A randomized placebo-controlled double blind trial of liraglutide 3 mg [Saxenda] on weight, body composition, hormonal and metabolic parameters in obese women with polycystic ovary syndrome (PCOS)

INVESTIGATOR-SPONSORED STUDY PROPOSAL

Universal Trial Number (UTN) is U1111-1198-4126

Woman's Health Research Department
Woman's Hospital
100 Woman's Way
Baton Rouge, Louisiana

Correspondence to:

Karen Elkind-Hirsch, PhD
Director of Research
Woman's Hospital -Support Services Building
100 Woman's Way
Baton Rouge, Louisiana
phone-(225) 231-5278
fax-(225) 231-5290

karen.elkind-hirsch@womans.org

ClinicalTrials.gov ID: NCT03480022

Background and Significance:

Polycystic ovary syndrome (PCOS), a common, heterogeneous, heritable condition, is characterized by disordered reproductive and metabolic function that accounts for the myriad of clinical features including androgen excess, chronic anovulation, hyperinsulinemia, adiposity, and dyslipidemia. Hyperandrogenism, ovarian dysfunction and metabolic abnormalities - the main determinants of PCOS – all appear to be involved in a synergistic way in the pathophysiology of PCOS. Women with PCOS are more likely to be obese although PCOS can also manifest in lean women. Obesity, particularly abdominal obesity, plays a central role in the development of PCOS, and exacerbates the reproductive and metabolic dysfunction. Rather than absolute body weight, it is the distribution of fat that is important with central adiposity being a risk factor. Compared with weight-matched healthy women, those with PCOS have a similar amount of total and trunk fat, but a higher quantity of central (visceral) abdominal fat. Visceral adipose tissue is more metabolically active than subcutaneous fat and the amount of visceral fat correlates with insulin resistance and hyperinsulinemia. Weight gain is also often an important pathogenic factor, with the PCOS condition becoming clinically manifest in women with a presumable genetic predisposition for PCOS who subsequently gain weight. Therefore, environmental (particularly dietary) factors are important. However, body mass is also influenced by genetic factors such as fat mass and obesity-associated protein, and obesity itself is a highly heritable condition. Therefore, the weight gain responsible for the manifestation of PCOS in many women with this condition is itself influenced by genetic factors. Ethnicity, genetic background, personal and family history, degree of obesity must all be taken into account because they might aggravate or even trigger metabolic disturbances women with PCOS. Moreover, the incidence of glucose intolerance, dyslipidemia, gestational diabetes, and type 2 diabetes (T2D) is increased in women with PCOS at all weight levels and at a young age. Several studies have demonstrated that T2D occurs with increased frequency in women with PCOS so that recently the American Diabetes Association and the International Diabetes Federation have identified PCOS as a significant non-modifiable risk factor associated with type 2 diabetes. PCOS may be a more important risk factor than ethnicity or race for glucose intolerance in young women. The exact factors responsible for this excess risk in women with PCOS have not been identified; family history of T2D, obesity, insulin resistance, beta cell (ß-cell) secretory dysfunction, and hyperandrogenism are possible candidates. With better understanding of its pathophysiology, the metabolic consequences of the syndrome are now evident.

Obesity is considered one of the most important features of PCOS and it exacerbates insulin resistance and impaired glucose tolerance (IGT) in women with PCOS. Its mean prevalence in diseased women varies between 61 and 76%. The prevalence of obesity reaches 80% in the United States and 50% outside which indicates that this figure depends on local environmental factors, ethnic backgrounds, and lifestyle, and not on the mere presence of PCOS

itself. The increased prevalence of obesity in PCOS is associated with an increased frequency of metabolic syndrome and T2D. Obesity has been associated with a number of diseases and metabolic abnormalities that have high morbidity and mortality. Obesity appears to exert an additive, synergistic effect on manifestations of PCOS and PCOS is more prevalent in obese than in lean women. Moreover, obesity itself is a common pathogenic factor in insulin resistance, lipid dysfunction and metabolic syndrome and is usually accompanied by hypertension. The degree of obesity is positively associated with an increase in the incidence and degree of insulin resistance. Obesity may play a pathogenic role in the development of PCOS in susceptible individuals and weight loss has been found to improve many clinical features of PCOS. Even a modest weight loss (5% of initial body weight) in overweight or obese women with PCOS improves ovulation frequency and conception, reduces miscarriage, hyperlipidemia, hypertension, hyperglycemia and insulin resistance. The loss of intra-abdominal fat is specifically associated with resumption of ovulation. Weight loss has beneficial effects on cardiovascular risk factors such as dyslipidemia and blood pressure. Features of PCOS (e.g., hirsutism, testosterone levels, insulin resistance, menstrual cyclicity and ovulation) showed marked improvements, and PCOS frequently resolved after substantial weight loss induced by bariatric surgery. Furthermore, studies show that women with PCOS who achieve reductions in weight and waist circumference after a diagnosis of prediabetes are twice more likely to regress to normal glycemia than those who maintained baseline weight or gained weight.

While PCOS is the most common endocrine disorder among women in their reproductive years, many aspects of the condition are not fully understood. The fundamental pathophysiological defect in PCOS is unknown, but women with PCOS often demonstrate insulin resistance with compensatory hyperinsulinemia. Insulin resistance occurs in around 50% to 80% of women with PCOs, primarily in the more severe NIH diagnosed PCOS and in those who are overweight. Hyperinsulinemia may be directly responsible for the development of androgen excess, through its effects in reducing sex hormone-binding globulin (SHBG) synthesis and circulating concentrations, and in stimulating ovarian androgen production rates. Androgen excess, in turn, represents one of the major factors leading to altered ovarian physiology and associated ovulatory disturbances. In addition to the association of hyperinsulinemia and insulin resistance with the reproductive disorders that characterize PCOS, a number of metabolic abnormalities have also been associated with insulin resistance. The insulin resistance syndrome has been characterized by glucose intolerance, hypertension and dyslipidemia.

Hyperandrogenism (HA) comprises the biochemical hallmark of PCOS with elevated free testosterone levels accounting for the majority of the abnormal laboratory findings in women with oligomenorrhea. Hyperandrogenism has also been linked with several components of metabolic syndrome. Metabolic syndrome (MetS) is characterized by a cluster of risk factors including hypertension, elevated triglycerides, low high-density lipoprotein cholesterol, glucose intolerance, and obesity, which also identifies those at risk for cardiovascular disease. Insulin

resistance with subsequent hyperinsulinemia plays a major role in the development of MetS. The association of carbohydrate metabolism abnormalities with androgen excess disorders, particularly PCOS, is a well-defined entity. In particular, androgen excess in PCOS may contribute to increased visceral fat, decreased lipolysis in subcutaneous fat, reduced insulin sensitivity in adipose tissue and skeletal muscle, decreased high-density cholesterol (HDL-C) levels, and increased low-density lipoprotein cholesterol (LDL-C) levels. Some studies have indicated a positive association between MetS and HA in women with PCOS. Metabolic syndrome and its individual components are common in PCOS, particularly among women with the highest insulin levels and body mass index (BMI). In women with PCOS, there is an insulin post-binding defect in receptor signaling due to increased insulin receptor substrate-1 serine phosphorylation that selectively affects metabolic, but not mitogenic pathways in classic insulin target tissues and in the ovary. Hyperandrogenism per se may have a role in the higher prevalence of glucose intolerance in these patients. In the United States, 33–47% of women with PCOS have MetS, a rate two to three times higher than that of age-matched healthy women without PCOS. An estimated 30–40% of PCOS patients have IGT and 7.5–10% have T2D. Studies suggest that the annual progression rate from normal glucose tolerance to IGT and from IGT to T2D in women is substantially enhanced among women with PCOS, with the highest risk in women who are also obese and have a family history of type 2 diabetes.

Excess body weight is associated with hyperandrogenism. Sex hormone binding globulin, synthesized in the liver, not only provides transport for steroids in the blood, but also regulates hormone access to target tissues through varied degrees of binding affinity. Furthermore, the hyperinsulinemia in obese women may directly increase free testosterone levels by lowering the SHBG synthesis in the liver. On the other hand, rodent models have shown that hyperandrogenism promotes insulin resistance, reduces energy expenditure, and accordingly, increases the risk of abdominal obesity and metabolic risk factors. In a multiethnic sample of more than 2500 U.S. women between 42 and 52 years of age, oligomenorrhea was associated with the MetS only when coincident with HA. Conversely, women with HA had a significantly increased risk of the MetS independent of the menstrual frequency status. Animal (rodent) studies indicate that androgens may produce IR by direct effects on skeletal muscle and adipose tissue, mediated by alterations in the insulin receptor–glycogen synthesis, by altering adipokine secretion, and by increasing visceral adiposity. Moreover, a small study of 13 obese and 30 non-obese women showed that anti-androgen treatment partly reversed the peripheral insulin resistance (IR) in non-obese women only, whereas central obesity may have a direct role in androgen hypersecretion. Also, a recent study of young, overweight women suggested that the association between body fat and HA was predominantly mediated by insulin resistance. The interrelationships between body fat, IR and HA contribute to the complex pattern making it a difficult task to specify the role of each component.

