The COMET-PCOS trial- Comparing the effects of Oral Contraceptive Pills versus Metformin in the medical management of overweight/obese women with Polycystic Ovary Syndrome

NCT03229057
November 30, 2021

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(COMET-PCOS)

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Version 10.1

Supported by:

Potential R01 from the NIH

Study Intervention Provided by: No outside support
Sponsor of IND(IDE): Not Applicable

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Last updated: November 30, 2021

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Acronyms

American College of Cardiology	**ACC**
Adverse Event	**AE**
Cardiovascular Disease	**CVD**
Data Monitoring Committee	**DMC**
Diabetes Mellitus	**DM**
Data Safety Monitoring Board	**DSMB**
Insulin Resistance	**IR**
Lifestyle Modification	**LSM**
Metabolic Syndrome	**MetS**
Oral Contraceptive Pills	**OCP**
Polycystic Ovary Syndrome	**PCOS**
Oral Glucose Tolerance Test	**OGTT**

Study Synopsis

Objectives

To determine the effect of Oral Contraceptive Pills (OCP) verses Metformin verses OCP and Metformin on the prevalence of Metabolic Syndrome (MetS) and its components in overweight/obese women with Polycystic Ovary Syndrome (PCOS).

Hypothesis

OCPs increase the risk of MetS specifically by producing an atherogenic lipoprotein phenotype, increasing blood pressure and/ or body weight while metformin modestly decreases MetS risk by decreasing body weight and improving lipid phenotype. The combination of OCP and metformin (OCP, through lowering androgens, and metformin, through improvement in insulin sensitivity) will likely decrease the prevalence of MetS, thereby altering the risk profile for the development of diabetes and possible cardiovascular disease (CVD) in young women with PCOS.

Patient Population

The population will consist of 240 overweigh/obese women with hyperandrogenic PCOS, age 18-40 years old. Subjects must have a body mass index (BMI) between 25-48 kg/m^2. Subjects will be diagnosed with PCOS defined by the Rotterdam criteria.

Study Design

This will be a three-arm, double-blind, double-dummy, multicenter, prospective, randomized clinical trial comparing OCP + placebo vs. Metformin + placebo vs. OCP + Metformin on the prevalence of MetS in women with PCOS. This 6-month study will consist of a screening visit, followed by 5 study visits. No longer term follow-up is planned.

Treatment

The intervention will consist of randomizing subjects to one of three arms. Subjects will either be assigned to OCP + Placebo, Metformin + Placebo or OCP + Metformin. Metformin will be initiated in a step-up fashion using extended release pills as they are associated with fewer gastrointestinal side effects. Subjects will begin with one tablet of metformin every night for 5 days, eventually building up to 4 tablets every night, with the maximum dose of metformin being 2000 mg. In regards to OCP, previous randomized clinical trials (RCTs) have shown that 20mcg ethinyl estradiol/norethindrone 1.0 mg was well tolerated. The study will utilize a 20mcg OCP but a less androgenic third generation progestin (desogestrel 0.15mg) with potentially lesser impact on lipids and insulin sensitivity. All subjects with no menses the 3 months prior to screening will be given medroxyprogesterone acetate, at the screening visit, after a negative pregnancy test (in order to induce menses). Placebo pills will be administered to individuals randomized to OCP or metformin only in order to maintain study blinding. Subjects will undergo 6 in person study visits and life style modification counseling regarding diet and exercise. Patient contact will be made via the subject's preferred contact method after randomization and at the end of each month when there is no in person visit to ensure study compliance with medications, keeping study logs and to review side effects.

Primary efficacy parameter

Determine the prevalence of MetS after randomizing to OCP, metformin or OCP+metformin for 6 months. MetS will be defined by NCEP ATPIII criteria as the presence of at least 3 of the following 5 criteria: TG≥150mg/dl, HDL-C<50mg/dl, BP≥130/≥85mmHg, WC>88cm and fasting glucose≥100mg/dl.

Secondary/Tertiary efficacy parameters

Assess change in HDL-C function, serum apolipoproteins, lipid particle size and number, body fat distribution, BMI, serum adipokines, HbA1c, glucose and insulin sensitivity, serum markers of inflammation, free fatty acids, androgens, quality of life parameters, cognitive testing and predictive factors for change in prevalence on MetS.

Subjects will have the option to consent to storage of their blood for future additional analysis as related to the disease of interest. No research/future analysis will take place, prior to gaining IRB approval.

Statistical Analysis

The primary analysis will use an intent-to-treat paradigm, wherein all randomized subjects are included according to their randomized treatment arm. Data will be summarized using descriptive statistics for continuous variables and frequency statistics for categorical variables. For the primary outcome of the presence of MetS at the end of the trial, logistic regression will be used with independent variables that include terms for the treatment arm and the 3 randomization stratification factors as covariates, with a contrast constructed to test for linear trend over the three treatment arms. For secondary continuous outcomes, linear mixed-effect models will be fit to assess differences between the treatment arms with respect to changes in these outcomes over time. The independent variables in the model will be treatment arm, time, the interaction of treatment and time and the 3 randomization stratification factors as covariates.

Anticipated time to completion

A total of 5 years (2017-2022) is anticipated. 3.5-year enrollment period (based on 3 subjects per site/month x 2 sites), 6-month treatment period followed by time for analyses and interpretation of the data.

Regulatory Compliance

A data safety and monitoring board, led by Dr. Kathleen Hoeger, has been established to ensure that patient safety and clinical study data and regulatory requirements are met regarding the Food and Drug Administration (FDA) code for federal regulations. *This trial is registered on http://www.clinicaltrials.gov*

Study Objectives

Primary Aim

Our primary goal is to determine the effect of 6 months' treatment with OCP vs. metformin vs. OCP + metformin on prevalence of MetS and its components in overweight / obese women. Implicit in the primary aim is clearly defining MetS, by NCEP ATPIII criteria as the presence of at least 3 of the following 5 criteria: TG≥150mg/dl, HDL-C<50mg/dl, BP≥130/≥85mmHg, WC>88cm and fasting glucose≥100mg/dl; and the goal of tracking safety of our interventions throughout the study (through safety lab evaluations, vital signs and diaries).

Secondary Aim

The study will assess secondary outcomes such as defining the effects of OCP (lowering androgens) verses metformin (improving insulin sensitivity) verses OCP + metformin on body fat distribution, glucose tolerance, adipokines and markers of inflammation.

1. Assess change in HDL-C function by measuring reverse cholesterol efflux capacity.
2. Compare changes in serum apoliproteins and lipid particle size and number.

Tertiary Aims

1. Compare changes in total and visceral body fat distribution (DXA) and serum adipokines in the 3 arms and correlate with changes in serum and androgens and markers of insulin sensitivity.
2. Identify changes in serum markers of inflammation and free fatty acids.
3. Compare changes in anxiety, depression and quality of life parameters in all 3 arms.
4. Compare changes in cognitive function scores in all 3 arms.
5. Comparing response to treatment with presence of DNA polymorphisms

Background

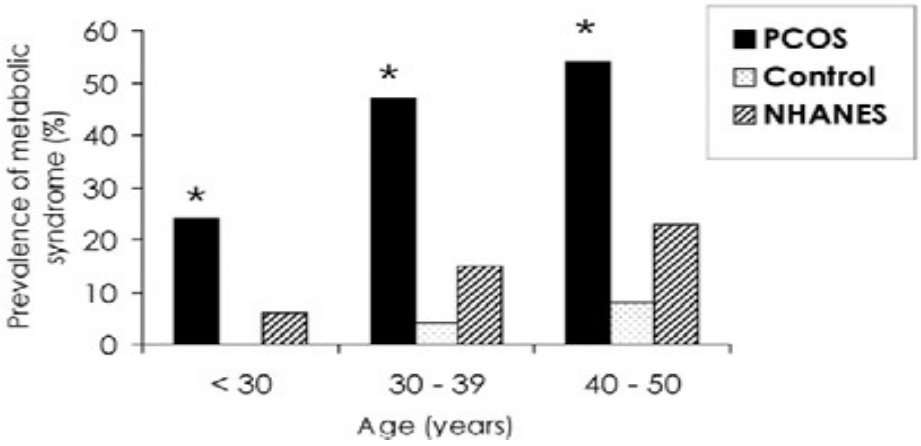
Rationale

Treatment of PCOS

Controversy exists regarding the optimal, long term management of overweight/obese women with polycystic ovary syndrome not attempting pregnancy. It is well known that these women are at increased risk for development of Type 2 diabetes, dyslipidemia, metabolic syndrome and possibly cardiovascular disease due to insulin resistance (IR) and maybe hyperandrogenism. In addition, the extent that racial differences in body fat distribution and dyslipidemia contribute to the differential burden of chronic disease in PCOS is unclear. Although preventive treatments with oral contraceptive pills or metformin are widely used, international surveys show prescribing patterns differ amongst treating physicians (i.e. gynecologist, endocrinologist, or pediatricians). In addition to different effects on menstrual cyclicity and hyperandrogenism, it is now clear that these different medical approaches also have varied metabolic effects possibly leading to adverse health consequences. For example, in a NICHD-funded study, the University of Pennsylvania, PCOS Center recently uncovered a greater than 2-fold increase in MetS after treatment with low dose OCP for 4 months in overweight/obese women with PCOS.

The few randomized clinical trials (RCTs) comparing OCP versus metformin use in overweight/obese women with PCOS show that with improvement in menstrual irregularity and hirsutism, OCP may increase metabolic risk by increasing triglycerides, blood pressure, body fat and weight. Metformin on the other hand, improves metabolic profile by decreasing serum glucose, insulin, triglycerides and body weight, although it has modest effects on menstrual irregularity and hyperandrogenism. These studies have several limitations including short study period, small numbers and the inclusion of Caucasian women only. Moreover, none have evaluated the effect of interventions on the composite risk of MetS or examined underlying mechanisms leading to change in metabolic risk. It is clear that there are no evidence-based recommendations for the optimum and comprehensive medical management of overweight/obese women with PCOS. The working hypothesis states that OCP, through lowering androgens, and metformin, through improvement in insulin sensitivity, will affect the prevalence of MetS thereby altering the risk profile for the development of diabetes and possible CVD in these young women.

Figure 1. Prevalence of MetS in women with PCOS, geographically matched controls and aged matched NHANES population



Metabolic Syndrome in PCOS

In 2005, we reported that the age-adjusted prevalence of MetS was higher in women with PCOS (47.3%, 95% CI 35.3-56.9%) compared to geographically matched controls (4.3 %, 95% CI 1.9-7.6%, p < .001). Even young women (< 30 years) had a high prevalence of MetS (Fig. 1). PCOS phenotypes with hyperandrogenemia have a significantly higher prevalence or MetS compared to controls[1,2,3]. African Americans (AA) in the general population have less visceral adipose tissue (VAT) and lower TG levels compared to Caucasians[4,5]and our recent data shows significantly lower visceral fat measured in AA women compared to white women with PCOS. (Table1).

