the early postpartum period.

When only OGTT glucose results were used, 24 (22%) fGDM women showed some form of glucose abnormality (IFG, IGT or IFG/IGT). Insulin resistance determined by the HOMA-IR model was observed in 46 (42%) fGDM of the assessed women within 3 months of delivery while the SI_{OGTT} and IGI/HOMA identified 98 (89%) fGDM women with a postpartum metabolic abnormality. Based on OGTT-derived indices of insulin secretion, it was quite clear that ß-cell function progressively deteriorates with worsening of glucose tolerance. Poor ß-cell compensation for insulin resistance was evident in all fGDM women with abnormal glucose tolerance (DM2, IFG and/or IGT). Furthermore, we observed that 68 (62%) fGDM women had one or more lipid level abnormalities (LDL, TRG or TRG/HDL ratio.). These abnormal lipid profiles were more prevalent in white fGDM women (65%) compared with non-white fGDM women (50%). However, non-white fGDM had significantly higher blood pressure than white fGDM women. The purpose of this study trial is to compare the efficacy of pharmacological treatment with liraglutide in combination with metformin to metformin monotherapy in a population of overweight women with a history of GDM who are at high risk for the development of diabetes. This study will examine if the addition of liraglutide to metformin therapy is more effective than metformin alone in improving metabolic parameters in overweight/obese women with prior GDM who are at risk of developing DM2. No studies to date have examined the use of GLP-1 receptor analogues in the treatment of women with previous GDM. We predict that the administration of liraglutide in combination with metformin will be more effective in improving ß-cell compensatory function than metformin alone. Given the ability of the combination therapy to prevent or delay a decline in glucose tolerance, we also will determine whether combination liraglutide-metformin treatment alters the development or progression of cardiometabolic risk more effectively than monotherapy with metformin.

Study Design

Subjects will be recruited using flyers distributed in the obstetric clinics and pathology laboratory associated with Woman's Hospital. Overweight females who are ≥18 years of age, had GDM in the previous 12 months (diagnosis of GDM using Carpenter-Coustan criteria according to Fifth International Workshop—Conference on Gestational Diabetes Mellitus[2]) and meet all inclusion/exclusion criteria will be offered participation in the study.

Major Inclusion Criteria

- Adult female ≥18 years to 45 years of age who experienced GDM within 52 weeks of index pregnancy
- Actual BMI ≥25 kg/ m2
- Written consent for participation in the study
- Patient completed lactation
- Dysglycemia (impaired fasting glucose [IFG}, impaired glucose tolerance [IGT} or IFG/IGT) and/or ß-cell dysfunction postpartum requiring pharmacological intervention (except type 1 or 2 diabetes)

Major Exclusion Criteria

- Personal or family history of medullary thyroid carcinoma or in patients with Multiple Endocrine Neoplasia syndrome type 2
- History of pancreatitis
- Significant cardiovascular, cerebrovascular, renal, or hepatobiliary diseases in the past (viral hepatitis, toxic hepatic damage, jaundice of unknown etiology)
- Serum AST and/or ALT level exceeding more than twice normal laboratory values
- Uncontrolled clinically significant hypertension Fasting serum triglycerides ≥800 mg/dl at screening.
- Lipid-lowering medications must have been maintained at the same dose for 3 months prior to enrollment
- Cholestasis during the past pregnancy
- Presence of contradictions for GLP-1 receptor agonist or metformin administration such as allergy or hypersensitivity
- Current use of metformin, thiazolidinediones, dipeptidyl peptidase-4 inhibitors or GLP-1 receptor agonist medications.
- Use of drugs known to exacerbate glucose tolerance.
- Use of prescription or over-the-counter weight-loss drugs
- Diabetes postpartum or history of diabetes or prior use of medications to treat diabetes except gestational diabetes

- Renal impairment (e.g., serum creatinine levels ≥1.4 mg/dL for women and eGFR less than 60 mL/minute/1.73 m^2
- History or currently undergoing chemotherapy or radiotherapy for cancer
- Pregnancy planned during the coming two years
- Currently breastfeeding
- Exclusion criteria include any condition, which in the opinion of the investigator would place the subject at increased risk or otherwise make the subject unsuitable for participation in the study

