Protocol
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Androgen Excess as a Cause for Adipogenic Dysfunction in PCOS Women

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LAY SUMMARY

The purpose of this research study is to collect specimen samples and study medical information from women with Polycystic Ovary Syndrome (PCOS) and women without PCOS. The goal is to learn more about the changes that take place in the body that result in PCOS.

We anticipate that 70 women will be enrolled in this study (20 without PCOS and 50 with PCOS). All patients will undergo a physical exam, blood tests, and transvaginal ultrasound of their ovaries. If they meet the criteria for this study, they will then undergo additional blood tests, removal of abdominal fat, transvaginal ovarian ultrasound, MRI, DXA scan, frequently-sampled intravenous glucose tolerance test (FSIGT), follicle stimulating hormone stimulation test, and Quality of Life surveys. The women without PCOS will be complete the study at this point.

The women with PCOS will be stratified according to whether they are insulin resistant or non insulin resistant based upon the results of their glucose tolerance test. Both groups will be randomized to receive the drug flutamide 125 mg/day or placebo. Subjects will take the drug/placebo every day for 6 - 28 day cycles and will continue to take the drug/placebo until all post treatment assessments are completed. Subjects will be asked to collect and store a urine sample once a week during the 6 - 28 day cycles. Subjects will also be asked to complete diaries. Once a month while they are taking the flutamide/placebo, they will return to the clinic and bring their urine samples. At that time they will undergo a physical exam, toxicity assessment, and blood draw. Quality of Life surveys will be performed at baseline, midpoint, and end of study for PCOS participants. Non-PCOS participants will complete Quality of Life surveys at baseline only.

After the 6 - 28 day cycles are completed the PCOS participants will continue to take study drug/placebo until they have completed end of treatment evaluations which include physical exam, additional blood tests, removal of abdominal fat, trasvaginal ovarian ultrasound, MRI, DXA scan, frequently-sampled intravenous glucose tolerance test (FSIGT), and follicle stimulating hormone stimulation test.

Per amendment #11 submitted 11-25-13, subjects who previously underwent an OGTT will be asked to undergo an FSIGT. Women enrolled on the PCOS arm may choose to decline the FSIGT and will undergo an OGTT post treatment.

Per Amendment #24 submitted 6/10/15, we requested permission to add a Family History Questionnaire to this study. Subjects who have been enrolled in the past as well as future participants will be asked to complete the questionnaire. Active study participants will be asked to complete the questionnaire at their next regularly scheduled study visit. Those who have completed all of their study visits will be contacted by phone or email to ask if they will complete the questionnaire. If they agree, it will be sent to the participant by US mail with a stamped return envelope or the questionnaire will be completed over the phone. New study participants will be asked to complete the questionnaire at their eligibility visit. If any participant(s) decline, their participation in

this study will not be affected nor will their future healthcare at UCLA.

BACKGROUND AND SIGNIFICANCE

Polycystic ovary syndrome (PCOS): PCOS, a common endocrinopathy affecting 6–10% of women, manifests by hyperandrogenism, ovulatory dysfunction and polycystic ovaries (PCO) in its complete phenotype. Abdominal adiposity occurs frequently in PCOS women vs. controls, including some, but not all, nonobese individuals. Consequently, the estimated prevalence of MBS in PCOS is 33-47%, about 2-3 times higher than that of age-matched controls.

Interplay between adipose function and androgen excess: In PCOS, intrinsic defects combine with endocrine factors to impair insulin sensitivity and induce adipocyte insulin resistance (IR). Conversely, physiological androgen administration to women induces IR. In PCOS women, therefore, androgen excess could decrease numbers of SC abdominal adipocytes and reduce capacity of this adipose to safely store fat. Thus, if energy intake exceeds this capacity, SC abdominal adipocytes would likely overfill with lipid to promote ectopic lipid deposition. We hypothesize that PCOS-related hyperandrogenism decreases adipogenic differentiation and increases adipocyte lipid content in a constrained SC abdominal adipose, inducing lipotoxicity, IR and ovulatory dysfunction.

Therefore, reducing PCOS-related androgen excess could improve SC abdominal adipogenic function and ovarian folliculogenesis, reducing use of ovulation-inducing agents in PCOS women wishing to conceive, and risks of multiple pregnancy.

Effect of flutamide on PCOS: Administration of flutamide, a nonsteroidal anti-androgen, to PCOS women lowers circulating androgen and triglycerides levels. Flutamide also restores ovulation in some, but not all, PCOS women. We will systematically compare SC abdominal adipogenesis, circulating adipogenic markers and SC abdominal as well as visceral (omental plus mesenteric) adipose content of lean PCOS women randomized to 6-month low-dose flutamide vs. placebo to determine how differences in key variables affect ovarian function and morphology, as well as in vivo E2 responsiveness to FSH.

We also are able to quantify SC abdominal and visceral adipose content by magnetic resonance imaging (MRI), as performed by Dr. Margolis. Finally low-dose flutamide vs, placebo administration to lean PCOS women will discover novel molecular and biochemical markers that predict efficacy of androgen blockade in restoring adipogenic and ovarian function.

AIMS AND OBJECTIVES

The purpose of this research study is to collect specimen samples and study medical information from women with PCOS and women without PCOS. The goal is to learn more about the changes that take place in the body that result in PCOS.

Our Aims are to:

Aim 1: Compare differences in SC abdominal adipose structure-function and regional abdominal adipose content in lean PCOS women vs. age- and BMI-matched controls. Hypothesis: SC abdominal adipose storage is constrained in lean PCOS women.
• Analyze adipocyte size, lipid accumulation, function and secretion of adipokines.
• Analyze same parameters indicated above and signaling pathways in new adipocytes originated from isolated adipose stem cells.
• Use cell culture to examine interactions between adipose stromal-vascular cells and adipocytes in the control of adipose development.
• Correlate key factors with insulin action in vivo; regional and percent body fat; circulating lipids, free fatty acids (FFAs), sex steroids, and adipokines; and ovarian volume, antral follicle number and serum AMH as well as serum E2 response to recombinant human (rh)FSH per antral follicle.