Table 1. Differences in BMI-matched AA and White women with PCOS Mean (SD)	**AA** N=48	**White** N=48	Difference of means	P value
Triglycerides mg/dl	96 (66-120)	117 (96.5-156.8)	0.7 (0.6, 0.9)	0.01
% Fat in largest Visceral Fat region	46.2 (5.4)	48.7 (5.1)	-2.5 (-4.3, -0.7)	0.01
% Fat in middle Visceral Fat region	41.2 (5.1)	44.4 (4.9)	-3.2 (-4.8, -1.6)	<.001
% Fat in inner-most Visceral Fat region	39.1 (4.9)	43.0 (4.9)	-3.9 (-5.5, -2.3)	<.001

Despite lower TG levels and less visceral adiposity, we found that AA women with PCOS ages 20-34 years have significantly increased risk of MetS compared to White women[6] indicating racial disparity in cardiometabolic risk factors (Table 2). Inclusion of AA women in studies examining metabolic outcomes in PCOS is therefore critical and we will stratify our randomization by race.

Table 2. Racial Disparity in MetS	N	**Metabolic Syndrome**	BMI≥30 kg/m2	TG≥ 150 mg/dL	HDL ≤ 50 mg/dL	BP≥ 130/85 mmHg	Glucose≥ 100 mg/dL
PCOS White	2	22.6 %	51.7	24.6	35.6%	31.9%	4.9%
PCOS Black	6	**40 %****	**72.7**	**10.9**	**76.6%**	**45.5% ***	**18.8%**
NHANES white	2	14.9%	66.1	15.5	39.9%	3.3%	9.0%
NHANES black	1	16.6%	75.4	9.9%	42.9%	10.6%*	8.3%

Effect of OCP on MetS

The OWL PCOS Study – We have recently completed an NIH funded study, OWL- PCOS at PENN and Hershey examining the effects of pretreatment with low dose OCP (20ug ethinyl estradiol) versus weight loss interventions for 16 weeks, on pregnancy rates in overweight/obese women with PCOS[7]. Surprisingly, the OCP arm showed a significant increase in MetS [OR=2.47; 95% CI 1.42, 4.27]; with no change in the intensive Lifestyle modification with pharmacotherapy arm [OR=1.18; 95% CI 0.63, 2.19)] or Combined arms [OR=0.72; 95% CI 0.44, 1.17]. The conversion rate to MetS was 28% in the OCP arm with significant increase in TG levels, and trend towards increase in BP and fasting glucose levels. Interestingly, we noted a significant decrease in VAT after treatment with OCP (Table 3) but an increase in glucose AUC. Our study suggests early onset metabolic dysfunction only in the OCP group, and this was attenuated in the Combined group with the addition of lifestyle modification associated with weight loss. We have previously shown that women with PCOS have a high risk for depressive disorders (OR 5.11 95% CI 1.26- 20.69; P<.03)[8] and this risk persists over time[9]. In the OWL-PCOS study there was in fact an improvement in depressive symptoms and health related quality of life scores with OCP treatment[7]. These findings underscore the need for a clinical trial to comprehensively compare the effectiveness of therapeutic alternatives with specific focus on metabolic outcomes in addition to gynecological, dermatological and mood changes. Understanding the associated pathophysiological changes will add great value to this clinical trial.

Table 3. Effect of OCP on Visceral Adipose Tissue	Mean Change from Baseline (95% CI)	P-valu
Fat Tissue in largest Visceral Fat region (g)	-66.3 (-127.1, -5.5)	0.03
Fat Tissue in middle Visceral Fat region (g)	-47.9 (-91.8, -4.1)	0.03
Fat Tissue in inner-most Visceral Fat region (g)	-40.0 (-78.4, -1.5)	0.04

HDL-C function and lipoprotein analysis in PCOS

There is increasing evidence showing measures of HDL-C function are more useful than HDL-C levels as predictors of CVD risk[10,11]. In collaboration with Dr. Nehal Mehta, Chief of Inflammation and CV Medicine at NIH/NHLBI, we reported no difference in HDL-C levels but a significant reduction in cholesterol efflux capacity in PCOS women[12] (Table 4). We found a significant negative association between testosterone levels and ApoA1 and HDL-C efflux. Women with PCOS also had significant elevation in the atherogenic particles, large VLDL and small LDL, independent of obesity[12].

Table 4. Standard lipid and Lipoprotein	PCOS n=124	Controls n=67	Difference of means	P value
Total Cholesterol	192.5±37.9	189.7±34.5	-2.8(-13.9, 8.2)	0.6
HDL-C mg/dL	**54.7 ±16.1**	**57.5± 17.9**	**2.8 (-2.2, 7.9)**	**0.2**
LDL-C mg/dl	167.1±50.8	154.9±43.9	-12.7(-27.4,2.07)	0.09
Triglycerides mg/dl	146.5±92.9	112.2±69.9**	-34.3 (-60.4,-8.2)	0.01
HDL-C function-Cholesterol efflux	**0.96 (0.86-1.06)**	**1.05 (0.91-1.18)***	**0.07 (0.17, 0.12)**	**0.005**
NMR spectroscopy Large VLDL nmol/L	4.04±3.7	2.37±1.73	-1.6 (-2.7,-0.63)	0.002
VLDL particle size	49.58±6.04	46.78± 5.28	-2.7(-4.7,-0.88)	0.004
Small LDL nmol/L	652.91 ±367.95	434.3± 280.7	-218.57(-330,-106)	0.0002

Impact of OCP vs. metformin on HDL-C function and lipoprotein analysis in PCOS

We have preliminary data using samples from the OWL PCOS study[7] (OCP arm) and PPCOS1 study (metformin arm)[111] showing significant increase in HDL-C efflux with both OCP and metformin treatments. (Table 5) However, this benefit is offset in the OCP group by a significant atherogenic effect, increased sLDL and VLDL particles.

Table 5. Impact of OCP versus metformin on	OWL PCOS-OCP arm n=34		PPCOS1-Metformin arm n=98	
	Mean Change from Baseline (95% CI)	P-value	Mean Change from Baseline (95% CI)	P-value
HDL-C efflux	**0.10 (0.05, 0.16)**	**0.001**	**0.08 (0.04, 0.11)**	**<.0001**
HDL-Cmg/dl	3.6 (0.1, 7.1)	0.04	2.2 (-0.2, 4.5)	0.07
HDL-Particles	4.2 (1.9, 6.5)	0.0004	0.9 (-0.6, 2.5)	0.23
LDL-Cmg/dl	11.7 (0.6, 22.7)	0.04	4.5 (-2.9, 11.8)	0.23
LDL-particles nmol/L	**222.8 (98.6, 347.1)**	**0.001**	51.4 (-31.4, 134.2)	0.22
Small LDL nmol/L	**190.1 (113.1, 267.1)**	**<.0001**	13.3 (-38.4, 64.9)	0.61
Large VLDL nmol/L	**206.2 (90.5, 321.9)**	**0.001**	47.8 (-29.3, 125.0)	0.22

PCOS and adipocyte dysfunction

We have reported significantly lower serum adiponectin and higher leptin[12], hsCRP[13] and FFA levels[12] in women with PCOS reflecting adipocyte dysfunction. Adipose tissue is a major source of FFA and inflammatory cytokines[14,15]which are drivers of CRP production in the liver [16,17].

Polycystic ovary syndrome (PCOS), which affects 5-15% of reproductive age women, has been linked to a high prevalence of cardiometabolic risk factors, namely type 2 diabetes (DM), obesity, dyslipidemia and hypertension[2]. The co-occurrence of these risk factors suggests the existence of metabolic syndrome (MetS), a clinically validated marker for identifying high-risk subjects predisposed to cardiovascular disease (CVD)[18,19]. The CVD risks of Mets and DM have been shown to be higher in women[20,21]and more importantly the association between MetS and CVD is shown to be more pronounced in younger women warranting aggressive preventive therapy[14]

. In our own studies, the prevalence of MetS in women with PCOS is significantly increased from a young age[23] and is higher in the hyperandrogenic phenotype[3]. In a meta-analysis, including 2,256 PCOS women and 4,130 controls, the OR for MetS was 2.2 (95% CI 1.36-3.56) independent of obesity[24].

Longitudinal studies in this population demonstrate a persistent increase in risk of DM, dyslipidemia and hypertension over a twenty-year period10, an increase in serum markers of CVD[25,26]and evidence of subclinical atherosclerosis[27]. In addition, perimenopausal and post-menopausal women with symptoms suggestive of PCOS have a higher risk of CV events[28,29]further linking PCOS to CVD later in life. Finally, it should be highlighted that, in addition to health consequences, these metabolic comorbidities along with gynecological and dermatologic concerns, contribute significantly to the financial (~$4.36 billion annually) and emotional burden of PCOS[30,31]. Collectively, these data support the notion that PCOS is associated with significant CV risk starting in early reproductive life and possibly continuing through menopause. It is striking that evidence based data on treatments for overweight/obese women with PCOS not seeking pregnancy is descriptive, limited and mixed. In fact, there are conflicting clinical guidelines from several medical societies[32,33,34]for first line management recommending either oral contraceptive pills (OCP) or metformin (insulin sensitizer). Surprisingly, the increased risk of MetS in this 'at risk' population as a consequence of taking these medications was unknown till recently. In the OWL-PCOS study, an NIH funded RCT21 we recently reported an increased risk of MetS (OR=2.47; 95% CI 1.42, 4.27) in obese women treated with OCP for 16 weeks. It is clear that comprehensive treatments addressing common complaints such as menstrual irregularity and hirsutism whilst improving metabolic risk are urgently needed. It is therefore proposed COMET PCOS, a randomized clinical trial to compare the effects of OCP vs. metformin vs. OCP+metformin on the risk of MetS in young overweight/obese women with PCOS**.**

OCP use in women with PCOS

OCP are effective first line therapy as they regulate menses and improve acne and hirsutism by significantly lowering bioavailable androgens in PCOS[35,36]. Decreasing androgens with OCP may have additional but relatively unexplored benefits in PCOS as elevated androgens may adversely affect insulin sensitivity, adipocyte function and fat distribution[37]. Some studies in women without PCOS suggest that OCP use is associated with glucose intolerance[38,39]hypertension[40], elevated CRP levels[41,42]and dyslipidemia (especially hypertriglyceridemia) [43,44]. Therefore, it can be hypothesized that OCP use may increase CVD risk especially given the high prevalence of obesity (50-80%) and hypertriglyceridemia in PCOS[2]. However, in a meta-analysis, treatment with OCP was associated with paradoxical changes in lipids, increase in triglycerides (TG) and high-density lipoprotein (HDL-C) levels, with no significant change in fasting glucose, low density lipoprotein (LDL-C) and insulin resistance (IR) in

women with PCOS[45]. Further the impact of only one abnormal metabolic risk factor versus clustering of metabolic components on long term risk of DM and CVD in this population is unknown. None of these studies have examined MetS, a validated predictor of cardiometabolic risk, as a composite outcome. In the OWL-PCOS study pre-conception treatment of obese women with OCP resulted in an increased risk of MetS[7].