All subjects will verbally provide a medical and gynecological history, and if they are eligible and sign a medical release form, their prenatal records will be obtained to confirm their pregnancy history and postpartum status. If eligible, participants will give written informed consent for participation in the Woman's Hospital Foundation Institutional Review Board-approved study. An oral glucose tolerance test (OGTT) with glucose (G) and insulin (I) measured at 0, 30, 60, and 120 after glucose load to assess diabetes, insulin resistance and pancreatic ß-cell function will be performed between 6-52 weeks postpartum. Glucose tolerance will be defined as normal, impaired glucose tolerance (IGT) or diabetic according to the criteria of the American Diabetes Association. [34] A measure of insulin sensitivity will be estimated using a single fasting G-I level by the homeostasis model assessment index (HOMA-IR). From the OGTT, we will use the derived composite whole-body insulin sensitivity index (ISI_{OGTT}). Beta cell function will be resolved by mathematical modeling from the OGTT. The insulinogenic index (IGI; $\Delta I/\Delta Glu_{30-0}$) divided by HOMA and corrected insulin response at glucose peak (CIR_{GluPea}); indexes of ß-cell activity, will be computed from insulin and glucose levels measured during the OGTT. An insulin secretion-sensitivity index (IS-SI) will be derived by applying the concept of the disposition index (which expresses the relationship between insulin sensitivity and insulin secretion) to measurements obtained during the 2-h OGTT .This index expresses the overall ability of the beta cell to increase its release rate relative to insulin resistance in response to a glucose stimulus. This approach of interpreting B-cell function is critical because the amount of insulin secreted by the ß-cell is very dependent on the prevailing degree of level of peripheral insulin sensitivity and reveals the progressive loss of ß-cell function in individuals with impaired glucose tolerance. [35-45] Only those women with previous GDM who

demonstrate dysglycemia (IFG ,IGT or IFG/IGT) and/or ß-cell dysfunction that necessitates medication added to diet and exercise intervention will be recruited for the study and thus will not be inclusive of women with diabetes postpartum. The baseline blood samples from the OGTT will also be analyzed for a lipid profile which includes total cholesterol, triglycerides, HDL and LDL cholesterol, TSH, liver enzymes, creatinine with eGFR, and quantitative ßhCG levels. A negative serum pregnancy test is a prerequisite for commencing treatment.

All consented patients will come into the outpatient tower for a clinic visit. A full physical examination will be performed including measurement of body mass index (BMI), waist: hip (W: H) ratio and blood pressure (BP). The total body adiposity (total fatness), defined as the accumulation of body fat without regard to regional distribution, will be expressed as BMI and calculated as weight (kg)/ height (m) ^{2}, whereas the W:H ratio is a measure of body fat distribution. Height will be measured to the nearest centimeter and weight measured by a sliding weight balance to the nearest 0.1 kg. Waist circumference will be measured at the minimum circumference between the rib cage and iliac crest (in centimeters) and hip circumference at the level of the largest circumference around the buttocks by trained clinicians using tape measures from the same manufacturer. The circumference measurements will be taken in the upright position using 15-mm width flexible metric tapes held close to the body but not tight enough to indent the skin. Cardiovascular risk will be assessed biochemically using lipoprotein profile (total cholesterol, triglycerides, HDL, calculated LDL), and by total body fat and its distribution W: H ratio.

Randomization and Allocation

At randomization, all study participants will be assigned to an 80 [+4 weeks] medication treatment group. A computer-generated randomization schedule using a block randomization method will be used. The treatment allocation ratio will be 1:1. Drug will be packaged and labeled according to the randomization schemes. Subjects who are randomized will be assigned the next sequential subject number. Once randomized, the subjects will begin treatment with the allocated study drug or placebo. All personnel involved in the study will be blinded to which treatment arm the patients have been randomized. The drug and placebo will look identical and not have any identifying characteristics.

Subjects will be equally randomized to one of two treatment groups: Metformin + Liraglutide or Metformin + Placebo. All patients will be taking either liraglutide 6 mg/ml or

