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

Karen Elkind-Hirsch (✉ karen.elkind-hirsch@womans.org)

Woman's Hospital <https://orcid.org/0000-0003-3577-4009>

Neil Chappell

Woman's Hospital

Donna Shaler

Woman's Hospital

John Storment

Woman's Hospital

Drake Bellanger

Woman's Hospital

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Abstract

Background

The efficacy of lifestyle modifications for established obesity is limited in women with polycystic ovary syndrome (PCOS) and more aggressive interventions are needed. We assessed the efficacy and safety of the GLP-1 analogue liraglutide 3mg (LIRA 3mg) versus placebo (PL) for reduction of body weight and hyperandrogenism in women with obesity and PCOS

Methods

This randomized, double-blind, placebo-controlled study enrolled women from a single-outpatient center diagnosed with PCOS (NIH criteria) with a body-mass index of at least 30 kg/m.² Participants were randomly allocated (2:1) to treatment with a subcutaneous injection LIRA 3mg or visually matching placebo, once daily for 32 weeks, plus lifestyle intervention. Study visits at baseline, and 32 weeks included weight, blood pressure (BP), waist (WC) measures and body composition evaluated by dual-energy X-ray absorptiometry (DXA). Oral glucose tolerance tests (OGTT) were done to assess glycemia, mean blood glucose (MBG), and compute insulin sensitivity (SI) and secretion (IS) measures. Sex steroids, free androgen index (FAI), complete metabolic profile and lipid profiles were measured in the fasting sample. Co-primary endpoints were change in body weight (BW) and FAI. Safety was assessed in all patients who received at least one dose of study drug. This study was registered with ClinicalTrials.gov NCT03480022

Findings

From October 2018 to June 2020, 88 patients were screened, of whom 82 were randomly assigned to LIRA 3mg (n = 55) or PL (n = 27). Change in mean BW from baseline to week 32 was - 5.7% (SE 0.75) with LIRA 3mg vs. -1.4% (1.09) with PL (P < 0.002). At week 32, more patients on LIRA 3 mg than on placebo achieved weight reductions of at least 5% (25[57%] of 44 vs. 5 [22%] of 23; (p < 0.007). LIRA 3mg resulted in significant reduction of FAI, improvements in SI and IS as well as OGTT MBG, and improved body fat by DXA. Gastrointestinal adverse events, which were mostly mild to moderate, were reported in 32 (58.2%) of 55 patients with LIRA 3mg, and 5 (18.5%) of 27 with PL.

Interpretation

In obese women with PCOS, LIRA 3mg once daily achieved a superior and clinically meaningful decrease in BW and androgenicity and improved cardiometabolic parameters compared with placebo.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder that affects up to 18% of women of reproductive age depending on the population studied and diagnostic criteria used (1). Obesity, a state of excess body fat accumulation, appears to be closely associated with PCOS (2). Obesity has

been reported to be threefold more prevalent in women with PCOS compared to healthy women [3] and is associated with an increased likelihood of metabolic sequelae. Excess adiposity in women with PCOS is associated with increased insulin resistance, and compensatory hyperinsulinemia in women with PCOS stimulates the ovarian production of androgens and inhibits the production of sex hormone-binding globulin (SHBG) (4).

Weight loss is fundamental to the management of obese women with PCOS. The goal for weight reduction in women with PCOS is to improve insulin resistance, reduce hyperandrogenism and alleviate PCOS clinical severity. First-line approaches to PCOS treatment include lifestyle modifications such as diet and physical activity; however, they are reported to be minimally effective in reducing weight or treating PCOS-related symptoms. (5) Lifestyle intervention strategies can often be insufficient in treating obesity; however, when combined with pharmacological treatments, clinically relevant weight loss and amelioration of obesity complications can be achieved (6). Glucagon-like peptide-1 (GLP-1) is an anorexigenic hormone released from the L cells of the small intestine in response to nutrient intake. GLP-1 has several physiological functions, including decreasing food intake and increasing satiety and satiation (7). Liraglutide is a GLP-1 receptor agonist that promotes sustained weight loss, as well as abdominal fat reduction, in individuals with obesity, prediabetes, and type 2 diabetes mellitus (T2D) (7–11). Antidiabetic therapy is approved at doses up to 1.8 mg, [8] whereas higher doses are required for maximum weight reduction [9,10]. The dose of liraglutide 3.0 mg (LIRA 3 mg), once daily, as an adjunct to lifestyle modification, was approved for obesity treatment with decreases in body weight of $\geq 5\%$ to as much as $\geq 10\%$ [10,11]. Among pharmacological therapies for obesity, liraglutide 3mg in non-diabetic obese or overweight subjects combined with a reduced calorie diet and increased physical activity was associated with increased weight loss with respect to placebo (– 5 to – 6 kg) with a demonstrated durability. Furthermore, it was associated with an improvement in waist circumference, blood pressure, inflammatory markers, and liver disease (11). Based on the positive results in patients affected by obesity, with or without diabetes, the administration of GLP-1 RA (mainly liraglutide) alone or in combination with metformin has been investigated in women with obesity and PCOS. The available studies of GLP-1 RA 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 (12–15). Treatment with high dose liraglutide alone in a small group of women with PCOS showed superiority in reducing BMI and waist circumference compared to low dose liraglutide combined with metformin. (16)

The weight loss effects of GLP1 RAs previously demonstrated in diabetic and obese non-diabetic patients, offer a unique opportunity to expand the medical options available to patients with PCOS. This randomized, double-blind, placebo-controlled study assessed the efficacy and safety of the GLP-1 analogue once daily subcutaneous liraglutide 3mg versus placebo for reduction of body weight and hyperandrogenism in women with obesity and PCOS

Results

From October 2018 to June 2020, 88 patients were screened, of whom 82 were randomly assigned to liraglutide (LIRA) 3mg (n=55) or placebo liraglutide 3 mg (PL) (n=27). Six participants were excluded from the study after consenting because they were diabetic, pregnant, hyperprolactinemic or not hyperandrogenic. Fifty-five non-Hispanic white (NHW) (67%) and 27 non-Hispanic black women (NHB) (33%) were started on medical treatment. Race was equally distributed between treatment arms. Sixty-seven (44 LIRA; 23 PL) of 82 (82%) participants completed the study per protocol [48 of 60 (80%) NHW and 19 of 22 (86%) NHB]. A total of 15 subjects did not complete the study; 11 participants were taking LIRA 3 mg while 4 subjects were on PL. A summary of participant flow and disposition over time is illustrated in Figure 1.

Baseline comparisons showed no statistically significant differences in any of anthropometric and hormonal characteristics and glycemic and cardiometabolic parameters between drug treatment groups for participants randomized to medication (intent-to-treat participants) (Supplementary Tables 1A and 1B). Similarly, when baseline characteristics of participants completing the trial (completers) were analyzed, no consistent differences between the treatment groups were observed (Supplementary Tables 2A and 2B).

The pretreatment and post-treatment anthropometric and hormonal characteristics and glycemic and cardiometabolic parameters between drug treatment groups in participants completing the trial are summarized in Tables 1A and 1B.

Anthropometric

Absolute body weight and BMI from first to last visit significantly decreased with LIRA 3mg with treatment with LIRA 3mg being more effective in promoting weight loss than PL ($p < 0.002$). Similarly, BMI was significantly decreased ($p < 0.001$) with LIRA 3mg compared with placebo (Table 1A).

Furthermore, when mean percent weight loss from baseline was compared, the LIRA 3mg participants showed significantly greater weight loss when compared to placebo; mean weight loss for LIRA 3 mg was 5.7% (+/-0.75) versus 1.4% (+/-1.09) for placebo ($p < 0.002$) as illustrated in Figure 2. This was further supported by significant differences in 5% and 10% loss from initial body weight when LIRA 3mg was compared to PL as seen in Table 2.