Metformin as an alternative treatment for women with PCOS

Women with PCOS have a greater degree of insulin resistance (IR) compared to age and weight matched controls[46] with defects in insulin-mediated glucose transport[47,48] and GLUT4 production[49,50]. Given the role of hyperinsulinemia/IR in the development of hyperandrogenism and disordered folliculogenesis, metformin is the most extensively used insulin sensitizing drug and often first line treatment of PCOS[51,52]. In the general population, it decreases the risk of DM and CV events in adults[53,54]and in children and adolescents with IR or pre-diabetes metformin improves insulin sensitivity[55] and may reduce BMI[56]. Metformin use in PCOS improves the frequency of menses and decreases androgens but less effectively compared to OCP making it less desirable as a first line agent[57]. However, metformin has a favorable impact on metabolic profile by decreasing fasting insulin and hsCRP levels, blood pressure[53,58] and is associated with modest weight loss in women with PCOS [59,60,61,62]. These studies, which are limited by size, randomization and short treatment duration, although suggestive of improvement in metabolic risk, are not conclusive. Lifestyle modification, another first line treatment in PCOS, is associated with poor adherence and sustainability. In a meta-analysis (608 women) use of metformin with lifestyle changes was associated with significantly lower BMI and improved menstruation compared to lifestyle changes alone[63].

OCP vs. metformin

Very few RCTs have examined the effects of metformin versus OCP specifically in overweight/obese women with PCOS. A Cochrane systematic review (4 studies, 104 subjects) concluded that treatment with OCP is associated with improvement in menstrual pattern and serum androgen levels compared with metformin; but metformin use decreases fasting insulin and TG levels compared with OCP use[64,65,66,67,68]. All these studies were conducted in Europe using a progestin that is not available in the US (cyproterone acetate) limiting the generalizability of these findings. These studies do not provide evidence for any single treatment of choice and lack data on important clinical outcomes such as the development of MetS or DM[69].

OCP vs. metformin vs. OCP + metformin

It is suspected that the combination of OCP+metformin may be an appropriate comparator to address gynecological, dermatologic and metabolic end points in PCOS. Only one clinical trial has included three arms namely OCP *vs* metformin *vs* OCP+metformin but included lean and obese women (n=65) with PCOS[70] showing improvement in body fat distribution in the OCP+metformin arm[71].

This clinical trial examined traditional CV markers, lacked mechanistic data and most importantly was not powered to provide clear recommendations. Overall these limited data present a clinical conundrum and underscore the need to answer important clinical questions; do OCP increase the risk of MetS in overweight/obese women with PCOS, can metformin mitigate these effects and therefore will the combination of OCP+metformin offer the best outcomes for improving menstrual regularity, decreasing hyperandrogenism and improving CV risk?

Further complicating matters, PCOS treatment practice patterns vary depending on the physician (i.e. pediatrician, gynecologist or endocrinologist) treating adolescent and adult women with PCOS not seeking fertility. In physician surveys metformin was noted to be preferentially used by Australian and European endocrinologists[72,73]while pediatricians and gynecologists practicing in the US frequently prescribed OCP as first line therapy[74]. Besides

competing guidelines from several international medical Societies, [32,33,34,75] there are no formal recommendations for CV risk stratification prior to prescribing these medications1. Although the best therapeutic approach for MetS is lifestyle modification, such programs alone have high dropout rates necessitating pharmacotherapy to achieve and maintain weight loss[76]. It is therefore critical to identify appropriate medical therapy whilst avoiding medications that might alter the risk of MetS in this young but high risk population. The overall goal of COMET-PCOS is to provide practitioners with evidence for the selection of the most appropriate treatment for overweight/obese women with PCOS by carefully defining the clustering of metabolic risks and benefits associated with three interventions - OCP, metformin or OCP+metformin.

Effects of OCP vs. metformin vs OCP + metformin on individual components of MetS

There is some controversy regarding the predictive value of clustering of risk factors i.e. MetS versus the individual risk factors being independently associated with DM/CVD[77]. Therefore, evaluating the effects of OCP and/or metformin on each component of MetS can provide critical and novel mechanistic insights about the pathways altered by these treatments.

Dyslipidemia

Although PCOS is associated with low HDL-C levels and high TG1 and OCP use increases both HDL-C and TG levels, it is not clear how the interaction of these diametrically opposite alterations impacts the potential risk of coronary artery disease (CAD). While it may be beneficial that the estrogenic component of OCP increases HDL-C[78,79], drugs such as the cholesterol ester transfer protein inhibitor torcetrapib and niacin that increase HDL-C, have failed to reduce CV events in clinical trials[80,81]. Two large studies recently indicated that HDL-C function may be a better marker of CVD outcomes independent of HDL-C levels[10,11]. We were the first to report significant reduction in cholesterol efflux capacity, a metric of HDL-C function, in PCOS[12]. While no gender differences have been described in HDL-C efflux[82], it is unclear if hyperandrogenemia, IR or inflammation affects HDL-C function in PCOS[83,84]. Both hypertriglyceridemia, the commonest lipid abnormality in PCOS, and loss of function mutations of Apo C3 are also predictive of CHD[85,86]. In IR states, adipocytes increase free fatty acid (FFA) production[14] and the liver subsequently increases TG and VLDL synthesis[15] providing the substrate for increased atherogenic small (s)LDL particles[87,88]. Lipoprotein subclass profiles measured by nuclear magnetic resonance (NMR) spectroscopy are not fully apparent on a conventional lipid panel. sLDL and sHDL are associated with incident DM[89] and IR[90,91]and are superior to standard LDL-C measurements in prediction of future CV events[92]. Of note, metformin therapy improved NMR measured lipoprotein profile in the Diabetes Prevention Study[93]**.** Therefore, as a secondary aim evaluating the effects of OCP, metformin and OCP+metformin on detailed lipid phenotyping will provide novel data to define the precise atherogenic impact of each study arm.

Visceral adiposity

Waist circumference is independently and strongly associated with DM, particularly in women[94,95]. Young women with PCOS have a twofold increased risk of DM9 independent of obesity[96]. It is however unclear if visceral adiposity in PCOS is similar[97,98,99]or increased [100,101,102] compared to controls. Studies examining change in body fat distribution after medical treatment in PCOS are also few and contradictory. While androgens may predispose to central body fat distribution[103], OCP use, despite a decrease in androgens, has been associated in some studies with an increase in total and visceral fat mass[104,105,106]. On the contrary, metformin increases insulin-mediated glucose uptake in visceral adipose tissue, thereby increasing the re-esterification of FFA[107,108]. However, clinical studies in PCOS also show mixed results on the effects of metformin on visceral adiposity[70,109,110]. These controversies underscore the need to systematically evaluate change in visceral fat distribution with OCP and/or

metformin use in order to understand its contribution to risk factors such as dyslipidemia, inflammation and altered glucose tolerance.

Adipokine and cytokine secretion

Visceral adiposity is associated with adipocyte dysfunction, characterized by inflammation and impaired adipokine secretion. Leptin secretion rises in parallel with fat expansion in adipocytes[111] and elevated leptin levels have been described in PCOS compared to weight matched controls[112]. Adiponectin, on the other hand, which has insulin-sensitizing, anti-atherogenic and anti-inflammatory properties, and is inversely associated with MetS and independently predicts DM[113], has been shown in a meta-analysis to be significantly lower in PCOS[114]. This observation was independent of obesity and testosterone, but associated with IR. The impact of metformin or OCP therapy on changes in adiponectin are mixed[67,68,69,115,116] possibly related to differential actions on the liver and adipose tissue. Adipocytes also secrete inflammatory cytokines and a meta-analysis confirmed significantly higher levels of hsCRP in PCOS[25]. Metformin treatment is associated with decreased hsCRP[117] and IL-6 levels in PCOS[65,118]. Precisely defining the effects of OCP and/or metformin use on adipokines and inflammatory cytokines will better assess the impact of these treatments on underlying pathophysiological processes associated with adipocyte dysfunction.

In summary, evaluation of MetS risk modification associated with treatments such as OCP and/or metformin in a high risk PCOS population is urgently needed and will have a major impact on current clinical practice.

Implications for the COMET-PCOS Trial

This project brings together a multidisciplinary exceptionally well-qualified team with demonstrated expertise in reproductive endocrinology, cardiometabolic risk assessment and inflammation and clinical trials to study two major health issues namely, PCOS and MetS. Our study includes four highly innovative components:

First Due to lack of evidence there is currently no consensus regarding the medical management of overweight/ obese women with PCOS. OCP and metformin, though currently in use, have differing treatment outcomes and safety profiles, resulting in conflicting guidelines for use in PCOS. COMET-PCOS will be the first 3 arm clinical trial to be adequately powered to provide evidence for single agent versus combination therapies for medical management of PCOS. It will have significant impact on current clinical practice paradigms much like the impact of the PPCOS1 trial (Clomid versus metformin versus clomid+metformin)[111].

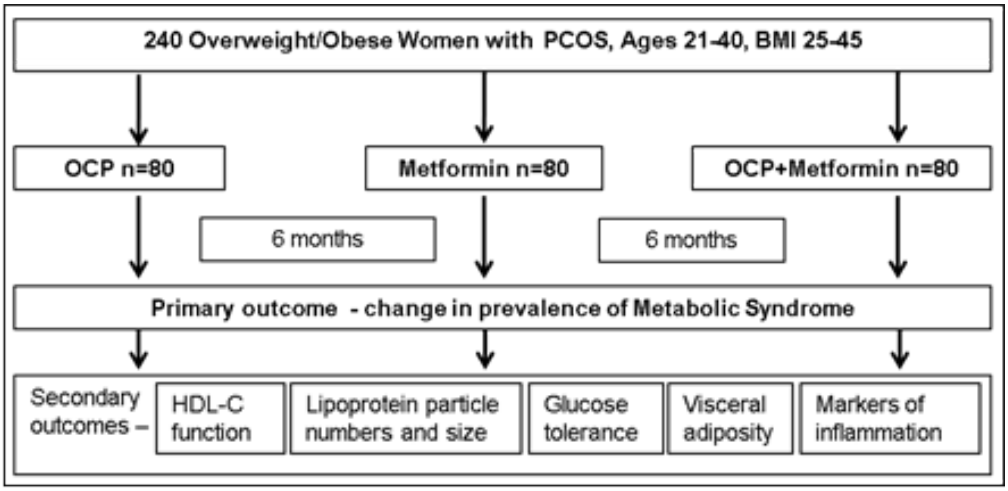
Second Based on our recent findings from OWL-PCOS we have included MetS as a composite endpoint to provide comprehensive assessment of early CVD risk in this young population.

Third We have included innovative mechanistic secondary aims and will apply novel concepts to the assessment of dyslipidemia, the commonest metabolic abnormality in PCOS. We will measure HDL-C function (cholesterol efflux capacity) and lipoprotein particle size and number using NMR spectroscopy to clearly define the atherogenic impact of the three therapeutic interventions.