Aim 2. Examine effect of flutamide on SC abdominal adipose structure-function, regional abdominal adipose content and ovarian folliculogenesis in lean PCOS women. Hypothesis: SC abdominal adipose storage is normalized in lean PCOS women receiving flutamide.
• Examine in vitro effects of sex steroids on adipogenesis.
• Determine changes in SC abdominal adipogenesis, circulating lipids, FFAs, sex steroids, and adipokines.
• Determine changes in SC abdominal and visceral adipose content, fat free mass as well as percent body fat.
• Determine changes in ovarian morphology and function as well as ovulatory frequency, as measured by weekly urinary pregnanediol glucuronide (UPDG).
• Determine key factors that predict ovulation by serial urinary UPDG measurements.

RESEARCH DESIGN AND METHODS

Aim 1. Compare differences in SC abdominal adipogenesis of lean PCOS women vs. age- and BMI-matched controls

Subjects and clinical assessment: We will recruit subjects with the intention of enrolling 90 women - 70 lean (18.5-25 kg/M2) PCOS subjects and 20 age- and BMI-matched controls with the assumption that there will be dropouts such that 36 women with PCOS and 12 controls will complete the study. Subjects will complete a standardized questionnaire emphasizing menstrual dating, abnormal hair growth and acne. The questionnaire also will annotate age, smoking status, medications, surgical history and family histories of excess hair growth in female relatives and of diabetes in parents or siblings for exclusion criteria and for inclusion of some basic traits as covariates. Subjects also will undergo a physical examination; hirsutism will be scored by the modified Ferriman-Gallwey (mFG) method. Transvaginal sonography (TVUS) will be performed to determine the presence or absence of polycystic ovaries by Rotterdam

criterion. A screening blood sample will be obtained for determinations of steroid hormones, TSH, and prolactin; biochemical hyperandrogenism will be defined as an elevated serum total or free T > 2 SD above the mean value.
Non-Hispanic Caucasian women between the ages of 18 and 35 years will be recruited to avoid confounding differences as a function of race. PCOS patients will be diagnosed by 1990 NIH criteria. Controls will have regular menstrual cycles at 21 to 35 day intervals, a luteal phase progesterone (P4) level > 3 ng/mL, and no evidence of hirsutism, acne, alopecia, polycystic ovaries or endocrine dysfunction. Exclusion criteria are: present/past history of smoking during the past year, cancer, alcohol abuse, drug addiction, severe depression, or post traumatic stress; diabetes; uncontrolled hypertension (≥ 165/100); clinically significant hepatic or renal disease, or other major medical illness; signs or symptoms of infection; recent (within 30 days) use of an experimental device; recent (within 6 months) use of androgens, anabolic steroids or non-steroidal anti-inflammatory drugs; recent (within 3 months) use of hormonal agents (including birth control pills, Plan B, Mirena IUD, or insulin sensitizers); use of the drug warfarin; beta blockers; CYP active medications or herbs.

Frequently sampled intravenous glucose tolerance test (FSIGT): All studies will be conducted in the follicular phase in controls and during amenorrhea in PCOS women. Each subject will be instructed to drink only water during the 12-hour fasting period before the FSIGT, and to avoid caffeinated fluid intake. Glucose in the form of a 50% solution (0.3 g/kg) and regular human insulin (0.03 units/kg) will be injected through an intravenous line at 0 and 20 min, respectively. Blood will be collected at -20, -15, −5, 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 min for glucose and insulin determinations. Approximately 2-3 tablespoons (28-42 ml) will be drawn for the FSIGT test.
Adipocytes isolation and culture: Approximately 1.0 gm of fat will be obtained from the lower SC abdomen using standard procedures under local anesthesia. Adipocytes and the stromal vascular fraction will be isolated to measure adipocyte cell number and diameter, lipid accumulation and function, adiponectin, and stem cell development.

Procedures: All procedures will be performed in normal and PCOS women at the start of study.

i). Venipuncture: Fasting blood will be collected for blood count, chemistry panel, LH, FSH, total/free T, DHT, A4, DHEAS, E1, E2, anti-mullerian hormone (AMH), SHBG, adiponectin, IL-6,lipid profile, C-reactive protein, glucagon, leptin, C-peptide, and FFAs. Blood samples for all participants will be shipped to Oregon State Health University (OHSU) for analysis of AMH, IL-6, adiponectin, glucagon, leptin, and C-peptide levels. The University of Michigan will perform a comprehensive hormone analysis by LC-MS/MS, including traditional (T, AD, DHEA, DHEAS) and emerging androgen biomarkers (11-oxygenated C19 steroids, 11 OHA, 11KA, 11OHT, 11KT). There will be no personal identifiers on the samples that sent to OHSU or University of Michigan.

Study numbers will be used.

ii). Body composition: Body composition will be assessed by BMI, waist-to-hip circumference and DXA scanning. Girths will be measured at the waist (narrowest section of the torso between ribs and umbilicus) and hips (largest protrusion of the hip region, above the gluteal fold). For total body fat and regional fat distribution (central vs. appendicular), whole body scans will be performed, utilizing DXA imaging (Hologics QDR 1500). A portable BIA will calculate lean body mass changes by computerized methods to measure resistance and reactance.

iii). Body fat distribution: Total body DXA will measure abdominal fat (i.e., the area between the dome of the diaphragm [cephalad limit] and the top of the greater trochanter [caudal limit]). Total body DEXA images also will determine % body fat; fat-free body mass; total body, abdominal, and leg fat; and abdomen/leg fat mass ratio. The leg region is that area below the top of the greater trochanter.
Multiple axial abdominal and pelvic MRI slices from the diaphragm to the pelvis will assess cross-sectional areas of SC abdominal and visceral fat.