Treatment with LIRA 3mg resulted in favorable changes in a variety of measures of truncal adiposity. All abdominal adiposity measures decreased with LIRA 3mg therapy compared with placebo therapy.

Statistical comparisons of waist size, waist/hip ratio and waist/height ratio showed a significantly greater reduction in mean WC ($p = < 0.011$), WHR ($P < 0.038$) and mean WHtR ($p < 0.048$) after 32 weeks in the LIRA 3mg group compared to PL (Table 1A).

Body fat distribution is one determinant of metabolic dysfunction that cannot be determined by BMI alone and the use of body composition measurement using DXA scans provides a comprehensive look at subjects' body fat, muscle mass and bone. Total body fat (TBF) percentage (%) looks at the participant's total body fat mass (TFM in kg) divided by the total body mass. Lean mass (kg) is the muscle tissue,

skeletal tissue, and water in the body. With LIRA 3mg treatment, TFM and TBF % decreased significantly ($p < 0.018$ and $p < 0.028$ respectively, Table 1A). There was a significant reduction in percentage TBF, trunk fat, upper body fat, and lower body fat in the liraglutide group compared to the placebo group (Table 1A). There was a significant decrease in total body fat mass (kg) assessed by DXA in the liraglutide group compared to placebo, consistent with data on scale measured weight (Supplementary Figure 1). There were no significant changes in lean body mass with either treatment (Table 1A).

The relative fat mass (RFM) index estimates whole-body fat percentage based using a simple linear equation based on the ratio of height and waist measurements. RFM was significantly decreased ($p < 0.05$) with LIRA 3mg compared with placebo (Table 1A). The relative fat mass (RFM) index has been postulated to be more accurate than BMI to estimate whole-body fat percentage (TBF %) and improve body fat-defined obesity. We found that the values of RFM positively correlated with whole body fat percentage (RFM-TBF% $r = 0.71$, $P < 0.0001$) but in this population of nondiabetic women with obesity and PCOS, BMI better predicted whole-body fat mass percentage, as measured by DXA (BMI-TBF% $r = 0.89$, $P < 0.0001$).

Levels of abdominal adiposity based on the android-to-gynoid-fat ratio (AGR) and trunk/leg fat ratio (TLR) is similar to WHR where body shape is described using a ratio but instead of waist and hips circumferences, body trunk/torso and leg fat are used. With LIRA 3 mg treatment, there was a decrease in the AGR (Table 1A; $P < 0.034$) compared to PL. A significant decrease in trunk/leg fat mass ratio (TLR) was also found with LIRA 3mg versus placebo treatment (Table 1A; $P < 0.035$).

Hormonal Changes

Treatment with LIRA 3mg resulted in a significantly decreased free androgen index ($P < 0.006$) compared with PL as illustrated in Figure 3. While total testosterone and dehydroepiandrosterone sulfate (DHEAS) were not consistently reduced with LIRA 3mg compared to placebo (Table 1A), SHBG levels were increased ($P < 0.049$) with LIRA 3mg treatment compared to PL treatment.

Menses occurrence was significantly increased following treatment ($P < 0.0001$) with LIRA 3mg compared to placebo which did change (Table 1A). The change in menstrual frequency with drug treatment is further illustrated in Figure 4. In the LIRA 3mg group, menses were more regular with mean frequency of menstrual cycles increased from baseline of 4.5 ± 0.3 cycles/year to 8.65 ± 0.4 compared with 4.8 ± 0.5 to 4.8 ± 0.65 cycles/year in the PL group (Table 1A).

Glucose Metabolism Measure

Fasting blood glucose levels ($P < 0.021$) and mean glucose levels during OGTT ($P < 0.009$) were significantly improved with LIRA 3mg treatment compared to placebo (Table 1B). All participants on LIRA 3mg showed significantly improved glucose excursion during an OGTT after 32 weeks of treatment compared with subjects on PL (Supplementary Figure 2).

Fasting insulin sensitivity as determined by the HOMA-IR significantly decreased with LIRA 3mg compared with placebo therapy ($P < 0.035$; Table 1 B). Similarly, as seen in Table 1B, the OGTT-derived insulin sensitivity index (SI_{OGTT}) and corrected early insulin response to a glucose challenge (IGI/HOMA) were significantly improved after 32 weeks with LIRA 3mg therapy ($P < 0.028$ and $P < 0.042$, respectively). As illustrated in Figure 5, whole body insulin sensitivity (SI_{OGTT}) was significantly increased with LIRA mg as compared to PL.

In evaluating overall carbohydrate metabolism, the insulin secretion-sensitivity index (IS-SI) derived from the product of insulin action (SI_{OGTT}) and insulin secretion (IGI) from the OGTT, showed a significantly higher mean score with LIRA 3mg treatment indicative of improved beta cell function with enhanced insulin sensitivity ($p < 0.033$; Table 1B). The subjects' mean IS-SI score in the LIRA 3mg treatment group was significantly higher than the mean score on PL (Supplementary Figure 3).

Cardiometabolic Measures

Total cholesterol, HDL-cholesterol levels, and LDL-cholesterol levels were not consistently altered with any treatment as shown in Table 1B. In contrast, TRG concentrations ($P < 0.016$) and TRG/HDL ratio ($P < 0.028$) were reduced significantly with LIRA 3mg compared with placebo (Table 1B). LIRA 3mg therapy was superior to PL in lowering TRG concentrations (Supplementary Figure 4) and improving the TRG/HDL ratio.

The product of triglyceride and glucose, the TYG index is a simple measure of insulin sensitivity which has been used as a surrogate of HOMA-IR for identifying insulin resistance in non-diabetic individuals. Consistent with the other surrogate measures of insulin resistance, the TYG index was significantly lowered with LIRA 3mg compared with placebo (Table 1B; $P < 0.01$),

Systolic blood pressure and diastolic blood pressure were not differentially affected by LIRA 3mg or placebo treatment (Table 1B). While systolic BP was not consistently altered with treatment, DBP was consistently lower in both groups after 32 weeks (pre-vs. post-treatment; $P < 0.002$)

Subgroup Analyses by Race

We performed ancillary subgroup analyses to look at possible racial differences in response to LIRA 3mg and PL in non-Hispanic White (NHW) and non-Hispanic Black NHB women enrolled in this trial. Baseline comparisons showed no consistent differences between NHW and NHB participants on any clinical, anthropometrical, and biochemical parameters in any of the treatment groups (Supplementary Tables 3A and 3B). For both NHW and NHB participants, a significant decline in weight (% change from baseline) was seen with LIRA 3mg treatment compared to placebo (Table 3). Greater percent weight loss was seen in NHW compared to NHB women on LIRA 3mg but the difference was not significant (group 1 vs. 3; $p > 0.05$). The percent weight loss between LIRA 3mg treated groups was significantly greater than placebo treated groups with NHB PL not losing weight on just lifestyle instructions alone. However, significantly more NHW women lost 10% of their body weight than NHB women on LIRA 3mg treatment

(1-NHW LIRA vs. 3-NHB LIRA; $P < 0.04$). Similar to % weight loss, when absolute body weight from pre-treatment to post-treatment was analyzed, both NHW and NHB participants on LIRA 3mg had significantly greater weight loss than the placebo treatment groups ($P < 0.01$) as shown in Table 3.

Likewise, when fat mass (kg) measured by DXA was analyzed, both NHW and NHB participants on LIRA 3mg had significantly higher reductions in fat mass than the placebo treatment groups ($p < 0.028$, Table 3). What was striking was in the PL group, NHW women lost weight but diet and lifestyle were not effective for the NHB group (Table 3). In contrast, there were no differences between NHW and NHB subjects on LIRA 3mg in terms of menstrual cyclicity, androgen levels and glycemic and insulin parameters. Both NHW and NHB participants on LIRA 3mg showed significant and equivalent improvements in androgenicity, cycle frequency and glucose and insulin measures compared to NHW and NHB participants on PL.