Fourth Recognizing that there are racial disparities in metabolic components in PCOS, we will stratify the randomization in our primary aim by race. This will provide unique observations of potential importance.

Study Design

Figure 2: COMET-PCOS Study Flowchart



Overview

The flowchart (**Figure 2**) summarizes this study which will consist of medical treatments commencing with randomization at equal allocation (1:1:1) to three interventions OCP, metformin and OCP+metformin for a duration of 6 months. The primary outcome will be change in prevalence of Mets in each group. The secondary outcomes include assessment of HDL-C efflux, lipoprotein particle size and number, glucose tolerance, body fat distribution and measurements of markers of inflammation.

Treatment Design

This will be a three-arm, multi-center, prospective randomized trial of three types of medication treatment in overweight/obese women with hyperandrogenic PCOS who will be randomized to either OCP + placebo, metformin + placebo or OCP + metformin. All subjects will also receive lifestyle modification counseling regarding diet and exercise.

The appropriate study candidates will be recruited from the University of Pennsylvania (Penn) or Pennsylvania State University, Hershey Medical Center (PSU). Recruited subjects will meet the inclusion and exclusion criteria detailed below. Monitoring of this trial at both sites will be conducted by the Penn designated monitor for the Women's Health Clinical Research Center at the University of Pennsylvania, with progress reports provided to the Data and Safety Monitoring Board (DSMB) no less than every twelve months in order to review trial progress and subject safety.

Study Population

Two-hundred forty (240) overweight/obese women with hyperandrogenic PCOS not seeking pregnancy, age 18-40 years, will be enrolled in the participating sites. The overall goal of the inclusion/exclusion criteria is to identify a population of healthy women who are between 25-48 kg/m². Subjects will have a history of androgen excess and chronic anovulation or PCO appearing ovaries. If existing medical records are used to verify inclusion or exclusion criteria, the site should keep a copy of these in the source documents. A one-month wash-out period (6 month wash out in the case of injectable hormonal contraceptive) will be required for medications prior to screening (most common OCP and metformin). Medications that subjects are required to wash-out are included in the MOP and questions can be directed to the Principal Investigator at each site.

Selection and Enrollment of Subjects

Inclusion Criteria

1. Women ≥ 18 to ≤ 40 years of age (at the time of screening), with hyperandrogenic PCOS.
2. Subjects will be diagnosed with PCOS defined by the most up to date Rotterdam criteria based on:
    a. Androgen excess

    And

    b. polycystic ovaries.

    Or

    c. A history of chronic anovulation or spontaneous periods.

3. BMI ≥ 25 kg/m² to ≤ 48 kg/m² obtained at screening visit.
4. In good general health according to the investigators discretion
5. Willing to avoid pregnancy for the duration of the study.

Exclusion Criteria

1. Current pregnancy or desire of pregnancy during course of study
2. Currently breastfeeding
3. Known 21 hydroxylase deficiency or any form of congential adrenal hyperplasia (CAH)
4. Untreated thyroid disease (TSH ≤0.45 mlU/mL and ≥ 4.5 mlU/mL)
5. Untreated hyperprolactinemia (2 Levels≥30 ng/ml at least one week apart)
6. Type 1 or type 2 Diabetes Mellitus currently receiving anti-diabetic agents, (subjects may wash out from metformin if taking the drug for another indication.)
7. Liver disease (AST/ALT≥2 times normal or a total bilirubin ≥2.5 mg/dL)
8. Renal disease (BUN≥30 mg/dL or serum creatinine ≥1.4 mg/dL)
9. Anemia (hemoglobin ≤10 mg/dL)
10. current history of alcohol abuse (≥ 22 drinks/week in the past 3 months)
11. Poorly controlled hypertension defined as average systolic blood pressure >= 150 mm Hg or average diastolic ≥100 mm Hg obtained on three measurements obtained 5 minutes apart. If treated, average systolic blood pressure ≥140 mm Hg or average diastolic ≥90 mm Hg
12. Patients with a history of, or suspected cervical carcinoma, endometrial carcinoma
13. TG≥250mg/dl
14. Current Use of lipid lowering or weight loss agents
15. Current use of hormonal contraceptives such as oral contraceptives, depo progestin, or hormonal implants
16. Participation in any study of an investigational drug or device or biological agent within 30 days
17. Suspected adrenal or ovarian tumor secreting androgens
18. Suspected Cushing's syndrome
19. Bariatric surgery procedure in the recent past (≤12 months)
20. Absolute contraindications to the use of hormonal contraceptives or metformin (details in MOP)
22. subjects who are unable to comply with the study procedures, (In the opinion of the investigator,)

***Note:** the study will allow rescreening of subjects. When subjects are re-screened a new consent form will be signed and a new study number will be assigned.*

Study Termination Criteria

1. Development of diabetes as defined in the manual of procedures
2. Development of hypertension as defined in the manual of procedures
3. Unable to tolerate study medications due to side effects
4. Pregnancy (women found to be pregnant will be referred to prenatal care)
5. Miss more than 2 consecutive visits (unless excused by the PI)

Study Enrollment Procedures

Recruitment

We do not anticipate difficulty with the completion of this trial as our study team has demonstrated the ability to recruit a similar population in several previous trials (PCOS studies completed by UPenn and PSU: OWL-PCOS/PPCOS1 /PPCOS2). At both sites we have expert investigators in PCOS to improve external validity and to help overcome any possible recruitment barriers. Subjects recruited at UPenn are drawn from a four state area including Pennsylvania, New Jersey, Delaware, and Maryland. Dr. Dokras directs the Penn PCOS center which is located at 2 sites (Philadelphia city and in a suburban location - Radnor) and she sees approximately 1000 visits/year exclusively for PCOS. UPenn also has a high patient volume in Reproductive Endocrinology with 22,000 patient visits per year. Hershey Medical Center is the only academic medical center between Philadelphia and Pittsburgh with a large referral area. They have recruited subjects from areas as far away as Scranton/Wilkes Barre (90 miles) to the northeast, and Altoona (150 miles) to the West, Harrisburg (12 miles), York (30 miles), Lancaster (20 miles), State College (90 miles), Williamsport (90 miles), and Allentown (85 miles). The location of the two sites, University of Pennsylvania (UPenn) in an urban setting, and Hershey Medical Center (PSU) in a rural/suburban setting will allow us to recruit from a representative sample of the population.

Clinical Practices of Investigators

Women presenting to the clinical practices of Dr. Dokras, Dr. Mainigi, and Dr. Legro for consultation for PCOS (after clinical confirmation of their potential eligibility for admission into the study based on inclusion and exclusion criteria) will be approached to participate by the research coordinator or non-care providing physician. In the recruitment visit, study details including the intervention will be explained. Risks and benefits will be thoroughly discussed and consents given to the patient prior to any study procedures being performed. The drugs being utilized in this study are commonly used in current practice and may be familiar to the women interested in participating in the study.

Hospital/Local Health Care Referrals

Subjects will be recruited at each site from individual practice(s) of the investigators as noted above as well as faculty/resident clinics. Ongoing contact with practice and faculty members as well as with residents will be made by the investigators and coordinators, reminding them of the inclusion criteria, importance of the study, etc. In addition, the investigators will describe the study to members of other departments in the hospital, primarily family practice, medical endocrinology, and gynecology who also see and treat these patients. Contact with local physicians will be made and/or grand rounds will be given to disseminate information about the study. Letters will be mailed or emailed to potential subjects which provides details of the study and contact information of the study team (to allow for subject to reach out for more information). Penn Datastore as well as electronic medical record (EMR) recruitment tools such as SlicerDicer and best practice announcements (BPA) (and PSU, CHOP equivalents) may be utilized to provide a list of potential subjects based on clinical practice information.

Referrals from Study Participants

Study participants often refer friends, acquaintances and colleagues to be potential participants.

A subject referral-based payment structure will be added to increase recruitment. Prior study participants who have given their permission for re-contact that are not Penn Employees will be contacted to give them the opportunity to refer individuals to our study. For each individual referred who qualifies as eligible, the prior participant will get $25. The referral period will last until the study team determines the budget to support this type of referral is no longer available. The study team will inform the prior participants at that time of the ending of the referral-based payment program.

Previous Study Participants

The trial will be offered to women who have previously participated in clinical trials offered by the study team, who have consented to be contacted for future participation via their preferred contact method (after confirmation of their potential eligibility for admission into the study based on inclusion and exclusion criteria).

Local Advertisements

Advertisements will be placed in local newspapers and run on local television networks / radio near Penn and PSU and will be continued on a regular basis if response is good. SEPTA advertising through regional rail, Market-Frankford line and Broad Street Line has been a top source of recruitment in the pass and may be utilized again.

Contact with PCOS support groups

Contact will be made with both national and local support groups to spread information about the study through informational brochures and/or participation in local meetings if necessary.

Web sites

The study will be prominently displayed on the local (Penn, PSU, CHOP) web site. Additionally, each center will have a web page devoted to this study with general as well as contact information. Information will also be available at clinicaltrials.gov. Social Media and Craigslist will also be utilized, as this has been a proven source of recruitment in the past. For ads placed on social media, specifically Facebook and Instagram, they will be one-way adds and the study team will not directly interact with subjects on social media. The ad will highlight a contact number for the interested individual to call to discuss the study. Social media ads will be monitored by the University of Pennsylvania study coordinator to ensure they are properly being featured and not causing inappropriate comments. This monitoring will occur bi-weekly. If any problems arise with the social media ad they will be immediately removed however this is not anticipated as they are one-way ads. This type of advertising has been previously used for studies within the department and has been well received.

IRB Approval

It is expressly acknowledged that all informational material that could be construed to be advertising will be approved by the central IRB prior to dissemination.

Procedures for Tracking Sources of Subjects and their Disposition

We will track all contacts from subjects interested in the study. We will develop a pre-screening list that documents date and point of contact, eligibility based on telephone screening or survey, and follow-up if subject meets prescreening and is interested in further participation. The consent form may be mailed or emailed prior to the screening visit which is the next point of contact.

Obtaining Informed Consent

Once potential women have been prescreened, they will be referred to the site clinical coordinator or his/her designee for a screening visit. The consent process will be conducted according to each site's standard operating procedures.

Inclusion and exclusion criteria will be reviewed. After the study has been completely explained to the woman, she will be given the informed consent documents to review. Some individuals may wish to complete the informed consent process at the time of this discussion. In these cases, the informed consent documents will be signed once all questions are resolved. In other cases, the subjects may wish to take the consent forms home for further consideration. In these cases, the coordinator will confirm the woman's willingness to be contacted, and set up a tentative timeframe to be back in touch with the subjects. The consent can be signed once all questions have been answered to the satisfaction of the potential subject. A signed informed consent document, approved by the central IRB, will be confirmed on all subjects prior to the baseline evaluation.

In order to be eligible for enrollment and randomization, the woman must be confirmed to meet all inclusion and exclusion criteria described above.