There is considerable heterogeneity in clinical studies among women with hyperandrogenism and there could be multiple clinical phenotypes, even in a single patient at different ages. Obesity significantly affects the circulating concentrations of total testosterone and SHBG. Body fat excess, particularly visceral fat accumulation, is another common finding in these women regardless of weight and even at a young age. Literature data consistently confirm that up to 80% of PCOS subjects are overweight or obese, with a typical central distribution of adipose tissue. It has been hypothesized that body fat could have a direct role in determining insulin resistance and possibly androgen hypersecretion in these women, by mechanisms such as increased lipolysis, abnormal adipokine secretion, and altered steroid hormone metabolism. Body weight status was the major factor determining the risk of IGT and MetS in women with PCOS. However, the intricate interrelationships between body fat excess, insulin resistance, and hyperandrogenism make it difficult to assess the specific role of each of them. Obesity-related insulin resistance and resulting hyperinsulinemia may cause a decreased SHBG and an increased ovarian androgen production, both of which contribute to the hyperandrogenism. However, this may form a vicious circle as hyperandrogenism may also contribute to the insulin resistance by increasing free fatty acid flux to the liver and muscle through visceral lipolysis and, in addition, by altering muscle structure toward less insulin-sensitive muscle fibers. Indeed, obese women with PCOS have more profound IR or T2D, gestational diabetes, dyslipidemia and risk of cardiovascular disease and greater level of androgens due to low levels of SHBG. Ethnicity, genetic background, personal and family history, degree of obesity must all be taken into account because they might aggravate or even trigger metabolic disturbances women with PCOS.

Women suffering from PCOS are subjected to a range of symptoms associated with menstrual dysfunction, excess of androgen, which significantly influence the quality of life. The sweet spot for intervention in PCOS occurs early in patients who don't yet desire pregnancy and who are experiencing the classic PCOS progressive weight gain. This occurs at an early age, before or around the time of puberty. Aggressive treatment at this stage will reduce the risk of a host of potential health problems later. In addition to infertility issues, these include increased long-term risks of diabetes, hypertension, dyslipidemia, metabolic syndrome, endometrial cancer, obstructive sleep apnea, and nonalcoholic fatty liver disease. Weight reduction is the most important treatment target when PCOS is linked to obesity. Obese women referred for assistance with weight loss had a prevalence of PCOS of 28.3%. Obesity is a great problem in women with PCOS and we do not have a conventional satisfactory treatment for it. Weight management by lifestyle intervention often remains unsatisfactory in obese women with PCOS. Lifestyle interventions remain essential to the management of women with PCOS; however, the majority of non-diabetic obese patients with PCOS do not reach their therapeutic goals with these interventions alone and require pharmacologic therapies.

A great deal of attention has been given to the metabolic disturbances that accompany PCOS as well as these disturbances later in life. The growing body of evidence linking PCOS to an inherited resistance to insulin action, aggravated by lifestyle problems such as obesity, poor diet and physical inactivity has led to trials of drug therapies in patients with PCOS. Over the last years, considering the importance given to insulin resistance in the pathogenesis of the syndrome, clinical studies have focused on insulin sensitizing drugs for the treatment of women with PCOS, with metformin being the drug most extensively studied in this syndrome. Although no antidiabetic agents have US Food and Drug Administration approval for the treatment of PCOS, metformin was preferred due to the fact that it had the safest risk-benefit ratio, and could cause weight loss, while thiazolidinediones increased weight as a result of fluid retention. Metformin acts by decreasing hepatic gluconeogenesis and free fatty acid oxidation while increasing peripheral glucose uptake. Early studies in PCOS suggested that metformin indirectly reduces insulin levels, dyslipidemia and systemic inflammation; however, recent placebo-controlled trials failed to demonstrate significant metabolic benefit. Considerable variability in the metabolic responses to metformin has been observed in women with PCOS, attributable to several potential factors such as different doses of the drug and genetic background. While metformin is not a weight loss drug it is possible that the weight loss that often accompanies protracted metformin therapy may account for some of the beneficial effects observed in many studies. Weight loss has been claimed to be a beneficial secondary effect of extended release metformin but the effect is not very consistent. Metformin has inconsistently demonstrated weight reduction. In addition, metformin has been shown to have no clinically significant effect in reducing abdominal adiposity. Interestingly, most studies have not found any beneficial effects of metformin treatment in obese patients with PCOS. Irrespective of treatment group (after adjustment for baseline BMI and age), only weight loss, but not the use of metformin, was associated with a significant improvement in metabolic and reproductive function in obese women with PCOS. Furthermore, a number of studies have substantiated the view that obesity may reduce the benefit of metformin treatment.

Novelty of Study

Polycystic ovary syndrome is now recognized as one of the most common endocrine system disorders among women of reproductive age. Earlier studies using National Institute of Health criteria estimated PCOS affects between 5% and 10% of the female population ages 18 to 44. The diagnostic criteria used to define PCOS are frequently being modified with the projected figure of affected women using the newer diagnostic criteria to be about one in every 10 to 15 women. .Most women are diagnosed during their twenties or thirties, but recent studies warn that PCOS may affect even prior to age of teens and as young as 11 years of age, much ahead of their puberty. The economic burden of PCOS is significantly huge. Around 4 billion dollars are spent annually in the United States to screen for the disease and treat its various morbidities,

including hirsutism, infertility, obesity, and diabetes mellitus.

The realization that hyperinsulinemia is a key component in the pathogenesis of PCOS provided a basis for advances in treatment strategies for women with the disorder. Lifestyle modification, including diet and exercise, is considered a cornerstone of the management of women with PCOS presenting with obesity, particularly the abdominal phenotype. PCOS is characterized by a vicious cycle whereby androgen excess favors abdominal fat deposition, which in turn aggravates insulin resistance and compensatory hyperinsulinism, further enhancing ovarian androgen secretion. Hence, therapeutic strategies ameliorating abdominal adiposity and weight excess may inhibit this vicious cycle, improving not only the metabolic co-morbidities of PCOS but also androgen excess and reproductive aberrations for overweight, anovulatory women with PCOS. Modest weight loss (5-10% of total body weight) can improve ovulation, decrease serum androgen levels and in some cases improve hirsutism. While weight loss is the key in the treatments of obese patients with PCOS, current non-pharmacologic management of body weight is hard to achieve. Thus, in the majority of patients with PCOS pharmaceutical intervention is an additional essential therapeutic aid to lifestyle changes.

The genetic disruption of insulin signaling in the brain has indicated that this pathway is important for the ovulation and body weight regulation. These insights have been directly translated into a novel pharmacotherapy aiming to achieve weight loss for obese PCOS patients with insulin-sensitizing drugs such as metformin and use of antidiabetes medications. The most widely used drug is metformin for women with PCOS and metabolic disturbances, but the weight loss effects of metformin are disputed. Several studies have shown an increase in insulin sensitivity and pregnancy rate accompanied by decreased insulin and androgen levels in PCOS patients taking metformin but it has limited efficacy in obese women. Other studies with orlistat and metformin showed a significant reduction in body weight, androgen levels and metabolic cardiovascular risk factors in obese PCOS women. Recently a number of antidiabetes drugs have been approved which facilitate weight loss and improve the underlying insulin resistance. Incretin mimetics evolved as therapeutic options for the treatment of T2D primarily because of their effects on insulin and glucagon secretion, with weight loss as an additional benefit. Early studies of human glucagon-like peptide-1 (GLP-1) showed that continuous peripheral infusion was associated with decreased appetite and increased satiety. Continuous infusion of GLP-1 also was shown to improve insulin sensitivity, glycemic control, and pancreatic beta cell function in individuals with T2D. Weight loss ranging from 2 to 6 kg has been a consistent finding in studies designed to investigate the glycemic benefits of GLP-1 agonists in individuals with T2D. Additionally, this therapy has produced progressive weight loss in obese people without diabetes. A recent meta-analysis concluded that GLP-1 receptor agonists not only had a significant effect on weight loss in overweight T2D patients but also in non-diabetic overweight persons, reducing subcutaneous fat areas in particular. The mechanisms of weight loss with GLP-1 agonists are not fully understood but may include changes in energy expenditure, changes

in leptin sensitivity, or nausea resulting in decreased food intake. Available clinical trials of GLP-1 receptor agonist therapy in the treatment of excess body weight in women with PCOS demonstrate that exenatide and liraglutide are effective in weight reduction either as monotherapy or in combination with metformin (**Elkind-Hirsch et al.2008; Jensterle et al, 2016)**. One small study has investigated the effect of liraglutide in a subset of obese patients with PCOS and higher metabolic risk profile reporting a significantly greater weight loss with liraglutide in combination with metformin than metformin alone (**Jensterle et al, 2015**). Another preliminary report confirmed that liraglutide had an add-on effect on weight loss in obese women with PCOS who had lost <5% body weight during a 6-month pre-treatment with metformin (**Jensterle et al, 2014a**). Similar to native GLP-1, liraglutide causes glucose-dependent insulin secretion, promotes weight loss and may subsequently improve insulin resistance. Short-term liraglutide treatment was associated with weight loss and significantly improved eating behavior in obese women with PCOS (**Jensterle et al, 2014b**). These studies in women with PCOS also showed that androgens may be modestly decreased and menstrual frequency may be increased (**Nylander et al, 2017**). Glucose parameters were generally improved. We reported that treatment with exenatide for 24 weeks was superior to single agent metformin treatment in improving insulin action and reducing body weight and hyperandrogenism in obese women with PCOS (**Elkind-Hirsch et al.2008**). We further found exenatide treatment in women with PCOS significantly improved first-phase insulin responses to oral glucose administration. Since aberrant first-phase insulin secretion and impaired suppression of endogenous glucose production are major contributors to postprandial hyperglycemia and development of T2D, the effects of the GLP-1 agonist, liraglutide, to target these defects, and normalize glucose excursions are likely to be clinically significant in obese patients with PCOS.