iv). Ovarian testing:

a). Ovarian morphology: TVUS using a 4- to 8-mHz vaginal probe will be performed in the follicular phase in controls and during amenorrhea in PCOS women. Summed ovarian volume will be calculated. Antral follicle number, defined as the total follicle number (2-9 mm in diameter) of both ovaries, will be determined by 1 investigator (D.A.D.). Polycystic ovaries will be defined by 12 or more follicles in each ovary measuring 2-9 mm in diameter, and/or increased ovarian volume (> 10 ml), with one such ovary sufficient to define PCO.

b). E2 response to recombinant human (rh)FSH. Blood samples will be drawn through an indwelling iv catheter at -90, -60, -30 minutes and time 0 as well as 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h after 150 U rhFSH injection (112). The E2 response to rhFSH will be assessed before and after adjusting for numbers of TVUS-detected ovarian antral follicles.

Aim 2. Examine effect of flutamide in lean PCOS women on SC abdominal adipogenesis, visceral adipose content and ovarian folliculogenesis.

Per amendment #29, we are requesting permission to change the randomization strategy since all PCOS subjects enrolled thus far have been non-insulin resistant

based upon the results of the Frequently Sampled Intravenous Glucose Tolerance Test. Therefore, the need to stratify the PCOS subjects into two groups (i.e. insulin resistant versus non-insulin resistant) is unnecessary. All PCOS subjects recruited will now be randomized to study drug versus placebo based upon non-insulin resistant status. Of note, the control subjects are not randomized since they do not receive study drug or placebo.

Adipogenic studies: All adipogenic studies performed at study initiation will be repeated at the end of the 6 - 28 day cycles of flutamide vs. placebo intervention in PCOS women.

Procedures: All procedures performed at study initiation also will be repeated at the end of the 6 - 28 day cycles of flutamide vs. placebo intervention in PCOS women. The end of treatment fat biopsy will be performed of the opposite side of the lower abdomen than where the first biopsy was taken. In addition, monthly liver function studies will be performed to detect possible elevations of serum transaminase levels above the normal range during flutamide vs. placebo therapy.

Menstrual records and urinary pregnanediol glucuronide (UPDG): Ovulatory frequency will be determined by having subjects keep a daily menstrual record and collect weekly first morning urine samples for UPDG and creatinine assay. Urine samples will be stored for later analysis. Urine samples will be analyzed at the University of Wisconsin-Wisconsin National Primate Research Center for the urinary metabolite of progesterone (urinary pregnanediol glucuronide [UDPG]) and creatinine testing.

Six months following the completion of protocol procedures, participants who received flutamide/placebo will be contacted by phone to check on the status of their health. Subjects will be asked if they have experienced any health problems or have become pregnant since they completed the study procedures.

Daily diaries will include self reporting on taking pills, menstrual flow, symptoms, concomitant medications, exercise patterns, and diet habits.

Quality of life assessments and ovarian size measurements will be conducted at baseline, and at the end of 3 and 6 months of treatment. The PHQ, Beck's Anxiety, Beck's Depression questionnaires will be completed at baseline. The Beck's Depression and Beck's Anxiety will be completed at 3 and 6 month visits. Scoring of the Quality of Life surveys (Beck’s anxiety and Beck’s depression questionnaires) will be at the time the subject is present for their study visit. If any areas of concern are identified, they will be addressed with the subject before they leave and the appropriate referrals

will be made.

In order to conduct quality control testing on the urine samples, we are requesting approval to utilize urine specimens from IRB #11-001454, "A Study to Collect Specimens and Medical Information for Use in Research" - PI - Joshua Cohen, MD and UCLA IRB #13-000430 "A study to learn more about the optimal conditions for storing and shipping urine samples for research purposes" - PI - Daniel Dumesic, MD. We will also utilize blood and tissue samples from participants in IRB #11-001454 in order to conduct further laboratory tests including immunohistological assessments which will allow us to integrate adipose/hormonal data to more fully investigate the pathophysiology processes of diseases under study. Specimens may be analyzed in Dr. Dumesic's lab at UCLA and/or shipped for analysis to David H. Abbott, Ph.D.; Wisconsin National Primate Research Center, 1223 Capitol Court, Madison, WI, 53715.

Participants will be given the opportunity to sign a minimal risk consent form if they wish to communicate by e-mail during their participation in this study.

In December, 2013 an amendment was submitted for this study requesting a change from using the OGTT test to an FSIGT test. Subjects in the control arm who have already undergone an OGTT test and do not wish to undergo an FSIGT test will not be affected and the data from their OGTT test will be used. Subjects in the PCOS arm who have undergone an OGTT test at baseline may choose to repeat a baseline FSIGT test and have an FSIGT test at the end of study. If they do not wish to have an FSIGT test, an OGTT test will be performed at the end of study.

Per amendment #34: To date, our preliminary data have shown as expected that serum levels of Anti-Mullerian Hormone (AMH), as a marker of ovarian follicle number, are greater in PCOS than control subjects. However, one PCOS subject has serum AMH levels that cannot be detected by the routine AMH assays performed at Oregon Health Sciences University (OHSU). Re-analysis of her serum AMH levels at ANSH Laboratories, Texas (per IRB approved amendment #33) using antibodies directed towards different regions of the AMH protein suggest that this PCOS subject has an AMH protein that is not biologically active. This exciting discovery offers an important scientific opportunity to identify the type of AMH abnormality in this PCOS subject, particularly because serum AMH measurement as a clinical marker of ovarian follicle number may be misleading in some PCOS women. Therefore, ANSH Laboratories and the University Medical Center Rotterdam, Netherlands have agreed to conduct tests to identify the gene mutation and protein structure/function abnormality of AMH in this PCOS subject. These findings will be compared with 5-10 PCOS and 5-10 control subjects who are either new subjects, currently participating or have previously participated in this study. For the analysis, frozen monocytes, frozen serum, and/or fresh whole blood samples are needed. As part of our current study design, we already have frozen monocytes and serum aliquots from all subjects on hand that can be analyzed immediately. However, an additional 20 mL of plasma collected in EDTA

plasma tubes for the newly proposed studies are needed. The additional blood will be drawn at the initial Frequently-Sampled Intravenous Glucose Tolerance Test (FSIGT) visit for new subject and at the convenience of the subjects who sign the addendum consent form. All collected specimens analyzed at ANSH Laboratories or University Medical Center Rotterdam will be de-identified and ANSH Laboratories will forward the specimens to the University Medical Center Rotterdam on our behalf. Any additional specimens remaining after analysis by AHSH Laboratories or University Medical Center Rotterdam will be destroyed.

