Inositol Supplementation to Treat Polycystic Ovary Syndrome: A Double Blind Dose Ranging RCT (INSUPP-PCOS)

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Clinical Intervention Study Protocol

PREFACE

Polycystic ovary syndrome (PCOS) is the most common endocrine abnormality in women in the U.S. and is characterized by both reproductive (anovulation and androgen excess) and metabolic dysfunction (insulin resistance). PCOS lacks a simple, safe and effective treatment for women of all ages and all weights. Recently a dietary supplement, inositol, has been used widely to treat women with PCOS. However there are no well-designed trials to address the risk/benefit ratio and identify the mechanism of action.

Polycystic ovary syndrome (PCOS) affects 7-8% of the U.S. female population,[1] and is the most common endocrinopathy among women.[2] It is a heterogeneous syndrome with reproductive aspects consisting of chronic anovulation and hyperandrogenism, as well as metabolic aspects consisting of insulin resistance, glucose intolerance and metabolic syndrome.[3] We have investigated and established multiple first line reproductive treatments for ovulation induction such as weight loss,[4] clomiphene or letrozole,[5] advanced infertility therapies including IVF[6] and examined long term health with other treatments such as oral contraceptives (OCP)[4] as well as metabolic treatments for infertility and long term health including metformin,[7,8] thiazolidinediones[9] and lifestyle modification.[4 9] Treatment often depends on the presenting complaint, for example OCPs have limited use for infertility and clomiphene has no role to treat hyperandrogenism. Further, we[4] and others[10] have shown that chronic OCP use in overweight/obese women with PCOS can result in a deterioration in glucose tolerance and exacerbation of the metabolic syndrome, such that treatments which favor insulin sensitization and weight loss such as metformin and lifestyle modification have become the preferred chronic treatment.[11] Chronic medications are needed for metabolically challenged patients with PCOS, especially those that are safe, inexpensive and have a low chance of harm.

One such medication, that is available throughout the world as a supplement, is inositol, and more specifically a combination of two stereoisomers, d-*chiro* and *myo*-inositol, which has been used extensively to treat women with PCOS. The combination is thought to improve insulin signaling by restoring an imbalance in inositolglycans which are second messengers in insulin (and gonadotropin) signaling. Although there has been a consensus statement advocating inositol use,[12] this statement also highlighted the lack of high quality randomized controlled trials (RCTs) to identify the risk-benefit ratio. *We propose here the first adequately powered and designed clinical trial to test prospectively the effects of inositol supplementation in a dose ranging double blind RCT, (the INSUPP PCOS study) according to CONSORT guidelines.*[13] This will be a four armed study of three doses inositol (2gm, 4gm and 6gm a day) vs placebo over a 3 month period with the reduction in hyperandrogenism (based on serum testosterone levels) as the primary outcome and key secondary outcomes of the change in sex hormone binding globulin(SHBG) and the related free androgen index, fasting insulin levels, and area under the curve glucose levels from an oral glucose challenge test (OGTT).

FULL PROTOCOL TITLE

Inositol Supplementation to Treat Polycystic Ovary Syndrome: A Double Blind Dose Ranging RCT (INSUPP-PCOS)

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Summary of Revisions Made: Minor typos and revision; compensation added to HRP-595 Protocol Site Addendum and Consent form
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Summary of Revisions Made: Minor typo to consent sebumeter assessment. Minor wording change to indicate protocol and consent clarification for visit length of time needed between screening and randomization visit, to include if medically necessary. MTH 1 and MTH 3 visit window clarification. Minor wording revision to Protocol site addendum.
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Summary of Revisions Made: Minor typo to inclusion criteria (P4) level indicates <3 ng/dL , should state <3 ng/mL
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Version Number: 1.7 Version Date:10Jul2020Summary of Revisions Made: Addition of Liver disease (AST/ALT≥2 times normal or a total bilirubin ≥2.5 mg/dL) to exclusion criteria. Minor typo correction to exclusion criteria, the #2 was entered twice. NCCIH reviewed version date 10Jul2020 and approved on 30Jul2020(submitted to IRB on 05Aug2020).
Version Number: 1.8
Version Date:19Aug2020
Addition of language to include If applicable due to unforeseen or extenuating circumstances outside of our control ie: pandemic, emergency or unexpected issue arises arrangements will be made to complete as many of the study specific visit parameters and data remotely.
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Received official approval by NCCIH of replacing Dr. Dodson on this study protocol with Dr. Butts. This protocol modification reflects this approval. Minor typo corrections.
Version Number: 1.10
Version Date: 08Jul2021
Addition of 3 month/90 day time window from obtaining consent to randomization. Addition of caffeine intake questionnaire to be completed at month 1 and month 3 visits. Minor typo corrections.

Version Number: 1.11
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Amendment to Section 4.1 Inclusion criteria to modify: Key inclusion criteria #1 Hyperandrogenemia is defined as a total testosterone of (T)>40 ng/dL. Adding University of Pennsylvania as an additional site for meeting target enrollment total. Adding PENN's study personnel. Adjustment of sample size as result of lower than anticipated drop out. Modification of stratification as a result of additional site. Minor typo corrections.
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Amendment to Section 4.2 Exclusion Criteria #8 wording revised. Also, clarifying that PENN site prescreening tool is done via REDCap or phone. And minor typo/edits.
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Omitting methods for an outcome under data analysis that does not pertain to the study.

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I. HRP-595 – Protocol Site Addendum for Human Subject Research (2/15/19)

II. HRP-598 – Research Data Plan Review Form Application Supplement (10/19/18)

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Dr. Legro is PI of the multi-site study and will oversee the conduct of this trial. He will participate fully in all aspects of the study, including the development of the final protocol and study forms. He will attend regular meetings of the steering committee. He will also participate in the care of subjects in the study at PSU, including examining patients during screening and baseline visits, performing imaging studies and providing expert decision-making for treatment implementation and progression.

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Dr. Dokras is PI of the University of Pennsylvania study site and will oversee the conduct of this trial at this site. She will participate fully in all aspects of the study, including the development of the final protocol and study forms. She will attend regular meetings of the steering committee. She will also participate in the care of subjects in the study at the PENN site, including examining patients during screening and baseline visits, performing imaging studies and providing expert decision-making for treatment implementation and progression.

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Dr. Butts will participate fully in all aspects of the study, including the development of the final protocol and study forms and attend regular meetings of the steering committee. She will also participate in the care of subjects in the study at

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Dr. Raja-Kahn will provide expertise as a Medical Endocrinologist and as a Clinical Researcher in the field of obesity and related disorders including Type 2 diabetes and Polycystic Ovary Syndrome (PCOS). She will participate in the development of the protocol, case report forms, manual of operations, participating in regular meetings, and contributing as an author to all abstracts and publications. She has collaborated with Dr. Legro on multiple randomized clinical trials and projects that include a focus on women with polycystic ovary syndrome making her well suited to contribute significantly to recruitment, patient monitoring, and also as a member of the Steering Committee.

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Allen will serve as a biostatistician for this protocol. His contribution includes study design, sample size and power calculations, randomization, data collection and organization, data monitoring, data summarization and visualization, data analysis and model building, and interpretation. He has collaborated with Dr. Legro for many years having been involved as a co-investigator and lead biostatistician on many research projects involving women with polycystic ovary syndrome (PCOS).

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PRÉCIS

Study Title

Inositol Supplementation to Treat Polycystic Ovary Syndrome: A Double Blind Dose Ranging RCT (INSUPP-PCOS)

Objectives

Primary Study Endpoints

Specific Aim 1: Identify the effect of inositol supplementation on serum testosterone levels during the study. *Hypothesis 1 (Primary Outcome):* Women with PCOS who receive inositol supplementation will have a significantly greater reduction in serum total testosterone than women on placebo, as well as our secondary markers of hyperandrogenism: SHBG and the free androgen index.

Secondary Study Endpoints

Specific Aim 2: Characterize the effects of insulin sensitization by inositol on glucose tolerance. *(Secondary Outcomes)***.** *Hypothesis 1:* Women with PCOS who receive inositol will experience a progressive improvement in glucose tolerance as determined by the AUC of a 2h 75g glucose tolerance test. *Hypothesis 2:* Women with PCOS will experience a decrease in fasting insulin.

Design and Outcomes

This multi-site, double-blind, randomized controlled trial will assess the change in serum testosterone from baseline to end of study (after 3 months of treatment) across 4 arms as the primary outcome. Serum testosterone levels will be batched and run at the Core Ligand Lab at the University of Virginia. Secondary outcomes of SHBG, calculated FAI and glucose and insulin levels from the 75g 2h OGTT will also be run at the Core Ligand lab. We will additionally phenotype subjects for a variety of clinical and biochemical measures of hyperandrogenism, assess body composition by DXA and quantify quality of life, sexual function and sleep by standardized instruments or validated questionnaires to examine a variety of exploratory outcomes.

Interventions and Duration

Subjects will be provided with daily treatment of an inositol supplement at either 2, 4 or 6 gm/day or an identical daily placebo over a 3 month period.

Sample Size and Population
We will recruit 108 women with PCOS (27 per arm) into this study. Randomization to the four treatment arms (placebo bid, inositol 1 gm bid, inositol 2 gm bid, and inositol 3 gm bid) will use 1:1:1:1 allocation. The randomization scheme for this study will use variable-size, random permuted blocks to ensure 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 site and obesity status at baseline (BMI < 30 kg/m^2 or BMI ≥ 30 kg/m^2). Although stratification may potentially yield unequal numbers between strata, within each individual stratum there will be approximately equal numbers and balance between the treatment groups.

1. STUDY OBJECTIVES

1.1 Primary Objective

Specific Aim 1: Identify the effect of inositol supplementation on serum testosterone levels during the study. *Hypothesis 1 (Primary Outcome):* Women with PCOS who receive inositol supplementation will have a significantly greater reduction in serum total testosterone than women on placebo, as well as our secondary markers of hyperandrogenism: SHBG and the free androgen index.

1.2 Secondary Objectives

Specific Aim 2: Characterize the effects of insulin sensitization by inositol on glucose tolerance. *(Secondary Outcomes)***.** *Hypothesis 1:* Women with PCOS who receive inositol will experience a progressive improvement in glucose tolerance as determined by the AUC of a 2h 75g glucose tolerance test. *Hypothesis 2:* Women with PCOS will experience a decrease in fasting insulin.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

Polycystic ovary syndrome (PCOS) is the most common endocrine abnormality in women in the U.S. and is characterized by both reproductive (anovulation and androgen excess) and metabolic dysfunction (insulin resistance). PCOS lacks a simple, safe and effective treatment for women of all ages and all weights. Recently a dietary supplement, inositol, has been used widely to treat women with PCOS. However there are no well-designed trials to address the risk/benefit ratio and identify the mechanism of action.

Polycystic ovary syndrome (PCOS) affects 7-8% of the U.S. female population,[1] and is the most common endocrinopathy among women.[2] It is a heterogeneous syndrome with reproductive aspects consisting of chronic anovulation and hyperandrogenism, as well as metabolic aspects consisting of insulin resistance, glucose intolerance and metabolic syndrome.[3] We have investigated and established multiple first line reproductive treatments for ovulation induction such as weight loss,[4] clomiphene or

letrozole,[5] advanced infertility therapies including IVF[6] and examined long term health with other treatments such as oral contraceptives (OCP)[4] as well as metabolic treatments for infertility and long term health including metformin,[7,8] thiazolidinediones[9] and lifestyle modification.[4 9] Treatment often depends on the presenting complaint, for example OCPs have limited use for infertility and clomiphene has no role to treat hyperandrogenism. Further, we[4] and others[10] have shown that chronic OCP use in overweight/obese women with PCOS can result in a deterioration in glucose tolerance and exacerbation of the metabolic syndrome, such that treatments which favor insulin sensitization and weight loss such as metformin and lifestyle modification have become the preferred chronic treatment.[11] Chronic medications are needed for metabolically challenged patients with PCOS, especially those that are safe, inexpensive and have a low chance of harm.

One such medication, that is available throughout the world as a supplement, is inositol, and more specifically a combination of two stereoisomers, d-*chiro* and *myo*-inositol, which has been used extensively to treat women with PCOS. The combination is thought to improve insulin signaling by restoring an imbalance in inositolglycans which are second messengers in insulin (and gonadotropin) signaling. Although there has been a consensus statement advocating inositol use,[12] this statement also highlighted the lack of high quality randomized controlled trials (RCTs) to identify the risk-benefit ratio. *We propose here the first adequately powered and designed clinical trial to test prospectively the effects of inositol supplementation in a dose ranging double blind RCT, (the INSUPP PCOS study) according to CONSORT guidelines.*[13] This will be a four armed study of three doses inositol (2gm, 4gm and 6gm a day) vs placebo over a 3 month period with the reduction in hyperandrogenism (based on serum testosterone levels) as the primary outcome and key secondary outcomes of the change in sex hormone binding globulin(SHBG) and the related free androgen index, fasting insulin levels, and area under the curve glucose levels from an oral glucose challenge test (OGTT).

2.2 Study Rationale

Scientific Background and Gaps:

The best long-term treatment to maintain health in women with polycystic ovary syndrome (PCOS) is unknown.[11] The best evidence-based clinical studies of therapy in women with PCOS have been infertility studies. These studies have a clearly defined outcome (i.e. live birth) and a limited duration (treatment up to 6 months plus 9 months of pregnancy with conception); whereas, chronic treatment studies have ill-defined endpoints and duration of follow-up. Given the myriad reproductive and metabolic endpoints of chronic treatment, mixed risk/benefit effects have been noted with chronic treatments. For example, oral contraceptives (OCP) may improve hyperandrogenism and anovulatory vaginal bleeding, but have the potential downside of exacerbating metabolic dysfunction.[21,22] Women with PCOS may also be at increased risk for thromboembolic events due to increased number of underlying risk factors.[23] In any case, age limits the use of OCP beyond 35-40 years of age, especially in women with PCOS, of whom up to 40% are current or intermittent smokers.[24] Metformin therapy may slow the progression to diabetes in other populations[25]

(though without an FDA indication for this), but in PCOS, metformin use is associated with modest decreases in testosterone levels and improvement in menstrual cyclicity, blood pressure, and improvements in circulating lipids.[26] Many women do not want to take a medication off label for decades, especially one, such as metformin, with a high rate of gastrointestinal side effects. Designing trials to address long term health and outcomes are more complicated as women are unlikely to develop the major morbidity (e.g. endometrial cancer, stroke, myocardial infarction) during the reproductive life span. Given the lack of a gold standard long-term medical treatment, many women resort to supplements to treat their PCOS symptoms.