Intervention Group Assignment

After screening is completed the information will be uploaded in RedCAP electronically, and randomization will be performed by IDS at the time of the randomization visit. Subjects and study coordinators will not be informed of their treatment assignment.

Study Interventions

Interventions, Administration and Duration

Study Medication: All subjects will be randomized to receive either OCP + placebo, Metformin + placebo or OCP + Metformin for 24 weeks. All medications will be self-administered. Once eligibility has been confirmed and subjects have been randomized, the site staff will distribute appropriate medications during each visit. The subject will receive enough pills to last until the next visit (additional pills for each arm will be distributed at the start of the study for situations if/when the visit window must be utilized; subjects will receive appropriate instructions regarding the extra medication). Subjects will be required to wash out of any excluded medications prior to enrollment in the study.

Medication Compliance: Medication bottles will be returned at visits 3 through 6 unless a remote visit is being conducted at the discretion of the PI. IDS will perform pill reconciliation during these visits to measure medication compliance. Medication compliance can also be assessed by reviewing the returned subject diaries. Compliance is assessed during the monthly visits on site or remote contact with the subject. Non-compliant subjects will be reminded of the importance of taking the medication properly.

Life Style Modification (LSM): In addition to taking study medication, all subjects will receive counseling (through the use of handouts) about diet and exercise at the randomization and 16 week visit. The diet is based on the lifestyle modification protocol of the look AHEAD study and POWER-UP trial. The study will follow the Diabetes Prevention Program recommendations for increasing physical activity. A similar protocol was utilized in OWL-PCOS and similar written materials will be provided to all subjects in the current trial. Assessment of dietary intake will be based on NCI created automated self-administered 24-hour (ASA24) food recall tool. This will allow us to access variability in dietary intakes in the 3 arms of the study.

Cognitive Testing: All subjects will undergo cognitive functioning testing via the NIH Toolbox, a comprehensive set of neuro-behavioral measurements that quickly assesses cognitive, emotional, sensory, and motor function. Audio-recording of cognitive testing is optional (all recordings will be deleted after transcription). The NIH Toolbox is assessing cognition for subject (age3-85), thus eliminating a ceiling effect. Staff will be trained to administer the tests. Training is performed online via the NIH Toolbox website. Any additional training or oversite will be done by a Clinical Neuropsychologist. During testing staff will audio record cognitive testing with subjects to verify answers given in a timely matter. All audio recordings will be consented for by the subject prior to recording and will remain on file for less than 24 hours at which point they will be deleted. One patient recording from each certified staff member will go to the Clinical Neuropsychologist to verify standardization. A description of the neuropsychological assessments includes:

Neuropsych assessments:

- *Controlled Oral Word Association (COWA):* subjects must spontaneously name as many words as possible beginning with a given letter within a 60 second span.
- *Category fluency:* subjects must spontaneously name as many objects as possible that fit a given category within a 60 second span.
- *Logical memory:* subject is read the first story and recalls as many details as possible. The subject is then read a second story and must recall as many details as possible about that story; second story and recall is repeated. Following a delay of 25-35 minutes, the subject must again recall as many details of each story as possible.
- *Digit Span:* subject is read a string of numbers and must repeat the numbers back to the examiner in either the exact same order (DS forward) or in reverse order (DS backward). Number strings increase in length by one number with each successful turn, and discontinued when patient makes an error. Score is the longest string a patient can successfully repeat back.
- *Digit symbol substation:* Testing page shows a key at the top, which contains the numbers 1-9, each paired with a special symbol. Below the key, subjects must fill in the numbers that match a grid of special symbols as quickly as possible, based on the key above.
- *Rey-Osterreith Figure:* subject copies the complex figure from the stimulus page. The stimulus is removed and the patient then immediately redraws as much of the figure as they can (immediate recall score).
- *Pegboard:* subject is tested with both dominant and non-dominant hands on how quickly they can fill in a pegboard. Each peg has a groove, which must be perfectly matched with the groove of the hole in order to fit. Score based on time to complete.
- *Trail making:* Numbers are scattered over a page and the subject must connect the numbers in numerical order; score derived from both number of errors and time to complete. Executive function can be tested by requiring patients to connect numbers in an alternating fashion between two colors, i.e. yellow 1-pink 2-yellow 3- pink 4, etc. Score still derived from both errors and time to complete.
- *Visual Puzzles:* Subjects are shown a 2D item that they are to imagine as a puzzle made from 3 pieces. The subject indicates which 3 pieces fit next to each other without stacking to make the given item. Each item has a 30 second time limit, with a discontinuation rule of 3 errors in a row. The score is based on time and errors.

Urine Pregnancy Test: A urine pregnancy test will be performed for all subjects at every visit. If a positive result is found, the subject will be informed by site staff of positive result and offered resources regarding prenatal care or alternative options. In the case of a positive pregnancy result, subjects will be withdrawn from the study. In the case of a subject becoming pregnant, the subject will have the choice to consent to allow the study team to track until outcome of the pregnancy.

Vital Signs and Biometrics**:** Vital signs and biometrics for this study include but are not limited to, measuring height and weight, blood pressure, pulse, waist and hip circumference. Vital signs and biometrics will be collected at every visit except height which will only be recorded at the screening visit.
Blood pressure and pulse will be measured in sitting position at all visits after the subject has rested for at least five minutes. Three blood pressure measurements will be taken 5 minutes apart and then averaged.

Laboratory Evaluations: Blood will be collected at Screening, Randomization, week 16 and week 24 visits (early termination visit). Subjects will be required to be fasting prior to Screening, Randomization, week 16 and week 24 visit (nothing to eat or drink besides water for a minimum of 8 hours prior to blood draw). Subjects who have used hormonal contraception must wait at least 30 days prior to the collection of screening and fasting blood l tests. Screening blood tests that were completed within the last year can be substituted for laboratory tests collected at screening visit.

Transvaginal Ultrasound: Transvaginal ultrasound examinations will be performed at screening if indicated, randomization and 24 week visit (or early termination visit). The transvaginal ultrasound examinations will be performed according to the standard procedure for the center and include finding regarding the uterus, the endometrium and the ovaries. If a transvaginal probe is not tolerated, then transabdominal ultrasound may be used. The same procedures were used in the OWL-PCOS study and are well known by the centers.

F-G Score: The Ferriman-Gallwey score is a measure for quantifying hirsutism in women. The Primary Investigator (or designee, which may be the subject) will assess each subject's hirsutism using the Ferriman-Gallwey scale at Screening, Randomization (if applicable and/or necessary) and 24 week visit. During the assessment for hirsutism, the PI (or designee) will also assess the subject's acne.

DXA: DXA scans will be completed on each patient at Randomization Visit and 24 week visit. The scanning will include a whole body scan, the lumbar spine and both hips.

Oral Glucose Test: OGTT will be performed at the randomization and at 24 week visit. Prior to the OGTT subject must fast overnight for minimum of 8 hours. If subject is not fasting, the blood work must be rescheduled. Patients will receive 75 g of an oral glucose solution at time zero (0) and blood will be sampled every 30 minutes for 2 hours for glucose, and insulin levels.

PCOSQ: The PCOSQ is a self-administered questionnaire for measuring health-related quality of life in women with PCOS. The PCOSQ will be administered to subjects at Randomization and 24 week visit.

STAI: The STAI (State-Trait Anxiety index) is a questionnaire for accessing trait anxiety and state anxiety. The STAI will be administered to subjects at Randomization and 24 week visit.

CES-D: The CES-D is a screening test for depressive symptoms. The CES-D will be administered to subjects at Randomization and 24 week visit.

FSDS-R: The FSDS-R (The Female Sexual Distress Scale-Revised) is a screening questionnaire for measuring sexually related personal distress in women with Female Sexual Dysfunction (FSD)

FSFI: The FSFI (Female Sexual Function Index) is a brief questionnaire measure of sexual function in women. It

was developed for the specific purpose of assessing domains of sexual function (e.g. sexual arousal, orgasm, satisfaction, pain) in clinical trials.

ASA24: The Automated Self-Administered 24-Hour Dietary Assessment Tool (ASA24). This 24-hour dietary recall captures detailed information about all foods, drinks, and supplements (including vitamins, minerals, herbals, and other dietary supplements) you consumed from midnight to midnight. You will be asked to complete this at 12 weeks and 24 weeks.

Concomitant Interventions

No other interventions, other than those specified in the protocol are allowed to treat PCOS or weight management during participation in the study.

Adherence Assessment

Adherence to the interventions will be monitored by the use of medication reconciliation, diary compliance and by monthly visits on site or contact with the subject.

Clinical and Laboratory Evaluations

Schedule of Evaluations

The table below summarizes the clinical and laboratory evaluations during the trial. A description of the visits follows. Note, prior to the screening visit, subjects may prescreen for potential eligibility.

Table 6: Study Visits and Procedures

Study Visit Schedule	Screening (half of the visit as a remote option)	Randomization	Randomization Patient Contact (PC)	4 Week Visit (remote option)	8 Week Visit (remote option)	12 Week PC	16 Week Visit	20 Week PC	24 Week Visit	Early Termination Visit (> or equal to 12 weeks of medication)	End of study PC
Sign Informed Consent	x										
Medical History Questionnaire	x										
Screening labs	**x***										
DNA Sample		x									
Safety Labs	**x***	x					x		x	x	
Menstrual History	x	x		x	x		x		x	x	
Urine Pregnancy Test	x	x		x	x		x		x	x	
Vitals and Biometrics	x	x		x	x		x		x	x	
Hirsutism Assessment	x	x							x	x	
Transvaginal Ultrasound	**x***	x							x	x	
Fasting labs		x					x		x	x	
OGTT labs		x							x	x	
DXA Scan		x							x	x	
Lifestyle Modification		x					x				
ASA 24						x			x	x	
Acne Assessment		x							x	x	
HRQOL – PCOS, STAI, CES-D, FSDS-R, FSFI &		x							x	x	

Cognitive testing Questionnaires											
Randomization		x									
Dispense Medication/Log		x		x	x		x				
Collect Medication/Log				x	x		x		x	x	
Review Concomitant Medications	x	x	x	x	x	x	x	x	x	x	x
Review Adverse Events			x	x	x	x	x	x	x	x	x

*As Needed

Timing of Evaluations

7.2.1 Screening visit:

1. Obtain informed, signed consent from subject.
    - Subject must specifically be made aware of potential drug side effects and adverse outcomes.
        * Remote option for e-consent via REDCap, utilization of this option at PI discretion

2. Complete medical assessment.
    - At the Screening Visit, a detailed general medical history for the former 6 months (longer in case of gynecological relevance) and history of concomitant diseases or interventions is to be reviewed and to be documented.
        * Remote option for completion, utilization of this option at PI discretion

3. Must obtain and record menstrual history.
    - Determine need for medroxyprogesterone acetate (if subject has had no menses in the last 3 months)
        * Remote option for completion, utilization of this option at PI discretion

4. Perform urine pregnancy test.

5. Record vital signs and biometrics

6. Blood draw for both screening labs (as needed) and safety labs (as needed)

6. Review Hirsutism (F-G) Score
    * Remote option for completion, utilization of this option at PI discretion

8. Transvaginal Ultrasound (if indicated)

9. Review electronic Medical Record (if available)
    - If subjects are patients through the University of Pennsylvania Health System or Hershey Medical Center, electronic medical records will be reviewed to verify eligibility. Screening blood laboratory tests that were completed within the last year can be substituted for laboratory tests collected at screening and randomization visit.