The findings of these additional tests will be conveyed only to the subject with the anomaly since it could impact her future clinical care. We anticipate that there will be no impact from the findings on the PCOS or control subjects who have measurable AMH levels.

Per amendment #42, epigenetic studies (RNA sequencing, DNA Methylation, and Assay for Transposase Accessible Chromatin Sequencing) on existing fat cell samples will be conducted at UCLA.

INCLUSION CRITERIA

Caucasian and Non-Hispanic women between the ages of 18 to 35 years.

Groups will be: 12 lean controls (Aim 1); 36 age- and BMI-matched PCOS women randomized to flutamide vs. placebo for 6 months (Aims 1 and 2).

i) Lean patients with PCOS: 36 subjects with PCOS (defined by 1990 NIH criteria [all Aims]), BMI 18.5-25 kg/m2. This BMI range is defined as normal and has been chosen to examine underlying mechanisms of PCOS-related androgen excess in the genesis of adipogenic and ovarian dysfunction, independent of obesity. Excessive course hair on upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arm and/or thigh.

ii) Lean control women: 12 healthy subjects, BMI 18.5-25 kg/m2. Controls will have regular menstrual cycles, and no evidence of hirsutism, acne, alopecia, polycystic ovaries, and/or endocrine dysfunction. This BMI range has been chosen to match that of the PCOS group.

Up to 70 lean PCOS patients and 20 lean controls will be recruited, for a total of 90 study participants over a 5-year interval, accounting for a 20% rate of patient drop-out or insufficient adipose procurement. Participants must be fluent in English.

EXCLUSION CRITERIA

Exclusion criteria for study participation are: present or past history of smoking within past 1 year, cancer, alcohol abuse within past 5 years, drug addiction within past 5 years, severe depression within past 5 years, or post traumatic stress disorder within

past 5 years; diabetes; uncontrolled hypertension (≥ 165/100); clinically significant hepatic or renal disease, or other major medical illness; recent (within 3 months) use of androgens, anabolic steroids or hormonal agents (including birth control pills or insulin sensitizers), Mirena IUD, or Plan B contraception. These exclusion criteria are chosen to avoid effects from medical conditions, environmental factors or exogenous agents. Women taking the drug warfarin, beta blockers, CYP active medications, or herbs will be excluded.

Since this study involves MRI testing, women with pacemakers, metal implants, or claustrophobia will be excluded.

The screener will assess the participants response to establish if depression or drug use exclude participation in this study.

If a woman becomes pregnant while participating in this study she will be excluded from further participation.

Non-English speaking individuals will be excluded from this study. The eligibility criteria includes non-Hispanic Caucasians only so it is assumed that the vast majority of participants will be English speaking. Since PCOS phenotype varies by ethnicity, eligibility was restricted to only one ethnic group in order to control for ethnic variability.

SUBJECT IDENTIFICATION AND RECRUITMENT

Initial contact may be made at the time the patient visits the clinic for non-research related indications. The treating physician, colleague physician, nurse, medical assistant or study coordinator will interface with the potential subject to obtain their consent to be contacted regarding the study. In addition, IRB approved scripts for contact recruitment, responding by e-mail, in person contact or referral contact will be used.

Additionally, patients will be recruited by obtaining a list of possibly eligible patients by extraction of electronic medical records as described in the "Review of Medical Records" section below. After reviewing their charts, verifying eligibility, and obtaining approval from their PCP or GYN provider, patients will be directly contacted by a member of the study team using the IRB approved screening script.

Since potential subjects may contact us directly in response to flyers, ads, etc., we will respond to all inquiries via phone or email as they prefer. If a potential participant contacts us directly by email and wishes more information without phone contact, the consent form will be sent to them via email for their consideration. If they are interested in the study, they will have the option to respond by phone for further screening. The study team will utilize the recruitment script/screening script. The recruitment script/screening script will not be sent via email to potential subjects.

Subjects who have already undergone an OGTT test will be contacted at their next

study visit or by phone to be given the opportunity to undergo FSIGT testing.

Per amendment #15 we will not include identifiers on the screening script worksheet. A screening code number will be assigned to each interested potential participant. A separate database on a password protected computer in a locked office will include the link between the personal identifiers and the code number so that we can contact the potential participant. In order to minimize the amount of time spent by potential participants as well as the study staff on potential participants who are not eligible for the study, we have added additional screening questions.

Flyers will be available in 100, 200, and 300 Med Plaza, West Medical, UCLA Clinics, Community Clinics, UCLA and other college campuses, off campus student groups, offices of community physicians, student health centers, and community locations. Information regarding study design and eligibility also will be available on the UCLA OB/GYN Departmental website and Craigslist as well as other internet postings. Newspaper ads that contain information from the IRB approved flyers will be used. Announcements of the study and flyers will be sent to UCLA and community physicians, UCLA and community clinics, hospitals, student health centers, college campuses, community health centers, Craigslist and internet postings.

Potential patients will be assured that their health care will not be affected by their decision whether or not to participate in this study.