Adverse Events

Participants were 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 (17). Participants were asked about the most common adverse events related to liraglutide such as nausea, headache, diarrhea, constipation and vomiting if not volunteered. No serious adverse events were reported during the trial. As seen in Table 4, nausea was the most frequent AE, occurring in 25.5% of the LIRA 3 mg group and 11.0% of the PL group, and it generally had early onset in the initial 5 weeks with increasing dose and was transient. Another common adverse event with LIRA 3mg was injection site reactions which was not seen with placebo. All side effects were generally mild to moderate and clinically manageable. Two participants on LIRA 3mg were exited from the trial prior to completion because they became pregnant and delivered healthy full-term babies.

Discussion

PCOS is uniquely expressed as a multisystemic condition often with varied phenotypes across affected women. The recent development of multiple new therapeutic agents for the management of type 2 diabetes (T2DM) has broadened the options for patient-specific treatment of PCOS. There has been increased interest in considering GLP-1 RAs (mainly liraglutide) as prospective therapeutic options for the management of PCOS (18,19). In addition to their glycemic reducing effect, GLP-1 RAs also have significant effects on weight reduction, lowering blood pressure and improving the lipid profile. This study clearly demonstrates that treatment with LIRA 3mg once daily over 32 weeks is associated with weight loss, improved hyperandrogenism, and menstrual cycle restoration in prediabetic women with obesity and PCOS.

As much as a 5–10% weight loss improves clinical (reproductive and metabolic) outcomes in women with PCOS (2,5). 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 T2DM (20). The few available trial

designed with weight loss as the primary endpoint in non-diabetic obese participants have shown GLP-1 RAs are efficacious for the treatment of obesity (11,21). Subjects had a dose-dependent mean weight loss of 5.7–9.2% (6.0–8.8 kg) versus 0.2–3.1% (0.2–3.0 kg) with placebo (i.e., on diet and exercise alone) (21). A recent meta-analysis concluded that GLP-1 receptor agonists not only had a significant effect on weight loss in overweight T2DM patients but also in non-diabetic overweight persons, reducing subcutaneous fat areas in particular (10.). In agreement, in this study LIRA 3mg reduced bodyweight more than placebo did resulting in a greater loss of absolute body weight and BMI in women with obesity and PCOS. Similarly, we found that LIRA 3mg therapy was more effective in promoting loss of total body fat mass and total fat mass percentage compared with placebo based on DXA measures.

Body composition has significant influence on metabolic activity. Increases in weight and larger waist circumference (WC) are significantly associated with diabetes incidence (22). At risk women with obesity and PCOS have a higher WC, waist/hip ratio (WHR), and waist/height ratio (WHtR) than their obese counterparts. Waist circumference, WHR and WHtR are measures that correlate better with body fatness and be more predictive of adverse metabolic effects because of obesity, as well as cardiovascular complications (23). The improvement in measures of central adiposity were most dramatic with LIRA 3mg therapy where mean WC, WHR and WHtR measurements significantly decreased over 32 weeks, whereas central adiposity was not reduced at 32 weeks on placebo treatment. This finding is in accordance with prior studies in non-diabetic patients that showed that liraglutide 3mg has a significant effect on decreasing abdominal adiposity (9,10).

There is now considerable evidence that cardiometabolic complications from adult obesity are closely related to body fat distribution, with a gender dimorphism (22). In men, adipose tissue tends to accumulate in the upper body (both subcutaneous and visceral), leading to a pattern of android fat distribution characterized by a convex belly and an apple-shaped body. Conversely, in women, adipose tissue accumulates mostly subcutaneously, especially thighs and hips, leading to a pear-shaped body (i.e., a pattern of gynoid fat distribution). Imaging techniques with DXA enable us to evaluate patterns of fat deposition, including levels of abdominal adiposity based on the android-to-gynoid-fat ratio (AGR) (24). Similar to WHR and where body shape is described using a ratio, but instead of waist and hips circumferences, body trunk/torso and leg fat are used. It has been shown that an increase in the android fat distribution (with values of AGR greater than 1) is associated with conditions such as dyslipidemia and insulin resistance, as well as other cardiovascular risk factors such as impaired glucose tolerance, hypercholesterolemia, hypertriglyceridemia, and hypertension (24). Women with a higher ratio of abdominal-to-peripheral fat deposition may be at greater risk for developing cardiometabolic disease. A lower trunk to leg fat ratio (TLR) generally means lower risk for certain health issue. A high ratio of trunk to leg fat showed a strong association to diabetes and mortality that was independent of total and regional fat distributions (25). Fat mass in the legs, in contrast to fat mass in the trunk, is negatively associated with glucose levels and HOMA-IR. Using DXA analyses, a significant reduction in both AGR and TLR was found with LIRA 3mg compared with placebo treatment. Treatment with LIRA 3mg was associated with a marked reduction in total body, trunk, and upper body and lower body fat with no significant change in lean body mass. The significant decrease in percentage upper body fat in the LIRA

3mg group compared to placebo indicated by DXA measurement is particularly relevant from a clinical standpoint, as this could be one mechanism whereby liraglutide ameliorates metabolic imbalances, by reducing the android fat stores, which increase metabolic and cardiovascular risks (26). In the literature, loss of fat mass in the android region is associated with improved cardiovascular risk factors (27).

In previous studies, the relative fat mass (RFM) index has been shown to be more accurate than BMI to estimate whole-body fat percentage based on DXA analyses among women and men (28). Woolcott et al (28) reported that the RFM was more accurate than BMI to estimate whole-body fat percentage and improved body fat-defined obesity misclassification among American adult individuals of Mexican, European or African ethnicity. In this study, the values of RFM positively correlated with whole body fat but in this population of women with obesity and PCOS, BMI better predicted whole-body fat percentage, measured by dual energy X-ray absorptiometry (DXA).

Women with PCOS demonstrate varying degree of resistance to insulin and frequently present with elevated levels of serum insulin (4). Obesity and high levels of androgens both may contribute to the observed resistance to insulin. Moreover, testosterone excess predisposes women to obesity and hyperglycemia (29). It is well established that insulin resistance (IR) and compensatory hyperinsulinemia are central etiological abnormalities in women with PCOS which lead to the overproduction of ovarian and adrenal androgens and an increase in androgen bioavailability through inhibition of sex hormone-binding globulin (SHBG) secretion (4). Even a small weight loss is associated with improvements in hormonal levels and beneficial effects on ovulation and menstrual frequency (30,31). It seems that the main role in the relationship between hyperandrogenism presented as free androgen index (FAI) and metabolic complications is played by SHBG, not by total testosterone (TT). Not surprisingly, SHBG is proposed as a clinically useful marker of IR and metabolic status in PCOS (32). Short-term monotherapy or combination (with metformin) treatment with GLP-1 RAs in overweight or obese patients with PCOS has been reported to result in significant weight loss and additional beneficial effects on biochemical hyperandrogenism (12,14). While TT and DHEAS levels did not significantly decline with treatment in the current study, a significant reduction in FAI consistent with a significant increase in SHBG levels was seen with LIRA 3mg compared to PL therapy. Notably, the most striking finding was the improvement in menstrual cyclicity which returned within a month of LIRA 3mg therapy initiation while no change was found with placebo. Although not directly evaluated, 2 participants conceived during the trial demonstrating return of ovulatory function with LIRA 3mg which was not observed in the placebo group.