Previous Data:

Dietary supplements are increasing being utilized by the U.S. Population. According to the 2007 National Health Interview Survey, 18 percent of American adults had used an oral dietary supplement (not including vitamins and minerals) in the previous year.[27] Dietary supplement use is more common among women than men. Data collected the National Health and Nutrition Examination Survey (NHANES) from 2003 to 2006 showed that 53% of women took a dietary supplement in the last year and the largest category was multi-vitamin (one third of all supplements taken).[28] These supplements have considerable cost to the U.S. population. It has been estimated that in the U.S., 44% of all out-of-pocket costs for Complementary and Alternative Medicine (CAM), or about $14.8 billion, was spent on the purchase of nonvitamin, nonmineral, natural products.[29]

It has been reported that supplement use is common among women with PCOS,[30] though definitive data are lacking on prevalence and types of supplements. One of the most common used supplements is inositol. [30] The rationale for the use of inositol goes back to a small randomized placebo controlled study of d-chiro-inositol published by John Nestler in the NEJM in 1999.[31] Inositolphosphoglycans are second messengers involved in insulin signaling. Inositol is incorporated into cell membranes as phosphatidyl-myo-inositol, the precursor of the second messenger, inositol triphosphate, which functions in insulin (and other protein-including gonadotropin) signalling.[32] D-chiro-inositol, one of the sterioisomers of myo-inositol was thought to be deficient contributing to insulin resistance in women with PCOS. This study of 44 women with PCOS showed that women who received d-chiro-inositol had improved ovulatory function (19 of 22 women receiving inositol ovulated vs 6 of 22 on placebo) with significant decreases in serum androgen and triglyceride concentrations as well as blood pressure.[31] Subsequently, the pharmaceutical company that developed the drug (INSMED), conducted two Phase II multi-center studies in women with PCOS (the PI, Dr. Legro was both a site investigator and a consultant to INSMED during the early 2000s). These studies showed no benefit (as did simultaneously conducted studies in type 2 diabetes) and the company discontinued development of the drug for PCOS and for Type 2 diabetes. The full results of the Phase II studies were never published, but the investigators presented and published an abstract with report of some of the data from one of the Phase II trials at the Annual Meeting of the American Society of Reproductive Medicine in 2001.[33] The trial was a randomized, double-blind, placebo-controlled, dose-ranging two month

trial preceded by a 4 week placebo run-in at 17 US clinical sites. The investigators did a responder analysis, based on patients who had evidence of hyperandrogenemia (elevated total testosterone and free testosterone at baseline). This analysis included 71 of the randomized 223 patients. They assessed the effects of a d-chiro-inositol formulation called INS-1, consisting of 300 to 1200 mg/day or placebo on ovulatory rate (based on weekly progesterone levels) and circulating total testosterone (T) and free T levels. The ovulatory rates during the two months of treatment were: placebo, 18%, 300 mg d-chiro-inositol, 6%, 600 mg d-chiro-inositol, 32%, 1200 mg d-chiro-inositol, 44%, ($p \leq 0.05$). In those women who ovulated while receiving INS-1 there was a 22% decrease in free T concentration (from 1.61 to 1.25 ng/dL) and a 20% decrease in T level (from 71 to 57 ng/dL; both $p \leq 0.05$). In those women who did not ovulate while receiving INS-1 there were only small, non-significant decreases in free and total T concentrations. This placebo response rate is lower than we are projecting for our study (i.e. we are using 30%) and the effect size in the higher doses is significantly higher (i.e. 78% increase at 600 mg and 122% increase at 1200 mg vs our projected 50% increase on study drug). A Cochrane meta-analysis analyzing the effects of d-chiro-inositol in PCOS reported on two RCTs (a second one was conducted in Italy[34]) and was unable to make any summary conclusions due to the small sample sizes and limitations of the study designs.[26] A recent systematic review of inositol treatments in PCOS (first authored by an employee of a company in Italy that manufactures inositol)[35], concluded, not surprisingly, that the treatment was beneficial, but there were a lack of RCTs. The authors were unable to perform a meta-analysis.

Study Rationale:

Subsequently, despite the lack of published data about the efficacy of d-chiro-inositol, experts proposed that there was *an excess of d-chiro-inositol* leading to an improper balance between myo-inositol and d-chiro-inositol in women with PCOS.[12] Myo-inositol is the predominant inositol in the human composing more than 90% of inositols, though there are thought to be tissue specific patterns in the relative expression of the isomers, which is controlled by an insulin sensitive epimerase.[36] This led to several studies (3 clinical trials)[37-39] with replacement of myo-inositol alone.[40] In the ovary of women with PCOS two independent labs[36,41] noted an excess of d-chiro-inositol relative to myo-inositol compared to normal follicular development in normal women (which was about 100 to 1). Similarly ratios in plasma were ~40:1 and this led to the current formulation of myo-inositol to d-chiro-inositol (Ovasitol® by Theralogix, which is a daily dose of 4000 mg of myo-inositol combined with 100 mg of d-chiro-inositol, as we propose to use as the middle dose in this dose ranging study)(**Table 1**). While the science behind this rationale is not strong as indicated by the low impact of the journals where these results have been published, the fact remains that this is a widely used treatment in the U.S. and the world. Clinical trials conducted with the proposed combination have shown favorable results on both reproductive and metabolic parameters, but the science behind these clinical trials is of the lowest quality, lending suspicion to publication bias. There are major methodologic concerns with the published trials to date as summarized in Table 1. These include a lack of clinical trial registration, no pre-specified primary outcome

and power analysis to justify the sample size, no pre-specified statistical analysis plan and failure to adequately compare treatment groups according to between group, intention to treat (ITT) analysis, no double blinding, no allocation concealment, no accounting for patient drop out, no systematic collection or reporting of adverse events to highlight the major issues.

Table 1 Studies to date of myo/chiro inositol (40:1 ratio) and effects on Reproductive/Metabolic Parameters in women with PCOS.

Reference, Year	Type of Study	N, Criteria	Primary Outcome	Length	Significant Change in Variables from baseline in Myo/Chiro group	Significant Change in Variables between Groups
Benelli et al, 2016[42]	Open label RCT of DCI/MCI versus folic acid placebo	46, OW	Not Specified	6 mos	-18% Free T +49% SHBG -42% HOMA-IR Ovulation not assessed	No between group analysis performed
Minozzi et al, 2013[43]	Open Label	24, OW/OB	Not specified	24 wks	-14% LDL-C +8% HDL-C Ovulation not assessed	No comparison group
Nordio et al, 2012[44]	RCT of DCI/MCI vs MCI alone	50, OW	Not specified	6 mos	-66% Total T -51% DHEAS -26%Androstenedione +43% SHBG -38% AUC Insulin and Glucose Ovulation not assessed	None

OW/OB= overweight or obese, N.A. = not applicable, T = testosterone, AUC = area under the curve on 2 hour OGTT, SHBG = sex hormone binding globulin

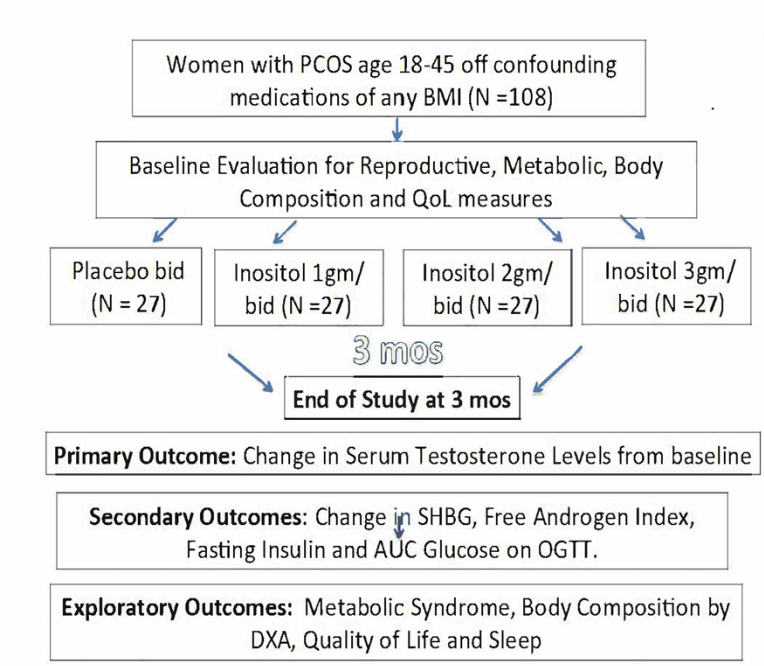
We propose that the primary mechanism for restoring ovulation will be the reduction in hyperandrogenism. This has been noted with all medications that have been used for ovulation induction including our previous studies with clomiphene citrate, letrozole and metformin in women with PCOS.[5,7] The marked reduction in total testosterone and androstenedione (primarily ovarian origin) as well as in DHEAS (adrenal origin) are likely mediated by direct ovarian and adrenal effects.[44] A secondary mechanism is the improvement in insulin sensitivity which is reflected by decreased insulin and glucose levels during the oral glucose tolerance test (OGTT) is associated with marked increases in sex hormone binding globulin (SHBG).[45] SHBG preferentially binds androgens, removing them from the peripheral androgen pool, and is found in high levels not only in blood but also in follicular fluid. It is difficult to tease out the relative contribution of decreased hyperandrogenism and improved insulin sensitivity on increased SHBG levels as both likely contribute to it. We have noted in our previous studies, however, that ovulation induction methods such as clomiphene citrate and letrozole which impact these reproductive parameters more than metformin have significantly improved ovulation rates (and pregnancy rates).[5,7] These factors are included in our predictive models of ovulation.[46,47] Further we noted in our Pregnancy in Polycystic Ovary Syndrome II (PPCOSII) trial a greater decrease in Anti-Mullerian Hormone (AMH) levels with letrozole vs clomiphene, [5] and we anticipate we will see similar related decreases in

AMH levels in our inositol treatment group correlating with decreased hyperandrogenism and improved menstrual frequency and ovulation rates. AMH is secreted by the granulosa cells of pre-antral and antral follicles and high intra-ovarian levels such as seen in women with PCOS with high antral follicle counts may suppress local follicular development.

Previous clinical studies of the combination myo/chiro inositol treatment in women with PCOS have suffered from a lack of rigor, reproducibility and transparency. There have not been any double-blind, placebo controlled studies to establish proof of efficacy. Further the studies to date have failed to register their trials (or at least reference the trial registration in their publications), design the trial around a pre-specified outcome, reveal the methods of randomization or the means to preserve allocation concealment. They have failed to report adverse events and serious adverse events. Most of the boxes in the CONSORT checklist of a randomized trial would remain unchecked.[13,48] We will remedy this in this trial by using a double-blind, placebo controlled study design, with a clear pre-specified primary outcome (i.e., change in serum testosterone levels), and by maintaining allocation concealment with the randomization scheme unknown to investigators. This rate is based on the extensive experience of the PI in using this as a primary or secondary outcome in studies of women with PCOS. It is also based on the FDA's acceptance of this as an important clinical outcome for industry sponsored studies in women with PCOS including troglitazone,[9] anastrazole[49,50] and with d-chiro-inositol[33]. It is uncertain what is the best risk factor/surrogate outcome (i.e. diabetes, blood pressure, intimal medial thickness, coronary artery calcification, etc.) for CVD events such as stroke and myocardial infarction. Further the FDA in their guidance for industry studying drugs for type 2 diabetes and metabolic syndrome has upped the ante requiring at some point in the approval process, studies examining CVD events instead of just risk factors. Such a focus is outside the scope of this or any RO1 study, though we will collect surrogate measures of CVD health in this study. However, hyperandrogenism in women has been identified not only as an overall vital sign in women's health, but within women with PCOS hyperandrogenism correlates closely with anovulation and metabolic dysfunction[51], and improvement in the hyperandrogenism is associated with improved ovulation rates. Additionally anovulation is a risk factor for endometrial cancer,[52,53] so Improvement in hyperandrogenism and restoration of ovulation would likely have a favorable impact on the long term risk for this common cancer. The risk/benefit ratio of any treatment must be carefully assessed before proceeding with such a study and there are no reported adverse events (though the rigor in which these were collected in previous studies is questionable), nor are there any fetal risks such as increased loss or anomalies noted, and the drug has been used in multiple IVF studies. There have been several studies in which inositol preparations (primarily myo- alone) have been used throughout pregnancy, including early pregnancy,[54,55] for a variety of indications, most commonly to prevent gestational diabetes[56,57] for which there is a suggestion of benefit from a Cochrane Systematic Review.[58] It has also been used, again with a suggestion of benefit to treat neonates with respiratory distress syndrome.[59] In this study we will collect all adverse events, including pregnancy-related, overseen by a Data Safety and Monitoring Board (DSMB) and IRB at Penn State.

3. STUDY DESIGN

Figure 1: Flow Chart of INSUPP PCOS Trial



This multi-site, double-blind, randomized controlled trial will assess the change in serum testosterone from baseline to end of study (after 3 months of treatment) across 4 arms as the primary outcome: daily treatment with an inositol supplement at either 2, 4 or 6 gm/day or an identical daily placebo over a 3 mos period (**Fig.1**).

Serum testosterone levels will be batched and run at the Core Ligand Lab at the University of Virginia and not shared with the participants during study participation. Secondary outcomes of SHBG, calculated FAI and glucose and insulin levels from the 75g 2h OGTT will also be run at the Core Ligand lab as we have done in multiple previous studies. We will additionally phenotype subjects for a variety of clinical and biochemical measures of hyperandrogenism, assess body composition by DXA and quantify quality of life, sexual function and sleep by standardized instruments or validated questionnaires to examine a variety of exploratory outcomes. Our experimental design, through allocation concealment, randomization and predetermined inclusion/exclusion criteria, is robust and easily replicated. In addition to publishing a protocol paper, we will upload the final protocol and case report forms (CRFs) with our main outcomes paper and make them readily available to other investigators seeking replication. We have also shared our protocols and CRFs with other researchers studying PCOS while our studies are ongoing resulting in impactful important studies.[6,19,20,60] All of these both ease and accelerate reproducibility of our studies. We note that the Cochrane meta-analyses of clomiphene vs metformin in PCOS and of letrozole vs clomiphene have not only upheld the primary findings of our results from the PPCOSI[7] and II[5] studies, but the effect size when all applicable trials are included is actually greater than our studies. This indicates the high level of reproducibility of the studies our team has designed and conducted.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

We will randomize 108 women with PCOS (27 per arm), age 18-45 years, of any BMI. All subjects must have evidence of an elevated serum testosterone (by local assay) and oligo- or amenorrhea since this is the classic NIH definition but also consistent with the Rotterdam criteria[61] and as per both our PPCOS II[5] and OWL PCOS[4] studies. Our baseline data from these studies establishes that such diagnostic criteria identify a group of women who are both hyperandrogenic AND have polycystic ovaries (over 90% of patients have polycystic ovaries either by increased antral follicle count or ovarian volume). Further when we compared the baseline characteristics of patients with PCOS diagnosed by the classic NIH criteria in PPCOSI (i.e. chronic anovulation and hyperandrogenism) versus the modified Rotterdam criteria in PPCOS II[5] (i.e. chronic anovulation with either hyperandrogenism or polycystic ovaries) we find very similar parameters for BMI, testosterone levels and hirsutism scores (by Ferriman Gallwey[62] with a score ≥ 8 indicative of hirsutism), and rates of enlarged polycystic ovaries (**Table 2**). Thus our population is generalizable across a wide variety of PCOS diagnostic criteria.