Table 7: Screening Blood Tests

Category	*Tests*
*Safety Labs***	Complete metabolic panel, lipid profile
Screening Labs	TSH*, Prolactin*, HbA1c*, CBC*, 17OH Progesterone^, DHEAS^, Total Testosterone#,

*If drawn within 1 year of screening, eligible for use to determine eligibility criteria.
^ If drawn within lifetime prior to screening, eligible for use to determine eligibility criteria.
# If FG≥6 (≥2 for women of Asian descent) do not need to draw lab. Eligibility criteria can be determined without this lab value.
** If drawn within 2 months of screening, eligible for use to determine eligibility criteria. These safety labs must be drawn at Randomization visit if not drawn at Screening visit.

7.2.2 Randomization Visit:

1. Review menstrual history
2. Complete urine pregnancy test
3. Record vital signs and biometrics
4. Complete fasting blood draw and Safety labs (as needed)
5. Optional whole blood draw for DNA testing
6. Start the oral Glucose Tolerance Test (this may be first since the subjects are fasting)
7. Review Lifestyle Modification Counseling
8. Complete PCOSQ, CESD-R, STAI, FSDS-R & FSFI
9. Cognitive testing
10. Complete DXA scan
11. Transvaginal Ultrasound (if indicated)
12. Randomize into study arm
13. Dispense Logs/Medications
    - Review medication instructions

*For more detailed information regarding the order in which study procedures should occur, please refer to the Manual of procedures.

****Complete patient contact with subject two weeks after this visit.***

Table 1: Randomization Fasting Blood Tests

Category	*Tests*
Fasting blood Labs	Total and free T, Apo A, Apo B, Apo.C3, hsCRP, FFA, IL-1,IL-6,1L-10, hsCRP, NMR lipoprotein analysis, HDL-C efflux, AMH, SHBG, TNF alpha, Estradiol

7.2.3 Follow- Up Visits

Follow up Visits will be scheduled as shown in Table 6. Initial visits are only one month apart to allow us to trouble shoot any side effects especially while increasing the metformin/placebo tablets to the full dose and ensure adherence with the protocol. Urine pregnancy test will be checked, adverse events assessed, study logs reviewed, and new medications and study logs will be dispensed at each visit. After randomization and at 12 and 20 weeks adverse events, menstrual history, medication compliance will be reviewed via patient contact. Patient contact has also been placed 2 weeks after randomization to ensure patients are taking the medication properly and have experienced no adverse side effects.

Table 9: Follow- Up Visits

Visit	Ideal Date	Lower Window (- 1 week)	Upper Window (+ 1 week)	Out of Window Limit (+/- weeks)
2 Weeks*	2 weeks	1 week	3 weeks	0 / 4 weeks
4 Weeks	4 weeks	3 weeks	5 weeks	2 / 6 weeks
8 Weeks	8 weeks	7 weeks	9 weeks	6 / 10 weeks
12 Weeks*	12 weeks	11 weeks	13 weeks	10 / 14 weeks
16 Weeks	16 weeks	15 weeks	17 weeks	14 / 18 weeks
20 Weeks*	20 weeks	19 weeks	21 weeks	18 / 22 weeks
24 Weeks	24 weeks	23 weeks	25 weeks	22 / 26 weeks
25 Weeks*	25 weeks	24 weeks	26 weeks	23 / 27 weeks

* Patient contact

7.2.4 Week 4 Follow- Up Visit

* Remote option for completing visit, utilization of this option at PI discretion

1. Collect menstrual log and review medical history
2. Perform urine pregnancy test
3. Record Vital signs and Biometrics
4. Review Adverse Events

5. Collect Logs/Medication

6. Dispense Logs/Medications

7.2.5 Week 8 Follow- Up Visit

* Remote option for completing visit, utilization of this option at PI discretion

1. Collect menstrual log and review medical history

2. Perform urine pregnancy test

3. Record Vital signs and Biometrics

4. Review Adverse Events

5. Lifestyle Modification Assessment
   Instruction to subject that the ASA 24 (online diet diary recall assessment) to be completed prior to the 12-week patient contact.

6. Collect Logs/Medication

7. Dispense Logs/Medications

7.2.6 Week 12 patient contact

This contact will be made to ensure subject compliance with medications, keeping study logs and to review any side effects.

7.2.7 Week 16 Follow- Up Visit

1. Collect menstrual log and review medical history

2. Perform urine pregnancy test

3. Record Vital signs and Biometrics

4. Fasting optional blood draw and safety labs

5. Lifestyle Modification Counseling

6. Review Adverse Events

7. Collect Logs/Medication

8. Dispense Logs/Medications

7.2.8 Week 20 patient contact

This contact will be made to ensure subject compliance with medications, keeping study logs and to review any side effects. Instruction to subject that the ASA 24 (online diet diary recall assessment) to be completed prior to the 24-week visit

7.2.9 Week 24 Final Follow- Up Visit or Early Termination Visit (≥ to 12 weeks of being on study medication)

1. Collect menstrual log and review medical history
2. Perform Urine pregnancy test
3. Record vital signs and biometrics
4. Review Hirsutism (F-G) Score/ Acne Assessment
5. Fasting blood draw and safety labs
6. Complete an oral Glucose Tolerance Test
7. PCOSQ, CESD-R, STAI
8. Review Hirsutism (F-G) Score/ Acne Assessment
9. Complete DXA scan
10. Obtain a Transvaginal Ultrasound (TVU)
11. Review Adverse Events
12. Collect Logs and medication
13. Must obtain and record menstrual history
    - Determine need for medroxyprogesterone acetate (if subject has had no menses during the course of this study)

7.2.10 Week 25 patient contact

This contact will be made one week after patient has completed taking the medication to review any side effects and answer any patient questions. This patient contact will close out the study adverse events.

Study Risk and Benefits

Risk

The risks of these studies include the risks of the study procedures, and the risk of the study interventions. These will be discussed separately below.

Risks of Study Procedures

These risks include the risk of phlebotomy, oral glucose tolerance test and DXA. The risks of phlebotomy include pain at the phlebotomy site, bruising and rarely bleeding, and potentially iatrogenic anemia. Risks of the oral glucose tolerance test include the risks of multiple phlebotomy or in some cases the risk of insertion of an IV. The risks of IV insertion include the risks of phlebotomy, and additionally the induction of anemia, and infection at the site. Glucose ingestion can cause nausea and in rare instances vomiting upon ingestion. We have never experienced an allergic reaction to the oral glucose solution, though this is possible. Some subjects also may experience an episode of hypoglycemia after the test which can present with increased heart rate and anxiety. In the most severe cases, fainting can occur. The DXA scan will involve exposure to radiation, on average 2-3 milliroentgens of radiation during the procedure. The average amount of radiation that a person would receive from a DXA scan is less than 1% of the background radiation experienced from living in the Philadelphia area for one year, for example.

Risks of Study Interventions

Oral Contraceptive Pill - OCP used in this study is low dose (20micrograms ethinyl estradiol), which may cause bloating, nausea, breast tenderness, mood changes, weight change and headaches. Common side effects include breakthrough bleeding and amenorrhea. OCP are also associated with increased risk for cardiovascular disease, especially venous thromboembolism and stroke. OCP can also cause hypertension and alter blood lipid and glucose levels. Interactions with drugs such as phenobarbital, phenytoin, and rifampin increase the metabolism of oral contraceptives, thus decreasing the effectiveness as a method of birth control. Table lists all side effects recorded in women in the OCP arm of the recently completed OWL-PCOS study (n=49). Roughly same numbers are expected in this current study.

OWL-PCOS study OCP side effects	**Number of subjects (%)**	**OCP side effects**	**Number of subjects (%)**
Headache	14 (28.6)	Dizziness/Vertigo	2 (4.1)
Upper Respiratory Infections	8 (16.3)	Fatigue	2 (4.1)
OWL-PCOS	N (%)	Side effects	N (%)
Nausea/Vomiting	7 (14.3)	Back Pain	1 (2.0)
Breast Pain	10 (20.4)	Acne	1 (2.0)
Abdominal Pain	1 (2.0)	Chest pain	1 (2.0)
Dysmenorrhea	8 (16.3)	Dental abscess	1 (2.0)
Constipation	1 (2.0)	Hot flushes	1 (2.0)
Abnormal uterine bleeding	4 (8.2)	Elevated BP w/o hypertension	1 (2.0)
Mood Swings	3 (6.1)	Gastroenteritis	1 (2.0)
Gas/Bloating	2 (4.1)	Otitis media	1 (2.0)
Musculoskeletal Pain	3 (6.1)	Anemia	1 (2.0)
Vaginitis/Vulvitis	4 (8.2)	Visual Changes	1 (2.0)
Pelvic Pain	3 (6.1)	Conjunctivitis	1 (2.0)
Bunion	1 (2.0)	Hemorrhoids	1 (2.0)
Myalgia/myositis	1 (2.0)	Bleeding, rectal	1 (2.0)
Fever	1 (2.0)	Cholelithiasis	1 (2.0)
Sweating excess	1 (2.0)	Galactorrhea	1 (2.0)

Treatment with metformin XR - Gastrointestinal symptoms (diarrhea, nausea, vomiting, abdominal bloating, flatulence, metallic taste in the mouth and anorexia) are the most common reactions to metformin XR and

approximately 30% more frequent in women taking metformin XR compared to placebo. These symptoms are generally transient and resolve shortly after initiation of treatment. These symptoms will be managed with a step down decrease in the daily metformin XR dose (1/2 to one tablet per day per week) until symptoms resolve. These symptoms alone will not be a reason for withdrawal from the study unless a patient is unable to tolerate any dose of the medication. There is a small risk of lactic acidosis among women taking this medication. This most commonly occurs in patients with poorly controlled diabetes and impaired renal function. Vitamin B12 deficiency has also been reported in metformin-treated adults with T2DM. Women with these pre-existing medical problems will be excluded from the study as per the exclusion criteria. The Table below shows adverse events from metformin use in women with PCOS in the PPCOS1 study and the same results are expected in this current study.