placebo solution for injection in a 3 ml pre-filled pen which is self-administered via subcutaneous injection once per day in the abdomen or thigh. For patients taking liraglutide, treatment will be initiated at 0.6 mg subcutaneous, increased to 1.2 mg and then to 1.8 mg subcutaneous, as tolerated, during the 4-wk non-forced dose-escalation period up to a maximum allowed dose of 1.8 mg subcutaneous daily. Placebo-treated subjects will undergo the same non-forced dose-escalation regimen, using matching injections. All patients will be receiving therapy with metformin which will be started at an initial dose of metformin of 500 mg daily (with dinner) for 2 weeks. They then will be increased to a metformin dose of 500 mg BID (breakfast and dinner). The dose will be increased to 500 mg am, 1000 mg pm (with breakfast and dinner) for 2 weeks and then increased to the final dose of 1000 mg BID (breakfast and dinner) until the end of the study. For patients who cannot tolerate the full dose of metformin in combination with liraglutide, the metformin dose will be adjusted at the discretion of the physician to a level that is tolerable. The physician investigator or research nurse will dispense a ten-week supply of the appropriate study medication to the subject at the time of randomization and every ten weeks thereafter. All patients will have a urine pregnancy test performed to document they are not pregnant prior to receiving the next 10 weeks of medication. Appointments will be made to return every ten weeks to receive a new supply of medication. All patients will receive the same counseling concerning the benefits of lifestyle modification through diet and exercise. Patients in each group will receive standardized dietary advice and appropriate written information on a balanced weight-reducing diet. The patients will be also encouraged to increase daily exercise (such as walking, using stairs), although this will not be formally assessed. The participants will receive further encouragement to adhere to the regime at their 32 (+4) week, 56 (+4) week and 80 (+4) week review visits and during follow-up phone calls.

Oral glucose tolerance tests with glucose (G) and insulin (I) measured at 0, 30, 60, and 120 after glucose load to assess diabetes, insulin resistance and pancreatic ß-cell function will be performed 32 (+4) weeks after study medications are started. Testing will be repeated at 56 (+4) weeks and 80 (+4 weeks) after treatment start. The baseline blood samples from the OGTT will also be analyzed for liver enzymes, TSH, creatinine with eGFR (baseline and 56 weeks only), and lipid profile. Subjects will remain on treatment unless they are diagnosed with DM2 during an OGTT or a random blood test at which time final testing will be performed. They will be withdrawn from the study and they will be referred to a specialist for additional treatment of

diabetes. In subjects who complete 80 (+4) weeks of treatment, all study medications will be stopped and subjects will be asked to return for routine OGTT testing by their physician.

Primary Study Endpoint:

- An index of *insulin secretion in relation to insulin resistance (IS-SI)* will be calculated. Thus, β-cell compensatory capacity will be evaluated by the insulin sensitivity-secretion index (IS-SI) defined as the product of composite insulin sensitivity index and first-phase insulin release index (insulinogenic index).

Secondary Study Endpoint(s):

- Indexes of insulin sensitivity and secretion using the serum glucose and insulin concentrations obtained in the fasting state and during the 2hr OGTT with INS will be computed by several measures previously validated in women. Changes in insulin resistance –baseline {HOMA-IR} and composite insulin sensitivity index [ISI_{OGTT}], and pancreatic ß-cell function (corrected insulin response [CIRglupeak] and insulinogenic index [IGI])/HOMA-IR
- Anthropometric measurements [BMI, absolute body weight, waist circumference, waist: hip ratio,
- Cardiometabolic risk measures [lipids, liver enzymes; blood pressure]
- Development of diabetes, impaired glucose tolerance or impaired fasting glucose based on positive OGTT. Glucose tolerance will be defined as normal, impaired glucose tolerance (IGT) or diabetic according to the criteria of the American Diabetes Association.[1] Patients diagnosed with diabetes will be withdrawn from the study and referred to a specialized physician.

Biochemical assays and calculations

Laboratory Measures: Hormonal and metabolic parameters will be measured at baseline (6-52 weeks postpartum), and after 32 (+4) weeks, 56 (+4) weeks and 80 (+4) weeks treatment intervention following an overnight fast. Blood samples will be obtained in the fasting state and ½, 1 and 2 h after a standardized 75-g oral glucose load. Blood samples will be centrifuged, divided into aliquots, and stored at –70°C until assayed. Plasma glucose levels will be determined with a glucose analyzer using the glucose oxidase method (Glucose Reagent Kit, Bayer Newbury, UK). Plasma insulin will be determined in all samples in duplicate by microparticle enzyme immunoassay (Abbott AxSYM System, Abbott Laboratories, Abbott Park,

IL). Levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides will be determined in the initial basal sample using standard enzymatic colorimetric assays on an automated clinical chemistry analyzer whereas low-density lipoprotein cholesterol (LDL-C) will be calculated according to the Friedewald equation. Serum creatinine, liver enzymes, AST, and ALT will be measured using standard automated kinetic enzymatic assay. Circulating levels of TSH and ßhCG will be measured using a two-site sandwich immunoassay with direct chemiluminometric technology (Diagnostic Products, Los Angeles, CA). The intra- and interassay coefficients of variation are less than 7 and 11%, respectively, over the sample concentration range.