Table 2: NIH criteria for PCOS (PPCOS I trial) and Modified Rotterdam Criteria (PPCOS II trial) identify similar populations of women with PCOS based on reproductive and metabolic parameters

Study	PCOS Diagnostic criteria	BMI kg/m²	Total Testos-terone ng/dL	SHBG nmol/L	Hirsutism Score Mean	Fasting Glucose mg/dL	Fasting Insulin µU/mL	Ovarian Volume (Left) cm³
PPCOS I	Anovulation and hyperandrogenemia	35.1 ± 9.3	62 ± 28.5	29.7 ± 17.8	14.4 ± 7.9	89.0 ± 79.5	23.0 ± 26.7	12.0 ± 7.0
PPCOS II	Anovulation and hyperandrogenism OR polycystic ovaries	35.2 ± 8.7	55 ± 28.8	33.9 ± 23.1	17.0 ± 8.5	86.0 ± 12.8	19.3 ± 27.0	12.6 ± 7.4

4.1 Inclusion Criteria

We will exclude subjects with World Health Organization (WHO) type 1 ovulatory dysfunction (hypothalamic hypoestrogenism) and WHO type III ovulatory dysfunction (ovarian failure). We will not exclude subjects with prior exposure to study drug in this study, as both the PPCOS I[7] and II[5] studies have shown prior exposure to study medication (i.e. clomiphene, letrozole or metformin) had no effect on ovulation or pregnancy rates compared with drug naïve subjects. This will allow also for a quicker recruitment and greater applicability of results to the larger population of women with PCOS. We also intend to stratify randomization by BMI category (BMI < 30 kg/m² or BMI ≥ 30 kg/m²)

<u>Key Inclusion Criteria (Women age 18 – 45, must have ovulatory dysfunction and either Hyperandrogenism or Polycystic ovaries)</u>

1. Hyperandrogenemia (HA). Hyperandrogenemia is defined as a total testosterone (T) > 40 ng/dL (M.S. Hershey Clinical Labs Testosterone assay).[63,64] Outside lab values obtained within the last year documenting elevated T levels are sufficient to meet criteria of hyperandrogenemia.

2. Chronic anovulation or oligomenorrhea defined as spontaneous intermenstrual periods of ≥45 days or a total of ≤8 menses per year. For women who have been on ovarian suppressive therapy or other confounding medication (i.e. insulin sensitizing agents) within the last year prior to the study, a history of ≤8 menses per year prior to the initiation of this prior therapy will qualify as evidence of oligomenorrhea. For women with more regular bleeding patterns, but who are suspected to be experiencing anovulatory bleeding, a midluteal progesterone (P4) level < 3ng/mL will be evidence of ovulatory dysfunction and qualify as anovulation.

4.2 Exclusion Criteria

Key Exclusion Criteria

1. Subjects with hyperprolactinemia (defined as 2 prolactin levels at least one week apart ≥30 ng/mL). The goal of eliminating subjects with documented hyperprolactinemia is to decrease the heterogeneity of the PCOS population. These subjects may be candidates for ovulation induction with alternate regimens (dopamine agonists). A normal level within the last year is adequate.
2. Women who are pregnant at study screening or randomization.
3. Subjects with known 21-hydroxylase deficiency or other enzyme deficiency leading to the phenotype of congenital adrenal hyperplasia. 21-hydroxylase deficiency will be excluded in all subjects by a fasting 17-hydroxyprogesterone (17-OHP) level >2 ng/mL and ACTH stimulation test as needed.[65]
4. Subjects with elevated FSH levels (>10 mIU/mL). A normal level within the last year is adequate.
5. Subjects with uncorrected thyroid disease as per American Society of Reproductive Medicine guidelines for nonpregnant subjects[66] (TSH ≤0.45 mIU/ML or ≥4.5 mIU/mL). A normal level within the last year is adequate.
6. Subjects with a suspected adrenal or ovarian tumor secreting androgens.
7. Subjects with suspected Cushing's syndrome.
8. Subjects on confounding medications which affect ovarian function including metformin, oral progestins, combination hormonal contraceptives, other medications for type 2 diabetes (i.e. pioglitazone, liraglutide, etc. or weight loss drugs).[67] A 2 month washout period for combination hormonal contraceptives, a 1 month washout for oral progestins and a 1 month for weight loss and type 2 diabetes drugs will allow participation if these medications are not medically indicated. Please refer to the study MOP for medication-specific washout periods.
9. Subjects with previous allergic reactions to the supplement or to the placebo maltodextrin or inulin.
10.Liver disease (AST/ALT≥2 times normal or a total bilirubin ≥2.5 mg/dL)

4.3 Study Enrollment Procedures

Identification of subjects

Subjects will be recruited at each individual site: Penn State College of Medicine site(PSU) and University Of Pennsylvania (PENN). The patients will be recruited

from a variety of sources within and outside our health care systems such as: fliers, social media outlets (Facebook, Instagram, or Twitter), radio advertising, Lions Eye, mass mailings, email blasts, search engine campaigns, daily news, brochure or rack card. Any of these materials used for advertising sources will be IRB approved prior to implementation.

At PSU site, one source will be subjects, with PCOS who may have participated in our prior studies, including our family studies, and treatment trials. Another source will be from our clinical subspecialty practices of reproductive endocrinology and medical endocrinology and our larger departmental practices of Ob/Gyn and Medicine at Penn State Hershey Medical Center. Additionally, this study will use EIM as a strategy for recruitment and identification of potential subjects. We will promote the study to departmental and medical center physicians. The study will also be posted on StudyFinder. We will register the randomized trial at clinicaltrial.gov, which also serves as a source of patients.

At PENN's site, one source will be subjects, with PCOS who may have participated in their prior studies. 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.

Recruitment process

We will track all contact from subjects interested in the study. We will develop a pre-screening list that documents date and point of contact, eligibility based on telephone/REDCap screening and follow-up if the subject meets prescreening and is interested in participation. The consent form document will be sent to provide ample time to review prior to the screening visit where the consent process will take place in person at that time.

Eligibility/screening of subjects

The study will use a telephone pre-screening form when potential subjects contact in response to the various ways of recruitment. The prescreening tool will help study coordinators to assess basic eligibility to warrant a screening visit. At PENN site, their prescreening survey can completed with potential subjects interested by telephone or REDCap.

Consent Process

Obtaining Informed Consent

Timing and Location of Consent

For each site, a subject who qualifies for further screening will be scheduled fora screening visit where the consent process will take place in a private room setting and prior to any study procedures. She will initially be counseled about the purpose of the study, the procedures, the risks, and the time commitment. Voluntariness will be stressed and ample time will be given for discussion and questions. Informed consent is obtained in person by study personnel, and the subject also has the opportunity to interact with one of the study investigators who performs the physical exam and reviews eligibility criteria. All subjects give written informed consent to participate in the study during this screening visit and will be given a copy of the signed document.

Coercion or Undue Influence during Consent

The study team will stress voluntariness of participation in the research study. Subjects will be made aware that participation will in no way affect their clinic care.

Waiver or alteration of the informed consent requirement

We request of waiver of the informed consent requirement for pre-screening purposes to review the medical records of those potential subjects that may already be patients within the healthcare system. These would be patient referrals from sources such as those listed in Section 4.1 above.

Consent Documentation

Written Documentation of Consent

Written documentation of consent will be obtained per HRP-091 – SOP: Written Documentation of Consent.

Waiver of Documentation of Consent (Implied consent, Verbal consent, etc.)

Waiver of documentation of consent is being requested for subjects to fast prior to the screening visit (see phone intake pre-screening document)

Consent – Other Considerations

We will not be recruiting Non-English speaking subjects, cognitively impaired adults or subjects who are not yet adults (i.e. infants, children or teenagers)

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Method for Assigning Subject to Treatment Groups

Randomization is a critical feature of a clinical trial because it prevents treatment-selection biases. The statistician involved in this study will develop the programs

necessary for the random number generator to create 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 this study's statisticians blinded as well. The randomization scheme for this study will use variable-size, random permuted blocks to ensure 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 site and obesity status at baseline (BMI < 30 kg/m^2 or BMI ≥ 30 kg/m^2). Although stratification may potentially yield unequal numbers between strata, within each individual stratum there will be approximately equal numbers and balance between the treatment groups.

Randomization to the four treatment arms (placebo bid, inositol 1 gm bid, inositol 2 gm bid, and inositol 3 gm bid) will use 1:1:1:1 allocation. As previously mentioned, the independent statistician will generate the final randomization scheme and it will be uploaded into the password protected, secure REDCap database. When a patient is ready to be randomized the study coordinator will contact their respective site's Investigational Drug Services (IDS), who are completely independent of the study, and provide them with the patient's name and BMI status at baseline for the stratified randomization. IDS will log into REDCap to perform the randomization and dispense the treatment medications. Only IDS will have access to the Randomization form in REDCap, in order to maintain blinding of the study team (PIs, study coordinators, and statisticians). Participants will be entered consecutively.

Study Drug/Placebo: Inositol is a supplement available throughout the world and more specifically a combination of two stereoisomers, d-*chiro* and *myo*-inositol, which has been used extensively to treat women with PCOS. The combination is thought to improve insulin signaling by restoring an imbalance in inositolglycans which are second messengers in insulin (and gonadotropin) signaling. More information about this supplement can be found here: https://theralogix.com/products/ovasitol-inositol-powder

Treatment Regimen

Arm A: Placebo Treatment bid: In the placebo group, women with PCOS (N = 27) will receive the daily placebo (maltodextrin and inulin) in an identical fashion as the active study group and will be monitored the same. Both the active supplement and placebo packets will each contain 2.6 grams of carbohydrates, which will equal roughly 10 calories per packet. This small amount of carbohydrate will have minimal if any impact on any of the study outcomes, such as glucose tolerance, dyslipidemia, or prevalence of the metabolic syndrome. Pill counts (or in this case packet counts) will be monitored at each study visit.

Arm B: Active Treatment with Inositol 1 gm/bid: In this group, women with PCOS (N = 27) will receive daily inositol BID as active drug (each dose with 1000 mg of myo-inositol and 25 mg of d-chiro-inositol) over the initial 3 mos RCT period. They will be advised to take it twice a day on a daily basis at the same time each day. Pill counts (or in this case packet counts) will be monitored at each study visit. We have

used this in a number of high impact trials to monitor compliance with study medication and found high adherence.[4,5,7,15]

Arm C: Active Treatment with Inositol 2 gm/bid: In this group, patients with PCOS (N = 27) will receive daily inositol BID as active drug (each dose with 2000 mg of myo-inositol and 50 mg of d-chiro-inositol) over the initial 3 mos RCT period. They will take it in an identical fashion to the other groups. Pill counts (or in this case packet counts) will be monitored at each study visit.

Arm D: Active Treatment with Inositol 3 gm/bid: In this group, patients with PCOS (N = 27) will receive daily inositol BID as active drug (each dose with 3000 mg of myo-inositol and 75 mg of d-chiro-inositol) over the initial 3 mos RCT period. They will take it in an identical fashion to the other groups. Pill counts (or in this case packet counts) will be monitored at each study visit.

Blinding of the Test Article

The inositol supplement and placebo will be provided in individual packets that will be identical. Both active drug and placebo are colorless, odorless and tasteless and dissolve in water or any liquid which will preserve the double-blinding of this trial. The drug will be kept and dispensed in the Investigational Pharmacy at each site and they will hold the randomization and unblinding key as needed. The investigators and biostatisticians will remain blinded to the allocation sequence and patient assignment.

Unblinding Procedures

The Investigational Pharmacy at each site will hold the randomization and unblinding key for their patients as needed. The investigators and biostatisticians will remain blinded to the allocation sequence and patient assignment. DSMB Chair will have the discretion of unblinding the DSMB at any time if it is warranted for safety and/or scientific reasons.

5.2 Handling of Study Interventions

Receiving, Storage, Dispensing and Return

Receipt of Test Article

Study drug and placebo will be provided by Theralogix, a Maryland-based nutritional supplement manufacturer (see letter of support from Jay Hammer, Theralogix). The inositol supplement and placebo will be provided in individual packets that will be identical. Each packet will contain 3 grams of powder. In Arm A, all 3 grams will be placebo powder, consisting of a mixture of maltodextrin and inulin. We are not using lactose as a placebo given the high prevalence of lactose intolerance in the U.S. population. In Arm B, each three gram packet will contain 2 grams of placebo powder mixed with 1 gram of inositol. In Arm C, each 3 gram packet will contain 1 gram of placebo powder and 2 grams of inositol powder. In Arm D, each 3 gram packet will contain no placebo powder and 3 grams of inositol powder. Subjects will be instructed to take one packet twice per-day, with instructions to dissolve the packet in 8-10 ounces of water.

Storage
Stability will be monitored and appropriate storage conditions will be assured. The drug will be kept and dispensed in the Investigational Pharmacy at each site.

The PI has had experience working with Theralogix through the Reproductive Medicine Network's MOXI study, a randomized double-blind study of Theralogix's male antioxidant supplement (Conception XR®) in males with abnormal semen analysis parameters seeking fertility (Clinicaltrials.gov # NCT02421887) which is completed running with 13 randomized subjects at Penn State. Additionally, Therologix has collaborated with NIH funded investigators studying the use of a cranberry extract (Theracran®) to prevent urinary tract infections (it halved the rate in a high quality RCT).[68] As per our agreement through the Reproductive Medicine Network, Theralogix will supply the study medication and placebo at cost, and will have no role in the design, interpretation, or write up of results. We will share the results of submitted abstracts and manuscripts with the company

Preparation and Dispensing
The study supplement packets, containing placebo and/or inositol, will be produced in a GMP-certified facility by Theralogix. The supplement will then be certified for content accuracy and purity by an independent, non-profit third party program at NSF International® (http://www.nsf.org/services/by-industry/dietary-supplements). The Investigational Pharmacy at Penn State Hershey Medical Center and University of Pennsylvania will dispense the study drug and placebo per institutional policy.

Return or Destruction of the Test Article
The Investigational Pharmacy at each site will destroy returned study drug packets as per institutional policy.

5.3 Concomitant Interventions
All concomitant medications will be collected. There are no prohibited medications or therapies throughout the study.

5.4 Adherence Assessment
Study medication (supply bags (including empty, partially used, and unopened bags) will be collected at each visit and packet counts performed to assess compliance.

6. STUDY PROCEDURES

6.1 Schedule of Evaluations

Assessment	Screening: Visit-1	Baseline, Randomization: Visit 1 (within 3 months/90 days of obtaining consent)	Treatment Visit 1 (Month 1 +/- 7 Days) **	Treatment Visit 2 (Month 3 +/- 7 Days) **
Informed Consent Form	X			
Demographics	X			
Medical History	X	X	X	X
Physical Examination (including PAP, if necessary)	X			
Concomitant Medications and Adverse Event Review	X	X	X	X
Blood Pressure, Weight, Waist/Hip Ratio	X	X	X	X
Perform Hirsutism Assessment		X	X	X
Distribute and Collect Daily Diary (if applicable)		X	X	X
Dispense and Collect Study Drug (if applicable)		X	X	X
Study Drug Compliance			X	X
Inclusion/Exclusion Labs (if not previously done): Testosterone, TSH, PRL, 17OHP,etc. (local)	X			
Safety Labs (fasting): CBC, CMP, Lipid profile (Hershey Medical Center)	X			X
Urine Pregnancy test (monthly home and study visits)	X	X	X	X
Primary Outcome: Serum Testosterone (UVA Core)		X	X	X
Other Outcome labs: SHBG, DHEAS, Androstenedione, AMH, CRP, HbgA1c, Progesterone, Estradiol (UVA Core)		X	X	X
Measure Sebum with Sebumeter		X	X	X
2-hour 75g OGTT (5 timepoints Glucose/insulin at UVA Core)		X		X
Transvaginal Ultrasound U/S(*)	X(*)	X(*)		X
DXA Scan for Body Composition		X		X
Questionnaires, including QOL Caffeine Intake Questionnaire		X	 X	X X

(*) – indicates this procedure may be done at Screening or Randomization(**)If applicable due to unforeseen or extenuating circumstances outside of our control ie: pandemic, *emergency* or unexpected issue arises and a subject *cannot make it to the research center for a study visit within the protocol-defined window*, *some of these visits may be conducted remotely via tele-health, HIPAA compliant conferencing or phone, etc. The determination to conduct the visit remotely will made on a case-by-case basis by the study Principal Investigator. The study team will make arrangements* with the subject to complete as many of the study specific visit parameters remotely if this situation applies to ensure subject safety and will not affect the integrity of the study data and outcomes. Any changes to study procedures that are made during these circumstances will still be reported to the IRB, per our institutional standards.