The source of referrals will be the health care providers of patients seen in the OB/GYN Clinics at 200 Med Plaza, West Medical, Simms Mann, the office of Dr. Heaps in 100 Med Plaza, community clinics and private practices, student health centers, and any additional individual interested in and eligible for participating in clinical research. A member of the health care team (i.e. treating physician, colleague physician, nurse, medical assistant, or study coordinator) will give the patient a brief overview of the study and ask their permission for the study physician to contact them. Please be assured that referring physicians will request and document patients' permission to provide us with their contact information.

PROCEDURES

The following information, as stated in the consent form, outlines the events that will take place at each visit and the anticipated length of time required by the subject. The sequence of the procedures may vary.

The study procedures will take place at UCLA Westwood Campus in the 200 and 300 Medical Plaza Buildings, the Clinical Translational Research Center, Ronald Reagan Medical Center, and/or Room 22-265 in the Center for Health Sciences.

Before subjects begin the study:

Before they begin the study, subjects will need to agree to use a non-hormone type of

birth control for the duration of their participation in this study. If subjects have not undergone permanent sterilization, examples of acceptable types of birth control are an IUD (without hormones) or spermicidal jelly in combination with a diaphragm or condoms.

During the study:

If subjects take part in this study, the researcher(s) will ask them to do the following:

All Study Participants

Initial Visit

• The consent form will be reviewed with the subject and anyone they wish to have present. If they choose to participate, they will sign the consent form and HIPAA Authorization form. Subjects will be given a copy for their records.
• The doctor will ask questions regarding their past medical history, family medical history, and lifestyle to determine if they are eligible to participate in this study.
• If subjects are not eligible to participate in this study, no further study related office visits or procedures will be performed and they will receive $10 for their time
• If subjects are eligible to participate, they will undergo the following:

- Physical Exam
- An assessment of their skin
- Height, weight, waist and hip measurements
- Transvaginal ultrasound of their ovaries
- Blood tests - approximately 1 teaspoon (6 ml) of blood will be drawn from their vein. All of the blood samples in this study will be used to detect any abnormal hormone levels. A pregnancy test will also be performed.
- Complete questionnaires regarding their feelings about various areas of their health, quality their life, and family history of PCOS and/or diabetes. Subjects have the right to refuse to answer any questions that they do not wish to answer.
- Whether subjects are eligible or not, they will be paid $20 for completing this visit.
    - It is estimated that this visit will take approximately 2 hours.

Women with and without PCOS

If subjects are eligible and choose to continue participation, they will undergo the following tests and procedures. Subjects will be given separate instructions on how to prepare for the tests. Subjects will complete a contact information sheet with the name of someone to contact in the event of an emergency.

Frequently-Sampled Intravenous Glucose Tolerance Test (FSIGT) and Blood Tests

• Approximately 5 tablespoons (64 ml) of blood will be drawn from their vein for research purposes.
• The first blood draw will be sent to the laboratory for an immediate test of their progesterone level, which indicates whether or not they are ovulating. If the progesterone test indicates that they are ovulating, the Frequently-Sampled Intravenous Glucose Tolerance Test will not be able to be performed that day and will need to be rescheduled. • A Frequently-Sampled Intravenous Glucose Tolerance Test (FSIGT) will be performed. Subjects will be given instructions on how to prepare for the test, which include not eating or drinking anything except water after midnight on the day of the test. A small amount of glucose will be injected into their vein. Shortly thereafter, a small amount of insulin will also be injected in their vein so that researchers can learn more about how their body reacts to glucose and insulin. Two small catheters will be attached to their veins in order to remove the blood samples so that they will not have to be stuck with a needle at each time interval. A total of approximately 2-3 tablespoons (28-42 ml's) will be removed from their vein for the FSIGT test. Their blood will be drawn 20, 15, and 5 minutes and at time 0 immediately **before** infusion of the glucose. Insulin will be injected 20 minutes after the glucose injection. Your blood will be drawn at 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 minutes **after** the glucose injection.

• It is estimated that this visit will take approximately 5-6 hours.

MRI and DXA Scan

In order to be sure that participants are not ovulating, approximately ½ teaspoon (2 ml's) of blood may be drawn and sent to the laboratory for an immediate test of their progesterone level before their MRI and/or DXA scan. If the progesterone test indicates that they are ovulating, the test(s) may not be able to be performed that day and may need to be rescheduled.

• Subjects will undergo a Magnetic Resonance Imaging (MRI) of their abdomen and pelvis. They will NOT be injected with a dye before the MRI.
• Subjects will be asked to ingest only a clear liquid diet beginning at midnight on the day of the MRI and DXA scan.
• Their bone density and body fat composition will be assessed by a test called a DXA Scan.
• It is estimated that this visit will take approximately 2 hours.

- The medical images will be performed at UCLA. The imaging studies are performed as part of this research study and will not be reviewed or interpreted by a UCLA radiologist. Any information regarding the imaging findings should be directed to the study PI

Removal of Abdominal Fat

- In order to be sure that participants are not ovulating, approximately ½ teaspoon (2 ml's) of blood may be drawn and sent to the laboratory for an immediate test of your progesterone level before the removal of fat. If the progesterone test indicates that you are ovulating, the fat removal may not be able to be performed that day and may need to be rescheduled.
- Subjects will be asked to ingest only a clear liquid diet beginning at midnight on the day of the procedure to remove abdominal fat.

• Subjects will be given local anesthetic and a small amount of fat approximately the size of a quarter will be removed from under the skin in their lower abdomen.
• Subjects will be asked to ingest only a clear liquid diet beginning at midnight on the day of the procedure to remove abdominal fat.
• It is estimated that this visit will take approximately 2 hours.