PPCOS1 metformin Side effects	Metformin(N=208)
Blood and lymphatic system disorders	1/208 (0.5%)
Cardiac disorders	1/208 (0.5%)
Congenital, familial and genetic disorders	0/208 (0.0%)
Ear and labyrinth disorders	3/208 (1.4%)
Endocrine disorders	2/208 (1.0%)
Eye disorders	3/208 (1.4%)
Gastrointestinal disorders	177/208 (85.1%)
General disorders and administration site conditions	61/208 (29.3%)
Immune system disorders	3/208 (1.4%)
Infections and infestations	43/208 (20.7%)
Injury, poisoning and procedural complications	3/208 (1.4%)
Investigations	6/208 (2.9%)
Metabolism and nutrition disorders	37/208 (17.8%)
Musculoskeletal and connective tissue disorders	29/208 (13.9%)
Nervous system disorders	108/208 (51.9%)
Pregnancy, puerperium and perinatal conditions	0/208 (0.0%)
Psychiatric disorders	41/208 (19.7%)
Renal and urinary disorders	7/208 (3.4%)
Reproductive system and breast disorders	84/208 (40.4%)
Respiratory, thoracic and mediastinal disorders	24/208 (11.5%)
Skin and subcutaneous tissue disorders	19/208 (9.1%)
Surgical and medical procedures	0/208 (0.0%)
Vascular disorders	33/208 (15.9%)

Protection Against Risks

Risks of adverse events will be reduced by the study personnel and the investigators regularly monitoring participants' progress, by oversight of the local IRBs and the DSMB (see section below).

Clinical staff at each site - Our reproductive endocrinology groups at UPenn and PSU staffs a daily infertility clinic that meets every day (365 days a year). The staffing doctor also covers inpatient responsibility and research aspects of our service. The subjects at UPenn will be seen in the WHCRC which is on the same floor and adjacent to the REI practice site. The daily clinic at PSU meets just below the GCRC where the subjects are studied. At both sites a physician is available at a moment's notice to see a subject. Therefore, the patients participating in the

trials will have access to same day consult or visit with an investigator involved in the study, regardless of weekends and holidays, for any potential problem that arises in a study. We have utilized this system successfully for a wide variety of clinical studies for over 10 years.

Review at research meetings - Both groups at UPenn and PSU currently hold weekly research meetings attended by all the research staff which allows for communication about study issues and problems with any subjects in the study. In addition to the weekly site research meetings, the entire investigative team will meet by phone conference monthly, Face to face meetings will be held 2 times a year, to discuss the results of patients' most recent study assessments as well as any adverse events. The research coordinators will inform the investigative team of any adverse medical events, or abnormal lab values, reported by study participants. All studies will be IRB approved and regular progress reports submitted to the IRB as well as adverse events (see below).

Protection against Study Procedure Risks

Phlebotomy - risks are reduced by the use of skilled nurses or study personnel who have had extensive experience with phlebotomy. The risks of an OGTT are minimized by utilizing trained nursing personnel with investigator back-up during the test. They are experienced in inserting IVs in study subjects. When we know of prior difficulties with these procedures, we will consult with Anesthesia to insert the IV and minimize pain and psychological trauma.

DXA - The whole body radiation dose to a female during a DXA is <1 mRem. The human race is continually being exposed to radiation from natural and man-made sources. The dose of background radiation to each person is 300-400 mRem so the exposure for these measurements is a minimal increase in exposure. For comparison purposes, this is less radiation exposure than from a routine chest x-ray and it is comparable to the radiation exposure from cosmic ray exposure during an airplane flight across the United States. The risk from this level of exposure is considered to be minimal. A negative pregnancy test will be obtained prior to these studies in all subjects. If an individual has participated in any other research study in the past 12 months that included exposure to ionizing radiation, we will assess overall exposure to radiation. If it exceeds 500 mrem for the calendar year by participating in this study, the potential participant will be excluded or moved to a later recruitment wave. Total ionizing radiation exposure will be evaluated with the help of the University of Pennsylvania and Penn State Radiation Protection Advisory Committees.

8.2.1 Protection against Risk of Study Interventions

Study subjects will be carefully screened for medical problems prior to randomization. The risks of the medications used in this study will be lowered through education and frequent follow-up. Study subjects will be monitored monthly in person or by telephone for adverse reactions. Only a limited supply of medication will be dispensed at each visit to encourage compliance with the protocol and prevent a prolonged exposure of an early gestation to medication.

Oral Contraceptive Pill -We will exclude subjects with all absolute contraindications to OCP including a history of thrombophlebitis, known or suspected clotting disorders, cerebrovascular or coronary artery disease or myocardial infarction, known or suspected uterine, cervical or breast neoplasia, history of a benign or malignant liver tumor that developed during the use of OCPs or other estrogen-containing products. Additionally, we will exclude other relative contraindications as listed in the exclusion criteria. Smoking will not be an absolute contraindication. Subjects will be counseled about the risks of OCP, and warning signs for serious adverse events including leg swelling, redness, pain, shortness of breath, racing heart, chest pain, severe and unremitting headache, unilateral weakness or speech disturbance. Subjects will have a scheduled visit or receive communication from the study team monthly and symptoms elicited and recorded as part of the visit.

Worrisome signs will be reported to study physicians immediately for evaluation and management. We will have a low threshold of suspicion for discontinuing medication. We should note that we had no serious adverse events among 49 obese women randomized to a low dose OCP in the OWL-PCOS study (see table 2 for side effects above). Although the OWL-PCOS study showed a significant increase in prevalence of MetS in the OCP arm, only a small proportion of subjects developed DM or hypertension (4.6% and 6.7% respectively after 16 weeks). A fasting glucose level will be evaluated at the 16 week visit and a value >200mg/dl will prevent a subject from continuing in the study. BP will be measured at each visit and a subject with BP>150/100mmHg x2 will be unable to continue in the study.

Metformin - There is a small risk of lactic acidosis among women taking this medication. This most commonly occurs in patients with poorly controlled diabetes and impaired renal function. Women with these pre-existing medical problems will be excluded from the protocol as per the exclusion criteria. There have been reports of lactic acidosis induced by exposure to iodine-containing radiocontrast agents, such as those used for an intravenous pyelogram. Metformin XR will be stopped prior to procedures involving exposure to radiocontrast agents to reduce the chance of developing lactic acidosis (one week prior to medication and resume one week after the test). The development of lactic acidosis for any reason will be a reason for discontinuing participation in the study. Metformin XR is pregnancy category B with no known human teratogenic risk and no known embryonic lethality in humans. Metformin XR has been used throughout pregnancy in a number of studies with no adverse maternal or fetal effects. If a subject is pregnant during any part of the study, her participation will be stopped.

Preventing Pregnancy - All subjects will be asked to use barrier contraception during this study. Pregnancy tests will be performed at each in person study visit.

Menstrual irregularity - All subjects will be asked to keep detailed menstrual log and study coordinators and site PIs will review symptoms. Subjects may present with break through bleeding on the low dose OCP. Some subjects in the metformin arm may not have any menses during the study period. We will induce menses by administering medroxyprogesterone acetate at the screening visit if a subject has not had menses for 3 months. There is no data to suggest that lack of menses in the metformin arm for 6 months will increase risk of endometrial hyperplasia. In fact, several studies have described the biological plausibility of metformin having a protective effect on the endometrium. If a subject has not had menses during the study, we will administer medroxyprogesterone acetate after confirming a negative pregnancy test at the end of the study.

Potential Benefits of the Proposed Research to Human Subjects and Others

The subjects in this study will not benefit directly. The results from the study can be applied in the future to patients who stand to benefit from this information.

Importance of the Knowledge to be Gained

The importance of this study is high for the following reasons:

1. Both MetS and PCOS are major health problems and the risks of common treatments (OCP or metformin) on development of MetS are poorly understood.
2. Knowledge gained from this investigation will identify optimal strategies for treatment of overweight/obese women with PCOS
3. Understanding the underlying pathophysiology for alterations in metabolic risk with OCP or metformin use, will provide important information for future studies.

Statistical Considerations

General Design Issues

This will be a randomized, double-blind, double dummy clinical trial of three types of medication, OCP + placebo, metformin + placebo or OCP + metformin. We will track all subjects to completion. Subjects will be randomized 1:1:1 to the three treatments.

Randomization

Randomization is a critical feature of a clinical trial because it prevents treatment-selection biases. The study statistician will develop the programs for the randomization; however, the final random seeds used to generate the randomization scheme will be prepared by a statistician in PSU's Department of Public Health Sciences independent of the study in order to keep the study statistician blinded as well. The randomization scheme for this study will use variable-size, random permuted blocks to ensure that the number of participants in each treatment arm is balanced after each set of *B* randomized participants, where *B* is the block size. Furthermore, the randomization will be stratified by recruitment site (UPenn/PSU), race (AA/non-AA), and the presence of MetS at baseline (yes/no). Randomization to the metformin or OCP arms will use 1:1:1 allocation.

Outcomes

Primary Outcome Measurements

The primary outcome for this trial is prevalence of MetS.

Secondary Outcome Measurements

We may assess change in HDL-C function, serum apolipoproteins, lipid particle size and number, body fat distribution, BMI, serum adipokines, HbA1c, glucose and insulin sensitivity, serum markers of inflammation, free fatty acids, androgens, quality of life parameters, cognitive testing and predictive factors for change in prevalence on MetS

Sample Size and Accruals

Sample Size and Power Calculations

The primary outcome of the COMET-PCOS trial will be to assess a linear trend in prevalence of MetS after 6 months' treatment over the 3 arms assuming a 30% prevalence of MetS at baseline (derived from PENN data and OWL-PCOS). We anticipate a 15% subject drop-out over the course of the trial (in OWL-PCOS it was 8.1% over 16 weeks). Based on these assumptions, a sample size of 240 (80 per arm) will provide 80% statistical power to detect a linear trend in the prevalence of MetS over the 3 arms at the end of 6 months of 26% in the metformin arm, 40% in the OCP+metformin arm and 50% in the OCP arm using a two-sided test for linear trend with a significance level of 0.05.

Table 10: Sample size scenarios based on varying assumptions

Proportion with MetS at the End of the Trial in Metformin Arm	Proportion with MetS at the End of the Trial in OCP+Metformin Arm	Proportion with MetS at the End of the Trial in OCP Arm	Total Sample Size (0% Drop-out)	Total Sample Size (15% Drop-out)	Power (%)	Type I Error (α)
0.25	0.38	0.50	204	240	84	0.05
0.25	0.38	0.52	204	240	89	0.05
0.26	**0.40**	**0.50**	**204**	**240**	**80**	**0.05**
0.26	0.40	0.52	204	240	86	0.05
0.28	0.42	0.52	204	240	80	0.05

Statistical Analysis

Primary analyses will invoke an intent-to-treat paradigm, wherein all randomized subjects are included according to their randomized treatment arm, regardless of actual treatment received, protocol violations, etc. Data will be summarized using descriptive statistics for continuous variables (mean, standard deviation, number of observations, and percentiles) and frequency statistics (frequencies and percentages) for categorical variables. The area under the curve (AUC) for glucose and insulin from the OGTT will be calculated using the trapezoidal rule. Univariate and bivariate distributions will be inspected in order to address any missing data, inconsistent responses, outliers, and data entry errors. The sample size estimates have taken into consideration a participant drop-out of 15%; however, every effort will be made during the studies to minimize any drop-out. If, however, study attrition appears to be an issue, we will use the observed data to determine if patients who completed the study differed from those who did not. To control for potential confounding factors for the association of the treatment effects with the metabolic syndrome, we have stratified the randomization by recruitment site, race, and the presence of metabolic syndrome at baseline. Although stratification may potentially yield unequal numbers between strata, within each individual stratum there will be approximately equal numbers and balance between treatment groups. All analyses will include these 3 randomization stratification factors as covariates in the statistical models. All hypothesis tests will be two-sided and all analyses will be performed using SAS software, version 9.4 (SAS Institute, Inc., Cary, NC), R software (open source), or Stata software, version 13 (StataCorp LP, College Station, TX).