The drug, liraglutide 3.0 mg was approved for chronic weight management in management in obese adults with an initial BMI of 30 kg/m^2 or greater or in overweight adults BMI of 27 kg/m^2 or greater with at least one weight-related co-morbid condition as an adjunct to a reduced-calorie diet and increased physical activity. Liraglutide is an acylated human GLP-1 analog that binds to and activates the GLP-1 receptor. It lowers body weight through decreased caloric intake while stimulating insulin secretion and reducing glucagon via a glucose-dependent mechanism. For obesity management, patients may lose weight with GLP-1 receptor agonists due to other unique actions. Glucagon-like peptide-1 receptor agonists (GLP-1RAs) can slow gastric emptying and increase satiety. While predictors of weight loss success for the general population are available (protein intake, weight loss medications), predictors of weight loss success may differ between normal and hyperandrogenic women. Glucagon-like peptide 1 agonists are linked with dose dependent weight lowering potential in different obesity related populations. The weight loss effects of GLP-1RAs previously demonstrated in diabetic and obese non-diabetic patients, offer a unique opportunity to expand the medical options available to patients with PCOS. Given this lack of information, the aim of the present study was to

investigate the effects of liraglutide 3mg vs. placebo on body composition as well as hormonal and metabolic features in non-diabetic obese women with PCOS.

Study Rationale

The non-diabetic obese female with PCOS offers a unique model to study the relationship between insulin resistance and adiposity. We propose a double-blind, placebo-controlled 30-week trial designed to directly examine the therapeutic effects of liraglutide 3 mg (LIRA 3 mg) compared to placebo on body weight, hormonal and cardiometabolic parameters in obese non-diabetic women with PCOS. All patients will receive diet and lifestyle counseling, including advice on exercise commencing during the lead-in period and continuing throughout the study. In this study, we will examine the efficacy of LIRA 3mg on body weight and body composition, reproductive function metabolic parameters and cardiovascular risk factors in a well-defined group of pre-menopausal obese non-diabetic women with hyperandrogenism, focusing on the relationship to obesity and insulin resistance. Women with PCOS demonstrate abnormal body composition characterized by a greater percent body fat, body fat mass, and increased ratio of fat to lean mass (F/L ratio). Studies using DEXA methodology report a higher degree of metabolic dysfunction in patients with PCOS which appears be directly associated with their higher F/L ratio. The use of DEXA technology that is simple, operator independent, safe, accurate and cost-effective will be used to assess fat quantity and distribution.

There is a growing need to develop pharmacologic interventions to improve metabolic function in women with PCOS. Given that PCOS is a frequent condition and weight loss is essential but difficult to achieve, it is important to study if the effect on body weight reported in other studies can be confirmed in a selected population of hyperandrogenic patients, especially with medications currently approved for weight reduction. **High dose liraglutide alone results in significant weight reduction in obese women without PCOS. There is limited data on weight loss with high dose liraglutide in non-diabetic females with PCOS treated with this agent (Jensterle et al, 2016).** Studies on the effect of anti-obesity medication combined with lifestyle changes on body weight and composition and androgen excess in obese women diagnosed with PCOS are lacking. The investigators aim to elucidate the most efficacious weight reduction regime in obese PCOS women. We hope to determine which treatment(s) addressing the multifaceted disturbances of this disorder in patients with PCOS and obesity emerges as the preferable therapy.

Benefit/Risk and Ethical Assessment

Glucagon-like peptide-1 receptor agonists (GLP-1RA) are peptides that mimic native GLP-1, binding to its receptors to elicit the same effects, but at much higher pharmacological levels than the physiological profiles. The most common treatment-related adverse effects of GLP-1RAs are gastrointestinal in nature and include nausea, vomiting, and diarrhea, which are usually

mild and tend to subside over time. The GLP-1RAs are usually well-tolerated, with nausea being the most significant adverse side effect. Other documented but infrequent concerns with GLP-1 receptor agonists include mild injection site reactions. When looking at the benefit–risk assessment, the GLP-1 receptor agonists demonstrate clinical advantages such as reduced risk for drug-related hypoglycemia and often favorable effects on body weight.

Women with PCOS are more likely to be overweight or obese. Research has increased the understanding of the persistent alterations in physiological and behavioral processes that contribute to weight gain and hamper weight loss. Evidence suggests that pharmacotherapy for the management of obesity may modify these processes and thereby help individuals adhere to diet and exercise regimens, to lose more weight and to maintain weight loss. Although no pharmacological agent is without some risk, LIRA 3mg therapy appears to have wide margins of safety when used appropriately. The robust clinical benefits observed with this pharmacologic agent may confer a significant advantage to improve outcomes in patients at high risk of developing T2D and cardiovascular disease.

Study Hypothesis

*Randomized, **Parallel,** Placebo-Controlled, Double-Blind Prospective Study Trial*

This is a prospective double-blind randomized outpatient drug efficacy study comparing the use of liraglutide (3 mg) to placebo in nondiabetic obese women with polycystic ovary syndrome. Seventy-two women will be allocated to treatment, in a 2:1-subject distribution ratio, with a daily regimen liraglutide 3.0 mg or placebo (see Figure 1-Flow of Patients through Trial) for 28-30 weeks of intervention. We hypothesize that the use of the GLP-1 agonist liraglutide 3.0 mg (LIRA 3mg) compared with placebo in obese women with PCOS will lead to a beneficial reduction in biochemical hyperandrogenism due to greater reduction in body weight. The resulting weight loss will assist in decreasing insulin resistance leading to improved hormonal and cardiometabolic parameters in this patient population., To investigate this, we will perform a randomized double-blind clinical trial (RCT) treating obese women with PCOS with either liraglutide or placebo for 28-30 weeks.

STUDY OBJECTIVES

Primary objective

The primary objectives of this study are to compare the therapeutic impact of liraglutide 3 mg versus placebo on reduction of body weight and biochemical hyperandrogenism (as determined by the free androgen index) in obese non-diabetic women with PCOS. We will 1) determine the percentage of participants achieving ≥5% reduction in baseline body weight with each treatment and 2) assess the inhibition of biochemical hyperandrogenism (ovarian androgen production and sex hormone binding capacity) in response to each treatment.

Secondary study objectives

The secondary study objectives are to determine the effect of treatment with anti-obesity medication versus placebo on anthropometric, **clinical**, hormonal and metabolic parameters in non-diabetic obese women with hyperandrogenism.

EFFICACY VARIABLES/MEASURES

Primary endpoints

The co-primary end points are to compare obese women with PCOS receiving liraglutide 3mg (LIRA 3 mg) with those receiving placebo on body weight and bioavailable ovarian androgen concentrations as determined by:

1a. percent change in body weight from baseline to week 30 and

1b percentage of participants achieving ≥5% reduction in body weight from baseline to week 30

2 reduction of free androgen index [FAI=testosterone (T)/sex hormone binding globulin (SHBG) levels] from baseline to 30 weeks

Secondary endpoints

We will further examine the impact of the administration of these pharmacotherapies in obese non-diabetic PCOS women on:

Anthropometric and Clinical Indices

1. Change from baseline of body mass index [BMI], absolute body weight, waist circumference (WC), waist: hip ratio (WHR) , waist-height ratio (WHtR), and whole-body dual-energy X-ray absorptiometry [DXA]) measures of body composition (trunk fat mass and truck fat/extremities fat ratio) to determine the relative contribution of changes in fat mass (FM) vs. lean mass (LM) to overall weight loss at week 30
2. Compare women with hyperandrogenism for frequency of patients achieving a body weight reduction of at least 10% [Time Frame from baseline to 30 weeks]
3. Change in menstruation frequency (normalized to the number of menstruations per year) from before and after 30 weeks of treatment

Metabolic Parameters

1. Change in glycemic values from baseline to 30weeks
2. Fasting and 2 hour glucose levels after an OGTT
3. Surrogate measures of insulin action derived from 75 gram OGTT [insulin sensitivity index (HOMA-IR, Matsuda index), corrected early insulin secretory response (insulinogenic index/HOMA-IR), area under the curve (AUC) for insulin and glucose, and oral disposition index (product of Matsuda index and insulinogenic index; SI_{OGTT} x Δinsulin 30–0 min to glucose 30–0 min)]

Cardiovascular Risk Factors (change from baseline to 30 weeks)

1. Plasma lipid fractions
2. Blood pressure

Other endocrine levels (change from baseline to 30 weeks)

1. Adrenal androgen concentration- dehydroepiandrosterone sulfate (DHEAS) levels

The following will be documented for each patient:

1. Presence of polycystic ovary syndrome (PCOS) will be recorded using modified National Institutes of Health (NIH) criteria which are inclusive of presence of oligo-/amenorrhea (cycle >35 days or <8 cycles year), and clinical and/or biochemical hyperandrogenism, after exclusion of related disorders. Other causes to bleeding irregularities and androgen excess will be excluded.
2. Metabolic syndrome (MetS) will also be documented and defined (2005 National Cholesterol Education Program, Adult Treatment Panel III) as the presence of at least three of the following criteria: abdominal obesity (waist circumference >80 cm in women); serum triglycerides ≥1.7 mmol/L; serum HDL <1.3 mmol/L; systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg; and fasting plasma glucose ≥7.0 mmol/L

Safety variables/Measures

Safety and tolerability will be assessed by collating data on treatment-emergent adverse events (AE), laboratory tests, physical examinations, and vital signs. Prevention of pregnancy will be monitored monthly by both laboratory and home pregnancy testing. All patients will be educated about not becoming pregnant and perform monthly urine home pregnancy tests during the months that they do not have laboratory evaluations. Patients will be educated about the side effects and use of liraglutide 3.0 mg and the injection delivery system. Liraglutide 3.0 mg is a well-tolerated long-term weight loss agent. The most common expected AEs (prevalence >5%) are nausea, diarrhea, constipation, vomiting, dyspepsia, fatigue, dizziness, and abdominal pain (see reference 77 - prescribing information)**.** Patients will be asked about the most common adverse events related to liraglutide such as nausea, headache, diarrhea, constipation and vomiting if not volunteered. This protocol and the associated Informed Consent as well as any addenda or amendments, must be reviewed and approved by the Woman's Hospital 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.