Follicle Stimulating Hormone (FSH) Stimulation Test and Ultrasound of the Ovaries

• Subjects will undergo a transvaginal ultrasound.
o Subjects will undergo a Follicle Stimulating Hormone (FSH) stimulation test. FSH is the hormone that normally induces the ovary to develop a follicle and a single injection of FSH predicts how sensitive the ovary is to FSH. Their blood will be drawn at -90, -60, -30 minutes and time 0 as well as at 1/2, 1, 1 1/2, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hours after the injection. In order to prevent multiple needle sticks for the blood draws, a catheter will be placed in a vein in their arm before the first blood draw. Their blood will be drawn at the intervals described above through the catheter.
• The first blood draw will be sent to the laboratory for an immediate test of their progesterone level which indicates whether or not they are ovulating. If the progesterone test indicates that they are ovulating, the FSH test will not be able to be performed that day and will need to be rescheduled. If the test cannot be performed, they will be paid $10 for this visit and another appointment will be scheduled.
• It will be necessary for them to stay at UCLA Medical Center in the Clinical Translational Research Center, which is located in the Center for Health Sciences building for approximately 26 hours in order to complete this test. Food will be provided to them during the testing.
• Approximately 1-2 tablespoons (21 mL) of blood will be drawn from their vein.

If subjects have PCOS, an additional 4 mL (approximately 1 teaspoon) of blood will be drawn for a complete blood count and comprehensive metabolic panel in order to assure that there are no abnormalities in these blood values before they begin taking flutamide or placebo.

Since some of the blood tests to confirm hormonal levels are timed around different phases of the menstrual cycle and menstrual cycles vary, it is possible some of the blood tests described above will need to be repeated. The Frequently Sampled

Intravenous Glucose Tolerance Test and the Follicle Stimulating Hormone Test will only be performed as described above.

Subjects will be paid $280 once they have completed the FSIGT, DXA, MRI, Fat Removal, and FSH stimulation visits. If they report to UCLA for a study visit and their blood tests indicate that they are not in the desired part of their menstrual cycle for a particular test, they will be compensated an additional $10 and the visit will be rescheduled. Participants will be asked to complete a form so that a check can be mailed to them within 2 months.

Women who do not have PCOS will conclude their participation in this study at this point. No further procedures for this study will be conducted.

Women with PCOS will continue participation as described below.

Women with PCOS will be asked to do the following:

• Take one pill (either placebo or flutamide) each morning before breakfast for 6 complete 28 day cycles. The drug flutamide has not been approved by the FDA for use in women or for the treatment of PCOS.
• Complete a diary regarding what time they took the pill, whether they had any menstrual bleeding, any changes in their health, any medications they took, if they exercised that day, and a brief summary of their diet.
• Collect a sample of their first morning urine once a week and store it until their next clinic visit. Subjects will be given instructions on the collection and storage of the samples.

• Return to the UCLA Clinic once a month for the following:

o A brief physical exam including a measurement of their height, weight, waist, and hips. Review any symptoms subjects may have experienced during the past month.
o Bring their prescription bottles so that the pills can be counted.
o Bring their weekly urine samples.
o Bring their completed diaries.
o Have blood tests (serum pregnancy test, complete blood count, and comprehensive metabolic panel) – approximately 1 teaspoon (5 ml) of blood will be drawn.
o After the third and sixth cycle, subjects will be asked to complete questionnaires regarding their feelings about various areas of their health and the quality of their life. Subjects have the right to refuse to answer any questions that they do not wish to answer.

• After the third and sixth cycle, subjects will also have an assessment of their skin, and a transvaginal ultrasound of their ovaries.
• It is estimated that each of these visits will take approximately 1 hour
• Subjects will receive be reimbursed for their parking expenses and receive $240after all 6 visits have been completed.
• Subjects will be asked to continue taking the flutamide or placebo until the procedures listed below are completed. Every effort will be made to complete all study visits as soon as possible.

After Completing Flutamide/Placebo

The FSIGT, MRI, DXA, Removal of Abdominal Fat, and FSH tests will be repeated as described above. Please note that a testosterone blood test, complete blood count, and comprehensive metabolic panel will be performed at the post-treatment FSH test.

30 Days after Last Pill

o Physical Exam
o Blood tests (pregnancy test, complete blood count, and comprehensive metabolic panel) - approximately 1 teaspoon (6 ml) of blood will be drawn from their vein.

Participants will be paid $300 once you have completed the FSIGT, DXA, MRI, Fat Removal, FSH stimulation, and 30 day after last pill visits. If they report to UCLA for a study visit and their blood tests indicate that they are not in the desired part of their menstrual cycle for a particular test, they will be compensated an additional $10 and the visit will be rescheduled.

6 months after completion of the study procedures

Participants who have received the flutamide or placebo will be contacted by phone 6 months after the study procedures have been completed. Subjects will be asked if they have experienced any health problems or become pregnant since they completed the study procedures. There will be no compensation for the phone call.

PRIVACY AND CONFIDENTIALITY

Subjects will be recruited with the clear understanding that they are participating in research. All information obtained from these studies will be maintained in charts separate from the hospital medical records. Only Dr. Dumesic and the study team will be privy to the study charts and the names of those individuals participating. All blood, urine and fat samples will be obtained and stored with careful attention to current HIPAA privacy regulations, and all study subjects will be assigned a unique identifier number. All data sent for analysis will be coded, in order to remove unique identifiers from the data. Nevertheless, the likelihood that the data generated in the study will be of a sensitive nature is minimal.

Regarding confidentiality, all information obtained from this study will be maintained in charts separate from the hospital medical records. Data will be maintained in locked file cabinets and in password protected files on computer systems. Data will be coded and all analyses performed using coded data only. Identifying information needed for cohort follow-up and retention will be kept separate from data analysis files. Only a limited number of individuals of the clinical team will be able to re-link the study codes to the identity of specific study participants. In the event that participants no longer want their blood samples to be included in the study, we will document this in the central database and destroy their samples if they have not already been used.

Study information will be kept in locked offices and password protected computers. Only members of the study team will have access to identifiable data.