GLP-1 facilitates glucose disposal in an insulin-independent fashion which could be attributed to the overall reduction of glucagon secretion and changing the insulin/glucagon ratio (7). With regard to the fasting blood glucose, our results showed that LIRA 3mg consistently lowered blood sugar compared to placebo. Participants on LIRA 3mg also showed significantly improved glucose excursion during an OGTT after 32 weeks of treatment with subjects on LIRA 3mg showing a significantly greater drop in MBG levels compared with placebo. In addition to its glycemic effect, there is considerable evidence that GLP-1 improves insulin sensitivity in peripheral tissues. In obese individuals, inflammation of the adipose tissue is the main driver for IR and by reducing the inflammatory response; GLP-1 facilitates insulin

sensitivity (33). We found that LIRA 3mg treatment of obese women with PCOS resulted in significantly improved insulin sensitivity, estimated by both the HOMA-IR and the OGTT-derived insulin sensitivity index compared to placebo. A significant increase in corrected first phase insulin secretion with treatment with LIRA 3mg. was also demonstrated. There is evidence that GLP-1 receptor agonists act directly on pancreatic beta cells to stimulate insulin secretion. Overall carbohydrate metabolism as estimated by subjects' mean insulin sensitivity-secretion index improved after 32 weeks with LIRA 3mg showing significant improvement over the treatment period indicative of improved beta cell function with enhanced insulin sensitivity with LIRA 3mg therapy versus placebo. This is likely due to direct effects of the GLP-1 receptor agonist on β -cells, as has been demonstrated in single-dose studies (34) but may also be due, in part, to some degree of amelioration of glucose toxicity over the 32 weeks of treatment. Treatment with liraglutide improves beta-cell function in patients with type 2 diabetes, as assessed by homoeostasis model assessment-B analysis and proinsulin: insulin ratio (35). Additionally, liraglutide is able to enhance first- and second-phase insulin secretion and is able to restore beta-cell sensitivity to glucose (34,35). Our finding confirms the beneficial effect of GLP-1 agonists on beta cell function in this obese prediabetic PCOS population.

Visceral adiposity is associated with elevated triglycerides, reduced HDL cholesterol and rise in blood pressure. The improved metabolic and cardiovascular profiles observed with liraglutide are likely multifactorial, including weight loss, and improved glycemia (71). Furthermore, although obesity affects lipid profile, PCOS seems to be a completely independent risk factor of dyslipidemia which affects 70% of patients (36). Dyslipidemia is a common problem among the PCOS-related metabolic abnormalities. Elevated triglycerides (TRG) and decreased high-density lipoprotein cholesterol (HDL-c) seem to be the most common pattern in PCOS (37). While levels of total cholesterol, high and low density lipoprotein cholesterol were not consistently altered after 32 weeks with any treatment, TRG concentrations were significantly reduced with LIRA 3mg compared to placebo. Improvements in TRG/HDL ratio were observed with LIRA 3mg which was not observed with placebo treatment alone. This is consistent with the majority of prior studies which demonstrated that GLP1 agonists significantly lower TRG which reduces atherogenic risk (12, 38,39). Levels of triglycerides decreased significantly from baseline after 32 weeks of treatment with liraglutide as demonstrated in a meta-analysis of the LEAD 1–6 trials (72). We previously reported similar reductions in TRG levels in non-diabetic women with prior GDM being treated with liraglutide and metformin therapy (40). The improvement in the mean TRG/HDL ratio, a marker of insulin resistance, with LIRA 3mg but not placebo further confirms the beneficial effect of GLP-1 agonists on β -cell function and increases in mean insulin sensitivity (41).

While no statistically significant reduction in systolic and diastolic blood pressure was observed with LIRA 3mg compared to placebo there was a significant reduction in diastolic blood pressure with both treatments with weight loss in both groups in this study. Weight loss has been postulated as a contributing mechanism for blood pressure lowering.

Within the adult PCOS population, racial and ethnic differences have been observed (42). Non-Hispanic black (NHB) women with polycystic ovary syndrome have a greater tendency for an adverse

cardiometabolic risk profile (increased insulin, HOMA-IR, and systolic blood pressure) despite lower triglycerides than non-Hispanic white women (NHW). NHB women with PCOS are very similar reproductively to NHW women with PCOS, but that metabolically there are factors that were more favorable in NHB women including lipid levels and bone density (75). However, results were mixed in that some parameters were not favorable in NHB with PCOS such as increased fasting insulin levels (43). NHB and NHW women with PCOS who were morbidly obese (BMI > 35) had WHR, total testosterone, and SHBG that were similar between groups [43]. Another study that compared NHW, NHB and Hispanic adults with obesity and PCOS found no difference in waist circumference and total testosterone by race/ethnicity (42). In this trial, we conducted secondary data analysis of our double-blind controlled clinical trial to determine if there were any racial differences in response to LIRA 3mg on weight loss in this prediabetic population of women with obesity and PCOS. We had 48 NHW and 19 NHB women in the trial with the sub-populations being 32 NHW and 12 NHB participants on LIRA 3mg and 16 NHW and 7NHB participants on placebo. While the numbers were small we did observe racial differences. Similar to prior findings, no consistent differences between NHW and NHB participants on any anthropometric or DXA parameters were observed at baseline. While both groups lost weight on LIRA 3mg, a greater percent weight loss was seen in NHW compared to NHB women on LIRA 3mg but the difference was not significant. However, significantly more NHW women lost 10% of their body weight than NHB women on LIRA 3mg treatment. What was striking was in the placebo group, NHW women lost weight but diet and lifestyle were not effective for the NHB group. One might postulate that the difference in weight loss observed was that NHW were more effective in changing their lifestyle. Our observations support that race/ethnicity should be considered in the management of PCOS and consideration of these racial disparities for future studies in this patient population. Notably, there were no racial differences in LIRA 3mg treated groups with equivalent changes in menstrual cyclicity androgen levels and glycemic parameters supporting an effect of GLP-1 RA liraglutide independent of weight loss in women with PCOS.

Strengths of this study include that the cohort of obese participants are well described and well phenotyped (1990 NIH consensus criteria- phenotypes A and B of the Rotterdam criteria [44]) and double-blind placebo controlled design. Limitations of the present study include the absence of gold-standard measures of insulin sensitivity such as the euglycemic–hyperinsulinemic clamp. Insulin sensitivity/resistance and β -cell function were assessed with OGTT-based surrogate indices rather than with clamp studies. We used the Matsuda index to estimate insulin sensitivity which has high reproducibility and is strongly correlated with the clamp. Additionally, concentrations of total T were obtained by second generation immunoassay so they may not be as sensitive or specific to detect differences between drug and placebo treatment groups if they had been measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Another weakness was the discontinuation rate. Dropout during weight loss program is unfortunately a common phenomenon. In addition, the occurrence of pregnancy during the program, which was the ultimate goal of many participants, was a reason to discontinue study participation. Attempts were made to limit and assess the effects of drop-outs. Last, serial assessments were over only 32 weeks of treatment.

Most of the medications were well tolerated. As previously found with LIRA 3mg, the most frequently reported side effects were gastrointestinal with nausea the most common adverse event. We found the nausea was generally mild to moderate and clinically manageable. The other finding with LIRA 3mg and with not placebo injection were reactions at the injection site. The greatest loss rate was LIRA 3 mg due to inability to tolerate medication and with placebo due to no weight loss.

There is growing interest in broadening the target population for incretin-based therapeutic agents to include individuals with prediabetes. This work provides the first evidence regarding the effects of short-term treatment with liraglutide 3mg versus placebo in prediabetic women with obesity and PCOS. Overall, our results show that treatment with LIRA 3mg is superior to PL in terms of clinical and metabolic benefits in this patient population. Liraglutide 3mg showed significant efficacy in weight reduction in non-diabetic patients with obesity and PCOS as well as improving the parameter of IR, together with improvement in β -cell function. In addition to recommended diet and physical activity, LIRA 3mg consistently resulted in a 5 to 7 kg weight loss, with a greater proportion of patients achieving a 5–10% weight loss compared with placebo. The use of LIRA 3mg over 32 weeks was well tolerated, induced weight loss, waist reduction, and improved certain obesity-related risk factors LIRA 3mg compared to PL in this patient population. The long-term safety and efficacy of this pharmacotherapy for women with PCOS has not been established. Metformin is currently the only recommended therapy for the treatment of prediabetes. Large, double-blinded, randomized clinical trials of longer duration are warranted to assess the long-term efficacy and safety of GLP1 agonists with/without metformin in this patient population. Future studies should include in their design consideration of the significant number of women with this disorder and the relatively young age of this population.