STUDY PLANS AND PROCEDURES

Subjects

Up to 92 non-diabetic women with PCOS, aged 18 years to 45 years of age, meeting BMI criteria, will be invited to participate. We will define hyperandrogenism using biochemical evidence (elevated testosterone and/or free androgen index with exclusion of androgen secreting tumors). Subjects will be recruited using flyers distributed in the metabolic clinic, gynecology clinics and pathology laboratory associated with Woman's Hospital. All participants will be provided a written informed consent and be asked to sign a copy before being enrolled in the study. The Woman's Hospital Institutional Review Board (WHIRB) will have approved both the protocol and consent. All subjects will undergo a verbal screen, and if they are eligible and sign a medical release form, their medical records will be obtained to confirm their medical history. All subjects will provide a medical and gynecological history including assessment of regularity and length of the menstrual cycle, with recording of menses in the 12-month period before the study. Patients will be specifically asked about the number of menses in previous 12 months (menstrual frequency). To be eligible for the study, subjects will be required to have irregular periods (cycle length outside 24–35 days or <8 cycles per year). All enrolled patients will then undergo baseline clinical and laboratory evaluations to exclude diabetes, thyroid disorder, significant hyperprolactinemia, elevated liver enzymes and/or severe hypertriglycidemia. A negative serum pregnancy test is a prerequisite for commencing treatment. Subjects will be instructed to use an IUD or double barrier methods of contraception (unless sterilized) during the study since hormonal methods are not permitted. Glycemic status will be measured at the beginning and end of each treatment period by a standard 75 g oral glucose tolerance test (OGTT). Obese women who meet study eligibility criteria (see inclusion and exclusion criteria) will be eligible to be randomized to treatment. We anticipate that 72 women will be randomized to treatment (this allows for 20 women to fail screening). 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.

Key Inclusion Criteria

- Female gender
- 18-45 years of age
- BMI ≥30 kg/m2 or BMI ≥27 kg/m2 with one or more obesity-associated co-morbid conditions (e.g. hypertension, and dyslipidemia)

- PCOS- NIH criteria hyperandrogenism and irregular menstrual cyclicity
- Non-diabetic as determined by a 75 gram oral glucose tolerance test (OGTT) and hemoglobin A1C. Non-diabetic is inclusive of women with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both (IFG/IGT). Participants with diabetes will be excluded
- Willing to use effective contraception consistently during therapy which is defined as:
    - an intrauterine device, tubal sterilization, or male partner vasectomy, or
    - combination of two barrier methods with one being male condom.
- Written consent for participation in the study

Key Exclusion Criteria

- Presence of significant systemic disease, cerebrovascular disease, clinically significant cardiac abnormalities or heart problems including congestive heart failure, unstable angina or acute myocardial infarction, current infectious liver disease, acute stroke or transient ischemic attacks, history of pancreatitis, or diabetes mellitus (Type 1 or 2)
- Any hepatic diseases in the past (infectious liver disease, viral hepatitis, toxic hepatic damage, jaundice of unknown etiology) or severe hepatic insufficiency and/or significant abnormal liver function tests defined as aspartate aminotransferase (AST) >3x upper limit of normal (ULN) and/or alanine aminotransferase (ALT) >3x ULN
- Renal impairment (e.g., serum creatinine levels ≥1.4 mg/dL for women, or eGFR <60 mL/min/1.73 m2) or history of unstable or rapidly progressing renal disease or end stage renal disease.
- Uncontrolled thyroid disease (documented normal TSH), Cushing's syndrome, congenital adrenal hyperplasia or clinically significant elevations in prolactin levels. The clinical significance of prolactin levels will be determined by the treating physician
- Significantly elevated triglyceride levels (fasting triglyceride > 400 mg %)
- Untreated or poorly controlled hypertension (sitting blood pressure > 160/95 mm Hg)
- Use of hormonal medications, the use of medications that cause clinically significant weight gain or loss (prescription or OTC) and medications known to exacerbate glucose tolerance (such as isotretinoin, hormonal contraceptives, GnRH analogues,

glucocorticoids, anabolic steroids, C-19 progestins) including herbal medicines for at least 8 weeks. Use of anti-androgens that act peripherally to reduce hirsutism such as 5-alpha reductase inhibitors (finesteride, spironolactone, flutamide) for at least 4 weeks

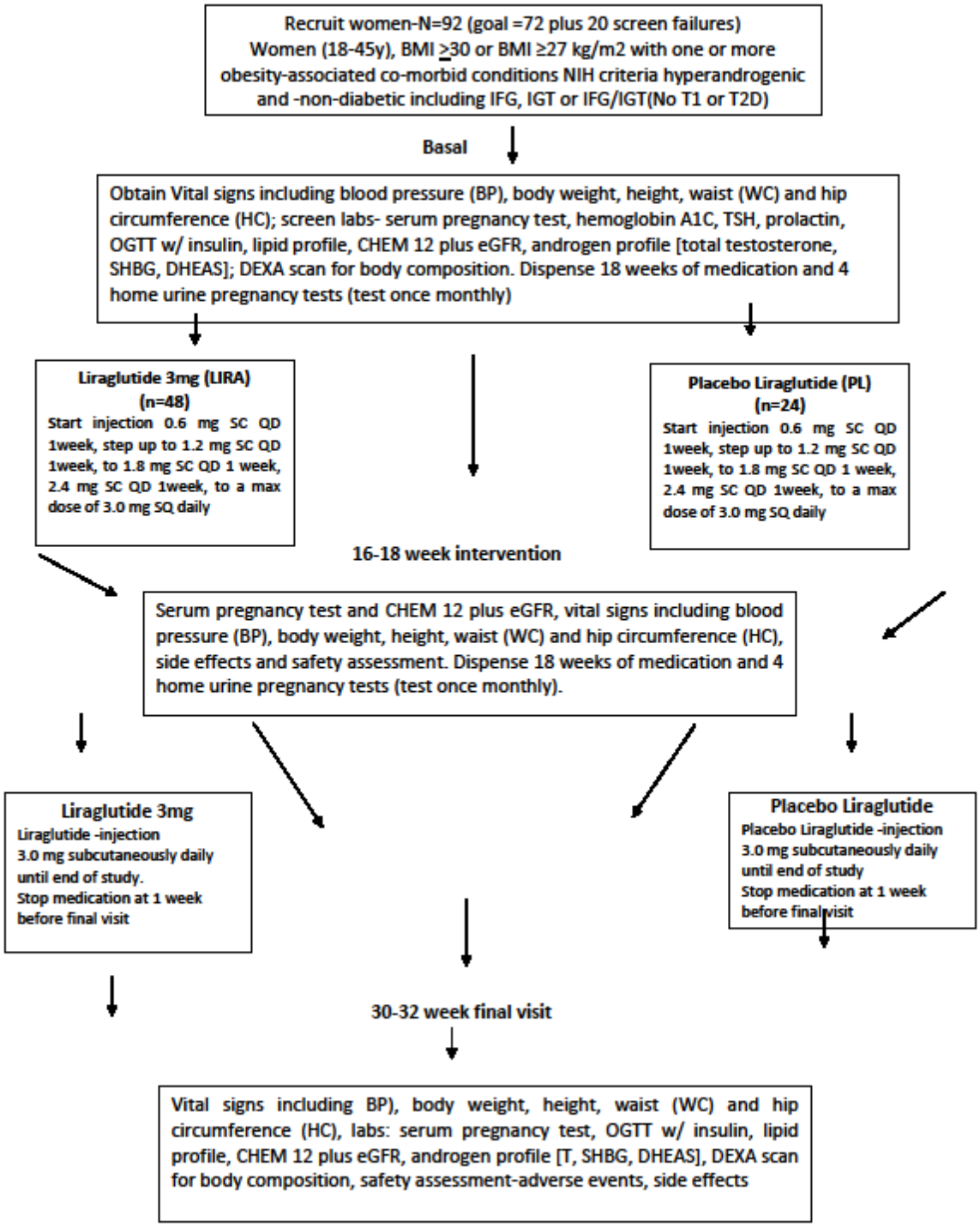
- Prior history of a malignant disease requiring chemotherapy
- Family or personal history of familial medullary thyroid carcinoma or multiple endocrine neoplasia type 2
- Known hypersensitivity or contraindications to use GLP1 receptor agonists
- Use of metformin, thiazolidinediones, GLP-1 receptor agonists, DPP-4 inhibitors, SGLT2 inhibitors or weight loss medications (prescription or OTC) stopped for at least 4 weeks
- Prior use of medication to treat diabetes except gestational diabetes
- Eating disorders (anorexia, bulimia) or gastrointestinal disorders
- Suspected pregnancy (documented negative serum ßhCG test), desiring pregnancy in next 15 months, breastfeeding, or known pregnancy in last three months
- Active or prior history of substance abuse (smoke or tobacco use within past 6 months) or significant intake of alcohol
- Previous bariatric surgery or device intervention for obesity
- Patient not willing to use barrier contraception during study period (unless sterilized or have an IUD)
- History of major depressive or other severe psychiatric disorders
- Inability or refusal to comply with protocol
- Currently participating or having participated in an experimental drug study in previous three months

STUDY PLANS AND PROCEDURES

Treatment Regimen

Obese non-diabetic women with PCOS will be treated for 28-30 weeks with either liraglutide 3 mg (LIRA 3mg) or placebo (see Figure 1-Flow of Patients through Trial).