Insulin sensitivity and secretion:

Indexes of insulin sensitivity and secretion using the serum glucose and insulin concentrations obtained in the fasting state and during the 2hr OGTT with INS will be computed by several measures previously validated in premenopausal women.[35] Insulin resistance/sensitivity will be calculated using homeostasis model assessment (HOMA-IR), which is based on fasting insulin and glucose concentrations[36] and the Matsuda insulin sensitivity index, which takes into account mean insulin and glucose levels following oral glucose stimulation. [37]

Basal insulin resistance measurement The homeostasis model assessment of insulin resistance (HOMA-IR) will be calculated from fasting plasma glucose and insulin concentrations according to the report by Matthews et al. [36] with the formula: HOMA-IR = FIRI x FPG divided by 22.5, where FIRI is the fasting insulin concentration (microunits per milliliter) and FPG is the fasting glucose level (millimoles per liter). Lower HOMA-IR values indicate greater insulin sensitivity, whereas higher HOMA-IR values indicate lower insulin sensitivity (insulin resistance).

Indexes of insulin sensitivity from the OGTT. Originally proposed by Matsuda and DeFronzo[37], the composite whole body insulin sensitivity index (ISI_{OGTT}) is an insulin sensitivity index that reflects a composite estimate of hepatic and muscle insulin sensitivity determined from OGTT data. The ISI_{OGTT} is a measure of peripheral insulin sensitivity derived from the values of insulin (microunits per milliliter) and glucose (milligrams per deciliter) obtained from the OGTT and the corresponding fasting values. $ISI_{(OGTT)} = 10{,}000/\sqrt{[(G_{fasting} \times I_{fasting}) \times (G_{OGTTmean} \times I_{OGTTmean})]}$, where fasting glucose and insulin data are taken from *time 0* of the OGTT and mean data represent the average glucose and insulin values obtained during the entire OGTT. The

square root is used to correct for nonlinear distribution of insulin, and 10,000 is a scaling factor in the equation. ISI_{OGTT} correlates reasonably well with estimates of whole body insulin sensitivity determined by the glucose clamp. The fasting component reflects hepatic insulin sensitivity, whereas the mean of the dynamic data primarily represents skeletal muscle insulin sensitivity. This partitioning concept has recently been validated using glucose clamp studies.[38]

Insulin secretion was estimated by two methods. **Insulin secretory function** will be estimated from the OGTT using the *corrected insulin response at peak glucose* ($CIR_{GluPeak)}$ calculated as (100 x Insulin $_{glucose\ peak}$[microunits per milliliter)]/{Glu $_{glupeak}$(milligrams per deciliter)] - [Glu $_{glupeak}$(mg/dl)])] –70 mg/dl)].]}.[39] Corrected insulin response is a useful indicator of ß-cell activity: the higher the CIR_{GluPea}, the greater the insulin secretion for the same glucose stimulus.

Early phase insulin secretion (*insulinogenic index [IGI]*) is calculated as the ratio between serum insulin and blood glucose concentrations 30 min after the glucose load.[40] The IGI is calculated from the OGTT data as the ratio of the increment of insulin to that of glucose 30 min after a glucose load {Δinsulin (0–30 min) in microunits per milliliter divided by the Δglucose (0–30 min) in mg per deciliter.[41] This index has been shown previously to correlate strongly with the first phase of insulin response following an intravenous glucose tolerance test.[42] Pancreatic β-cell function [IGI/HOMA-IR] is best estimated by the acute insulin response to glucose corrected for by the relative insulin resistance.[43]

To evaluate beta cell function in relation to insulin sensitivity, an *insulin secretion-sensitivity index* (IS-SI) will be calculated from the product of the insulinogenic index (IGI) and the ISI_{OGTT} based on the existence of the /predicted hyperbolic relationship between these two measures.[44] Based on this relationship of insulin sensitivity and insulin secretion, changes in insulin secretion may reflect one of three scenarios regarding ß-cell function. No change in the relationship would result in ß-cell function moving along the curve. The second would be one in which ß-cell function was improved; this would be characterized by a change in the relationship between insulin sensitivity and the insulin response with a shift to the right of or above the curve. Finally, a decrease in ß-cell function would be characterized by a shift to the left of or below the curve. The insulin secretion/insulin resistance (disposition) index calculated as the product of insulin secretion measured with ($\Delta I_{0-30}/\Delta G_{0-30}$) and ISI_{OGTT} has been shown to have excellent power to predict onset of type 2 diabetes.[45]