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Methods

Participants

This was a randomized, double-blind, placebo-controlled study designed to compare the efficacy of liraglutide 3mg therapy with that of placebo therapy in nondiabetic, premenopausal women, aged 18 to 45 years of age diagnosed with PCOS with a body-mass index of at least 30 kg/mg.² PCOS was defined according to modified National Institutes of Health [NIH] 1990 criteria (44). Participants were recruited

from Woman's Hospital outpatient Endocrinology and Weight Management Clinic. After a verbal screen, all subjects signed a release and their health records were obtained to confirm their medical history. All subjects provided a gynecological history assessing the regularity and length of their menstrual cycle; participants were specifically asked to record the frequency of menses in previous 12 months. Eligible subjects were required to have the combination of irregular periods (cycle length outside 21–35 days or <8 cycles per year) together with biochemical evidence of hyperandrogenism (total testosterone (T) >50 ng/dL, or free androgen index [FAI] >3.87) [45]) and exclusion of known disorders for bleeding irregularities and androgen excess. The main inclusion criteria also included obese class I, II, and III (BMI >30 mg/kg²) and agreement to use effective contraception consistently during therapy. Adequate contraception during the study was defined as an intrauterine device, tubal sterilization, or combination of two barrier methods with one being male condom since hormonal methods were not permitted.

Exclusion criteria consisted of diabetes diagnosis, smoking within 6 months, pregnancy or lactation, clinically significant systemic disease, uncontrolled hypertension, acute pancreatitis or gall bladder disease, injectable hormonal contraceptive use within 6 months and use of oral contraceptives, other steroid hormones, drugs that affect gastrointestinal motility or carbohydrate metabolism, and/or anti-obesity drugs within 3 months prior to study entry. The Institutional Review Board of the Woman's Hospital Foundation approved the study.

Prior to participation in this trial, each subject had an opportunity to ask questions and signed (and dated) a written informed consent, which was witnessed. The signed consent forms were filed with the investigator's study charts for each subject. A total of 88 participants provided written informed consent to enroll in the study. Following written consent, all study participants underwent clinical, anthropometrical, and biochemical assessments. All subjects were screened at the initial lab evaluation with a complete metabolic profile (CMP) and calculated eGFR, thyroid stimulating hormone (TSH), prolactin, total testosterone (TT) dehydroepiandrosterone sulfate (DHEAS), sex hormone binding globulin (SHBG) and calculated free androgen index (FAI), and qualitative beta human chorionic gonadotropin (β -hCG) to exclude diabetes, thyroid disorder, significant hyperprolactinemia, elevated liver enzymes and/or severe hypertriglyceridemia. A negative serum pregnancy test was a prerequisite for commencing treatment. Six participants were excluded from the study after consenting because they were found to be ineligible based on findings at their laboratory screening. Eighty-two non-diabetic women with obesity and PCOS who met criteria were enrolled. To ensure that participants remained anonymous, all study subjects were assigned an individual study identifier.

Procedures

At baseline and study completion, oral glucose tolerance tests (OGTTs) were carried out (starting at 6:30-9:30 AM) after an 8- 12-hour overnight fast. After the collection of a baseline blood sample, a 75-g oral glucose load was administered; additional blood samples were drawn 30, 60, and 120 minutes later for analysis of glucose and insulin levels. Oral glucose tolerance tests (OGTT) were done to assess glycemia, mean blood glucose (MBG), and compute insulin sensitivity (SI) and secretion (IS) measures. Glucose tolerance was defined as normal, impaired, or diabetic according to the criteria of the American

Diabetes Association (19). Fasting baseline blood specimens were also used for measures of an androgen profile (TT, DHEAS, SHBG, FAI), a lipid panel (total cholesterol [CHOL], high-density lipoprotein [HDL-C], low-density lipoprotein [LDL-C], and triglycerides [TRG]) and complete metabolic profile (CMP).

Trained personnel using standardized protocols at the baseline and follow-up examinations obtained anthropometric measurements. At the initial clinic visit, a full physical examination was performed with body weight (BW), height, waist circumference (WC) and hip size measured and vital signs (blood pressure [BP], respiration and temperature) collected. Body weight was measured to the nearest 0.1 kg using a calibrated digital scale with participants in light clothing and no shoes. Height was measured to the nearest centimeter. Body mass index (BMI) was calculated as body weight in kilogram divided by height in meters squared (kg/m^2). WC was measured at the narrowest level midway between the rib cage and the iliac crest and hip circumference measured at the widest level over the buttocks while the subjects were in the standing position using a flexible measuring tape. The waist/hip ratio (WHR) and waist/height ratio (WHtR) were calculated and used to estimate abdominal adiposity (47). BP was measured using a sphygmomanometer and measured in mmHg. Study visits at baseline, and 32 weeks included body composition evaluated by dual-energy X-ray absorptiometry (DXA).

Randomization and masking

Participants were randomly allocated (2:1) to treatment with a subcutaneous injection liraglutide 3mg (LIRA 3mg) or visually matching placebo (PL), once daily for 32 weeks, plus a lifestyle intervention. All subjects were assigned to one of these 2 groups based on computer-generated random numbers using a block randomization method. The study biostatistician provided a randomized list where every 2 patients were randomized to treatment and 1 to control to guarantee balanced treatment assignment within both strata. LIRA 3mg and PL were provided by NovoNordisk A/S in identical pre-filled pens labeled with serial numbers and accompanied by a dispensing unit list. Printed directions for use (DFU) were provided by Novo Nordisk A/S and were handed out to participants at the first dispensing visit. The research nurse instructed study subjects on how to take their medication. All subjects received the same instructions on **how to take the medicine**. As participants, investigators, and those assessing outcomes were blinded to drug assignment, an independent unblinded research assistant instructed the investigators as to which serial numbers of drug to supply each participant

All participants were dispensed medication and home pregnancy tests for 18 weeks. They were required to perform monthly home pregnancy tests which they sent a texted picture of to the study coordinator until the mid-study visit. The participants were instructed to bring back medication at each visit.

Participants were seen at 16-18 weeks for mid-study clinical and laboratory evaluations including a CMP and pregnancy test. BW, BMI, waist (WC) and hip circumference and BP were measured at every visit. All side-effects of treatments and reason for any withdrawal from the study were recorded. Adherence to medical treatment was also evaluated. Participants were dispensed another 18 weeks of medication and home pregnancy tests and required to perform monthly home pregnancy tests until the final visit.

At study completion (32 weeks of treatment), all clinical and laboratory tests were repeated. All anthropometric parameters and physical measurements, including vital signs and DXA, were again performed and calculations repeated for post-treatment effects.

All participants received the same counseling concerning the benefits of lifestyle modification through diet and exercise. The study subjects were also encouraged to increase daily exercise (such as walking, using stairs), although this was not formally assessed. The participants received further encouragement to adhere to the regime during follow-up phone calls.

During the whole study period, compliance to the treatment was documented. Adherence to treatment, side effects of the treatment, secondary events and reason for any withdrawals from the study were recorded from baseline and throughout the follow-up visits. Questioning regarding the occurrence of adverse events and use of concomitant medication took place all through the trial. Each adverse event was evaluated by all the researchers and when necessary (e.g. pregnancy) immediately reported to the WHIRB, the company, and to the FDA for follow-up and recommendations.