6.2 Description of Evaluations

6.2.1 Screening Evaluation

Consenting Procedure
Prescreening and consenting will take place prior to any study procedures or visits take place as described above in Section 4.3.

Screening Visit and procedures
After eligibility has been assessed with the prescreening tool, we will schedule a screening visit. For the PSU site, this will be conducted at the Clinical Research Center (CRC) at Hershey Medical Center (HMC). (Dr. Legro is Director of the Clinical Services Core (CSC), now part of the Hub Resource Capacity (HRC) which includes the CRC of the Penn State NIH funded Clinical and Translational Science Institute (CTSI). For the PENN site, this will be conducted at the Reproductive Research Unit(RRU). We will obtain informed consent and screen patients to establish a PCOS diagnosis (see criteria above).

- We will collect demographic information and ask about any medications the subject is currently taking.
- Patients will present in a fasted state for a minimum of 10 hours (no food or drink, except for water) so we can send safety labs which will consist of a fasting complete metabolic profile with electrolytes and glucose, renal and liver function, a CBC and a lipid profile with total cholesterol, HDL-C and triglycerides.
- We will also obtain baseline medical history, perform a full exam and will collect a pap smear as needed according to ASCCP (The American Society for Colposcopy and Cervical Pathology) guidelines.
- A transvaginal ultrasound will also be performed at this visit, since ovarian morphology and volume are part of the Rotterdam diagnostic criteria for PCOS. We will use our standard case report form which tracks endometrial thickness, any uterine abnormalities (mullerian, leiomyomas, polyps, etc), ovarian size and volume, antral follicle count, measurement and description of all follicles and/or cysts > 10 mm in average diameter. There are no obvious findings on transvaginal ultrasound at screening other than pregnancy (which would presumably be detected by the urine hCG screen) which would preclude participation. However, if in the opinion of the physician performing the transvaginal ultrasound visit find suggestive pelvic malignancy, further participation in the study will be deferred until this possibility has been ruled out. Because ultrasound is not diagnostic for genital track malignancies this will rely on physician judgement.

6.2.2 Baseline and/or Randomization

Because these patients are anovulatory, it will not be possible to time this visit timed to a specific menstrual cycle phase. This Randomization visit must occur within 3 months (90 days) of obtaining informed consent and after eligibility has been assessed from the screening procedures.

Patients may become eligible after seeking treatment for a medical condition and having been re-evaluated, and the Principal Investigator approves continued study participation. Therefore, where possible will avoid unnecessary duplication of tests that could be burdensome to the patient. The procedures performed at the randomization visit will be repeated at the end of the 3 months RCT.

- Patients will present after an overnight fast.
- Vital signs including blood pressure, waist/ hip measures
- Hirsutism will be obtained. Hirsutism is determined by a modified Ferriman-Gallwey Score ≥ 8 (based largely on midline hair distribution and density) at screening exam.[69]
- We will perform a urine pregnancy test at this visit and every other visit. Urine pregnancy tests will be dispensed to the patient to check on a monthly basis or if they experience withdrawal vaginal bleeding.
- We will perform a transvaginal ultrasound at this visit, only if it was not performed at the screening visit.
- We will perform a standardized acne assessment and measure facial sebum via the sebumeter as we done in multiple studies AND has been used extensively in clinical trials of acne. We have used it in multiple trials in women with PCOS[70] and have not found changes in sebum with the short term use of ovulation induction agents such as clomiphene or letrozole,[4,5] but we recently found a significant decrease in sebum (>25%, $P < .001$, $N = 50$) with the combined use of oral contraceptives and lifestyle and a nearly significant decrease (~20%, $P = 0.06$, $n = 49$) with oral contraceptives alone in a 16 week intervention. There may be a similar magnitude in decrease of sebum with chronic inositol therapy, and a similar power with our sample size to detect a ~25% decrease. In our studies, the mean baseline levels of sebum in women with PCOS are around 100 mcg/cm^3 and standard deviation is close to 50mcg/cm^3.[4,70]
- A Reproductive /Metabolic serum panel (Testosterone (T), Progesterone (P4), Andostenedione (A4), DHEAS, Sex Hormone Binding Globulin (SHBG), Anti-Mullerian Hormone (AMH) and glycohemoglobin (HgbA1c) will be obtained. These will be assayed at the NICHD supported University of Virginia Core Ligand lab run by Dan Haisenleder as we did for OWL PCOS and PPCOS I and II using well validated assays with low intra- and inter-assay CVs.[70]
- Patients will present to the visit after an overnight fast for a standard 2 hour oral glucose tolerance test (OGTT) consisting of timed fasting and glucose challenged (75 g oral glucose load) with glucose and insulin levels at baseline and every 30 minutes.
- Daily diaries will be dispensed to all subjects (as is standard in our studies).[5,7] The diary records the days and severity of bleeding[71] as well as study drug intake, side effects and concomitant medications throughout the study. Obese women will be offered standard weight loss therapy by referral to a Dietitian and recommendations to restrict calories and increase physical activity.

Because this is standard practice, we do not believe that it will bias study results as a concurrent lifestyle therapy, since we will stratify randomization by obesity to allow for equal allocation between treatment groups.

- Validated questionnaires (i.e. the SF-12, a validated shorter version of the SF-36, a validated medical quality of life questionnaire,[72] the Female Sexual Function Index (FSFI),[73] the PCOSQ, a validated PCOS quality of life questionnaire[74]) will be administered at this visit. We will use two simple validated scales to evaluate sleep, the STOP BANG (for sleep apnea)[75] and the Epworth Sleepiness Scale (daytime sleepiness).[76] Dysfunctional sleep including sleep apnea has been attributed to insulin resistance, and therefore tracking the effects on sleep by questionnaire will examine this relationship. We anticipate questionnaires will be completed during the 2 hour OGTT.
- Afterwards, a DXA scan for body composition will be obtained.[8,77] Bone mineral density and body composition data will be determined by dual energy x-ray absorptiometry (DXA)(Hologic Discover, Hologic Inc., Bedford, MA). Subregion analysis of android (abdominal) and gynoid (hip) fat will be modeled according to our previous studies.[4,78,79]

Since a transvaginal ultrasound was obtained at the screening visit, we do not see the need to repeat it in such a short period of time, however this will be obtained at the subsequent 3 month end of study visit to assess ovarian and uterine effects.

Conception during treatment: Patients who are sexually active and at risk for pregnancy (i.e. no permanent sterilization of patient or partner) will be asked to use barrier contraception during the 3 mos study. We will be dispensing urine pregnancy tests so patients can test monthly at home to identify any unexpected pregnancies as soon as possible.

Women who do conceive on study medication will immediately discontinue from the study, but we will continue to follow them until the pregnancy ends. We will collect at the first subsequent visit all of their study drug. We will follow serum levels of hCG until location and viability of the pregnancy can be determined in the clinic by transvaginal ultrasound. We will request update on the outcome on all pregnancies, both maternal, fetal/infant up to one month after delivery or pregnancy termination. At the last obstetrical ultrasound visit prior to referral for prenatal care, we will ask the patient to sign a release of medical records form from her obstetric provider so that we can track any adverse events related to pregnancy or childbirth by review of the complete records. If we do not receive any information about the pregnancy outcome from the patient or medical record request, we will contact the patient at 1 month after her due date. We will revise the protocol accordingly.

6.2.3 Blinding Procedures

See section 5.1 above for Blinding procedures.

6.2.4 Follow-up Visits

FIRST MONTH – VISIT (MONTH 1 +/- 7 DAYS)
A first monthly visit will be conducted for the following:

- Review adverse events,
- Update the medical history and medications
- Obtain biometric data including weight, blood pressure, waist/hip measures,
- Hirsutism scores
- Sebum measurements.
- Caffeine Intake Questionnaire
- Reproductive/Metabolic panel above that will be run at the UVA core lab. Patients will not have to fast for this visit, and we will have greater flexibility to schedule this around their needs.
- Study medication packets (preferably empty) will be collected and packet counts performed to assess compliance.
- New study medication for two months will be dispensed at this visit by prescription through our investigational pharmacy.
- A urine pregnancy test will be performed at this visit and we will also dispense urine pregnancy tests for patients to test monthly at home.

6.2.5 Completion/Final Evaluation

END OF RCT VISIT (MONTH 3 +/- 7 DAYS)
At this visit we will:

- Repeat the complete phenotyping performed at the Randomization Visit.
- Biometric data including weight, blood pressure, waist/hip measures,
- The Reproductive/Metabolic panel
- Safety labs,
- 2h OGTT,
- Questionnaires, including caffeine intake
- DXA scan
- Transvaginal ultrasound scan will be repeated.
- Final packets of used study medication will be collected and packet counts performed.

7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

Risks of Inositol and Placebo: There are no known serious adverse effects, pattern of adverse effect related to the drug or interactions with other drugs. Similarly there is no pattern of adverse effects to maltodextrin and inulin mixture in the placebo. Both the active supplement and placebo packets will each contain 2.6 grams of carbohydrates, which will equal roughly 10 calories per packet. This small amount of

carbohydrate will have minimal if any impact on any of the study outcomes, such as glucose tolerance, dyslipidemia, or prevalence of the metabolic syndrome. We chose the higher dose of inositol to identify if there is a better risk/benefit ratio at this dose. We are not aware of any studies using the dose in women with PCOS. However, our proposed 6 gm daily dose of inositol (40:1 myo to d-chiro-inositol) is not unprecedented in the literature. In fact, there are studies, primarily in the psychiatric literature, that have used up to 18 gms a day. There are multiple case studies and observational studies, primarily in the psychiatric literature, that have used up to 18 gms a day. There are multiple case studies and observational studies, but we have identified three higher quality studies in these populations using large doses of inositol. (12-18 gms/day) far exceeding our upper daily dose. We summarize these studies and the side effect profile on the Table below:

Reference	Type of study	Indication	Total N Subjects	Inositol Dose	Comparator	Side Effect Profile
Leppink et al, 2017 [86]	Double blind RCT	Trichotillomania	38	6-18 grams/d for 10 weeks, dose began at 6 gm/day and was escalated each week by 6 gm until 18gm/day	Placebo	On Inositol, 4/19 with nausea/upset stomach, 2/19 with stomach pain, 2/19 with headache, 2/19 with diarrhea, 1/19 with gas, and 1 SAE, an ectopic pregnancy
Palatnik et al, 2001 [87]	RCT crossover	Panic Disorder	21	18 gm myo-inositol/day x 1 mos., first week was 12 gm/day	Fluv-oxamine up yo 150 mg/day	16/21 on fluvoxamine and 8/21 on inositol c/o nausea, 9/21 on fluvoxamine vs 1/21 on inositol complained of Tiredness
Fux et al, 1999 [88]	Double blind RCT crossover	Obsessive-Compulsive Disorder	13 (10 Completed)	18 gm myo-inositol/day x 6 weeks	Placebo (glucose)	No side effects reported

Our conclusion from the review of these higher quality studies is that higher doses beyond 6gm/day of inositol are safe and generally well-tolerated, though mild GI side effects are possible, as we have noted on our IND application. The one SAE of an ectopic pregnancy in the Leppink et al [37] study was felt to be unrelated to the inositol, in fact she continued on the study drug and in the study after treatment of the ectopic.

Risks of Blood Draw: The risks of phlebotomy include pain at the phlebotomy site, bruising and rarely bleeding, and potentially iatrogenic anemia.

Risks of Transvaginal Ultrasound**:** The risks include abdominal or pelvic discomfort.

Risks of DXA Scan: The DXA scan will involve exposure to radiation, on average 2-3 milliroentgens of radiation during the procedure. This is equivalent to the exposure the average person receives on a cross country airplane flight.

Oral Glucose Test: Possible side effects from this test include anemia (lack of red blood cells), infection at the site of the IV if used for blood draws, nausea, vomiting, allergic reaction, hypoglycemia (low blood sugar), as well as the standard blood draw discomforts and risks.

Risk of Randomization: Subjects are assigned to a treatment program by chance. The treatment received may prove to be less effective or to have more side effects than the other research treatment(s) or other available treatments.

Questionnaires: Subjects may get tired or bored when completing questionnaires and are free to skip any questions they do not want to answer.

Reproductive Risks: The risks to an unborn baby or a nursing child from the study drug are not known at this time and may cause problems to an unborn baby. Women who are pregnant or are nursing a child may not participate in this research study. Women capable of becoming pregnant, will be required to take a pregnancy test before beginning the research and must agree to take reasonable and necessary precautions against becoming pregnant during the period of the investigation. The investigator will discuss appropriate precautions with subjects and advise to notify the investigator immediately if pregnancy is suspected.

Risk of Loss of Confidentiality: There is a slight risk of loss of confidentiality associated with participation in the study. To mitigate this risk, all subjects will assigned a unique study ID that will be used throughout the study to identify each subject. The list linking identifiable information from the study ID will be maintained in the REDCap database designed for this study. REDCap uses secure passwords and access to the database would be restricted to study personnel.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

See section 7.1 above

7.3 Adverse Events and Serious Adverse Events

7.3.1 Adverse Event Definitions

For drug studies, incorporate the following definitions into the below responses, as written:	
Adverse event	Any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related
Adverse reaction	Any adverse event caused by a drug

Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction". • *Reasonable possibility.* For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
Serious adverse event or Serious suspected adverse reaction	Serious adverse event or Serious suspected adverse reaction: An adverse event or suspected adverse reaction that in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Life-threatening adverse event or life-threatening suspected adverse reaction	An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that had it occurred in a more severe form, might have caused death.
Unexpected adverse event or Unexpected suspected adverse reaction.	An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure, general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

7.4 Reporting Procedures

Adverse events are defined as unfavorable medical changes that occur during or after the study initiation, that may or may not be related to or caused by study participation. Adverse events are not procedures or surgeries (the medical condition that caused the need for the procedure or surgery is the adverse event or pre-existing conditions or illnesses that do not worsen during the study period.

All adverse event (serious or non-serious) and abnormal test findings observed or reported to study team believed to be associated with the study drug(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.

An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy. **NOTE:** Simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study
- The test finding is considered an adverse event by the investigator.

Causality and Severity Assessments

The Site Principal Investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s) or device(s); and 3) if the adverse event meets the criteria for a serious adverse event.

The intensity of the event will be evaluated and recorded as one of the following:

Mild intensity – events may or may not be volunteered by the patient. The patient is aware of the event but it is easily tolerated.
Moderate intensity – signifies discomfort sufficient to interfere with normal activities. A change in therapy may or may not be indicated.
Severe intensity – side effects are almost always brought up by the patient, definitely interfere with functioning, and require a medical intervention.

If the Site Principal Investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s) or device(s)", the adverse event will be classified as associated with the use of the study drug(s) or device(s) for reporting purposes. If the Site Principal Investigator's final determination of causality is "unknown but not related to the study drug(s) or device(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

7.5 Followup for Adverse Events

Reporting of Adverse Reactions and Unanticipated Problems to the FDA

7.5.1 Written IND/IDE Safety Reports

The Sponsor-Investigator will submit a written IND Safety Report (i.e., completed FDA Form 3500A) to the responsible new drug review division

of the FDA for any observed or volunteered adverse event that is determined to be a serious and unexpected, suspected adverse reaction. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Sponsor-Investigator will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., "Follow-up IND Safety Report").