Concomitant Medications and Therapy:

None of the patients will be allowed to take medications likely to influence metabolic profiles except thyroid supplementation. The following medications cannot be used immediately prior to or concomitant with the treatment therapy; drugs known to affect gastrointestinal motility, lipid lowering agents (statins), other medications known to affect carbohydrate metabolism (glucocorticoids, anabolic steroids) or anti-diabetes medications (metformin, thiazolidinediones, GLP-1 receptor agonists, pramlintide; DPP4 inhibitors). Vitamins and topical medications can continue to be used. Barrier contraception, IUD and hormonal contraceptives are also permitted.

Safety Measures:

This protocol and the associated Informed Consent as well as any addenda or amendments, must be reviewed and approved by the Woman's Hospital Foundation Institutional Review Board (WHIRB) review committee prior to the start of the study. All revisions to this Protocol are considered "protocol amendments" these must be approved in advance, in writing, by the WHIRB. Every patient will have given her written informed consent prior to participating in the study. Prior to participation in this trial, each subject will have an opportunity to ask questions and will sign (and date) a written Informed Consent, which must be witnessed. The signed consent forms will be filed with the investigator's study charts for each subject. Any subject may voluntarily withdraw from the study at any time without prejudicing treatment.

Patients will be educated about the side effects and use of liraglutide 6.0 mg/ml for injection in a 3 ml pre-filled pen and the injection system. The most common adverse events related to liraglutide are headache, nausea, dyspepsia and vomiting, and are generally of mild-to-moderate intensity. Liraglutide is classified as a pregnancy category C drug. The GLP-1 receptor agonist exenatide has been shown to cause reduced fetal and neonatal growth in mice. Irregular fetal skeletal growth has been reported in rabbit and mouse models at dosages significantly higher than the normal dosage range. Four unintended pregnancies were reported during GLP-1 receptor agonist exenatide clinical development program through 30 June 2004. GLP-1 receptor agonist treatment was discontinued immediately. The pregnancies resulted in healthy babies born at 35, 37, and 39 weeks of gestation (one woman had an elective abortion). Two of the subjects were using oral contraceptives and also received antibiotic agents approximately 1 month prior to discovery of the pregnancies. Based on this limited experience, there is no indication of any detrimental effects of GLP-1 receptor agonists on either the mother

or the fetus during pregnancy. In the event of pregnancy, it is unlikely that liraglutide would cause any direct untoward fetal effects, given the very low potential for liraglutide to cross the placental barrier. The drug has not been adequately studied in nursing mothers or pediatric patients. In previous studies, the GLP-1 receptor agonist exenatide has been found in the milk of lactating mice in low concentrations, so it should be used with caution in nursing mothers. If patients become pregnant during the study, liraglutide will be stopped immediately.

Patients starting metformin therapy will be advised that they may have minor gastrointestinal side effects. The most common side effects of metformin are diarrhea, nausea or vomiting, flatulence, indigestion, abdominal discomfort and rarely, a metallic taste in the mouth. These acute reversible adverse effects occur in 5–20% of patients treated with metformin. The symptoms are dose related and remit if the dose is reduced, sometimes an increase in the dose can later be tolerated. Taking the drug with or after food, and starting therapy with low dosages that may be increased slowly can minimize these. The dose can then be increased slowly at intervals of two weeks. Lactic acidosis is the biguanide-related adverse effect of most concern with an estimated incidence of less than 0.01 to 0.08 cases. Should a patient have lactic acidosis attributable to metformin, the drug can be removed by hemodialysis. Other contraindications to the use of metformin include concurrent liver disease and a previous history of lactic acidosis. Metformin therapy will also be stopped if the blood lactate concentration is substantially increased by any illness. Metformin therapy will be temporarily suspended for all major surgical procedures that involve restriction of fluid intake. Metformin is classified as a United States Food and Drug Administration category B drug (i.e., "no evidence of risk in humans"). This means that, while there is no evidence of teratogenesis or adverse fetal effects, insufficient data exist to state that harm does not occur. Metformin does cross the placenta, prompting a cautious approach to its use in pregnancy. There have been several published reports of the use of metformin during pregnancy, predominantly in women with insulin resistance and PCOS. Some clinicians routinely use metformin to treat diabetes in pregnant women. However, most experts believe that if medication is needed to control blood sugar during pregnancy, insulin is the drug of choice, not an oral antihyperglycemic agent. If patients become pregnant during the study, metformin will be stopped immediately.