This study was being conducted when COVID-19 became a public health emergency. The study protocol was amended to add an option for telemedicine visits following the COVID 19 pandemic. Forty-one participants enrolled prior to COVID had completed or dropped out of the trial. The remaining 41 participants were either already enrolled or enrolled following the COVID pandemic. The COVID-19 shut down of in-person interactions affected the ability to complete clinic study visits beginning in March 2020 through the first week of May 2020. Ten participants were scheduled for their final study visits the end of April; final visits were moved to the first week of May. Each of these ten participants had adequate study medication to continue in the trial which was verified by the study coordinator and they were still within compliance of protocol. Three patients had a mid-study visit scheduled for March-April 2020. Since an in-clinic visit could not occur, a mid-study virtual visit was completed (N = total of 3 visits for 3 participants). The participants received medications and home pregnancy tests from the study coordinator and were instructed to do an extra pregnancy test at home in place of a laboratory evaluation.

Assessment of Body Composition

Total and regional body composition was determined using dual-energy x-ray absorptiometry (DXA) (Hologic Discovery A model, Hologic, Inc., Waltham, MA) at the start and completion of the study trial.

Participants in hospital gowns were positioned in supine position on the DXA table, and instructed to keep their arms separated from their trunk, hands placed flat on the table, palms facing down, away from their thighs adjacent to the side of the body and their legs separated from one another. Average scanning time was approximately 8 minutes. With this method, body composition consisting of body fat (kg) and lean (kg) soft tissue was estimated. Data were analyzed with Hologic QDR Software for Windows (version 12.5), which integrates whole-body measurement and standard body regions, such as the trunk, arms, and legs, delineated by specific anatomical landmarks. For each region of the whole body (head,

trunk, arms, and legs), fat and lean body mass were determined, expressed as mass (g). The total fat mass percentage (TBF %) was calculated by dividing the weight of the total fat mass (TFM; kg) by bodyweight. Lean mass (kg) is the muscle tissue, skeletal tissue, and water in the body. Ratios of regional DXA mass compartments (like android to gynoid fat mass) have also been used to stratify risk for metabolic disease (48). Android region is typically defined as the region between the last thoracic rib and the upper part of iliac wings. Gynoid fat deposition was assessed by lower limb fat percentage and includes the gluteo-femoral region. Android-to- gynoid fat ratio (AGR) was determined by using fat percentage in lower limbs and in the abdominal region (49). Trunk fat was the amount of fat measured by DXA from below the neck to the pelvis excluding limbs. Trunk-to-leg fat ratio (TLR) was calculated with DXA data as trunk fat mass divided by the sum of left and right leg fat mass, multiplied by 100. A ratio of truncal-to-leg fat also has been associated with cardiometabolic disease-related outcomes in adults (50). This novel body shape measure provides additional information regarding central adiposity to better stratify individuals at risk for diabetes and mortality, even among those with normal BMI (50).

Laboratory Measurements

Plasma glucose levels were determined with a glucose analyzer using the glucose oxidase method (Glucose Reagent Kit, Bayer Newbury, UK). Serum insulin was determined in all samples in duplicate by microparticle enzyme immunoassay (Abbott AxSYM System, Abbott Laboratories, Abbott Park, IL). Levels of CHOL, HDL-C, and TRG were determined using standard enzymatic colorimetric assays on an automated clinical chemistry analyzer whereas LDL-C was calculated according to the Friedewald equation. Electrolytes, serum creatinine, and liver enzymes were measured using standard automated kinetic enzymatic assay. Circulating levels of TSH, prolactin, β hCG, SHBG and DHEAS were analyzed using competitive binding immunoassays with direct chemiluminometric technology (Beckman Coulter Access 2, Brea CA). Total testosterone concentration was measured with an automated chemiluminescent micro-particle immunoassay, the Architect® second-generation testosterone assay (Abbott Diagnostics, Abbott Park, IL) with 5 ng/dL as the minimum detectable concentration of TT. The intra- and interassay coefficients of variation were estimated less than 7 and 11%, respectively, over the sample concentration range for all assays.

Assessment of Insulin Sensitivity and Secretion

To evaluate insulin sensitivity and secretion, indexes derived from either fasting or OGTT-stimulated concentrations of glucose and insulin were used: The Homeostatic Model Assessment of Insulin Resistance Index (HOMA-IR) was calculated using the equation: Fasting insulin concentration (μ U/mL) $\times$ fasting glucose concentration (mmol/L) 22.5 (51). Matsuda's insulin sensitivity index (SI_{OGTT}) was calculated according to the formula: $10,000/\sqrt{[\text{fasting glucose (mg/dL)} \times \text{fasting insulin (}\mu\text{U/L)} \times [\text{MPG} \times \text{MSI during OGTT}]}$ where MPG (mg/dL) is mean plasma glucose OGTT, and MSI (μ U/mL) is mean serum insulin during OGTT (52). Early pancreatic β -cell response was 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 ($\text{IGI}/\text{HOMA-IR}$) (53,54). An oral disposition index (estimation of β -cell compensatory function,) the insulin secretion-sensitivity index (IS-SI) was derived by applying the concept of the disposition index to measurements obtained during the 2-h OGTT and calculated as the index of insulin secretion factored by insulin sensitivity ($\Delta\text{INS}/\Delta\text{PG 30} \times \text{Matsuda SI}_{\text{OGTT}}$) during the OGTT (55).

Collateral Research

To evaluate abdominal adiposity, a WC > 35 inches, WHR > 0.8 and WHtR > 0.5 were considered to be elevated indicating increased cardiometabolic risk (56). Fasting blood glucose (FBG) and mean blood glucose (MBG) concentrations were calculated in mg/dL from glucose levels obtained during the OGTT. MBG concentrations were calculated by summing glucose values obtained at 0, 30, 60 and 120 minutes during the OGTT and dividing by 4. Hyperinsulinemia was considered when fasting levels were >10 mU/l and >40 mU/l, 2 h post-load. The FAI was calculated from the total T concentration (nmol/l)/concentration of SHBG (nM/L) $\times 100$ (45). Dyslipidemia was defined as the presence of at least one lipid parameter abnormality on the described standard lipid panel. A TRG/HDL-C ratio greater than 3.0 was used as an indirect measure of insulin resistance (57).

Safety and tolerability was assessed by collating data on treatment-emergent adverse events (AE), laboratory tests, physical examinations, and vital signs. Prevention of pregnancy was monitored monthly by both laboratory and home pregnancy testing. All participants were educated about not becoming pregnant and performed monthly urine home pregnancy tests. Safety was assessed in all patients who received at least one dose of study drug

Outcomes

The co-primary endpoints of this study were to compare the therapeutic impact of liraglutide 3 mg versus placebo on reduction of body weight and bioavailable ovarian androgen concentrations (as determined by the FAI) in non-diabetic women with obesity and PCOS. We measured 1) the percent change in body weight from baseline to week 32 and the percentage of participants achieving $\geq 5\%$ reduction in baseline body weight with each treatment and assessed 2) the reduction of free androgen index in response to each treatment. We further examined the impact of the administration of these pharmacotherapies on secondary outcome measures of insulin sensitivity, first phase insulin response, glycemic parameters determined with a 75-g OGTT, total fat mass and fat distribution evaluated by DXA and other anthropometric parameters and cardiometabolic markers (lipid fractions and blood pressure) in these obese non-diabetic PCOS women

Statistical analyses

The measurement of changes in body weight and/or hyperandrogenism after treatment utilizing LIRA 3mg vs. placebo as a primary outcome in randomized controlled trials of prediabetic women with obesity and PCOS has not been previously reported. 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 was designed to recruit 92 participants, enroll 48 liraglutide and 24 placebo participants to ensure that the number of subjects completing the study (38 LIRA/19 PL) as derived by the sample size calculation was met.

