Primary Ovarian Insufficiency: Phenotype and Optimal Treatment

Protocol

Version Date: 6/2/2020

1. General Information
1.1 Protocol Signature Page

I have read the foregoing protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study in accordance with the design and specific provisions outlined herein; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment.
I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the conduct of the study.

I will use the informed consent form approved by the CCHMC IRB and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board or Ethics Committee responsible for this study.

I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse experiences as defined in Section 11 of this protocol.

The below signed confirm herewith to have read and understood this study protocol and/or amendment and appendices; furthermore, to accomplish this study in accordance to the protocol and Good Clinical Practice guidelines, as well as local regulations and regulatory authorities.

PRINTED OR TYPED NAME(S)	SIGNATURE	DATE
Investigator		
Investigator		
Study Coordinator		
Co-Investigator		
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Co-Investigator		

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2. Abstract

Background: Primary ovarian insufficiency (POI) is an enigmatic condition that affects ~1/10,000 women by age 20. Sometimes referred to as "early menopause," POI is characterized by estrogen deficiency among other hormonal abnormalities that resemble the menopause. POI is a serious chronic condition with no cure. The clinical presentation or 'phenotype' in adolescents is not well understood. Health consequences may include delayed or arrested puberty, skeletal losses, and the threat to reproductive health. Both the metabolic and emotional sequelae are substantial, and one of the most concerning is compromised bone health. The optimal hormone replacement therapy (HRT) regimen for these young women is debated and practice varies among health providers. Importantly only sparse data exist to guide clinicians to make evidence-based decisions regarding the management of these patients. If initiated early, HRT may prevent estrogen-associated bone loss.
Impact: Better understanding of POI may lead to improved treatments for this underserved population and have significant implications for the treatment of estrogen deficiency in other populations of adolescents and young women, and for all women going though natural menopause later in life. Little is known about the effects of HRT on bone health, body composition, cognition, and health-related quality of life, especially among adolescents. Understanding how this therapy affects these multiple health outcomes will fill knowledge gaps regarding treatment for young patients with POI, with potential implications for adolescents and young women with estrogen deficiency in other clinical settings. We will define the clinical presentation (i.e., phenotype) of adolescent POI. The pilot data collected will be used in a future application to the National Institutes of Health, to fund a larger trial that builds on observations from this initial study. The information gained from this pediatric model may also provide insights on management of the natural menopause that occurs in all women later in life.
Methods: Ten adolescents with idiopathic POI (i.e., from unexplained causes) will be recruited through the CCHMC Teen Health Center, Endocrine or Pediatric/Adolescent Gynecology Clinics. Ten healthy controls will be recruited from the Teen Health Center. Participants with POI will receive transdermal estrogen replacement (beginning at 25 μg/patch applied weekly), with the dose increased at subsequent study visits that will occur at 3, 6, and 12 months. All data collection will take place at the CCHMC Schubert Research Clinic. We will measure bone density of the central skeleton and body composition by dual-energy x-ray absorptiometry. To evaluate the peripheral skeleton, bone and muscle measures will be obtained by peripheral quantitative computed tomography. At each visit, the adolescents will have blood drawn to measure circulating hormone levels that are characteristically altered in adolescents with POI, along with safety assays. Cognitive functioning will be assessed using standardized tools. Participants will complete quality of life assessments, along with nutrition and physical activity surveys. Lastly, all will also complete a detailed medical history and health assessment.
Implications/Future Directions: Once the phenotype of adolescent POI is more clearly defined, a logical next question will be to determine whether negative health outcomes can be prevented or modified. Data from the proposed trial will guide the design of future prospective studies that evaluate the effects of traditional treatments (e.g., HRT), including a longer study to monitor HRT therapy, as well as more experimental treatments (e.g., skeletal agents) that may benefit young women with this rare condition. In addition, findings are expected to open avenues of research for adolescents and women with estrogen deficiency in other clinical settings.

3. Study Information

3.1 Background and Study Significance

Primary ovarian insufficiency (POI), sometimes termed "early menopause," is a condition in which young women under the age of 40 experience amenorrhea or irregular menses for 4 months or more, in association with elevated circulating levels of follicle stimulating hormone (FSH), approaching the menopausal range.[1-3] In most cases, the underlying cause remains a mystery even after a complete medical evaluation. POI is a serious chronic condition with no cure. It qualifies as a rare disease and as such, presents special challenges for patients, parents, and clinicians. While the diagnosis of POI is often delayed due to the assumption and myth that irregular menses are common among adolescents, early detection is critical for the maintenance of bone, cardiovascular, and reproductive health. Treatment options have focused on hormonal therapy and fertility preservation, but importantly, most studies to date have been carried out in adult women. Therefore, little is known about the clinical presentation of adolescents with this condition. Additionally, only sparse data exist regarding the effect of a standardized hormone replacement therapy (HRT) regimen, used commonly in practice to provide estrogen replacement, on bone and other tissues in affected young adolescents.