For safety, all subjects who enter the study are evaluable. Subjects will be monitored for safety by assessment of adverse events, physical exams, vital signs and laboratory values.

Continued patient safety assessment will be carried out and all adverse events documented and reported to the WHIRB. On each visit, compliance with treatment will be checked with questions about the side-effects and a subjective evaluation of the tolerability of the administered drug; the patients will also asked about incidental missed administrations.

Adverse Event Procedures

An adverse event is defined as any untoward medical occurrence in a clinical study subject administered a pharmaceutical product, and which does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease that is temporally associated with the use of a pharmaceutical product, whether or not it is considered related to that product. All observed or volunteered adverse events regardless of treatment group or suspected causal relationship to study drug will be recorded in the patient's record. Events involving adverse drug reactions, illnesses with onset during the study, or exacerbations of pre-existing illnesses will be recorded. Exacerbation of pre-existing illness may include worsening or increase in <u>severity</u> of signs or symptoms of the illness, increase in <u>frequency</u> of signs and symptoms of an intermittent illness, or the appearance of <u>a new manifestation/complication.</u> Exacerbation of a pre-existing illness should be considered when a patient/subject requires new or additional concomitant drug or non-drug therapy for the treatment of that illness during the trial. Lack of or insufficient clinical response, benefit, efficacy, therapeutic effect, or pharmacologic action, will not be recorded as an adverse event. The investigator will make the distinction between exacerbation of pre-existing illness and lack of therapeutic efficacy. In addition, clinically significant changes in physical examination findings and abnormal objective test findings (e.g., laboratory, x-ray, ECG) will also be recorded as adverse events. Any abnormal test result that is determined to be an error does not require reporting as an adverse event.

In order to avoid vague, ambiguous, or colloquial expressions, the adverse event term will be recorded using standard medical terminology rather than the subject's own words. Every attempt will be made to describe the adverse event in terms of a diagnosis. All related signs, symptoms and abnormal test results will be grouped together as a diagnosis if applicable and recorded as a single adverse event. All adverse events will be evaluated for <u>intensity</u> and <u>causal relationship</u> with use of the study medication. For all adverse events, sufficient information

should be obtained by the investigator to determine the causality of the adverse event (i.e., study drug or other illness). Follow-up of the adverse event, after the date of therapy discontinuation, is required if the adverse event or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator or his/her designated representative. Clinically significant changes, in the judgment of the investigator, in physical examination findings (abnormalities) will be recorded as adverse events. All subjects who have adverse events, whether or not the adverse events are considered associated with the use of the study medication, will be monitored until the adverse event resolves, stabilizes, or becomes chronic. The clinical course of the adverse event will be followed according to accepted standards of medical practice, even after the end of the observation period, until a satisfactory explanation for the adverse event is found or the investigator considers it medically justifiable to terminate follow-up.

SERIOUS ADVERSE EVENTS: A SERIOUS ADVERSE EVENT (SAE) IS ANY ADVERSE DRUG EXPERIENCE OCCURRING AT ANY DOSE THAT:

1. results in death;
2. is life-threatening (adverse drug experience that places the patient/subject at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death);
3. results in inpatient hospitalization or prolongation of existing hospitalization;
4. results in a persistent or significant disability/incapacity (defined as a substantial disruption of a person's ability to conduct normal life functions); or
5. results in congenital anomaly/birth defect.
6. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse drug experiences when, based upon appropriate medical judgment, they may jeopardize the patient/subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe pain); the event itself, however, may be of relatively minor medical

significance (such as severe headache). By contrast, the term "serious" is used to describe an event based on an event outcome or actions usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. The collection of serious adverse event information will begin at the signing of informed consent and continue through 30 days after administration of the last dose of study medication. Regardless of the above criteria, any additional adverse experiences which an investigator considers serious should be immediately reported. If an SAE occurs, the investigator should initiate appropriate support procedures.