The general features of the participants (quantitative variables) are presented as number of cases and mean and standard error of the mean unless otherwise mentioned. Categorical variables are presented as frequencies or percentages. Continuous variables were tested for normality of distribution using the Kolmogorov-Smirnov test. When necessary, non-normally distributed data were subjected to logarithmic transformation to obtain a normal distribution where necessary for subsequent analyses. The primary endpoints were 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 included changes in surrogate measures of insulin action (HOMA-IR, SI_{OGTT} , IGI/HOMA-IR and IS-SI; TRG/HDL-c ratio, TyG) and glycemic parameters (fasting blood glucose [FBG], MBG, and 2-hour post OGTT glucose) anthropometric parameters anthropometric measurements (body weight, BMI), fat distribution (WC, WHR and WHtR), BP, androgen and lipid profiles. For all analyses, in which the measures were continuous, data from evaluable subjects were 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 32 wks.) as the within-subjects effect. To evaluate the racial differences in the response to LIRA 3mg and PL in NHW and NHB women over visits, the interaction effect was calculated using a repeated-measures general linear model (SS/ (race x drug treatment) x repeated measures ANOVA). Only where a statistically significant interaction effect was found ($P < 0.05$) was the contrast test applied to locate the differences between the 4 treatment groups. Baseline comparisons between groups (intent-to-treat and completers), baseline comparisons between racial groups and change from baseline with treatment (absolute and percent) for continuous variables were analyzed using one-way ANOVAs to compare means between groups. If differences between the 4 treatment groups were significant ($P < 0.05$ for two-sided tests was set as the level of significance); post hoc comparisons were performed with the Bonferroni test to analyze the variation among the treatment groups. Simple linear associations between quantitative anthropometric and DXA variables were evaluated using Pearson's correlation coefficients with high collinearity defined as $|r| \geq 0.5$. Frequency of participants achieving a body weight reduction of at least 5% and 10% before and after treatment were compared with the Mann-Whitney U test which formally tests whether there is a difference in the dependent variable for categorical independent groups.

No formal statistical analyses were planned or conducted for the safety analyses. All participants who received at least one dose of study drug or placebo were included in the safety analyses. Data were reported in accordance with the sponsor reporting standards which included summarizing AEs and laboratory abnormalities by treatment group.

The completer population was defined as all randomized subjects who completed treatment through week 32 weeks. Data were analyzed on completed treatment parameters where relevant (evaluable

population). All analyses were conducted using IBM SPSS Statistics for Windows, Version 25.0. (Armonk, NY: IBM Corp). Results as determined by 95% confidence intervals (95% CIs) and $P < 0.05$ (2 sided) defined statistical significance.

Data availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available. Patient-related data not included in the paper were generated as part of clinical trials and may be subject to patient confidentiality. Upon request and subject to certain criteria, conditions and we will provide access to individual de-identified participant data from our investigator initiated trial. Data may be requested 12 months after study completion. De-identified participant data will be made available to researchers whose proposals meet the research criteria and other conditions and for which an exception does not apply, via a secure portal.

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Declarations

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Author Contributions

KEH conceived and designed the study, performed background research, carried out statistical analysis and interpretation of the data and drafted the manuscript. DS and DB were responsible for data collection and contributed to the conduct and supervision of the clinical study. NC and JS assisted in participant recruitment and provided administrative and technical support. NC contributed to writing, review and editing of the manuscript. All the authors critically reviewed the manuscript and approved the final draft for submission. KEH is the guarantor of this work and, as such, had full access to all the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis

Competing Interests

All authors are employed at Woman’s Hospital and have no financial ties to NovoNordisk. This was an investigator-initiated trial in which the supporters of the study had no responsibility for study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to all the data in the study, was responsible for data analysis and interpretation and had final responsibility for the decision to submit the report for publication.

The authors declare the following financial competing interests: K.E.H reports receiving grant support from Novo Nordisk, Astra-Zeneca, and Ortho Diagnostics, serves on advisory board for NovoNordisk and Astra Zeneca and is a consultant for EMD Serono. N.C. and J.S. report grant support from Ferring Pharmaceuticals. The authors, E.S. and D.B. have no competing interests. No other potential conflict of interest relevant to this article exists.

Additional Information

Supplementary Information-Additional tables and figures can be found online.

Tables

Table 1A: Pre- and Post-Treatment Anthropometric and Hormonal Characteristics of Participants who completed the 32 week Trial

Parameter	Liraglutide 3mg (n=44)		Placebo-LIRA 3mg (n=23)		P Value
<i>Anthropometric</i>	Pre	Post	Pre	POST	
Body weight (kg)	111 +/- 2.8	104.7 +/-2.9	119 +/- 4.7	117.9 +/-5	0.002
BMI (kg/m ²)	41.6 +/- 1.1	39.1 +/-1.1	43.9 +/- 1.7	43.4 +/- 1.8	0.001.
WC (cm)	111 +/- 2.2	100.9 +/-2.0	116 +/- 3.3	109.9 +/-3.3	0.011
WHR	0.85 +/- .01	0.81 +/-0.009	0.84 +/- 0.02	0.83 +/-0.015	0.038
WHtR	0.68 +/- 0.02	0.62 +/- .014	0.7 +/- 0.02	0.67 +/-0.02	0.048
RFM	46.1 +/- 0.6	43 +/-0.7	47.2 +/- 0.8	45.8 +/-1.04	0.05
<i>DXA</i>					
TFM (kg)	53 +/- 2.0	49.3 +/-2.1	57 +/-2.9	56.8 +/- 3.3	0.018
TBF (%)	47.6 +/- 0.82	46.0 +/- 0.9	48.2 +/- 0.77	47.9 +/-0.96	0.028
AND/GYN R	1.08 +/-0.01	1.05 +/- .01	1.09 +/- 0.02	1.08 +/-0.03	0.034
Trunk/Leg Fat R	1.05 +/- 0.03	1.02 +/- .03	1.06 +/- 0.05	1.07 +/-0.04	0.035
Lean BM	55 +/- 1.05	54.1 +/- 1.1	58.5 +/- 1.8	58.2 +/- 1.9	NS
<i>Hormonal</i>					
TT (ng/dL)	49 +/- 2.9	45.4 +/- 3	45 +/- 3.3	46.8 +/-4.1	NS
FAI	6.9 +/- 0.6	5.98 +/- 0.6	5.6 +/-0.4	6.4 +/-0.75	0.006
DHEA-S (mcg/dL)	176 +/- 13	177.1 +/-14.2	152 +/- 11	171.3 +/-16.8	NS
Menses/year)	4.5 +/- 0.3	8.65 +/-0.4	4.8 +/-0.5	4.8 +/-0.65	0.0001

Values are presented as Mean +/- SEM. P value reflects LIRA 3mg versus Placebo-LIRA. BW-absolute body weight; BMI-body mass index; WC-waist circumference; WHR- waist-to-hip ratio, WHtR- waist-to-height ratio, RFM-relative fat mass; TFM- total fat mass; TBF-total body % fat; AND/GYN R-android/gynoid ratio; Trunk/Leg Fat R-trunk/limb fat mass ratio, Lean BM-total lean body mass, TT- total testosterone; FAI- free androgen index; DHEA-S-dehydroepiandrosterone-sulfate, TSH-thyroid stimulating hormone, Menses/yr refers to frequency of menstrual cycles annualized to a year

Table 1B: Pre- and Post-Treatment Glycemic and Cardiometabolic Parameters of Participants who completed the 32 week Trial