If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor-Investigator will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

7.5.2 Telephoned IND Safety Reports – Fatal or Life-threatening Suspected Adverse Reactions

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor-Investigator will notify the responsible review division of the FDA by telephone or facsimile transmission of any unexpected, fatal or life-threatening suspected adverse reaction.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

7.5.3 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the Sponsor-Investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual at each site, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

7.6 Safety Monitoring

Quality Assurance and Quality Control

A well-defined and comprehensive data quality assurance program is essential to ensure complete, accurate, and valid data in the trial database, while limiting variability in data collection and recording. The Penn State Department of Public Health Sciences, of which the statistician is a Faculty member, has developed an effective quality assurance program for many research networks that incorporates processes for the prevention, detection, and reporting of procedural and recording errors. The statistician will collaborate with the PI in providing leadership in developing the appropriate oversight, reference materials, standard procedures, and performance reports to maintain a high level of data quality, identify areas of poor quality, and implement remedial action quickly. The statistician along with the PIs and clinic coordinator will create, update, and maintain trial a manual of procedures (MOP) detailing: the structure, roles, and responsibilities of the entities within the trial; policies and procedures for informed consent, participant confidentiality, source documentation, data access, publications and presentations, protocol violations, etc.; standardized protocol procedures where appropriate; randomization procedures; adverse event identification, recording, and reporting; data collection, entry, and quality control procedures; and instructions for completing standard data collection forms.

Data & Safety Monitoring Plan

7.6.1 Periodic evaluation of data

We will not perform an interim analysis or a futility analysis. One reason is that this is a dose ranging study with multiple treatment groups of limited sample size and thus we want to avoid any type 2 errors by prematurely shutting down recruitment before the complete sample size has been recruited. We anticipate also delayed recognition of trends in the primary outcome in this trial as we are batching serum testosterone levels which will be run at the core lab at UVA and not using them in the management of patients. Thus it may be some time before results of the primary outcome are available for data entry.

7.6.2 Data that are reviewed

The site investigator will be obligated to pursue and provide safety information as requested by the DSMB or NIH. In general, this will require a description of the

event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, including concomitant medications and illnesses, must be provided. The site investigator's assessment of causality must also be provided. If causality is unknown, it should be attributed to study participation. The certainty of the relationship of the event to study participation will be recorded as "Possible Related" or Not Possibly Related." The situation surrounding the event should be assessed to determine whether it is related to the study.

Adverse events will be categorized and % of patients experiencing adverse events and serious adverse events (SAEs) during the treatment period will be detailed. The Data and Safety Monitoring Board (DSMB) and IRB will be notified immediately of SAEs as per our safety plan. For each DSMB report, a summary of the reported adverse events will be presented in aggregate fashion, unless otherwise formally requested by the DSMB Chair.

7.6.3 Method of collection of safety information

We have adapted our adverse event collection and reporting methods from our other NIH funded clinical trials and will develop similar adverse event CRFs for tracking and tabulating adverse events during the study. At each site, adverse events will be recorded at each study visit and followed for outcome information until resolution or stabilization. Adverse events will also be entered in the REDCap database.

> The **Principal Investigator for the site,** will confirm that all adverse events (AE) are correctly entered into the AE case report forms by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; and will notify the IRB, FDA, sponsor and/or DSMB of all applicable AEs as appropriate. All assessments of AEs will be made by a licensed medical professional who is an investigator on the research.
>
> **The Research Coordinator** for the site, will complete the appropriate report form and logs; assist the PI to prepare reports and notify the IRB, FDA and/or DSMB of all Unanticipated Problems/SAE's.

7.6.4 Frequency of data collection

All data collection will begin immediately upon enrollment.

7.6.5 Individuals reviewing the data

The DSMB will receive immediate notification of all serious adverse events, and a regular report, at six month intervals, summarizing recruitment and all adverse events. The DSMB will meet via conference call after the receipt of the scheduled reports or after any major serious adverse event. The statistician will serve as the point of contact with the DSMB. The statistician will provide reports on accrual,

quality control, and patient safety to the DSMB during its meetings. The statistician and the DSMB will remain blinded during these meetings, but the DSMB Chair will have the discretion of unblinding the DSMB at any time if it is warranted for safety and/or scientific reasons. If requested, the DSMB Chair will be provided a sealed envelope containing information on treatment identity. DSMB and IRB will be notified immediately of SAEs as per our safety plan and a summary of the reported adverse events will be presented in aggregate fashion, unless otherwise formally requested by the DSMB Chair.

7.6.6 Frequency of review of cumulative data
Refer to Section 7.6.5 above

7.6.7 Statistical tests
Not applicable

7.6.8 Suspension of research
Not applicable

7.7 Potential Benefits to Subjects and Others

7.7.1 Potential Benefits to Subjects
The potential benefits to subjects may be a greater understanding of the cause of her anovulation. There may also be an improvement in her acne or hirsutism.

7.7.2 Potential Benefits to Others
This knowledge is important for establishing not only efficacious ovulation restoration therapies for women with PCOS, the most common cause of anovulatory infertility, but also important for better understanding of mechanisms leading to this disorder. These studies will also hold important implications for the treatment of women with PCOS not seeking pregnancy and may provide important information about the relative effects of this supplement on a variety of reproductive and metabolic parameters.

8. INTERVENTION DISCONTINUATION

Early Withdrawal of Subjects

8.1 Criteria for removal from study
Subjects will be removed from the study for safety reasons, failure to adhere to protocol or she withdraws consent.

8.2 Follow-up for withdrawn subjects
The statistical methods allow for withdraw/screen fails. Subjects will be asked to return the study drug to the site if withdrawn or removed from the study. No other safety follow-up planned.

The DSMB will receive immediate notification of all serious adverse events, and a regular report, at six month intervals, summarizing recruitment and all adverse events. The DSMB will meet via conference call after the receipt of the scheduled reports or after any major serious adverse event (as per the charter they may be adjudicated by email) and Dr. Chuang will send summaries of the IMC minutes to the Sponsor/PI, Dr. Legro, PENN's Site PI, Dr. Dokras and to the appointed NCCIH Program Official.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The statistical methodology we will be using for intent-to-treat analyses, which are restricted to available data only, is fairly robust to data missing at random as long as the amount of missing data is not too large. That said, for the primary outcome, subjects lost to follow-up will subsequently be conservatively assumed to have not changed their serum testosterone value since the baseline visit, i.e., last observation carried forward. Similar findings between both the intention-to-treat restricted to available data only analysis and this conservative last observation carried forward analysis approach will increase our confidence in the interpretation of the data. Additionally, if missing data appears to be a major issue we will use the observed data to determine if patients who completed the study differed from those who did not. We also may consider re-analyzing the data using missing data techniques, such as multiple imputation, if missing data is a major concern for this trial.

9.2 Sample Size and Randomization

The primary outcome of this double-blind, placebo-controlled, dose-response randomized trial will be to assess the change in serum testosterone levels from baseline to end of study (after 3 months of treatment). Sample size and power for this dose-response study combines multiple comparisons procedures and modeling procedures (i.e., MCP-Mod) as described by Pinheiro et al.[80] This methodology allows comparisons of active inositol doses to placebo (i.e., via Dunnett's test) as well as assessing the underlying dose-response model trend. As we do not have reliable data on the underlying dose-response model (i.e., shape) for inositol, we have considered the following six dose-response models for sample size estimation: linear, linear in the log, exponential, E_{max} (i.e., maximum change in effect), logistic, and quadratic. For this study, the 2 gm bid dose of inositol was considered the ED_{50} (i.e., the effective dose (ED) that provides half of the maximum change). Initial estimates of the outcomes, e.g. changes in serum testosterone, were taken from two PCOS studies by Legro et al.[4] and Banaszewska et al[81]. and these data were used to estimate inositol's maximum effect over placebo in the power analysis. For the primary outcome of the change in serum testosterone at 3 months of treatment, the maximum effect over placebo for inositol is expected to decrease by 15 ng/dL with a standard deviation of 18 ng/dL. Furthermore, we anticipate 10% subject drop-out for this study; therefore, sample size estimates have been inflated to account for this drop-out. Including the 10% subject drop-out, a sample size of 27 subjects per group (i.e., 108 total) will provide at least 80% power to detect a decrease in the primary outcome of

15 ng/dL in serum testosterone comparing inositol to placebo using MCP-Mod methods having a familywise error rate of 0.05. This sample size of 27 per group also provides sufficient power (>90%) for examining a dose-response trend over 5 of the 6 dose-response models considered (Figure 2 and Table 3). Additionally, given our sample size of 27 per group, which includes factoring in a 10% drop-out rate, we have considered power for secondary outcomes of free androgen index, fasting insulin, SHBG, and area under the curve (AUC) glucose under the six proposed dose-response models (Table 3).

Table 3: Power to detect a trend over various dose-response models, as well power for comparisons of inositol doses to placebo for various outcomes for a sample size of n=27 per group (108 total) which includes adjustment for 10% subject drop-out. The "maximum effect over placebo" was the effect used for the power calculations. The "mean change OCP" and "mean change resveratrol" columns are simply for informational purposes based on data reported by Legro et al[82] and Banaszewska et al[81], which helped guide our expected maximum effect of inositol over placebo estimates.

Outcome	Mean Change (SD) OCP	Mean Change (SD) Resveratrol	Maximum Effect over Placebo Mean Change (SD) **Inositol**	N per Group (includes 10% drop-out)	Dose-Response Model	Power to Detect a Trend (%)	Power for Comparisons to Placebo (%) (Dunnett's Test)
Testosterone (ng/dL)	-30.5 (20.9)	-12.0 (15.5)	-15.0 (18.0)	27	linear	90%	82%
					linear in log	92%	90%
					exponential	90%	81%
					E_{max}	91%	87%
					logistic	93%	80%
					quadratic	65%	54%
Free Androgen Index	-6.3 (5.4)	----	-4.5 (5.5)	27	linear	89%	81%
					linear in log	91%	89%
					exponential	89%	80%
					E_{max}	90%	85%
					logistic	92%	79%
					quadratic	63%	53%
Fasting Insulin (μU/mL)	1.2 (8.3)	-4.6 (5.8)	-5.0 (7.0)	27	linear	81%	70%
					linear in log	83%	80%
					exponential	81%	69%
					E_{max}	82%	76%
					logistic	85%	68%
					quadratic	53%	44%
SHBG (nmol/L)	52.2 (38.7)	2.1 (16.8)	10.0 (18.0)	27	linear	62%	50%
					linear in log	64%	61%
					exponential	62%	49%
					E_{max}	63%	56%
					logistic	66%	47%
					quadratic	38%	30%
AUC Glucose (mg/dL × minute)	22.9 (47.9)	---	-25.0 (40.0)	27	linear	71%	59%
					linear in log	73%	70%
					exponential	71%	58%
					E_{max}	72%	65%
					logistic	75%	56%
					quadratic	45%	36%

Treatment Assignment Procedures

Treatment randomization assignment and blinding procedures are previous described above in Section 5.1 and 5.2.

9.3 Definition of Populations

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, compliance, 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 sample size estimates have taken into consideration a participant drop-out of 10%; however, every effort will be made during the studies to minimize any drop-out.

9.4 Interim Analyses and Stopping Rules

There will be no formal stopping rules or planned interim analyses for this trial. See Section 8.0 above for more information.

9.5 Outcomes

9.5.1 Primary Outcome

The change in serum testosterone levels from baseline to end of study (after 3 months of treatment).

9.5.2 Secondary Outcomes

- The change from baseline at both months 1 and 3 for the following serum parameter levels: SHBG, free androgen index, DHEAS, androstenedione, AMH, CRP, HbgA1c, progesterone, and estradiol.
- The change from baseline to month 3 for the following serum parameters: fasting insulin, fasting glucose, area under the curve (AUC) insulin, and AUC glucose.
- The change from baseline to month 3 for the following: body composition data as measured using DXA, quality of life (as assessed using the questionnaires SF-12, PCOS questionnaire, and Female Sexual Function Index), quality of sleep (as assessed using the Epworth Sleepiness Scale and STOP-BANG), weight, systolic and diastolic blood pressure, sebum and Ferriman-Gallwey scores, ovarian volume, and antral follicle count.
- The presence of metabolic syndrome at the end of the trial.

9.6 Data Analyses

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, compliance, 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. We have stratified the randomization by site and BMI status (BMI < 30 kg/m^2 or BMI ≥ 30 kg/m^2) to account for this potential confounder and 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 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 (R Foundation for Statistical Computing, Vienna, Austria), or Stata software, version 13 (StataCorp LP, College Station, TX).

Design and analyses of dose-response studies generally consists of two techniques: (1) multiple comparisons procedures (MCP) that regards the dose as a qualitative factor and makes few assumptions regarding the underlying dose-response relationship and (2) modeling (Mod) that regards dose as quantitative and assumes some underlying dose-response relationship according to a pre-specified parametric model. For the primary outcome of the change in serum testosterone levels, we will be using MCP-Mod methodology that combines the MCP and Mod techniques.[80,83] Using the MCP-Mod approach, a set of pre-specified candidate models will be selected that cover a suitable range for the dose-response relationship. In the proposed inositol study, our candidate models will be linear, linear in the log, exponential, E_{max} (i.e., maximum change in effect), logistic, and quadratic. Each of these candidate models will be tested using appropriate contrasts and utilizing MCP techniques that preserve the familywise error rate, e.g., Dunnett's test. An overall dose-response effect will be established if at least one of the candidate model tests is significant. Once an overall dose-response effect is established, the best model among the significant candidate models will be selected to fit the data and to determine estimated doses using modeling techniques, i.e., dose-finding which helps determine an efficacious and safe dose for a future trial. The MCP-Mod methodology will be carried out for this study using the R package "Dose Finding". As a sensitivity analysis, following our assessment of the initial fit of the model we will add covariates that correspond to other potential confounders (e.g., age) to assess their impact, if any, on the treatment effects.

There are a variety of secondary analyses for this longitudinal trial and we acknowledge that the power of our study may be low for some of these outcomes. However, even if not significant, examination of these secondary endpoints will guide future research in this area. These secondary outcomes are related to reproductive and metabolic parameters and quality of life parameters as noted in Section 9.5.2. For continuous secondary outcomes, the MCP-Mod methodology will be applied as previously described. In addition to the MCP-Mod analysis for these secondary continuous outcomes, linear mixed-effects models[84], which are subject-specific models, also will be fit to assess differences among the treatment arms with respect to changes in these outcomes over time. Linear mixed-effects models are an extension of ordinary regression models that account for the between- and within-subject correlation inherent in longitudinal trials. For these mixed-effects models, a first-order antedependence covariance structure will be considered to assess the

residual error associated with the repeated measurements over time per participant. If the first-order antedependence covariance structure fails to provide model convergence then other covariance structures will be considered in the following order: unstructured, heterogeneous compound symmetry, and compound symmetry. Independent variables in the model will be treatment arm, time, the interaction of treatment and time, and the randomization stratification factors (site and BMI status) 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. 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% CIs will be used to quantify the magnitude of the effects. For binary secondary outcomes (e.g., presence of metabolic syndrome at the end of the study) logistic regression will be used with independent variables that include terms for the treatment arm and the 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.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Well-designed data collection forms (i.e. CRFs) will be developed to minimize data collection and recording errors. Separate data collection forms will be designed based on who is collecting the data and when and where the data are collected. Forms will be designed to collect cumulative information such as adverse events and the use of concomitant medications.

10.2 Data Management

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).