Figure 1: Flow of Patients Through Trial



Study Assessments

Following written consent, all participants will undergo the following clinical, metabolic and laboratory evaluations before, during and after 30 weeks of treatment. To ensure that

patients remain unidentified, all study subjects will be assigned an individual study identifier which includes the study acronym, patient initials, and unique number. All blood samples will be obtained and results identified and reported using this unique study identifier.

A. *Baseline (Pretreatment) assessment*- A full physical examination will be performed and vital signs (blood pressure, respiration and temperature) determined. Trained personnel using standardized protocols at the baseline and follow-up examination will obtain anthropometric measurements and blood specimens. Absolute body weight, height, waist and hip circumference, body fat distribution (waist/hip {WHR}) and waist/height ratio ({WHtR}) and blood pressure (BP) will be determined. Body weight will be measured to the nearest 0.1 kg using a calibrated digital scale with participants in light clothing and no shoes. Height will be measured to the nearest centimeter. 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}. The circumference measurements will be taken in the upright position using a 15-mm width flexible metric tape held close to the body but not tight enough to indent the skin. Waist circumference (WC) will be measured at the narrowest level midway between the lowest ribs and the iliac crest and hip circumference measured at the widest level over the buttocks while the subjects are standing and breathing normally. The WHR and WHtR will be calculated for measure of body fat distribution.

Oral glucose tolerance tests (OGTTs) with glucose (G) and insulin (I) measured at 0, 30, 60, and 120 after glucose load to assess diabetes, fasting (FBG) and mean blood glucose (MBG) concentrations, insulin resistance and pancreatic beta-cell function will be determined prior to randomization and at 30-32 weeks after full doses of study medications are reached. Mean blood glucose (MBG) concentrations will be calculated by summing glucose values obtained at 0,30,60 and 120 minutes during the OGTT and dividing by 4. At the initial lab evaluation**, a** complete metabolic profile (Chem 12) and calculated eGFR, TSH, prolactin, hemoglobin A1C, and beta-hCG levels will be determined for study inclusion. A baseline blood sample will also be used to measure an androgen profile (total testosterone [T], dehydroepiandrosterone sulfate [DHEAS], sex hormone-binding globulin [SHBG]), and a lipid panel (total cholesterol, high-density lipoprotein [HDL-C], low-density lipoprotein [LDL-C], and triglycerides [TRG].

Body composition analyses will be performed using dual-energy x-ray absorptiometry (DXA) (Hologic Discovery A model, software version 12.5; Hologic, Inc., Waltham, MA) at the start and completion of the study trial. For the scan, the participants will be asked to change into a hospital gown and asked to lie supine on on the table with hands by the side palms facing down away from the thighs and look at the ceiling to maintain head position. DXA can estimate 3 body compartments consisting of fat mass, lean body mass, and bone mass. The relative attenuation of two different x-ray energies by body tissues produces a three-component model comprising total fat mass (FM), total lean mass (LM including fluid and muscle), and total body bone mineral content (BMC) and density. DXA also has the ability to determine body

composition in defined regions such as the arms, legs, and trunk. DXA measurements are based in part on the assumption that the hydration of fat-free mass remains constant at 73%. Total body fat mass [FM]) and fat content of head, trunk and extremities (arms+ legs) is provided by the software. Default software readings provide lines positioned to divide the body into six compartments, i.e. head, trunk, arms and legs. The trunk is defined by a horizontal line below the chin, vertical lines between trunk and arms and oblique lines passing through the colli femuri. The region below this lower border of the trunk, including both legs and the hip region is called lower body region. For each region of the whole body, fat and lean body mass and BMC are determined. Standard software options are used to calculate the total fat-free mass (FFM), fat mass (FM) *vs.* lean mass (LM).

For a better description of the sex specific fat distribution the fat distribution index (FDI) will be calculated as:

FDI = Upper body fat mass in kg/Lower body fat mass in kg

A fat distribution index below 0.9 indicates a gynoid fat distribution, i.e. the fat mass of the lower body surpassed the fat mass of the upper body. A fat distribution index >1.1 defines an android fat distribution. In this case the amount of fat tissue of the abdominal region surpassed the fat mass of the lower body. An FDI between 0.9 and 1.1 is classified as an intermediate stage of fat distribution. We will use the FDI for further quantification of the fat distribution compared to the widely used waist to hip ratio. The WHR describes body shape and silhouette while the FDI provides the quantitative amount of fat distribution. Nevertheless we have to be aware that the FDI describes not the ratio of abdominal fat to gluteal-femoral fat, but the ratio between upper body fat, including abdominal fat and breast fat mass, and lower body fat.

Following baseline screening, eligible patients will be randomly assigned, in a 2:1 ratio, to receive once-daily subcutaneous injections of liraglutide, starting at a dose of 0.6 mg with weekly 0.6-mg increments to 3.0 mg, or placebo; both groups will also receive counseling on lifestyle modification. All subjects will be allocated to one of these 2 groups based on computer-generated random numbers using a block randomization method. The randomization list will be generated at the study site by the unblinded research assistant. Liraglutide and placebo will be provided in pre-filled pens (Novo Nordisk). The study drug (liraglutide and placebo) will delivered in identical prefilled pens, labeled with serial numbers and accompanied by a dispensing unit list. Printed directions for use of the medication will be handed out to subjects before administration of trial drug**.** All patients will receive the same instructions on how to take the medicine. As investigators and participants are blinded to drug assignment, an independent unblinded research assistant will instruct the investigators as to which serial numbers to supply each woman with. The participants will be randomized in a 2:1 ratio (liraglutide: placebo), as we believe that this will facilitate the recruitment to the study.

B. *Treatment*- all patients will be dispensed 5 months (18 weeks) of liraglutide 3 mg (LIRA 3 mg) treatment or placebo and 4 home pregnancy test kits.

Patients on LIRA -**S**tart injection 0.6 mg SC QD 1week, step up to 1.2 mg SC QD 1 week, to 1.8 mg SC QD 1 week, 2.4 mg SC QD 1week, to a max dose of 3.0 mg SQ daily.

Patients on PL -**S**tart injection 0.6 mg SC QD 1week, step up to 1.2 mg SC QD 1 week, to 1.8 mg SC QD 1 week, 2.4 mg SC QD 1week, to a max dose of 3.0 mg SQ daily.

All patients will be called monthly to document the results of their home pregnancy tests and to assess compliance with the medication**.** Patients will receive the same counseling concerning the benefits of lifestyle modification through diet and exercise. 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 by frequent contact using follow-up phone calls. Side effects of the treatment and reason for any withdrawals from the study will be recorded.

C. *Week 16-18 assessment*- Patients will return to clinic for an 18-week re-evaluation of clinical and anthropometric variables (height, weight, body mass index [BMI], waist and hip circumference and blood pressure) and a safety assessment. A serum pregnancy test and complete metabolic profile (Chem 12) and calculated eGFR will also be performed. Side effects of the treatment and reason for any withdraws from the study will be recorded. Another 18 weeks of medication will be dispensed and 4 home pregnancy test kits following a negative serum pregnancy test.

D. *Final (week 30-32) assessment*- After 28 weeks of treatment, patients will be scheduled for final evaluation. They will be instructed to stop medications 1 week prior to their laboratory assessment visit. All laboratory tests (except prolactin, TSH and hemoglobin A1C) will be repeated. All anthropometric parameters and physical including vital signs and DXA will again be performed and calculations will be repeated for post-treatment effects.

During the study period, cycle control will be assessed daily by the subjects using a menstrual diary. Vaginal bleeding will be classified by the subject as either spotting (requiring at least one pad/tampon per day) or bleeding (two or more pads/ tampons per day). The effects of treatment intervention on menstrual abnormalities will be evaluated by assess**in**g post-treatment changes **in** menstruation frequency over 30 weeks from the patient's menstrual cycle diary and normalized to the number of menstruations per year (52 weeks).

All side-effects will also be recorded and summarized for the 30 week-treatment period. During the whole study period, compliance to the treatment will be documented. Compliance with treatment will be checked by questioning about the side-effects and a subjective evaluation of the tolerability of the administered drug; the patients will also asked about incidental missed administrations and whether they had correctly followed the scheduled treatment. Questioning regarding the occurrence of adverse events and use of concomitant medication will take place throughout the trial.

Study Medication

Study Drug Storage- All investigational products (study drugs) will be stored under appropriate storage conditions in a secure area according to local regulations. The investigator is responsible for ensuring that it is dispensed only to study subjects and only from official study sites by authorized personnel, as dictated by local regulations. The investigator is responsible for ensuring that the investigational product is stored under the appropriate environmental conditions (temperature, light, and humidity), as noted in the product labeling. Novo Nordisk will supply all investigational products. **The distribution of all supplied medications is the investigators' responsibility.**

Study Drug Records- It is the responsibility of the investigator to ensure that the unblinded study coordinator maintains a current disposition record of investigational product. Records or logs must comply with applicable regulations and guidelines and should include:

- amount received and placed in storage area; amount currently in storage area
- label ID number or batch number
- amount dispensed to and returned by each subject, including unique subject identifiers
- non-study disposition (e.g., lost, wasted)
- amount destroyed at study site
- dates and initials of person responsible for Investigational Product dispensing/ accountability.

Destruction of Investigational Product- If the study drugs are to be destroyed on site, it is the investigator's responsibility to ensure that arrangements have been made for disposal, and that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal will be maintained.