All serious adverse events regardless of treatment group or suspected relationship to study drug must be reported to MedWatch in an expedited manner. The investigator will determine expectedness by referring to the most current version of the Investigator's Brochure in conjunction with any sponsor-generated IND Safety Reports. Since the trial is being conducted under a physician's IND, Drs. Harris and Elkind-Hirsch are responsible for submitting all IND Safety Reports to the Food and Drug Administration (FDA). The FDA 3500A Form with built-in instructions will be downloaded at: http://www.fda.gov/medwatch/getforms.htm. They will also be reported immediately to the Woman's Hospital Foundation Institutional Review Board at (225) 924-8516 and Woman's Health Research Department at (225) 231-5275. After submitting an expedited MedWatch 3500A report to the FDA, a courtesy copy will be sent by fax to NovoNordisk. NovoNordisk Safety personnel may fax a follow-up form to the study site to request additional information or clarification of information that was included on the FDA 3500A Report. Queries that address medical issues must be signed by a medically qualified (MD, DO) principal or sub-investigator. In cases where the investigator learns of the SAE after its occurrence and resolution, the time and circumstances of the event will be recorded. The reporting requirements will still be followed.

Any serious adverse event or death will be reported immediately independent of the circumstances or suspected cause if it occurs or comes to the attention of the investigator at any time during the study through the last follow-up visit required by the protocol or 30 days after the last administration of study drug, whichever comes later. Any serious adverse event occurring at any other time after completion of the study will be promptly reported if a causal relationship to study drug is suspected. The only exception to these reporting requirements are serious adverse events that occur during a pre-randomization/washout run-in period, during

which placebo alone or no active study drug or no protocol-specified background drug is administered.

Discontinuations:

The reason for a subject discontinuing from the study will be recorded in the patient chart. A discontinuation occurs when an enrolled subject ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The investigator must determine the primary reason for discontinuation. Withdrawal due to adverse event will be distinguished from withdrawal due to insufficient response according to the definition of adverse event noted earlier. The final evaluation required by the protocol will be performed at the time of study discontinuation. The investigator will record the reason for study discontinuation, provide or arrange for appropriate follow-up (if required) for such subjects, and document the course of the subject's condition. They will also to be reported to Woman's Hospital Foundation Institutional Review Board at (225) 924-8516 and Woman's Health Research Department at (225) 231-5275.

Sample Size Computation and Power Analysis

A priori sample size analysis was performed using the online calculator provided by the Massachusetts General Hospital Mallinckrodt General Clinical Research Center (http://hedwig.mgh.harvard.edu/sample_size/size.html). Because the possible advantages of combination treatment over metformin alone rely on the presumed positive effects of liraglutide on pancreatic ß-cell function parameters, we used the difference in a surrogate marker of insulin secretion relative to insulin resistance as the primary outcome for sample size analysis. An experimental design including a total of 118 patients would have power above 0.80 to detect differences in the IS-SI index between treatments such as those previously reported using a similar experimental design.[46]

We plan on recruiting 150 overweight women with a recent history of GDM to be studied. The estimated enrollment: of 75/group for an average follow up of 60 weeks of treatment (range 32-84 weeks) will be our intent-to-treat population. This sample size is projected to provide power ≥0.8 to detect a ≥20% difference in insulin action indices between treatment groups at a median follow-up of 24 months. The calculation was adapted to allow for significant dropout rate (20-22%) for completion of the full trial of 80 (+4) weeks of treatment. Subjects who drop out of the trial will not be replaced

Statistical Analysis Plan:

The primary outcome measure is the insulin sensitivity-secretion index (IS-SI) and secondary outcome measures include changes in lipid and hepatic enzyme levels, anthropometric parameters, insulin sensitivity and pancreatic ß-cell function, and glucose tolerance status. Continuous variables will be tested for normality of distribution using the Kolmogorov-Smirov test. Natural log transformations of skewed variables will be used where necessary in subsequent analyses. For variables with highly skewed distributions, a logarithmic transformation will be done first and the geometric means reported instead. Differences in effects of drug intervention for continuous variables (in their raw or transformed state) will be compared using Subjects/Drug Treatments by trials analyses of variance (ANOVAs). This analysis provides *p* values for differences between groups/drug treatments (A), differences over time (B), and the interaction of group/drug treatment with time (AB). Otherwise, changes between baseline and follow-up measurements will be compared between groups by Wilcoxon's nonparametric methods. Anthropometric measurements (body weight, BMI), blood pressure, direct and indirect measurements of carbohydrate metabolism (HOMA, SI_{OGTT}, CIRgp, IGI, and ISSI), and measurements of lipid profile (total cholesterol, HDL, LDL), and liver enzymes (ALT, AST) will be considered as dependent variables. The simultaneous effect of various baseline characteristics and characteristics at certain study treatment intervals between women who did not develop diabetes and women who did during follow-up will be studied with the use of a linear multiple regression analysis. Diabetes and IGT occurrence before and after different treatment will be compared with the McNemar test (complex chi square for paired data), which formally tests for a change between the observed proportions of 2 related samples.