10.3 Quality Assurance

10.3.1 Training

Dr. Legro has been devoted to quality PCOS research for many years. Weekly research team meetings provide a platform for study updates, training and valuable information for the ongoing conduct of the trial.

10.3.2 Quality Control Committee

The project will be governed by a steering committee (SC) with Dr. Legro as chair and Dr. Butts as Vice Chair (they will be responsible for the recruitment, management, and safety of subjects in the trial along with co-investigators). The SC will consist of all the funded investigators and study coordinators and meet weekly as part of their regularly scheduled research meeting. These weekly meetings provide a platform for study updates, training and valuable information for the ongoing conduct of the trial.

10.3.3 Metrics

Our site has a proven track record of enrolling women with PCOS in our research studies. We randomized 105 subjects in the earlier study for OWL PCOS and over the same time period we randomized 243 subjects in the PPCOS II trial for a total of 392 subjects. We have expanded our study investigators to include other investigators in alternative medicine (mindfulness) in PCOS who has an extensive practice in women with PCOS seeking chronic therapy. Currently we have no competing trials in PCOS that are ongoing at our site, and we filled a smaller Vitamin D study in record time, given the interest of patients in supplements. Thus, we do not anticipate difficulty in recruiting the subjects for this study or retaining them.

10.3.4 Protocol Deviations

Protocol Deviations for each site will be reported in accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB).

10.3.5 Monitoring

The study will be monitored by the NIH / National Center for Complementary & Integrative Health (NCCIH) on-site Monitoring Team. The study will not begin until the study site initiation visit has been completed and the NCCIH has approved all documents and site readiness for study initiation. The NCCIH will provide an independent review of the regulatory and subject records and the data collected to assure compliance with the protocol, GCP, and applicable federal regulations. The monitoring will occur at regular intervals after the enrollment of the first subject. Refer to the Manual of Procedures (MOP) for additional information.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This and any subsequent modifications to the protocol and the informed consent will be reviewed and approved by The Pennsylvania State University College of Medicine Institutional Review Board serving as the single IRB responsible for oversight of the multi-site study.

11.2 Informed Consent Forms

The Informed Consent Process has been outlined above in Section 4.3. In brief, the consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or

legal guardian and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

See Supplement – HRP-598 Research Data Plan Review Form Application Supplement as required by The Pennsylvania State University College of Medicine Institutional Review Board.

11.4 Study Discontinuation

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

12. COMMITTEES

Steering Committee: Dr. Legro as chair and Dr. Butts as Vice Chair (they will be responsible for the recruitment, management, and safety of subjects in the trial along with co-investigators). The SC will consist of all the funded investigators and study coordinator and meet weekly as part of their regularly scheduled research meeting. These weekly meetings provide a platform for study updates, training and valuable information for the ongoing conduct of the trial.

13. PUBLICATION OF RESEARCH FINDINGS

Publication of any results of this trial will be governed by the policies and procedures developed by the Steering Committee. Any presentation, abstract, or manuscript will be made available for review by the sponsor and the NCCIH prior to submission.

14. REFERENCES

1. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. The Journal of clinical endocrinology and metabolism 2004;89:2745-9.
2. Hull MG. Epidemiology of infertility and polycystic ovarian disease: endocrinological and demographic studies. Gynecological Endocrinology 1987;1:235-45.
3. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome. Endocrine reviews 2015;36:487-525.
4. Legro RS, Dodson WC, Kris-Etherton PM, et al. Randomized Controlled Trial of Preconception Interventions in Infertile Women With Polycystic Ovary Syndrome. The Journal of clinical endocrinology and metabolism 2015;100:4048-58.
5. Legro RS, Brzyski RG, Diamond MP, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. The New England journal of medicine 2014;371:119-29.
6. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. The New England journal of medicine 2016;375:523-33.
7. Legro RS, Barnhart HX, Schlaff WD, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. The New England journal of medicine 2007;356:551-66.
8. Ladson G, Dodson WC, Sweet SD, et al. The effects of metformin with lifestyle therapy in polycystic ovary syndrome: a randomized double-blind study. Fertility and sterility 2011;95:1059-66 e1-7.

9. Azziz R, Ehrmann D, Legro RS, et al. Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. The Journal of clinical endocrinology and metabolism 2001;86:1626-32.
10. Essah PA, Arrowood JA, Cheang KI, Adawadkar SS, Stovall DW, Nestler JE. Effect of combined metformin and oral contraceptive therapy on metabolic factors and endothelial function in overweight and obese women with polycystic ovary syndrome. Fertility and sterility 2011;96:501-4 e2.
11. Legro RS, Arslanian SA, Ehrmann DA, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism 2013;98:4565-92.
12. Facchinetti F, Bizzarri M, Benvenga S, et al. Results from the International Consensus Conference on Myo-inositol and d-chiro-inositol in Obstetrics and Gynecology: the link between metabolic syndrome and PCOS. European journal of obstetrics, gynecology, and reproductive biology 2015;195:72-6.
13. Harbin Consensus Conference Workshop G, Conference C, Legro RS, et al. Improving the reporting of clinical trials of infertility treatments (IMPRINT): modifying the CONSORT statementdaggerdouble dagger. Hum Reprod 2014;29:2075-82.
14. Guzick DS, Carson SA, Coutifaris C, et al. Efficacy of superovulation and intrauterine insemination in the treatment of infertility. National cooperative reproductive medicine network New England Journal of Medicine 1999 340:177-83.
15. Diamond MP, Legro RS, Coutifaris C, et al. Letrozole, Gonadotropin, or Clomiphene for Unexplained Infertility. The New England journal of medicine 2015;373:1230-40.
16. Katcher HI, Legro RS, Kunselman AR, et al. The effects of a whole grain-enriched hypocaloric diet on cardiovascular disease risk factors in men and women with metabolic syndrome. The American journal of clinical nutrition 2008;87:79-90.
17. Wadden TA, Volger S, Sarwer DB, et al. A two-year randomized trial of obesity treatment in primary care practice. The New England journal of medicine 2011;365:1969-79.
18. Wadden TA, Berkowitz RI, Womble LG, et al. Randomized trial of lifestyle modification and pharmacotherapy for obesity. The New England journal of medicine 2005;353:2111-20.
19. Shi Y, Wei D, Liang X, et al. Live birth after fresh embryo transfer vs elective embryo cryopreservation/frozen embryo transfer in women with polycystic ovary syndrome undergoing IVF (FreFro-PCOS): study protocol for a multicenter, prospective, randomized controlled clinical trial. Trials 2014;15:154.
20. Kuang H, Li Y, Wu X, et al. Acupuncture and clomiphene citrate for live birth in polycystic ovary syndrome: study design of a randomized controlled trial. Evidence-based complementary and alternative medicine : eCAM 2013;2013:527303.
21. Vrbikova J, Cibula D. Combined oral contraceptives in the treatment of polycystic ovary syndrome. Human reproduction update 2005;11:277-91.
22. Korytkowski MT, Mokan M, Horwitz MJ, Berga SL. Metabolic effects of oral contraceptives in women with polycystic ovary syndrome. The Journal of clinical endocrinology and metabolism 1995;80:3327-34.
23. Diamanti-Kandarakis E, Baillargeon JP, Iuorno MJ, Jakubowicz DJ, Nestler JE. A modern medical quandary: polycystic ovary syndrome, insulin resistance, and oral contraceptive pills. The Journal of clinical endocrinology and metabolism 2003;88:1927-32.
24. Legro RS, Chen G, Kunselman AR, et al. Smoking in infertile women with polycystic ovary syndrome: baseline validation of self-report and effects on phenotype. Hum Reprod 2014;29:2680-6.
25. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. New England Journal of Medicine 2002;346:393-403.

26. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev 2012;5:CD003053.
27. Barnes PM, Bloom B, Nahin RL. Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 2008:1-23.
28. Bailey RL, Gahche JJ, Lentino CV, et al. Dietary supplement use in the United States, 2003-2006. J Nutr 2011;141:261-6.
29. Nahin RL, Barnes PM, Stussman BJ, Bloom B. Costs of complementary and alternative medicine (CAM) and frequency of visits to CAM practitioners: United States, 2007. Natl Health Stat Report 2009:1-14.
30. Vitek W, Alur S, Hoeger KM. Off-label drug use in the treatment of polycystic ovary syndrome. Fertility and sterility 2015;103:605-11.
31. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of d-chiro-inositol in the polycystic ovary syndrome. . New England Journal of Medicine 1999 340:1314-20.
32. Thomas RM, Nechamen CA, Mazurkiewicz JE, Ulloa-Aguirre A, Dias JA. The adapter protein APPL1 links FSH receptor to inositol 1,4,5-trisphosphate production and is implicated in intracellular Ca(2+) mobilization. Endocrinology 2011;152:1691-701.
33. Nestler JE, Gunn R, Bates S, Gregory J, Jacobson W, Rogol AD. D-chiro-inositol (INS-1) enhances ovulatory rate in hyperandrogenemic,
oligomenorrheic women with the polycystic ovary syndrome. Fertility and sterility 2001;76:S110-S1.
34. Gerli S, Mignosa M, Di Renzo GC. Effects of inositol on ovarian function and metabolic factors in women with PCOS: a randomized double blind placebo-controlled trial. Eur Rev Med Pharmacol Sci 2003;7:151-9.
35. Unfer V, Nestler JE, Kamenov ZA, Prapas N, Facchinetti F. Effects of Inositol(s) in Women with PCOS: A Systematic Review of Randomized Controlled Trials. International journal of endocrinology 2016;2016:1849162.
36. Heimark D, McAllister J, Larner J. Decreased myo-inositol to chiro-inositol (M/C) ratios and increased M/C epimerase activity in PCOS theca cells demonstrate increased insulin sensitivity compared to controls. Endocrine journal 2014;61:111-7.
37. Costantino D, Minozzi G, Minozzi E, Guaraldi C. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. Eur Rev Med Pharmacol Sci 2009;13:105-10.
38. Genazzani AD, Lanzoni C, Ricchieri F, Jasonni VM. Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2008;24:139-44.
39. Papaleo E, Unfer V, Baillargeon JP, et al. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2007;23:700-3.
40. Unfer V, Carlomagno G, Dante G, Facchinetti F. Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2012;28:509-15.
41. Unfer V, Carlomagno G, Rizzo P, Raffone E, Roseff S. Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. Eur Rev Med Pharmacol Sci 2011;15:452-7.
42. Benelli E, Del Ghianda S, Di Cosmo C, Tonacchera M. A Combined Therapy with Myo-Inositol and D-Chiro-Inositol Improves Endocrine Parameters and Insulin Resistance in PCOS Young Overweight Women. International journal of endocrinology 2016;2016:3204083.

43. Minozzi M, Nordio M, Pajalich R. The Combined therapy myo-inositol plus D-Chiro-inositol, in a physiological ratio, reduces the cardiovascular risk by improving the lipid profile in PCOS patients. Eur Rev Med Pharmacol Sci 2013;17:537-40.
44. Nordio M, Proietti E. The combined therapy with myo-inositol and D-chiro-inositol reduces the risk of metabolic disease in PCOS overweight patients compared to myo-inositol supplementation alone. Eur Rev Med Pharmacol Sci 2012;16:575-81.
45. Nestler JE, Powers LP, Matt DW, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. The Journal of clinical endocrinology and metabolism 1991;72:83-9.
46. Rausch ME, Legro RS, Barnhart HX, et al. Predictors of Pregnancy in Women with Polycystic Ovary Syndrome. The Journal of clinical endocrinology and metabolism 2009.
47. Kuang H, Jin S, Hansen KR, et al. Identification and replication of prediction models for ovulation, pregnancy and live birth in infertile women with polycystic ovary syndrome. Hum Reprod 2015;30:2222-33.
48. Chauhan SP, Blackwell SC, Saade GR, Society for Maternal-Fetal Medicine Health Policy C. A suggested approach for implementing CONSORT guidelines specific to obstetric research. Obstetrics and gynecology 2013;122:952-6.
49. Tredway D, Schertz JC, Bock D, Hemsey G, Diamond MP. Anastrozole single-dose protocol in women with oligo- or anovulatory infertility: results of a randomized phase II dose-response study. Fertility and sterility 2011;95:1725-9 e1-8.
50. Tredway D, Schertz JC, Bock D, Hemsey G, Diamond MP. Anastrozole vs. clomiphene citrate in infertile women with ovulatory dysfunction: a phase II, randomized, dose-finding study. Fertility and sterility 2011;95:1720-4 e1-8.
51. Azziz R, Carmina E, Dewailly D, et al. Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. The Journal of clinical endocrinology and metabolism 2006;91:4237-45.
52. Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. [Review] [247 refs]. Cancer Epidemiology, Biomarkers & Prevention 2002 Dec;11:1531-43.
53. Barry JA, Azizia MM, Hardiman PJ. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction update 2014;20:748-58.
54. Greene ND, Leung KY, Gay V, et al. Inositol for the prevention of neural tube defects: a pilot randomised controlled trial. The British journal of nutrition 2016;115:974-83.
55. Rago R, Marcucci I, Leto G, et al. Effect of myo-inositol and alpha-lipoic acid on oocyte quality in polycystic ovary syndrome non-obese women undergoing in vitro fertilization: a pilot study. J Biol Regul Homeost Agents 2015;29:913-23.
56. Celentano C, Matarrelli B, Mattei PA, Pavone G, Vitacolonna E, Liberati M. Myo-Inositol Supplementation to Prevent Gestational Diabetes Mellitus. Curr Diab Rep 2016;16:30.
57. D'Anna R, Di Benedetto A, Scilipoti A, et al. Myo-inositol Supplementation for Prevention of Gestational Diabetes in Obese Pregnant Women: A Randomized Controlled Trial. Obstetrics and gynecology 2015;126:310-5.
58. Crawford TJ, Crowther CA, Alsweiler J, Brown J. Antenatal dietary supplementation with myo-inositol in women during pregnancy for preventing gestational diabetes. Cochrane Database Syst Rev 2015;12:CD011507.
59. Howlett A, Ohlsson A, Plakkal N. Inositol in preterm infants at risk for or having respiratory distress syndrome. Cochrane Database Syst Rev 2015;2:CD000366.
60. Wu X, Stener-Victorin E, Wu T, et al. ACUPUNCTURE AND CLOMIPHENE FOR INFERTILITY IN THE POLYCYSTIC OVARY SYNDROME: A MULTICENTRE RONDOMIZED CONTROLLED TRIAL. Fertility and sterility 2016;106:e375.

61. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertility and sterility 2004;81:19-25.
62. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. American journal of obstetrics and gynecology 1981;140:815-30.
63. Legro RS, Driscoll D, Strauss JFr, Fox J, Dunaif A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. . Proceedings of the National Academy of Sciences of the United States of America 1998;95:14956-60.
64. Legro RS, Schlaff WD, Diamond MP, et al. Total Testosterone Assays in Women with Polycystic Ovary Syndrome: Precision and Correlation with Hirsutism. The Journal of clinical endocrinology and metabolism 2010;95:5305-13.
65. Azziz R, Zacur HA. 21-Hydroxylase deficiency in female hyperandrogenism: screening and diagnosis. The Journal of clinical endocrinology and metabolism 1989;69:577-84.
66. Practice Committee of the American Society for Reproductive M. Subclinical hypothyroidism in the infertile female population: a guideline. Fertility and sterility 2015;104:545-53.
67. Legro RS, Kunselman AR, Brzyski RG, et al. The Pregnancy in Polycystic Ovary Syndrome II (PPCOS II) trial: rationale and design of a double-blind randomized trial of clomiphene citrate and letrozole for the treatment of infertility in women with polycystic ovary syndrome. Contemporary clinical trials 2012;33:470-81.
68. Foxman B, Cronenwett AE, Spino C, Berger MB, Morgan DM. Cranberry juice capsules and urinary tract infection after surgery: results of a randomized trial. American journal of obstetrics and gynecology 2015;213:194 e1-8.
69. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. . American Journal of Obstetrics & Gynecology 1981 Aug 1;140:815-30.
70. Legro RS, Brzyski RG, Diamond MP, et al. The Pregnancy in Polycystic Ovary Syndrome II study: baseline characteristics and effects of obesity from a multicenter randomized clinical trial. Fertility and sterility 2014;101:258-69 e8.
71. Legro RS, Pauli JG, Kunselman AR, et al. Effects of continuous versus cyclical oral contraception: a randomized controlled trial. The Journal of clinical endocrinology and metabolism 2008;93:420-9.
72. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Medical care 1996;34:220-33.
73. Rosen R, Brown C, Heiman J, et al. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. Journal of sex & marital therapy 2000;26:191-208.
74. Cronin L, Guyatt G, Griffith L, et al. Development of a health-related quality-of-life questionnaire (pcosq) for women with polycystic ovary syndrome (pcos). . Journal of Clinical Endocrinology & Metabolism 1998;83:1976-87.
75. Chung F, Yang Y, Liao P. Predictive performance of the STOP-Bang score for identifying obstructive sleep apnea in obese patients. Obesity surgery 2013;23:2050-7.
76. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-5.
77. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. Obesity (Silver Spring) 2012;20:1109-14.
78. Carey DG, Jenkins AB, Campbell LV, Freund J, Chisholm DJ. Abdominal fat and insulin resistance in normal and overweight women: Direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. Diabetes 1996;45:633-8.
79. Legro RS, Dodson WC, Gnatuk CL, et al. Effects of gastric bypass surgery on female reproductive function. The Journal of clinical endocrinology and metabolism 2012;97:4540-8.

80. Pinheiro J, Bornkamp B, Bretz F. Design and analysis of dose-finding studies combining multiple comparisons and modeling procedures. J Biopharm Stat 2006;16:639-56.
81. Banaszewska B, Wrotynska-Barczynska J, Spaczynski RZ, Pawelczyk L, Duleba AJ. Effects of Resveratrol on Polycystic Ovary Syndrome: A Double-blind, Randomized, Placebo-controlled Trial. The Journal of clinical endocrinology and metabolism 2016;101:4322-8.
82. Dokras A, Playford M, Kris-Etherton PM, et al. Impact of hormonal contraception and weight loss on high-density lipoprotein cholesterol efflux and lipoprotein particles in women with polycystic ovary syndrome. Clinical endocrinology 2017;86:739-46.
83. Bretz F, Pinheiro JC, Branson M. Combining multiple comparisons and modeling techniques in dose-response studies. Biometrics 2005;61:738-48.
84. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982;38:963-74.
85. Koch GG AS, Stokes ME. Poisson regression. In: NL KSaJ, ed. Encyclopedia of Statistical Sciences. 7 ed. New York: : John Wiley & Sons, Inc.; 1986:32-41.
86. Leppink EW, Redden SA, Grant JE. A double-blind, placebo-controlled study of inositol in trichotillomania. Int Clin Psychopharmacol 2017;32:107-14.
87. Palatnik A, Frolov K, Fux M, Benhamin J. Double-blind, controlled, crossover trial of inositol versus fluvoxamine for the treatment of panic disorder. J of Clin Psychopharmacol 2001;21:335-88. Fux M, Benjamin J, Belmaker RH. Inositol versus placebo augmentation of serotonin reuptake inhibitors in the treatment of obsessive-compulsive disorder: a double-blind cross-over study. Int J Neuropsychopharmacol 1999;2:193-5.

15. SUPPLEMENTS/APPENDICES

Version Date: 25Jul2023

CONSENT FOR RESEARCH
Penn State College of Medicine
The Milton S. Hershey Medical Center

Title of Project: Inositol Supplementation to Treat Polycystic Ovary Syndrome: A Double Blind Dose Ranging RCT (INSUPP-PCOS)

Principal Investigator: Richard S. Legro, MD

Address: Penn State Hershey Medical Center
Department of Obstetrics and Gynecology
500 University Drive, H103
Hershey PA, 17022

Telephone Numbers: Weekdays: 8:00 a.m. to 5:00 p.m. (717) 531-8478.
After hours call (717) 531-8521. Ask for the Obstetrics and Gynecology doctor on 24-hour call.

Subject's Printed Name: _____

We are asking you to be in a research study.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you.

This form gives you information about the research. Please ask questions about anything that is unclear to you and take your time to make your choice.

1. Why is this research study being done?

We are asking you to be in this research because you have Polycystic Ovary Syndrome (PCOS). PCOS is a hormonal condition in which your reproduction hormones are out of balance. Hormones are substances your body makes to help different processes happen. Some are related to your ability to have a baby, and also affect your menstrual cycle. Those hormones involved in PCOS include:

- **Androgens:** Often called "male" hormones, women have them, too. Those with PCOS tend to have higher levels, which can cause symptoms like hair loss, hair in places you don't want it (such as on your face), and trouble getting pregnant.
- **Insulin:** This hormone manages your blood sugar. If you have PCOS, your body might not react to insulin the way that it should.
- **Progesterone:** With PCOS, your body may not have enough of this hormone. That can make you to miss your periods for a long time, or to have periods that are hard to predict.

There is no cure for PCOS, but it can be managed and treated based on symptoms which can include hormonal birth control, medicines to block the effect of androgens and/or medication to help improve insulin's ability to lower blood sugar. Studies have shown that a nutrient call inositol may improve the way the body uses insulin and promote hormone balance. Inositol is commonly found in foods such as fruits, beans, grains and nuts. Inositol has not been approved by the Food and Drug Administration and is considered experimental in this study.

This research is being done to find out how a nutritional supplement called Inositol affects these reproduction hormones. Approximately 108 women with PCOS will take part in this multi-site research study.

2. What will happen in this research study?

SCREENING VISIT and procedures

We will schedule a screening visit at the Clinical Research Center (CRC) at Hershey Medical Center (HMC) to assess eligibility. You may need to stop taking certain medications (wash-out) to participate in the study which may include oral contraceptive pills and oral diabetes medications. Likewise, some medications will be prohibited during the study. Please discuss any medications you are taking with the study team and doctor; they will inform you of any medications that you need to stop.

You will present fasting meaning nothing to eat or drink (except for water) for at least 10 hours prior to your visit so we can send safety labs like complete metabolic profile with electrolytes and glucose, kidney and liver function, a CBC, total cholesterol triglycerides. If needed, we may also collect eligibility labs like testosterone, thyroid hormones and other reproductive hormones. The total amount of blood collected will be no more than 4 tablespoons.

We will also obtain baseline medical history, perform a full exam and will collect a pap smear, if needed and collect your vital signs (blood pressure, weight and waist/hip measurements). A pap smear will be collected if you are 21 or older and have not had one within the time period specified by current guidelines. A transvaginal ultrasound will also be performed at this visit to look at your uterus, ovaries and tubes.

If you are sexually active and have the ability to get pregnant (e.g. you or your partner have not been surgically sterile), you will be asked to use barrier contraception during the 3 months participation. Accepted methods of barrier contraception may include condoms, diaphragm or cervical cap.

If you do become pregnant while on study medication, you stop taking study medication immediately and be removed from the study. We will ask to follow up with you until the end of the pregnancy. This follow-up would include checking pregnancy levels in your blood, doing transvaginal ultrasound(s) and following the outcomes of both you and your baby up to one month after delivery.

RANDOMIZATION VISIT

This Randomization visit must occur within 3 months (90 days) of obtaining your informed consent and after eligibility has been assessed from the screening procedures completed above

You may become eligible after seeking treatment for a medical condition and having been re-evaluated, and the Principal Investigator approves your continued study participation. This is a double-blind study, meaning that you and your study doctor and team will not know which study treatment you are receiving, but we will be able to get this information quickly if it is needed to ensure your safety. You will be randomly assigned to 1 of 4 treatment groups. This means that

whichever study treatment you receive will be determined purely by chance, like flipping a coin. You will have a 1 out of 4 change of receive any one of the following study treatments:

Arm A: Placebo Treatment Twice a day: In the placebo group, women will receive the daily placebo (inactive ingredients - maltodextrin and inulin) in an identical fashion as the other study groups and will be monitored the same.

Arm B: Active Treatment with Inositol 1 gm/ Twice a day: In this group, women will receive daily inositol two times a day (each dose with 1000 mg of myo-inositol and 25 mg of d-chiro-inositol) at the same time each day.

Arm C: Active Treatment with Inositol 2 gm/ Twice a day: In this group, women will receive daily inositol two times a day (each dose with 2000 mg of myo-inositol and 50 mg of d-chiro-inositol).

Arm D: Active Treatment with Inositol 3 gm/ Twice a day: In this group, women will receive daily inositol two times a day (each dose with 3000 mg of myo-inositol and 75 mg of d-chiro-inositol).

You will take your study medication, one packet two times every day dissolved in 8 – 10 ounces of water at the same time each day. Both the active supplement and placebo packets will each contain 2.6 grams of carbohydrates, which will equal roughly 10 calories per packet. Packet counts will be monitored at each study visit.

A transvaginal ultrasound will also be performed at this visit to look at your uterus, ovaries and tubes, only if you have not received one at the screening visit.

You will present after an overnight fast for at least 10 hours. Vital signs including blood pressure, waist/ hip measures and hirsutism (looking at your hair) will be done. Urine pregnancy tests will be dispensed to you to check on a monthly basis if you think you may be pregnant or if you experience unexpected bleeding not associated with your period.

We will perform an acne assessment and measure facial sebum (oil) via the sebumeter. The sebumeter is a small, hand-held probe lightly placed on the middle of your forehead that measures the amount of oil. We will check different levels like testosterone, progesterone, hormones and glycohemoglobin (HgbA1c) (test for diabetes).

You will complete a 2 hour oral glucose tolerance test (OGTT) consisting of timed fasting and glucose challenge (75 g oral glucose load). This test measures the body's ability to use glucose (your body's main source of energy) and your blood will be sampled at baseline and every 30 minutes for 2 hours. For this testing, you may have a catheter (tube) inserted into your arm vein to avoid multiple blood draws. The total amount of blood collected for all of the tests at this visit will be no more than 8 tablespoons.

A daily diary will be given to you to record the days and severity of your period, study drug intake and any side effects or additional medications you take. If warranted, we will offer standard weight loss therapy by referral to a Dietitian and recommendations to restrict calories and increase physical activity.

We will ask you to complete several questionnaires at this visit more than likely to be completed during the 2 hour OGTT. These questionnaires ask about your health in general, your quality of life having PCOS, your sexual function (like arousal, orgasm, satisfaction and pain) and about your daytime sleepiness and sleep habits. You are free to skip any questions that you prefer not to answer.

You will have a DXA scan to look at your bone mineral density and body composition.

VISIT #1 (MONTH 1 +/- 7 Days)

At this visit we will update the medical history and medications, ask you if you have had any issues or problems since the last visit (adverse events), and obtain weight, blood pressure, waist/hip measures, hirsutism scores and sebum measures. We will repeat the reproductive/metabolic panel that was done at the randomization visit. The total amount of blood collected will be no more than 4 tablespoons. You will not have to fast for this visit. Study drug bags (including empty, partially used, and unopened bags) and unused/ full study drug packets will be collected to assess compliance. New study medication for two months will be given to you at this visit. A urine pregnancy test will be performed at this visit and we will also give you more urine pregnancy tests to test monthly at home. We will ask you to complete a questionnaire about your caffeine intake at this visit. You will also be given a new daily diary to continue recording the days and severity of your period, study drug intake and any side effects or additional medications you take.

END OF TREATMENT / FINAL VISIT (MONTH 3 +/- 7 Days)

At this visit we will repeat a lot of the procedures performed at the Randomization visit.
You will present after an overnight fast for at least 10 hours. Vital signs including blood pressure, waist/ hip measures, hirsutism (looking at your hair), an acne assessment and measure facial sebum (oil) using the sebumeter will be done.

We will check different levels like testosterone, progesterone, hormones and glycohemoglobin (HgbA1c) (test for diabetes). The total amount of blood collected will be no more than 4 tablespoons. Urine pregnancy tests will also be performed.

We will repeat a 2 hour oral glucose tolerance test (OGTT) to measure the body's ability to use glucose. Your blood will be sampled at baseline and every 30 minutes for 2 hours. For this testing, you may have a catheter (tube) inserted into your arm vein to avoid multiple blood draws.

We will ask you to complete several questionnaires at this visit more than likely to be completed during the 2 hour OGTT. These questionnaires ask about your health in general, your quality of life having PCOS, your sexual function (like arousal, orgasm, satisfaction and pain), your caffeine intake, and about your daytime sleepiness and sleep habits. You are free to skip any questions that you prefer not to answer.

You will have a DXA scan to look at your bone mineral density and body composition.

Additionally, a transvaginal ultrasound scan will be repeated.

Final packets of unused/full study medication will be collected as well as the daily diaries and packet counts performed to assess compliance.

Parameters	Screening	Randomization (within 3 months/90 days of obtaining informed consent)	Month 1(+/- 7 Days) RCT**	Month 3 (+/- 7 Days) RCT**
Obtain Informed Consent	**X**			
Collect Demographics Information	**X**			
Medical History and Meds	X	X	X	X
Physical Exam (PAP smear if indicated)	X			
Concomitant Medications and Adverse Event Review	X	X	X	X
Blood Pressure, Weight, Waist/Hip Ratio	X	X	X	X
Perform Hirsutism Assessment		X	X	X
Distribute and Collect Daily Diary (if applicable)		X	X	X
Dispense/Collect Study Drug, check compliance (if applicable)		X	X	X
Inclusion/Exclusion Labs (if not previously done): Testosterone, TSH, PRL, 17OHP,etc. (Local)	X			
Safety Labs (fasting): CBC, CMP, Lipid profile (Hershey Medical Center)	X			X
Urine Pregnancy test (monthly home and study visits)	X	X	X	X
Primary Outcome: Serum Testosterone (UVA Core)		X	X	X
Other Outcome labs: SHBG, DHEAS, Androstenedione, AMH, CRP, HgbA1c, Progesterone, Estradiol (UVA Core)		X	X	X
Measure Sebum with Sebumeter		X	X	X
2 hour 75 g OGTT (5 timepoints Glucose/Insulin at UVA Core)		X		X
Transvaginal Ultrasound U/S (*)	X*	X*		X
DXA Scan for Body Composition		X		X
Questionnaires, including QOL		X		X
Caffeine Intake Questionnaire			X	X

(*) – indicates this procedure may be done at Screening or Randomization

- *(**)If applicable due to unforeseen or extenuating circumstances outside of our control ie: pandemic, emergency or unexpected issue arises in which you cannot make it to the research center for a study visit within the protocol-defined window, some of these visits may be conducted remotely via tele-health, HIPAA compliant conferencing or phone, etc. The determination to conduct the visit remotely will made on a case-by-case basis by the study Principal Investigator. The study team will make arrangements with you to complete as many of the study specific visit parameters remotely if this situation applies to ensure your safety and will not affect the integrity of the study data and outcomes. Any changes to study procedures that are made during these circumstances will still be reported to the IRB, per our institutional standards.*

What are my responsibilities if I take part in this research?