POTENTIAL RISKS

Postmarketing data report the need for hospitalization and rare death events from liver failure in patients receiving flutamide. The flutamide dose chosen for this study is lower than the flutamide dose previously reported to harm the liver, with long-term (>1 year duration) flutamide studies showing no harmful liver effects at a dose of flutamide of 125 mg orally daily. Therefore, we do not anticipate that study participants with PCOS who receive flutamide (125 mg orally daily) in our study are at significant risk for harmful liver toxicity.

Study participants with PCOS who receive flutamide or placebo (125 mg orally daily) will be monitored with monthly blood tests. We will utilize the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Should a patient experience any hematologic or non-hematologic toxicity at grade 3 or higher, flutamide/placebo will discontinued and weekly blood tests will be performed until values return to normal.

If a patient experiences an acute life threatening adverse event, we will immediately contact the Investigational Drug Pharmacist and the blind will be broken.

As stated in the consent form, the risks include:

The possible risks and/or discomforts associated with the procedures described in this consent form include:

Flutamide:

Symptoms of liver injury, including loss of appetite, nausea and vomiting, fatigue, stomach or abdominal pain, flu-like symptoms and jaundice (yellowing of the skin or whiteness of the eyes), have occasionally been reported with the use of flutamide. Such liver injury, however, is related to the dose of flutamide and is reversible following discontinuation of the drug. For this reason, the flutamide dose chosen for this study is below the dose previously reported to harm the liver, with long-term (>1 year) flutamide studies showing no harmful liver effects at 125 mg orally on a daily basis. Additional risks of flutamide therapy include anemia, photosensitivity, breast tenderness, retention of fluid, discharge of fluid from your nipples, rash, hypertension, hot flashes, central nervous system reactions, decreased interest in having sexual relations, diarrhea, and anorexia. For these reasons, monthly blood tests will be done to monitor the safety of flutamide and this medication will be discontinued if there are any temporary changes in blood or liver function. Brownish urine also can occur and is due to a metabolite of flutamide.

Placebo Pills

There are no known risks from taking a placebo pill.

Risks of an Ultrasound:

Participants may experience some discomfort from having the ultrasound probe placed in their vagina and moved during the exam.

Ultrasound imaging has been used for over 20 years and has an excellent safety record. It is non-ionizing radiation, so it does not have the same risks as x-rays or other types of ionizing radiation.
Even though there are no known risks of ultrasound imaging, it can produce effects on the body. When ultrasound enters the body, it heats the tissues slightly. In some cases, it can also produce small pockets of gas in body fluids or tissues (cavitation), although this is extremely rare. The long-term effects of tissue heating and cavitation are not known.

Risks of Blood Draw:

Drawing blood may cause temporary pain from the needle stick, bruising or swelling at the site, and rarely, infection or fainting.

Risks of Procedure to Remove Fat from Under the Skin:

Potential problems during the collection of the subcutaneous abdominal fat specimens include a temporary discomfort from the local injection of anesthetic and/or pain, bleeding, and infection at the site of the lower abdominal incision. In very rare circumstances, local injection of anesthetic and/or the removal of abdominal fat may be associated with fainting with or without convulsions. Only experienced surgeons will perform the procedure to remove the small amount of fat, which should not significantly distort the contour of the abdominal skin.

Risks of Frequently-Sampled Intravenous Glucose Tolerance Test:

Insulin (introduced into your body during the intravenous glucose tolerance test) is the 1body's natural hormone for lowering blood sugars. If your blood sugars fall rapidly, you may experience mild dizziness, nausea, vomiting, shortness of breath, increased heart rate or sweating. If these effects occur, they are easily detected and treated with administration of supplemental sugar through the indwelling catheter, thereby raising the blood sugar. This condition is only temporary and has no known long-term health impact. The glucose (commonly called sugar) infused through the catheter as part of the intravenous glucose tolerance test may cause an initial feeling of warmth and flushing of the face, which are both transient symptoms that rapidly resolve. You will be asked to arrive for your testing after having fasted from midnight the night before. In light of the prolonged fast, you may feel hungry during the testing.

Risks of Follicle Stimulating Hormone (FSH) Stimulation Test.

Participants may experience lower abdominal bloating and/or vaginal spotting from this test.

Risks of DXA Scan:

Since the radiation procedures are all standard of care, the amount of radiation Subjects will receive is the same as that for similar patients who are not participating in this study. Therefore, they will not be exposed to any additional radiation by participating in this study.

Risks of Magnetic Resonance Imaging (MRI):

The MRI procedure uses a powerful magnetic field to generate detailed images of the body. The magnet could move objects within their body that contain metal, such as implants, clips and pacemakers. Subjects will be asked to tell the doctor if they have any metal items within their body.

MRI scanning is painless but they might experience discomfort in the machine. In particular, loud beeping and hammering noises occur during the study when the

scanner is collecting measurements. Subjects also may be bothered by feelings of claustrophobia when placed inside the MRI, or by lying in one position for a long time. Subjects might also experience stimulation of the nerves in their body, which feels like a gentle tap or sensation of mild electric shock.

Because the risks to a fetus from MRI are unknown, subjects cannot participate in this study if they are pregnant.

Known Reproductive Risks:

Subjects should not become pregnant while on this study because the drugs in this study and the DXA scan can affect an unborn baby. Women should not breastfeed a baby while on this study. Therefore, subjects will need to use effective birth control while on this study. Examples of acceptable types of birth control are a diaphragm with spermicidal jelly, condoms, or an IUD (without hormones). Subjects should use birth control starting at the beginning of their participation in this study.

Unknown Risks to Women of Child Bearing Potential and Pregnant Women:

Flutamide can occasionally induce ovulation in a woman with PCOS who does not ovulate. This is important because this medication blocks androgen action and can impair the ability of androgen to normally induce development of the external genitalia in the male fetus. For this reason, if subjects believe that they are pregnant or have a chance of becoming pregnant they should not participate in this study. A serum pregnancy test will be performed before the start of study procedures and monthly throughout their participation in this study. If they are pregnant, the will not be allowed to participate in the study. If women do participate in this study, they must use a medically effective form of birth control before entering the study, while participating in the study, and for at least one month after stopping the study. If they become pregnant during the study, they will be asked to tell the researchers right away.