Biological Sampling Procedures

Laboratory Measures

Hormonal and metabolic parameters will be measured at baseline and 30 weeks of treatment. All participants will undergo a standard 2-h oral glucose tolerance test (OGTT) after an overnight fast (10–12 h). Blood samples for the determination of glucose and insulin levels will be obtained in the fasting state (time 0) and collected at 1/2, 1, and 2 h after a standardized 75 g oral glucose load (OGTT with INS). Blood samples will be centrifuged, divided into aliquots, and assayed. Plasma glucose levels will be determined with a glucose analyzer using the glucose oxidase method (Glucose Reagent Kit, Bayer Newbury, UK). Serum 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. Electrolytes, serum creatinine, and liver enzymes will be measured using standard automated kinetic enzymatic assay. Circulating levels of TSH, prolactin, ß human chorionic gonadotropin (ßhCG), testosterone, sex-hormone binding globulin (SHBG) and DHEAS 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.

Assessment of Insulin Sensitivity and Secretion

Indices of insulin sensitivity and secretion using the serum glucose and insulin concentrations obtained in the fasting state and during the 2hr OGTT will be computed by several previously validated measures. Fasting and glucose-stimulated insulin sensitivity will be estimated by homeostasis model assessment of insulin sensitivity (HOMA-IR) and by Matsuda's insulin sensitivity index (SI_{OGTT}). Early pancreatic beta (β)-cell response will be estimated as the insulinogenic index (IGI) derived from the ratio of the increment of insulin to that of glucose 30 minutes after a glucose load (insulin 30 min − insulin 0 min/glucose 30 min − glucose 0 min) corrected for by the relative level of insulin resistance (IGI/HOMA-IR). The area under the curve (AUC) for glucose and insulin will be calculated using the mathematical model developed by Tai using measures obtained during the OGTT. An estimation of β-cell compensatory function, the insulin secretion-sensitivity index (IS-SI) will be derived by applying the concept of the disposition index to measurements obtained during the 2-h OGTT. The composite IS-SI is defined as the product of *1*) insulin secretion as measured by insulinogenic index (IGI) and *2*) insulin sensitivity as measured by the Matsuda index (ΔINS/ΔGLU 30 x Matsuda SI_{OGTT}). The IS-SI is a validated OGTT-derived measure of β-cell function analogous to the disposition index obtained from the intravenous glucose tolerance test. Improving β-cell compensatory function (increasing insulin sensitivity and enhancing insulin release after an oral glucose load) is reflective of improvement and/or delays in declining glucose tolerance.

The most frequent biochemical parameters of androgen excess include elevated total testosterone or free androgen index (FAI). Baseline blood samples will be collected for measurement of total testosterone (T) and sex hormone-binding globulin (SHBG) concentrations. The free androgen index (FAI) is calculated from the total T concentration (nmol/l)/ concentration of SHBG (nM/L) x100. While clinical markers of hyperandrogenism in females include cutaneous manifestations such as the presence of acne, hirsutism and/or male pattern alopecia, many of these will not be altered with 30 weeks of therapy.

Collateral Research

Several other endpoints will be assessed at each study visit. Baseline blood samples will also be collected for measurement of lipid profiles (cholesterol, HDL and LDL cholesterol, and

triglycerides), adrenal androgens (DHEAS), and liver enzymes (AST/ALT). Dyslipidemia is defined as the presence of at least one of the mentioned lipid parameters abnormalities.

Safety assessments

The safety and tolerability assessments will include incidence and intensity of adverse events, withdrawals because of adverse events, physical exams, vital sign measurements and clinical laboratory parameters. Patients will be seen at 16-18 weeks and 30-32 weeks for laboratory evaluation for a complete chemistry profile and to confirm they are not pregnant. Patients will also be required to perform monthly home pregnancy tests.

STATISTICAL/ANALYTICAL PLAN

Statistical Methods

Statistical analysis will be performed using SPSS version 15.1 for Windows (SPSS, Inc.; Chicago, IL). Continuous variables will be tested for normality of distribution using the Kolmogorov-Smirov test. When necessary, non-normally distributed data will be subjected to logarithmic or square-root transformation to obtain a normal distribution where necessary for subsequent analyses. The primary endpoints are comparison of percent change in body weight and therapeutic impact on biochemical hyperandrogenism (as determined by FAI) from baseline to week 30 of treatment. The secondary endpoints include changes in surrogate measures of insulin action (HOMA-IR, SI_{OGTT}, IGI/HOMA-IR and IS-SI) and glycemic parameters (fasting blood glucose [FBG] and 2 hour post OGTT glucose), glucose and insulin AUC, and mean blood glucose (MBG), anthropometric parameters (BMI, absolute weight, WC and fat distribution by DXA), blood pressure, lipid profiles, and adrenal androgen levels (DHEAS). Direct and indirect estimates of insulin sensitivity and secretion (HOMA, SI_{OGTT}, IGI/HOMA, β-cell compensatory function, glycemic parameters (FBG, MBG, 2 hour post OGTT glucose level, AUC) anthropometric measurements (body weight, BMI), fat distribution (WC, WHR and WHtR), BP, androgen and lipid profiles will be considered as dependent variables.

For all analyses, in which the measures are continuous, data from evaluable subjects will be submitted to a repeated-measures general linear model (SS/ Drug treatments x repeated measures ANOVA) including the arm of drug treatment (liraglutide 3mg vs. placebo) as the between-subjects effect, and the visit (baseline and 30 wks) as the within-subjects effect. To evaluate the differences in the response to each treatment over visits, the interaction effect will be calculated. Baseline comparisons between groups will be made by one-way ANOVAs.

Frequency of patients achieving a body weight reduction of at least 5% and 10% before and after treatment will be compared with the McNemar test (complex chi square [χ^2] for paired data), which formally tests for a change between the observed proportions of k related samples. Dysglycemia occurrence before and after different treatment will also be compared

with the McNemar test. The difference in frequency of menstruation before and after treatment will also be compared using the McNemar test.

Data will be analyzed on completed treatment parameters where relevant (evaluable population). The evaluable population is defined as all randomized subjects who complete treatment through week 30-32 week. Results will be reported as mean +/- S.E.M for normally distributed data and as median (interquartile range) if the distribution is not normal. Categorical data will be presented as percentage. $P < 0.05$ is considered statistically significant.

Sample Size and Justification

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*). To calculate sample size, we used the standard formula suggested for clinical trials by considering a type one error (α) of 0·05 and type two error (β) of 0·20 (power = 80%). Sample size calculation revealed that 57 participants randomized in a 2:1 ratio (liraglutide: placebo) were needed. Using a 30% drop-out rate, the study is designed to recruit 92 patients, enroll 48 liraglutide and 24 placebo to ensure that the number of subjects completing the study (38 LIRA/19 PL) as derived by the sample size calculation is met.

Ethical and Regulatory Requirements

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. Recruitment materials and advertising must be reviewed and approved by the WHIRB prior to use. 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. A copy of the informed consent will be provided to the subject. Any subject may voluntarily withdraw from the study at any time without prejudicing treatment.

Good Clinical Practice - This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonization (ICH) and in accordance with the ethical principles underlying the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50). The study will be conducted in compliance with the protocol. All potential serious breaches must be reported to Novo Nordisk (NOVO) immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the

protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study. Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure; debarment).

The United States Food and Drug Administration (FDA) have assigned pregnancy category X to Saxenda (3 mg liraglutide). Studies in animals or humans have demonstrated there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits. Safer alternatives exist. If patients become pregnant during the study, all medications will be stopped and the patient will discontinue from the study.

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 events will be evaluated on a continuous basis while the patient is on study and until 30 days after the last dose of study drug. Patients should be followed until all treatment-related adverse events have recovered to baseline or are deemed irreversible by the principal investigator.

Adverse Event Procedures

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

An Adverse Reaction (AR) is defined as any untoward and unintended responses to an investigational medicinal product related to any dose administered Thus, for an AR, a causal relationship must be at least suspected by the medical practitioner. Unexpected Adverse Reaction (UAR) is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an investigational product or summary of product characteristics for an authorized product).

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

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

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse event (experience) or adverse reaction is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see NOTE below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.) Potential drug induced liver injury (DILI) is also considered an important medical event.

Serious adverse reaction (SAR): an adverse event fulfilling both the criteria for a Serious Adverse event (SAE) and the criteria for an Adverse Reaction (ADR).

A Suspected Unexpected Serious Adverse Reaction is known as a SUSAR. Sometimes during a clinical trial, there may be serious adverse reactions in subjects given the drug, which may or

may not be dose related, but are unexpected, as they are not consistent with current information and regarded as possibly or probably related to the trial/study product by the investigator.

Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs.

NOTE:

The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

1A. Serious Adverse Event Collection and Reporting

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur during the screening period and within 30 days of discontinuation of dosing. The investigator should report any SAE that occurs after these time periods and that is believed to be related to study drug or protocol-specified procedure. All SAEs, whether they are related or not related to study drug, and pregnancies must be reported to Novo Nordisk (or designee) within 24 hours. They will also be reported immediately to the Woman's Hospital Foundation Institutional Review Board at (225) 231-5296 and Woman's Health Research Department at (225) 231-5275. SAEs must be recorded on an SAE Report Form or similar form (e.g. CIOMS, MedWatch); pregnancies on a Novo Nordisk approved Pregnancy Surveillance Form. Reports are to be transmitted via email or confirmed facsimile (fax) transmission.

Investigators and other site personnel must inform the FDA, via a MedWatch/AdEERs form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32, and will concurrently forward all such reports to Novo Nordisk. A copy of the MedWatch/AdEERs report must be be transmitted via email or confirmed facsimile

(fax) transmission to Novo Nordisk at the time the event is reported to the FDA. It is the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to Novo Nordisk at the same time.