Parameter	Liraglutide 3mg (n=44)		Placebo-LIRA 3mg (n=23)		P Value
<i>Glycemic</i>	Pre	Post	Pre	POST	
FBG (mg/dL)	96 +/- 1.7	90.2 +/- 1.3	95 +/- 2.4	94.3 +/- 2.2	0.021
MBG (mg/dL)	129 +/- 4.9	109.4 +/- 2.9	127 +/- 4.9	125.5 +/- 4.8	0.009
HOMA-IR	4.8 +/- 0.6	4.1 +/- .6	5.1 +/- 1.03	5.2 +/- 1.1	0.05
IS _{OGTT}	3.25 +/- 0.46	3.7 +/- .43	2.8 +/- 0.42	3.0 +/- .48	0.028
IGI/HOMA	0.69 +/- 0.09	1.01 +/- .18	0.61 +/- 1.2	0.8 +/- .16	.0042
IS-SI	345 +/- 44	532 +/- 91	326 +/- 87	416 +/- 69.7	0.033
<i>Cardiometabolic</i>					
CHOL (mg/dL)	181 +/- 4.9	176 +/- 5.3	183 +/- 8.7	178 +/- 8.8	NS
HDL (mg/dL)	42.5 +/- 17	41 +/- 1.8	42.2 +/- 1.5	42 +/- 2.3	NS
LDL (mg/dL)	113.5 +/- 4.5	113.4 +/- 5	117.7 +/- 7.2	112.4 +/- 8.2	NS
TRG (mg/dL)	131 +/- 10	109 +/- 7.7	117 +/- 12	114 +/- 11	0.016
TRG/HDL R	3.3 +/- 0.3	2.9 +/- .26	2.9 +/- 0.4	3.0 +/- .45	0.028
TYG index	8.8 +/- 0.08	8.39 +/- .07	8.7 +/- 0.10	8.5 +/- .09	0.01
SBP (mmHg)	122 +/- 1.7	116.8 +/- 2.7	126 +/- 2.0	123.3 +/- 2.4	NS
DBP (mmHg)	82 +/- 0.96	77.6 +/- 1.6	83 +/- 1.5	78.1 +/- 1.3	NS

Values are presented as Mean +/- SEM. P value reflects LIRA 3mg versus Placebo-LIRA. FBG-fasting blood glucose, MBG-mean blood glucose; HOMA-IR-homeostasis model assessment-insulin resistance; IS_{OGTT} -Matsuda insulin sensitivity index; IGI/HOMA--insulinogenic index divided by HOMA; IS-SI- insulin sensitivity-secretion index; CHOL- total cholesterol; HDL-C- high density lipoprotein cholesterol, LDL-C-- low density lipoprotein cholesterol; TRG-triglyceride; TRG/HDL-C R—triglyceride to high density lipoprotein cholesterol ratio; TYG index-triglyceride-glucose index, SBP-systolic blood pressure; DBP-diastolic blood pressure

Table 2- Change in Weight from Baseline to Study Completion

Parameter	LIRA 3mg	Placebo LIRA 3mg	P value
Number of subjects	44	23	
% weight loss	5.7+/-0.75	1.4+/-1.09	<0.002
Frequency of 5% weight loss	25 (57%)	5 (22%)	<0.007
Frequency of 10% weight loss	13 (29.5%)	2 (8.7%)	<0.05

Values are presented as Mean +/- SEM.

P value reflects LIRA 3mg versus Placebo-LIRA.

Table 3-Changes in Anthropometric and DXA Measures from Baseline to Study Completion analyzed by Race and Treatment

Parameter (Group No.)	NHWLIRA 3mg (1)	NHWPlacebo- LIRA 3mg (2)	NHB LIRA 3mg A (3)	NHBPlacebo LIRA 3mg (4)	P value
Number of subjects	32	16	12	7	
Weight Loss from baseline (%)					
% weight loss	6.5+/-0.95	2.2+/-1.5	3.5+/-0.8	-.43+/-1.02	M=p<0.0003 1 vs. 2- p<0.016 1vs 4- p<0.008 1vs 3-NS 2vs.4-NS 3 vs. 2- P<0.05 3 vs. 4-p < 0.37
Frequency of 5% weight loss	21 (65.6%)	5 (31%)	4 (33.3%)	0 (0%)	M=p<0.005 1 vs. 2- p<0.025 1vs 4- p<0.002 1vs 3- p=0.057 2 vs. 4-NS 3 vs. 2- NS 3 vs. 4-NS
Frequency of 10% weight loss	12 (37.5%)	2 (12.5%)	1 (8.3%)	0 (0%)	M=p<0.039 1 vs. 2- p≤0.05 1vs 4- p<0.032 1vs 3-p<0.04 2 vs. 4-NS 3 vs. 2- NS 3 vs. 4-NS
Weight (kg)					
Weight pretreatment (kg)	110+/- 17.6	120+/- 23.7	112.5+/-20.8	117.3+/-21.1	M=NS
Weight Post- treatment (kg)	103+/-19	118+/-25	108+/-21.5	118+/-22	M=p<0.002 1,3 vs. 2,4 =P<0.01
Fat Mass (kg) by DXA					
Fat Mass pretreatment (kg)	53.5+/- 2.3	58.8+/- 3.6	51.5+/-3.9	54+/-5.05	M=NS
Fat mass Post- treatment (kg)	49.4+/-2.5	57.3+/-4.4	49.2+/-4.2	55.7+/-4.7	M=p<0.018 1,3 vs. 2,4 =P<0.028

Values are presented as Mean +/- SEM.

M=main drug treatment effects; NS=no significant effect.

Group 1-NHW- Non- Hispanic White LIRA 3mg; 2-NHW PL LIRA 3mg, 3-NHB- Non-Hispanic Black LIRA 3mg, 4-NHB PL LIRA 3mg

Table 4-Summary of Adverse Events during Trial

Adverse event	Liraglutide 3mg (n=55)	Placebo Liraglutide (n=27)
Nausea	14 (25.5%)	3 (11%)
Vomiting	5 (9%)	0
Diarrhea	4 (7.3%)	0
Constipation	3 (5.5%)	1 (3.7%)
Heartburn	2 (3.6%)	1 (3.7%)
Reflux	2 (3.6%)	0
Indigestion	2 (3.6%)	0
Injection site reaction (bruising redness, itching)	3 (5.5%)	0
Prolong menstrual bleeding	3 (5.5%)	1 (3.7%)
No menstrual cycles	0	1 (3.7%)
COVID 19	0	1 (3.7%)

Figures

Participant Flow and Disposition Diagram

Figure 1

Participant disposition. Enrollment diagram (compliant with CONSORT flow diagram) shows participant flow and patient disposition over 32 weeks of the trial.

Figure 2

Changes in percent weight loss from baseline to 32 weeks. The mean reduction (percent decrease) in body weight (+S.E.M) after 32 weeks of treatment in prediabetic obese PCOS women treated with liraglutide 3mg (LIRA 3mg; n=44) is compared to placebo LIRA 3mg (PL LIRA 3mg; n=23). While both groups showed body weight reduction (% change from baseline), LIRA 3mg treatment was superior when compared with PL LIRA 3mg ($p<0.002$).

Figure 3

Changes in free androgen index (FAI) compared to baseline after 32 weeks of treatment. FAI was significantly decreased in prediabetic obese subjects with PCOS with LIRA 3mg where no change in FAI was found with PL LIRA 3mg therapy ($p < 0.006$). Data shown are the mean $\pm$ SEM.

Figure 4

Changes in menstrual cycle frequency compared to baseline after 32 weeks of treatment. Return of menses to a normal monthly pattern was significantly better with LIRA 3mg therapy in prediabetic obese participants with PCOS compared to treatment with PL LIRA 3mg which showed little improvement($p < 0.0001$).). Data shown are the mean $\pm$ SEM.

Figure 5

Changes in whole body insulin sensitivity compared to baseline after 32 weeks of treatment. Insulin sensitivity indices obtained from oral glucose tolerance tests (SIOGTT) in prediabetic obese PCOS women were significantly increased after 32 weeks with LIRA 3mg treatment in contrast to PL LIRA 3mg therapy ($p < 0.028$). Data shown are the mean $\pm$ SEM.

Supplementary Files

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