Unknown Risks to Infants:

The side effects of Flutamide on infants are not known, therefore if women are currently breastfeeding they cannot participate in this study.

Loss of Confidentiality:

As this study involves the use of identifiable, personal information, there is a chance that a loss of confidentiality will occur. The researchers have procedures in place to lessen the possibility of this happening.

Unknown Risks and Discomforts:

The experimental treatments may have side effects that no one knows about yet. The researchers will inform subjects if they learn anything that might make them change

their mind about participating in the study.

POTENTIAL BENEFITS

Society will benefit from the knowlege gained by this study.

PCOS affects at least 7% of the general female population, and these women have an increased risk of diabetes, hypertension and dyslipidemia, infertility, dysfunctional uterine bleeding and endometrial carcinoma. The proposed studies will have the potential benefit of improving our understanding of how altered adipogenesis in PCOS adversely affects metabolic and reproductive function, allowing for the development of newer, and more targeted, therapies. Specifically, clinically relevant knowledge gained by these studies will be a decisive step in improving reproduction in PCOS women and the safety of ovulation-inducing agents when necessary by diminishing the risks of ovarian hyperstimulation syndrome and multiple pregnancy.

DATA AND SAFETY MONITORING PLAN

A physician member of the OB/GYN Department (Dr. Brian Koos), who is not affiliated with the study, will monitor the study on a regular basis. In addition, Dr. Dumesic will review on a monthly basis the status of recruitment and the occurrence of any adverse events. If any untoward events occur, necessary systems changes will be promptly instituted.

STOPPING RULES

All Grade 3 toxicities will be immediately evaluated by Dr. Dumesic and Dr. Koos. The study will be terminated if two patients experience Grade 3 NCI-defined adverse hepatic events (i.e., serum alkaline phosphatase > 5-20 ULN, alanine aminotransferase > 5-20 ULN, aspartate aminotransferase > 5-20 ULN, total bilirubin > 3-10 ULN) or if one patient experiences a single Grade 4 NCI-defined adverse hepatic event (i.e., serum alkaline phosphatase > 20 ULN, alanine aminotransferase > 20 ULN, aspartate aminotransferase > 20 ULN, total bilirubin > 10 ULN).

RULES FOR WITHDRAWING PARTICIPANTS FROM STUDY INTERVENTIONS

Reasons participants may be withdrawn from the study include but are not limited to the following:

1) Participant chooses for any reason to discontinue her participation in the study.

2) It is deemed by the Principal Investigator that continued participation is not in the best interests of the participant.

3) Blood samples cannot be obtained by a health care provider licensed to perform

phlebotomy..

4) The subject has a persistent adverse reaction to blood sampling, such as dizziness, anxiety or pain.

5) During the frequently sampled glucose tolerance test (FSIGT), the subject experiences nervousness, irritability, headache, pale and clammy skin or excessive sweating, which are not promptly reversed according to the protocol stated on the signed physician's orders.

6) During the entire duration of procedures involving blood draws, nursing staff will be present to monitor for any of the above adverse symptoms. If significant symptoms of hypoglycemia develop, oral or intravenous glucose will be administered.

7) The surgeon performing the removal of the fat deems the fat acquisition procedure unsafe due to pre-existing anatomic distortion of the abdomen, or to unrelated complications arising during the procedure. Because a low yield of adipocytes may result from small adipose sampling, and poor sample handling or cellular fragility, only an experienced surgeon (Drs. Dumesic or Jarrahy) will perform the subcutaneous fat biopsy procedure.

8) Elevation in liver function studies above the normal range.

9) If the participant becomes pregnant.

10) Noncompliance

11) Subjects will be monitored for hypertension. If a subject has persistent increase in diastolic blood pressure of 20 mm Hg or more, or if a previously normotensive subject experiences persistent increase in blood pressure to > 150/100, flutamide/placebo will be discontinued and the subject will be withdrawn from study treatment.

FINANCIAL OBLIGATIONS

Neither the subject nor their insurance carrier will be charged for their participation in this study.

Control participants may receive up to $300 for their participation in this study and PCOS participants may receive up to $840.
Participants will be reimbursed for their parking expenses. Meal vouchers will be provided during the FSH visit.

STATISTICAL CONSIDERATIONS

We will use one-way factorial analysis of variance (ANOVA) methods to compare continuous outcomes including hormone levels; adipocyte size, lipid content and glycerol release; numbers of ASCs and preadipocytes; mRNA levels. If data are not normally distributed on any scale, we will carry out nonparametric ANOVA.

Our previous comparison of cFOS gene expression in PCOS women vs. controls showed an increase in ΔCt (6.0 ± 1.4). Based on this, we hypothesize that the mean difference between controls and IR PCOS is 1.7 SD units. Assuming this, a sample size of 8/group (IR vs. non-IR PCOS) provides over 80% power using the usual two-sided $p < 0.05$.

We also will compare all outcomes before vs. after 6 months of placebo or flutamide treatment controlling for IR status, using either the parametric paired t test or non-parametric Wilcoxon signed rank test. Insulin action and sensitivity will be assessed by calculating a Matsuda Index from blood glucose and insulin levels during the oral glucose tolerance test. Ovarian estradiol sensitivity to FSH will be calculated from the 24-hr area-under-the-curve (AUC) serum estradiol response to FSH infusion.

Based on our in vitro data, we observed a mean 0.17 log scale change in ΔCt PPARγ (SD=0.26 log units) after treatment with T + Flutamide vs. T alone. A sample size of 14 (7 non-IR, 7 IR) provides more than 80% power for the IR adjusted mean change.

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