When reporting to Novo Nordisk, a cover page should accompany the MedWatch/AdEERs form indicating the following:

- Investigator Sponsored Study (ISS)
- The investigator IND number assigned by the FDA (if applicable)
- The investigator's name and address
- The trial name/title and Novo Nordisk ISS reference number
-

Investigative site must also indicate, either in the SAE report or the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator. An SAE report should be completed for any event where doubt exists regarding its seriousness. If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form. **All SAE reports and accompanying cover page will** be transmitted to Novo Nordisk via email or confirmed facsimile (fax) transmission.

Serious adverse events that do not require expedited reporting to the FDA need to be reported to Novo Nordisk preferably using the MedDRA coding language for serious adverse events. In the case of blinded trials, the **investigator will provide a copy of the randomization list** or unblind those SAEs which require expedited reporting.

All SAEs **will be** reported to Novo Nordisk, whether or not considered causally related to the investigational product. All SAEs will be documented. The investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements. If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to Novo Nordisk (or designee) using the same procedure used for transmitting the initial SAE report.

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. All SAEs should be followed to resolution or stabilization.

<u>Nonserious Adverse Events</u>

A nonserious adverse event is an AE not classified as serious.

2A. Nonserious Adverse Event Collection and Reporting

The collection of nonserious AE information should begin at initiation of study drug. Nonserious AE information should also be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects.

Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate. All identified nonserious AEs must be recorded and described on the nonserious AE page of the study record.

Completion of supplemental study records may be requested for AEs and/or laboratory abnormalities that are reported/identified during the course of the study.

Laboratory Test Result Abnormalities

The following laboratory test result abnormalities should be captured on the nonserious AE study record page or SAE Report Form as appropriate:

- Any laboratory test result that is clinically significant or meets the definition of an SAE
- Any laboratory test result abnormality that required the subject to have study drug discontinued or interrupted
- Any laboratory test result abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g., anemia versus low hemoglobin value).

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued.

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated.

The investigator must immediately notify Novo Nordisk (or designee) Medical Monitor of this event and complete and forward a Pregnancy Surveillance Form to Novo Nordisk (or designee) within 24 hours and in accordance with SAE reporting procedures described in Section 1A.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form.

Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE (see Section 1A for reporting details.).

1. Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs (see Section 1A for reporting details).
Potential drug induced liver injury is defined as:

AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)

AND

Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),

AND

No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

2. Adverse Events of Special Interest

Certain serious adverse events are informative as single cases because they are uncommon and are known to be strongly associated with drug exposure (in accordance with the reporting obligations of 21 CFR 312.32). The occurrence of even one case of such adverse events would meet the definition of *suspected adverse reaction* (i.e., there is a reasonable possibility that the drug caused them).

In this study, the following adverse events are to be reported to Novo Nordisk, regardless of whether these reports are classified as serious or unexpected:

1. liver test abnormalities accompanied by jaundice or hyperbilirubinemia
2. opportunistic infections
3. pancreatitis
4. anaphylaxis
5. angioedema
6. Steven-Johnson's Syndrome

When one of these events meets the criteria for a serious adverse event, report the event using SAE reporting procedures. When one of these events does not meet the criteria for a serious adverse event, report the event within 24 hours as a non-serious event.

3. Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiogram, x-ray filming, any other potential safety assessment required or not required by protocol should also be recorded as a nonserious or serious AE, as appropriate.

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) 231-5296 and Woman's Health Research Department at (225) 231-5275.

Subjects MUST discontinue investigational product for any of the following reasons:

- Withdrawal of informed consent (subject's decision to withdraw for any reason).
- Any clinical adverse event, laboratory abnormality, or intercurrent illness, which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject.
- Pregnancy
    - Instruct subjects to contact the investigator or study staff immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of study pregnancy tests for subjects enrolled in the study.
    - The investigator must immediately notify Novo Nordisk if a study subject becomes pregnant.
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

All subjects who discontinue should comply with protocol-specified follow-up procedure. The only exception to this requirement is when a subject withdraws consent for all study procedures or loses the ability to consent freely (i.e., is imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness).

References cited in alphabetical order:

1. Andreoli A, Scalzo G, Masala S, Tarantino U, Guglielmi G. Body composition assessment by dual-energy x-ray absorptiometry (DXA). Radiol Med 2009; 114:286–300.
2. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005; 90:1929–1935.
3. Astrup A, Carraro R, Finer N, et al. Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. Int J Obes (Lond) 2012; 36(6):843–854.
4. Astrup A, Rossner S, Van Gaal L et al. Effects of liraglutide in the treatment of obesity: a randomized double-blind, placebo-controlled study. Lancet 2009; 374: 1606-1616.
5. Asunción M, Calvo RM, San Millán JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab. 2000; 85:2434–2438
6. Azziz R, Carmina E, Dewailly D, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006; 91:4237–4245.
7. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab. 2004; 89:2745–2749.
8. Barber TM, McCarthy MI, Wass JA, Franks S. Obesity and polycystic ovary syndrome. Clin. Endocrinol. (Oxford) 65(2), 137–145 (2006).
9. Berneis K, Rizzo M, Lazzarini V, Fruzzetti F, Carmina E. Atherogenic lipoprotein phenotype and low-density lipoprotein size and subclasses in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2007; 92:186–189
10. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update. 2006; 12:673–683
11. Boyle JA, Cunningham J, O'Dea K, Dunbar T, Norman RJ. Prevalence of polycystic ovary syndrome in a sample of Indigenous women in Darwin, Australia. Med J Aust. 2012; 196:62–6.
12. Brown CV Primary care for women: the role of the obstetrician-gynecologist. Clin Obstet Gynecol. 1999; 42(2):306-13.
13. Buse JB, Rosenstock J, Sesti G, et al.; LEAD 6 Study Group Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomized, parallel-group, multinational, open-label trial (LEAD-6). Lancet 2009; 374:1606–1616.

14. Carmina E, Bucchieri S, Esposito A, Del PA, Mansueto P, Orio F, Di FG, Rini G. Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. J Clin Endocrinol Metab 2007; 92:2500-2505
15. Campbell IW. Comparing the actions of older and newer therapies on body weight: to what extent should these effects guide the selection of antidiabetic therapy? Int J Clin Pract. 2010; 64(6):791–801.
16. Cascella T, Palomba S, De Sio I, Manguso F, Giallauria F, De Simone B, Tafuri D, Lombardi G, Colao A, Orio F: Visceral fat is associated with cardiovascular risk in women with polycystic ovary syndrome. Hum Reprod 2008;23:153–159
17. Christakou CD, Diamanti-Kandarakis E (2008) Role of androgen excess on metabolic aberrations and cardiovascular risk in women with polycystic ovary syndrome. Women's Health (Lond Engl) 4: 583–594. doi: 10.2217/17455057.4.6.583
18. Clark AM, Ledger W, Galletly C, Tomlinson L, Blaney F, Wang X, et al. Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. Hum Reprod 1995;10: 2705-12
19. Cussons AJ, Watts GF, Burke V, Shaw JE, Zimmet PZ, et al. Cardiometabolic risk in polycystic ovary syndrome: a comparison of different approaches to defining the metabolic syndrome. Hum Reprod 2008; 23: 2352–2358
20. DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (Exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. Diabetes Care 2005; 28: 1092 – 1100.
21. De Leo V, la Marca A, Petraglia F. Insulin-lowering agents in the management of polycystic ovary syndrome. Endocr Rev 2003; 24: 633–667
22. Diamanti-Kandarakis E. Role of obesity and adiposity in polycystic ovary syndrome. Int J Obes (Lond). 2007;31 Suppl 2:S8–13
23. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, et al. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab. 1999; 84:4006–4011.
24. Drucker DJ: Glucagon-like peptides. Diabetes 1998; 47:159 –169.
25. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocrine Rev 1997; 18: 774-800
26. Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R: The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 1996; 81:3299–3306.
27. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999; 22:141–146.

28. Ehrmann DA, Bread E, Corcoran MC et al. Impaired beta-cell compensation to dexamethasone-induced hypoglycemia in women with polycystic ovary syndrome. Am J Physiol Endocrinol Metab 2004; 287: E241-246.
29. Elkind-Hirsch K, Marrioneaux, O, Bhushan M, Vernor D, Bhushan R. Comparison of single and combined treatment with exenatide and metformin on menstrual cyclicity in overweight women with polycystic ovary syndrome J Clin Endo Metab, 2008; 93: 2670-8.
30. Essah PA, Nestler JE. The metabolic syndrome in polycystic ovary syndrome. J Endocrinol Invest. 2006; 29:270–280.
31. Fidler MC, Sanchez M, Raether B, et al. A one-year randomized trial of lorcaserin for weight loss in obese and overweight adults: the BLOSSOM trial. J Clin Endocrinol Metab. 2011; 96:3067–3077.
32. Flegal KM, Kalantar-Zadeh K. Overweight, mortality and survival. Obesity (Silver Spring) 2013; 21(9):1744–1745.
33. Flegal K, Kruszon-Moran D, Carroll M, Fryar C, Ogden C. Trends in obesity among adults in the United States, 2005 to 2014. JAMA 2016; 315(21):2284-2291
34. Frayling TM, Timpson NJ, Weedon MN et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007; 316(5826), 889–894.
35. Garvey WT, Ryan DH, Look M, et al. Two-year sustained weight loss and metabolic benefits with controlled-release phentermine/topiramate in obese and overweight adults (SEQUEL): a randomized, placebo-controlled, phase 3 extension study. Am J Clin Nutr. 2012; 95:297–308.
36. Harlass FE, Plymate SR, Fariss BL, Belts RP. Weight loss is associated with correction of gonadotropin and sex steroid abnormalities in the obese anovulatory female. Fertil Steril 1984; 42: 649–652
37. Hoeger K, Davidson K, Kochman L, Cherry T, Kopin L, Guzick DS. The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. J Clin Endocrinol Metab. 2008; 93(11):4299–4306.
38. Hollander PA, Elbein SC, Hirsch IB, et al. Role of orlistat in the treatment of obese patients with type 2 diabetes. A 1-year randomized double-blind study. Diabetes Care 1998;21:1288–1294
39. Holmang A, Larsson BM, Brzezinska Z, Bjorntorp P. Effects of short-term testosterone exposure on insulin sensitivity of muscles in female rats. Am J Physiol. 1992;262:E851–5
40. Holte J. Disturbances in insulin secretion and sensitivity in women with the polycystic ovary syndrome. Baillieres Clin Endocrinol Metab 1996; 10: 221–247.
41. Jendle J , Nauck MA , Matthews DR , Frid A , Hermansen K , Düring M , Zdravkovic M , Strauss BJ, Garber AJ. Weight loss with liraglutide, a once-daily human glucagon-like

peptide-1 analogue for type 2 diabetes treatment as monotherapy or added to metformin, is primarily as a result of a reduction in fat tissue. Diabetes Obes Metab 2009; 11:1163–1172