For the primary outcome of the presence of the metabolic syndrome at the end of the 6-month trial, logistic regression will be used with independent variables that include terms for the treatment arm and the 3 randomization stratification factors as covariates, with a contrast constructed to test for linear trend over the three treatment arms.

A variety of secondary continuous outcomes will be collected during this longitudinal trial. These secondary outcomes include serum androgens, cholesterol efflux (HDL-C function) parameters, serum apolipoproteins, lipid particle size and number, anthropometric measures (BMI, adipokines, biomarkers of inflammation (e.g., hsCRP),

measures of adipose tissue, abdominal adiposity, and quality of life measures (PCOSQ). For these continuous outcomes, linear mixed-effects models will be fit to assess differences between the treatment arms with respect to changes in these outcomes over time. The independent variables in the model will be treatment arm, time, the interaction of treatment and time, and the 3 randomization stratification factors as covariates. From the mixed-effects models, contrasts will be constructed to test the hypotheses of interest with respect to changes over time in the outcomes. Linear mixed-effects models are an extension of ordinary regression models that account for the between- and within-subject correlation inherent in longitudinal trials. Further, linear mixed-effects models do not drop patients with incomplete data and are easily extended to nonlinear mixed-effects models for ordinal data and count outcomes. Following our assessment of the initial fit of the models, we will add covariates to the models that correspond to other potential confounders (e.g., age) to assess their impact, if any, on the treatment effects. Residual diagnostics will be assessed to determine the appropriateness of the model fit and, if necessary, transformations of the response will be used to meet modeling assumptions. Differences in means and associated 95% confidence intervals (CIs) will be used to quantify the magnitude of the effects.

For any binary outcomes collected at each visit, analyses will be based on generalized estimating equations (GEE) with a logit link, an extension of logistic regression that accounts for correlated data within-subjects inherent in longitudinal trials, with independent variables that include terms for the treatment arm, time, the interaction of treatment and time, and the 3 randomization stratification factors as covariates. Following our assessment of the initial fit of the models, we will add covariates to the models that correspond to other potential confounders (e.g., age) to assess their impact, if any, on the treatment effects. The effect size will be quantified using the odds ratios (OR) and corresponding 95% CI.

Accrual

A total of 5 years will be required to complete the study after start up; 36-month enrollment period (based on 3 subjects per site/month x 2 sites), 1-month screening/randomization period, 6-month treatment period and time built in for data analysis and interpretation.

Data Collection, Monitoring and Adverse Experience Reporting

Records to be kept

Data will be collected prospectively by designated research personnel at each study site, supervised by the site PI. Original source documents will be kept in the study subject folder. Well-designed data collection forms will be developed to minimize data collection and recording errors. Administrative forms will be designed, such as visit procedure checklists, to assist the research staff in complying with protocol procedures. We will be collecting a medical history on each subject. We will also be obtaining biometric data, clinical data (hirsutism), imaging data from ultrasound, DXA scanning and biochemical data from blood. All data are being collected solely for the purpose of research and do not become part of the subject's medical record.

All tissue samples and images will be labeled with the code number assigned to each subject and only de-identified samples will be sent to laboratories for testing. Specimens will be stored in Women's Health Clinic Research Center's freezers at UPenn and banked for batched analysis.

Study data will be managed using REDCap (Research Electronic Data Capture), a secure web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g., for data types and range checks), audit trails, a randomization module, and a de-

identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The database is hosted at the University of Pennsylvania, which will be used as a central location for data management. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team. REDCap is flexible enough to be used for a variety of types of research and provides an intuitive user interface for database design and data entry.
Database access for REDCap is granted on a study-by-study basis. At the start of the trial when the database is created all research personnel will be given defined user roles and assigned a unique username and password. There is a 90-day password update policy for all REDCap users. The REDCap database sits behind an application firewall and the data is stored on a virtual machine at Penn which is backed-up nightly.

Maintenance/Retention of site records

In order to comply with Good Clinical Practice (GCP) requirements, the investigators must maintain the master patient log that identifies all patients entered into the study for a period of two years after the study ends so that the subjects can be identified by audit. The PI must maintain adequate records pertaining to subjects' files and other source data for a minimum of 5 years after completion of the study. Each clinical site will be responsible for ensuring study personal are trained and follow the data management guidelines of GCP and internal site policies.

Data Security

Study staff will make clear that subjects are not obligated to participate in the study and that their answers will be held strictly confidential. Questionnaires will not contain any identifying information, thus, ensuring the confidentiality of subjects' responses. Code numbers will be assigned to each subject to maximize anonymity when entering and analyzing data. Any linking list of patient names and codes will be maintained separately, and destroyed at the earliest possible time. Only the investigators and research staff at each individual study center will have access to this information for patients recruited at that center. The second site and the research coordinator will not have access to the linking list. The security of the research project data will be maintained through network hardware and user authentication (usernames and passwords). Back-ups of the project data files will occur every night, with user data backed-up incrementally Monday through Thursday and complete back-ups every Friday. Archival back-ups, stored indefinitely, are cut on the last weekend of every month. All back-up data are stored in a secure off-site location. The number and variety of back-ups ensure ample data redundancy and protection. In addition, each participant will be assigned a unique subject identification number. Only the study coordinators will have the log linking this identification number with the participant's personal information.

Adverse Event Reporting

Serious Adverse Events

All serious adverse events (SAEs) that occur from randomization through thirty days after the last dose of study medication must be reported. A serious adverse event is defined as: fatal or immediately life-threatening; severely or permanently disabling; requiring or prolonging inpatient hospitalization; overdose (intentional or accidental); ; or, any event adversely affecting the study's risk/benefit ratio. Additionally, any event that, based on appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above is considered an SAE.

If an SAE occurs and is thought to be related to the study medication, the study medication will be discontinued.

The site PI will report the SAE by completing and signing the Serious Adverse Event Report Form Subjects will be identified by subject identification number only. No other identifying information will be included on the form.

The site PI must determine and record on the SAE form whether the SAE is unanticipated or anticipated, and if it is related, possibly related, or unrelated to participation in the research.

The DSMB and FDA (as applicable) will be notified and provide a determination regarding the SAE.

These determinations will dictate timeframes for sites' submission to the DSMB (**Table 12**):

Table 11: Types of Serious Adverse Events and their reporting requirements

TYPE	SITE
Unanticipated and related/possibly related SAE, fatal or life-threatening	Report to DSMB within 1 business day of discovery
Other unanticipated and related/possibly related SAE	Report to DSMB within 1 business day of discovery
Anticipated and related/possibly related SAE	Report to DSMB within 5 business days of discovery
Unrelated SAE (anticipated or unanticipated	Report to DSMB within 10 business days (no more than 3 weeks) of discovery

Upon receiving notification of an SAE, the DSMB will review it via a closed-session email or conference-call discussion.
The PI will evaluate the frequency and severity of the SAEs and determine if modifications to the protocol and consent form are required. Site PIs will report the SAE to their site IRB according to local IRB requirements.

Adverse events deemed non-serious will also be recorded throughout study participation from the start of study drug through one week after the last dose of study medication. If an anticipated serious adverse event occurs at a frequency greater than expected, the DSMB will be notify the PI by the end of the next business day of discovery and follow the procedures for reporting serious and unanticipated and related adverse events. If an adverse event not initially determined to be reportable to the FDA under 21CFR312.32 is so reportable, the PI will report the adverse event to the FDA within 15 calendar days after the determination is made.

Data Monitoring
There will be no pre-determined stopping rules and no interim analysis.

Study Monitoring
A monitoring plan that satisfies the ICH/GCP guidelines for clinical monitoring will be used. The Penn designated monitor at the Women's Health Clinical Research Center, will lead this effort, and report findings to the PI of both sites and the DSMB when necessary. The Penn designated monitor will have full knowledge of the study protocol, Manuals of Procedures, and is familiar with the database system redcap and is trained to review patient charts. The Penn designated monitor along with the Project Manager/Lead Study Coordinator at each site will be responsible for training and supervising other personnel.

Once personnel at participating site are trained to recruit patients, the Penn designated monitor will be sent to the site to help initiate the study according to the study protocol, and to ensure that the clinical site meets the scientific, clinical, and regulatory requirements. For example, the Penn designated monitor will review all signed and dated forms (such as financial disclosure forms), the curriculum vitae and certifications of the investigators and personnel, CRF training, and the written IRB approval of the protocol and consent form.

The Penn designated monitor will return to the clinical site after a defined number of patients are recruited (recruitment of the first 5 patients) and yearly thereafter.

During the site visit, the clinical sites should provide to the monitor(s) a space and access to all relevant records including medical records and regulatory binders, and there would be immediate verbal feedback provided to the site after original source documents are compared to entries in the CRF. The clinical sites must agree to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved. The on-site monitor will conduct an audit of a random sample of entered information against the source documents, a review of all regulatory documents, a review of all informed consents, and a review of all pharmacy logs. The clinical site PI and study coordinator should be available to meet the monitor during the visit. The monitor will review electronic data from all sites, providing a method for identifying systematic errors or problems.

To assure Good Clinical/Laboratory Practice, the monitor will control adherence to the protocol at the clinical sites and evaluate the competence of the personnel at the clinical sites including the ability to obtain written informed consents and record data correctly. The monitor will inform the PI, and DSMB regarding problems relating to facilities, technical equipment, or medical staff. A thorough written report will follow each site-visit and will include a detailed itemization of discrepancies and items requiring follow-up or reconciliation. The monitor will be responsible for maintaining regular contacts between the investigators in the clinical sites. When the study ends, the monitor will also visit the clinical site to provide assistance for close-out.

Human Subjects Protection

Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study. A signed consent form will be obtained from the subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and this fact will be documented in the subject's record.

Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using SIDs only to prevent the loss of confidentiality. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the OHRP, the sponsor, or the sponsor's designee.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period

Study Modification/Discontinuation

The study may be modified or discontinued at any time by the IRB, the NIH, the OHRP, or other government agencies as part of their duties to ensure that research subjects are protected.

Data and Safety Monitoring Board

Kathy Hoeger from the University of Rochester Medical Center will chair the DSMB for this clinical trial. The DSMB will review safety information, especially SAEs that may occur, on a quarterly basis while the study is active and ongoing. Kathy will be consulted for any safety questions that may arise during the trail.

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