If you take part in this research, your major responsibilities will include:

- Complete diaries and bring them back to every visit
- Take study medication and bring back all study drug bags(including empty, partially used, and unopened bags) and unused/ full study drug packets. Packet counts are performed to assess compliance.
- Take the proper precautions against pregnancy
- Let the study team know of any changes in your health, new medications or any other issues while participating

3. What are the risks and possible discomforts from being in this research study?

Risk of Inositol and Placebo

There are no known serious adverse effects, pattern of adverse effect related to the drug or interactions with other drugs. Similarly there is no pattern of adverse effects to maltodextrin and inulin mixture in the placebo. Both the active supplement and placebo packets will each contain 2.6 grams of carbohydrates, which will equal roughly 10 calories per packet. This small amount of carbohydrate will have minimal if any impact on any of the study outcomes, such as glucose tolerance, dyslipidemia (unhealthy levels of fat in the blood – either too high or too low), or prevalence of the metabolic syndrome. Metabolic syndrome is a cluster of conditions — increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels — that occur together, increasing your risk of heart disease, stroke and diabetes.

There have been other studies where inositol was used in much higher doses (up to 12 – 16 grams/day). The side effects seen in these studies where mild and included:

- Gastrointestinal (nausea, upset stomach, diarrhea and gas)
- Stomach pains
- Headache
- Tiredness

One serious side effect was reported of an ectopic pregnancy (pregnancy outside of the uterus) which was felt to be unrelated to inositol and the drug was not discontinued. Overall, inositol at much lower doses like in this study is felt to be safe and generally well-tolerated.

If you are in the group that receives placebo, your condition will go without active treatment for 3 months and your symptoms may get worse. The research treatment may involve risks to you that are currently unforeseeable.

Radiation for DXA scan

If you take part in this research, you will have one or more medical imaging studies or treatments. The tests or treatments you will have include a total body bone mineral measurement. These tests involve a small amount of radiation. The radiation exposure from this research is approximately 8 microsievert. To give you an idea about how much radiation you will get, we will make a comparison with an every-day situation. Everyone receives a small amount of unavoidable radiation each year. Some of this radiation comes from space and some from naturally-occurring radioactive forms of water and minerals. This research gives your body the equivalent of about 1 extra days worth of this natural radiation. The radiation dose we have discussed is what you will receive from this study only, and does not include any exposure you may have received or will receive from other tests.

Oral Glucose Test
Possible side effects from this test include anemia (lack of red blood cells), infection at the site of the IV if used for blood draws, nausea, vomiting, allergic reaction, hypoglycemia (low blood sugar), as well as the standard blood draw discomforts and risks.

Transvaginal Ultrasound
Possible side effects include abdominal or pelvic discomfort.

Venipuncture
The discomfort associated with removing blood by venipuncture (by needle from a vein) is a slight pinch or pin prick when the sterile needle enters the skin. The risks include mild discomfort and/or a black and blue mark at the site of puncture. Less common risks include a small blood clot, infection or bleeding at the puncture site, and on rare occasions fainting during the procedure.

Risk of Randomization
You will be assigned to a treatment program by chance. The treatment you receive may prove to be less effective or to have more side effects than the other research treatment(s) or other available treatments.

Risk of loss of confidentiality
There is a risk of loss of confidentiality if your information or your identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening. The confidentiality of your electronic data created by you or by the researchers will be maintained to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

Questionnaires
You may get tired or bored when we are asking you questions or you are completing questionnaires. You do not have to answer any question you do not want to answer.

Reproductive Risks
The risks to an unborn baby or a nursing child from the study drug are not known at this time. If you are pregnant or become pregnant, the study drug may cause problems to your unborn baby. Women who are pregnant or are nursing a child may not participate in this research study. If you are a female capable of becoming pregnant, a pregnancy test will be required before you begin the research. You must agree to take reasonable and necessary precautions against becoming pregnant during the period of the investigation. The investigator will discuss appropriate precautions with you. If at any point during the research you believe there is any possibility that you may be pregnant, you must notify the investigator immediately.

Unforeseen Risks
It is possible that the study procedures could detect a possible unknown medical problem that is unrelated to the purpose of this study. If the research procedures uncover findings that may be important for you to know about, such as the possibility of a previously unknown medical condition, if this happens, a member of the study team may contact you to find out if you would like to learn more. These findings may require additional testing or treatment. The cost of any additional tests or related treatment will be your responsibility.

The Data and Safety Monitoring Board (DMSB), an independent group of experts, will be reviewing the information from this research throughout the study. If any important new information about the study

develops that may affect your health, welfare, or willingness to stay on the study, your study doctor will tell you. You may be asked to sign another consent form at that time.

4. What are the possible benefits from being in this research study?

4a. What are the possible benefits to me?

The potential benefit to you is that the treatment you receive may prove to be more effective than the other study treatment or than other available treatments, although this cannot be guaranteed.

4b. What are the possible benefits to others?

The results of this research may guide future treatment of PCOS.

5. What other options are available instead of being in this research study?

You do not have to take part in this study to be treated for your condition. Instead of participating in this research, you could:

- Receive commercially available treatments to treat PCOS and its symptoms, including hormonal therapy, anti-androgens (medicines that block the effect of androgens - "male hormones") or metformin (often used to treat type 2 diabetes but may have other positive effects for some women with PCOS)
- Be part of a different research study, if one is available.
- Choose not to be treated for your medical condition.

Before you decide if you want to be in this research, we will discuss the other choices that are available to you. We will tell you about the possible benefits and risks of these choices.

Because it is investigational, the therapy offered in this research is only available to you if you take part in the research study.

6. How long will I take part in this research study?

If you agree to take part, it will take you about 4 months to complete this research study. You will be asked to return to the research site 4 times. Each visit will last approximately 1.5 hours but no more than 3 hours depending on what procedures are completed at each time point. For example, those visits that include a 2-hour OGTT will take up to 3 hours.

7. How will you protect my privacy and confidentiality if I decide to take part in this research study?

7a. What happens to the information collected for the research?

Efforts will be made to limit the use and sharing of your personal research information. If the principal investigator moves to another institution, your data and/or samples may be transferred to the new institution. In our research files at The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) we will include these identifiers: your name, address, email address, phone numbers, date of birth, medical record number and a code number.

- A list that matches your name with your code number will be kept in a locked file in Dr. Legro's research office.
- Your research records will be labeled with your code number, your initials and date of birth and will be kept in a safe area in Dr. Legro's research office.
- Your research samples will be labeled with a code number, date of collection and will be stored in freezers in Dr. Legro's research lab.

- A copy of this signed consent form will be included in your HMC medical record. This means that other HMC healthcare providers will know you are in this study.
- Results of some of the research-related tests (including but not limited to blood tests, transvaginal ultrasound) will be kept in your HMC medical record.

For research records and specimens sent to the Core Ligand Lab at the University of Virginia, you will be identified by your code number and date of collection.

To help protect your privacy, a Certificate of Confidentiality will be obtained from the National Institutes of Health. This Certificate means that the researchers cannot be forced (for example by court subpoena) to share information that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below. The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally-funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). A Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

7b. How will my identifiable health information be used?
If you give your consent, health information that can be traced to you will be collected for this research study. In general, under federal law, health information is private. However, there are exceptions to this rule, and you should know who may be able to see, use, and share your health information for research and why they may need to do so. We will use and disclose your information only as described in this form and in the HMC Privacy Notice.

The research team may use the following health information:

- Past, present, and future medical records
- New health information from tests, procedures, visits, interviews, or forms filled out as part of this research study.

The following people/groups may see, use, and share your identifiable health information:

- HMC/PSU research staff involved in this study
- The HMC/PSU Institutional Review Board (IRB), a group of people who review the research study to protect subjects' rights and welfare
- The HMC/PSU Human Subjects Protection Office
- The HMC/PSU Research Quality Assurance Office
- Non-research staff within HMC/PSU who need this information to do their jobs (such as for treatment, payment (billing), or health care operations)
- Federal and state agencies (such as the U.S. Food and Drug Administration, the Office for Human Research Protections, the Department of Health and Human Services, the National

Institutes of Health, and other U.S. or foreign government bodies that oversee or review research)
- NIH and Westat groups which are part of the NIH
- The HMC/PSU pharmacy
- A group that oversees the data (study information) and safety of this research
- People or groups that we hire to do work for us, such as data storage companies, insurers, and lawyers
- Public health and safety authorities (for example, if we learn information that could mean harm to you or others, we may need to report this, as required by law)
- Organizations that provide independent accreditation and oversight of hospitals and research
- The Core Ligand Lab at the University of Virginia

These groups may also review and/or copy your original PSU/HMC records while looking at the results of the research study. It is possible that some of the other people/groups who receive your health information may not be required by Federal privacy laws to protect your information. We share your information only when we must, and we ask anyone who receives it from us to protect your privacy.

Because research is an ongoing process, your permission for the use, storage and sharing of your health information will continue indefinitely.

Your privacy rights:
- You have the right to refuse to sign this form that allows us to use and share your health information for research; however, if you don't sign it, you will not be able to take part in this research study.
- You have the right to withdraw your permission for us to use or share your health information for this research study. If you want to withdraw your permission, you must notify the person in charge of this research study in writing using the address on the front of this form. Once permission is withdrawn, you cannot continue to take part in the study.
- If you withdraw your permission, we will stop collecting health information about you for this study; we may continue to use and share your health information that we already have if it is necessary for safety and scientific soundness of the research study; and we will not be able to take back information that has already been used or shared with others.
- You have the right to see and get a copy of your health information that is used or shared for treatment or for payment. However, you may not be allowed to see or copy certain health information that is a part of this research study. This is only for the period of the study. You will be allowed to see that information when the entire research study is complete.

8. What are the costs of taking part in this research study?

8a. What will I have to pay for if I take part in this research study?

For costs of tests and procedures that are <u>only</u> being done for the research study:
- The inositol and/or placebo in this study will be provided by the study at no cost to while you take part in this study.
- You and/or your insurance company will not be charged for the cost of any tests or procedures that are required as part of the research and are outside the standard of care (what is normally done) for your condition.

- The research-related tests and procedures that will be provided at no cost to you include: blood work, pregnancy tests, 2 hour OGGT testing, transvaginal ultrasound and DXA scans.

For costs of medical services for care you would receive even if you were not in this research study:

- You and/or your insurance company will be responsible for the cost of routine medications, tests and procedures that you would receive even if you were not in this research.
- You and/or your insurance company will be billed for the costs of these routine tests and procedures in the usual manner.
- You will be responsible for any co-payments, co-insurance and deductibles that are standard for your insurance coverage.
- You will be responsible for any charges not reimbursed by your insurance company.
- Some insurance companies may not pay for routine costs for people taking part in research studies. Before deciding to be in this research you should check with your insurance company to find out what they will pay for.

If you have any questions about costs and insurance, ask the research study doctor or a member of the research team.

8b. What happens if I am injured as a result of taking part in this research study?

It is possible that you could develop complications or injuries as a result of being in this research study. If you experience a side effect or injury and emergency medical treatment is required, seek treatment immediately at any medical facility. If you experience a side effect or injury and you believe that emergency treatment is not necessary, you should contact the principal investigator listed on the first page of this consent form as soon as possible and the principal investigator will arrange for medical treatment.

<u>HMC/PSU compensation for injury</u>

- There are no plans for HMC/PSU to provide financial compensation or free medical treatment for research-related injury.
- If an injury occurs, medical treatment is available at the usual charge.
- Costs will be charged to your insurance carrier or to you.
- Some insurance companies may not cover costs associated with research injuries.
- If these costs are not covered by your insurance, they will be your responsibility.

When you sign this form you are not giving up any legal right to seek compensation for injury.

9. Will I be paid to take part in this research study?

You will receive compensation for being in this research study as described below for a possible total of $300. If you do not complete the study for any reason, you will be paid for the visits you have completed. The payment will be issued by an external company called Greenphire. You will be provided a ClinCard, which is a debit card that your funds are loaded onto and can be used at your discretion. The research team will give Greenphire some personal information about you, as described below. Greenphire will only use your personal information to process this reimbursement and will not share it with anyone for any other purpose. Details of the debit card system are explained on an additional sheet. If you lose the card, you may be responsible for the replacement fee.

When a visit is completed, funds will be approved and loaded onto your card. The funds will be available within 2-3 business days. In order to assign a ClinCard to you and load funds onto the ClinCard, Greenphire will need your Study/Subject ID, Name, DOB, Address, and Social Security Number.
You will have the option to receive updates related to payment alerts via text message and/or email message. Standard text messaging rates will apply. In order to send you messages Greenphire will need your Mobile Phone Number and/or E-mail Address. Payments received as compensation for participation in research is considered taxable income. If payments from Greenphire exceed $600 in any one calendar year, Greenphire will file a 1099 (Miscellaneous) form on behalf of Penn State.

Screening Visit:	$50
Randomization Visit:	$100
Month 1:	$50
Month 3:	$100

It is possible that your research information and/or specimens (both identifiable and de-identified) may be used to create products or to deliver services, including some that may be sold and/or make money for others. If this happens there are no plans to tell you, or to pay you, or to give anyfinancial compensation to you or your family.

10. Who is paying for this research study?

The institution and investigators are receiving a grant from the National Center for Complementary & Integrative Health to support this research.

11. What are my rights if I take part in this research study?

Taking part in this research study is voluntary.

- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.

If you stop being in the research, already collected data may not be removed from the study database. You will be asked whether the investigator can collect medical information from your routine medical care. If you agree, this data will be handled the same as research data. If you withdraw completely from the research study, no further information will be collected and your participation will end. You may discontinue taking part at any time without penalty or loss of benefits to which you are otherwise entitled.

Your research doctor may take you out of the research study without your permission.

- Some possible reasons for this are: continuing the research would be harmful, your condition has become worse, you become pregnant, you did not follow the instructions of the study doctor, you experience serious side effects.
- Also, the sponsor of the research may end the research study early.
- If your participation ends early, you may be asked to visit the research doctor for a final visit.

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

12. If I have questions or concerns about this research study, whom should I call?

Please call the head of the research study (principal investigator), Dr. Richard Legro at 717-531-8478 or the Obstetrics and Gynecology doctor on 24-hour call at 717-531-8521 if you:

- Have questions, complaints or concerns about the research.
- Believe you may have been harmed by being in the research study.

You may also contact the research protection advocate in the HMC Human Subjects Protection Office (HSPO) at 717-531-5687 if you:

- Have questions regarding your rights as a person in a research study.
- Have concerns or general questions about the research.
- Have questions about your privacy and the use of your personal health information.
- You may also call this number if you cannot reach the research team or wish to offer input or to talk to someone else about any concerns related to the research.

You may visit the Penn State College of Medicine's Clinical Research web site at http://med.psu.edu/clinical-research/faqs for:

- Information about your rights when you are in a research study;
- Information about the Institutional Review Board (IRB), a group of people who review the research to protect your rights; and
- Links to the federal regulations and information about the protection of people who are in research studies. If you do not have access to the internet, copies of these federal regulations are available by calling the HSPO at (717) 531-5687.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

INFORMED CONSENT AND AUTHORIZATION TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative and have answered any questions he/she has about the research.

_____	_____	_____	_____
Signature of person who explained this research	Date	Time	Printed Name

(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent and Authorization

Before making the decision about being in this research you should have:

- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.

Version Date: 25Jul2023

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

Signature of Subject

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

_____	_____	_____	_____
Signature of Subject	Date	Time	Printed Name