42. Jensterle M, Salamun V, Kocjan T, Vrtacnik B, Janez A. Short term monotherapy with GLP-1 receptor agonist liraglutide or PDE 4 inhibitor roflumilast is superior to metformin in weight loss in obese PCOS women: a pilot randomized study. J Ovarian Research 2015; 8:32-40.
43. Jensterle Sever M, Kocjan T, Pfeifer M, et al. Short-term combined treatment with liraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. Eur J Endocrinol 2014a; 170:451–9.
44. Jensterle M, Kocjan T, Kravos NA, Pfeifer M, Janez A. Short-term intervention with liraglutide improved eating behavior in obese women with polycystic ovary syndrome, Endocrine Research, 2014b; 40:3, 133-138.
45. Jensterle M, Kravos NA, Goricar K, Janez A. Short-term effectiveness of low dose liraglutide in combination with metformin versus high dose liraglutide alone in treatment of obese PCOS: Randomized trial. Endocrine Reviews 2016; 37 (2) Supplement, #185.
46. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002; 346(6):393–403.
47. Kraschnewski JL, Boan J, Esposito J, et al. Long-term weight loss maintenance in the United States. Int J Obes (Lond) 2010; 34(11):1644–1654.
48. Ladson G, Dodson WC, Sweet SD, et al. The effects of metformin with lifestyle therapy in polycystic ovary syndrome: a randomized double-blind study. Fertil Steril. 2011; 95(3): 1059 –1066. .
49. Lamos, EM, Malek R, Davis SN. GLP-1 receptor agonists in the treatment of polycystic ovary syndrome. Expert Review of Clinical Pharmacology 2017; 10(4): 401-408.
50. Legro RS, Arslanian SA, Ehrmann DA, et al. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2013; 98:4565–4592.
51. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab. 1999; 84:165–169.
52. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med. 2001; 111:607–613.
53. Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Rev. 2013;14:95–109

54. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS. Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. J Clin Endocrinol Metab. 2006; 91:1357–1363.
55. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010; 25:544–51.
56. Marre M, Shaw J, Brandle M, et al. Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with type 2 diabetes (LEAD-1 SU) Diabet Med. 2009;26(3):268–278.
57. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care (1999) 22:1462–70.
58. Metwally M, Amer S, Li TC, Ledger WL. An RCT of metformin versus orlistat for the management of obese anovulatory women. Human Reproduction 2009 24(4): 966-975.
59. Moghetti P, Tosi F, Castello R, Magnani CM, Negri C, Brun E, et al. The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women. J Clin Endocrinol Metab. 1996; 81:952–60.
60. Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the United States. JAMA. 2001; 286(10):1195–1200.
61. Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2011; 7(7):CD007506.
62. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 2008; 294:E15–26.
63. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993;36:741–744
64. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene induced ovulation in the polycystic ovary syndrome. N Engl J Med. 1998; 338:1876–1880.
65. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1991;72:83–9
66. Nielsen LL, Young AA, Parkes DG: Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. Regul Pept 2004; 117: 77 –88

67. Nohara K, Laque A, Allard C, Munzberg H, Mauvais-Jarvis F. Central mechanisms of adiposity in adult female mice with androgen excess. Obesity (Silver Spring). 2014;22:1477–84
68. Norman RJ, Clark AM. Obesity and reproductive disorders: a review. Reprod Fertil Dev. 1998; 10:55–63.
69. Norman RJ, Davies MJ, Lord J, Moran LJ. The role of lifestyle modification in polycystic ovary syndrome. Trends Endocrinol Metab. 2002; 13:251–257.
70. Nylander, M, Frossing S, Clausen HV, Kistorp C, FaberJ, Skouby SV. Effects of liraglutide on ovarian dysfunction in polycystic ovary syndrome: a randomized clinical trial. Reproductive Biomedicine Online 2017: 35: 121-127.
71. Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D et al. Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. J Clin Endocrinol Metab 2000; 85: 2767–2774.
72. Pollock NK, Bernard PJ, Gower BA, Gundberg CM, Wenger K, Misra S, et al. Lower uncarboxylated osteocalcin concentrations in children with prediabetes is associated with beta-cell function. J Clin Endocrinol Metab 2011; 96:E1092–9
73. Polotsky AJ, Allshouse A, Crawford SL, Harlow SD, Khalil N, Santoro N, et al. Relative contributions of oligomenorrhea and hyperandrogenemia to the risk of metabolic syndrome in midlife women. J Clin Endocrinol Metab. 2012;97:E868–77
74. Prelipcean MS, O'Neil PJ, Bell DS. Hyperinsulinemic hypoglycemia precipitated by weight loss. South Med J. 2005; 98(7):726–728.
75. Rajkhowa M, Neary RH, Kumpatla P, et al. Altered composition of high density lipoproteins in women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1997; 82: 3389–3394.
76. Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. Diabetes. 1998; 47:699–713.
77. Saxenda prescribing information. URL:www.accessdata.fda. gov/drugsatfda_docs /label/2014/206321Orig1s000lbl.pdf (accessed 22 Sept 2017).
78. Salley KES, Wickham EP, Cheang KI, Essah PA, Karjane NW, Nestler JE. Position Statement: Glucose intolerance in polycystic ovary syndrome—A position statement of the Androgen Excess Society J. Clin. Endocrinol. Metab 2007; 92: 4546-56.
79. Sever MJ, Kocjan T, Pfeifer M, Kravos NA, Janez A. Short-term combined treatment with liraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. European Journal of Endocrinology 2014; 170; 451–459.

80. Scholle SH, Chang JC, Harman J, McNeil M. Trends in women's health services by type of physician seen: data from 1985 and 1997-98 NAMCS. Women's Health Issue 2002; 12(4):165-77.
81. Snow V, Barry P, Fitterman N, Qaseem A, Weiss K. Pharmacologic and surgical management of obesity in primary care: a clinical practice guideline from the American College of Physicians. Ann Intern Med. 2005; 142:525–531.
82. Tai MM. A mathematical model for the determination of total area under glucose tolerance and other metabolic curves. Diabetes Care 1994;17:152–154
83. Talbott E, Clerici A, Berga SL, et al. Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of a case-control study. J Clin Epidemiol. 1998; 51:415–422.
84. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010;8:41
85. Thomson RL, Buckley JD, Noakes M, Clifton PM, Norman RJ, Brinkworth GD. The effect of a hypocaloric diet with and without exercise training on body composition, cardiometabolic risk profile, and reproductive function in overweight and obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2008; 93(9):3373–338.
86. Tong J, Sandoval DA. Is the GLP-1 system a viable therapeutic target for weight reduction? Rev Endocr Metab Disord 2011; 12:187–195.
87. Tosi F, Di SD, Kaufman JM, Bonin C, Moretta R, Bonora E, et al. Total body fat and central fat mass independently predict insulin resistance but not hyperandrogenemia in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2014;2:661–9
88. Tsai AG. Randomised controlled trial: liraglutide for weight loss: more research is needed. Evid Based Med. 2010; 15(2):46–47.
89. Van Can J, Sloth B, Jensen CB, Flint A, Blaak EE, Saris WH Effects of the once-daily GLP-1 analog liraglutide on gastric emptying, glycemic parameters, appetite and energy metabolism in obese, non-diabetic adults. Int J Obes 2014; 38:784–793.
90. Utzschneider KM, Prigeon RL, Faulenbach MV, Tong J, Carr DB, Boyko EJ, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. Diabetes Care 2009; 32:335–41.
91. Velasquez EM, Mendoza, Harner T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovarian syndrome reduces hyperinsulinaemia insulin resistance, hyperandrogenaemia and systolic blood pressure while facilitating normal menses and pregnancy. Metabolism 1994; 43:647-54.
92. Vilsboll T, Christensen M, Junker AE, Knop FK, Gluud LL. Effects of glucagon-like peptide 1 receptor agonists on weight loss: systematic review and metanalyses of randomized controlled trials. BMJ 2012; 344: d7771-7782

93. Wadden T, Butryn M, Wilson C. Lifestyle modification for the management of obesity. Gastroenterology. 2007; 132(6):2226–2238.
94. Zhong X, Zhang T, Liu Y, et al. Effects of three injectable antidiabetic agents on glycaemic control, weight change and drop-out in type 2 diabetes suboptimally controlled with metformin and/or a sulfonylurea: a network meta-analysis. Diabetes Res Clin Pract. 2015; 109(3):451–460.