All analyses will be conducted with subjects assigned to their initial treatment group, using all available data for the period of follow-up relevant to the particular analysis. Data will be analyzed on the basis of intention to treat and also on completed treatment parameters where relevant. Differences of $P < 0.05$ will be considered statistically significant. Results will be reported as mean +/- standard error of the mean (S.E.M) for quantitative variables and percentages for categorical variables unless otherwise noted. We will perform all statistical analyses using SPSS 15.0 for Windows (SPSS, Inc.; Chicago, Ill).

Study Drugs

Description

Both active drug (Liraglutide 6.0 mg/ml injection) and placebo will be supplied by Novo Nordisk A/S. as a pre-filled pen for subcutaneous injection. Each liraglutide pre-filled pen contains 18 mg/3 ml (6 mg/ml) and will deliver 30 doses of 0.6 mg; 15 doses of 1.2 mg or 10 doses of 1.8 mg; the placebo pen will deliver equal volumes of vehicle solution only. Liraglutide and placebo are visually identical.

Metformin Hydrochloride XR (extended-release) in 500 mg strength oral tablets will be purchased in bulk through the Woman's Hospital Infusion Pharmacy. Metformin hydrochloride XR is combined with a drug release controlling polymer so that drug is released slowly from the dosage form. The medication will be allocated into appropriate patient dosage regimens by the hospital pharmacy personnel. Dosage of metformin XR will be prescribed according to standard directions and not exceeding the maximum recommended daily dose in adults of 2000 mg.

Trial Supplies

Double-blind Liraglutide or placebo

Liraglutide 6.0 mg/ml or placebo solution for injection in a 3 mL pre-filled pen will be supplied by Novo Nordisk A/S. The pre-filled pen will be provided as a pen-injector. The Investigator will provide each subject with Direction for Use for the liraglutide pen-injector at each dispensing visit.

Packaging and Labelling of Trial Products

Liraglutide and placebo will be packed and labelled by Novo Nordisk A/S and provided in non subject specific boxes. Labelling will be in accordance with Annex 13, local law and trial requirements.

Storage, Handling, Accountability and Destruction of Trial Products

The trial product will be dispensed to each subject as required according to treatment group and under the direction of the study investigator(s). The Investigator will ensure availability of proper storage conditions, and study staff will record and evaluate the temperature (at least every working day). Storage facilities should be checked frequently. A log to document the temperature must be kept on file. No trial product will be dispensed to any person not enrolled in the study.

Storage and in-use conditions

The investigator will provide appropriate instructions to study subjects as to the in-use and not in-use conditions. These instructions will be in accordance with the local regulatory and legal requirements and consistent with the product prescribing information approved by the appropriate regulatory authorities.

Study Timelines

We anticipate that the proposed study will take 36 months (three years) to complete, including 12 months for patient recruitment. Table 1 presents the timetable and key activities in the proposed research.

Target Start Date: January 2011

Table 1

Phase	Tasks
I (Months 1-3)	FDA filing, IRB application, staff training, protocol development, drug and placebo receipt
II (Months 4-16)	Recruitment of women*
III (Months (4-34)	Patient enrollment, treatment, database and data entry, interim analyses if needed
IV (Months (24-36)	Final data entry, data analyses and publication

ADDITIONAL INFORMATION

Woman's Hospital is one of the largest, not-for-profit women's specialty hospitals in the United States. The hospital's most valuable asset is its patient population. Woman's statistics of over 8100 births (2009), 75,000 pap smears, 47,000 mammograms, 300,000 lab tests, and 13,000

biopsies annually in a centralized location make it ideal for clinical research studies in women's health. The ethnic mix is about 60% Caucasian and 40% African American. In the calendar year of 2009, 600 patients with gestational diabetes delivered at Woman's hospital. The rate of GDM was 7.4% for the year 2009.

The Physician Tower, which is on the hospital campus, offices approximately 60 private obstetrician-gynecologist offices that actively participate in Woman's Health Research Department clinical trials and encourage patient participation. This study will utilize the research staff (nurses, physicians, PhD) of the Woman's Hospital Health Research Department for recruitment and patient consenting. The Woman's Hospital Pathology laboratory facility will be utilized for all metabolic and glucose tolerance testing. Study administrative work (IRB approval and updates) and statistical analyses will be done through the Woman's Health Research Department. The staff of the Woman's Metabolic Health Clinic will be used for all patient follow-up, medication instruction and medical care.

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