POI affects ~1/10,000 women by age 20.[4] Although not as common in adolescents as adults, the disorder significantly challenges both the health and well-being of all individuals. The major emotional impact of the diagnosis for most patients is shock at the news of loss of normal fertility and grief over the loss of associated dreams. Upon receiving this news, many young women do not know where to turn, and health providers have often never encountered this rare diagnosis in their clinical practice. A long-term serious medical consequence is the increased risk for osteoporosis as these young patients may fail to accrue their peak bone mass during a critical period for bone accretion.[5] Adolescents and women with POI generally have a lower bone mineral density (BMD) than healthy age-matched peers, and available evidence suggests the incidence of osteoporosis to be higher among patients with POI compared to postmenopausal women.[6,7]

Menopause is defined as permanent menstrual cessation that results from the depletion of functional ovarian follicles. The average age of natural menopause is 52 years, but can vary depending on socioeconomic, lifestyle, and other factors.[8] In contrast, most women with POI do not exhibit permanent cessation of ovarian function and may experience unpredictable ovarian activity for many years.[9] By definition, POI has its onset before the age of 40, and in some cases, may occur as early as age 11 years.[10,11] The focus of this project is this younger patient group, adolescents with POI, about whom little is known.

3.2 Previous Experience of Key Personnel

Our team of investigators represent a skilled, experienced, multi-disciplinary research team with a track record of successful collaboration, and is well-equipped to meet the challenges of the proposed protocol. The group's expertise includes successful clinical trial for patients with eating disorders and several years of experience in clinical investigation and data analysis. Therefore, the proposed recruitment, enrollment, and evaluations for the current project will be feasible for our investigative team.

4. Study Aims and Hypothesis

Our overarching hypothesis is that estrogen deficiency in adolescents with POI (due to unknown or 'idiopathic' causes) leads to: subnormal bone density (axial and peripheral), altered bone structure and geometry, increased truncal body fat; cognitive deficits, and compromised health-related quality of life (HRQL). We hypothesize that in this pilot study, standard care HRT, provided as transdermal estrogen, will improve these outcomes for adolescents with this rare diagnosis.

Specific Aim 1: To identify the clinical features associated with idiopathic POI and define the phenotype of adolescents at their initial diagnosis.

- To define the phenotype of adolescents with POI by comparing axial and peripheral bone mineral density (BMD), peripheral bone structure and cross-sectional geometry, body composition, cognitive functioning, and HRQL versus healthy control subjects at presentation.
  Hypothesis: *Adolescents with POI will present with lower BMD; altered bone structure and geometry; increased truncal fat; and decreased cognitive processing speed, measures of episodic/working memory, and HRQL compared to age-matched controls.*

Specific Aim 2: To assess the response of adolescents with POI to HRT over 24 months.

- To compare the change in axial BMD and body composition; peripheral BMD, bone structure and cross-sectional geometry; cognitive functioning; and HRQL in adolescents with POI after initiation of transdermal estrogen therapy compared to untreated healthy control subjects, matched for age, sex and race/ethnicity.
  Hypothesis**:** *Adolescents with POI (due to idiopathic causes) who receive transdermal estrogen as HRT will have beneficial changes in each of these physical and psychological outcomes after treatment.*

5. Design and Methods

5.1 Design Overview

This pilot study will observe the progression of newly diagnosed POI patients physical and psychology outcomes after initiating standard of care HRT treatment in comparison to healthy female control participants' physical and psychology health over 24 months. Ten POI patients will be recruited aged 11-18 years. Ten healthy female control participants age 11-18 years will be recruited. The control group will reflect a comparison group similar to the POI patient group. As bone density, body composition, and cognitive domains continue to mature throughout the teenage years, this comparison group will provide an important metric of normal growth and development. At the time of recruitment, we will only recruit and enroll patients with POI patients secondary to idiopathic or select organic etiologies. We may learn throughout the course of the study that lab results may indicate an underlying organic cause. We have excluded in our recruitment and enrollment cases of primary POI secondary to trauma, radiation or chemotherapy.

5.2 Patient Selection

In total, 20 adolescent females aged 11-18 years will be recruited.

5.2.1 Inclusion Criteria for POI patients

The participant must:
1. Be willing to give informed consent/assent
2. Have a diagnosis of POI based on 2 elevated serum FSH levels obtained >1 month apart
3. Be English-speaking

5.2.2 Exclusion Criteria for POI patients

The participant must not:
1. Have other chronic disease known to affect bone health (e.g., cystic fibrosis, celiac disease, etc.)
2. Have an identified secondary cause of ovarian insufficiency (eg radiation, chemotherapy, etc.)
3. Have POI in the setting of Turner syndrome, Fanconi Anemia, galactosemia, or Perrault syndrome (as associated neurological/medical sequelae could confound baseline measures)
4. Have used medications known to affect bone metabolism over previous three months (e.g. anticonvulsants, chronic use of glucocorticoids, Depo-Provera, oral contraceptive pills)
5. Be currently pregnant (to be confirmed by pregnancy testing)

5.2.3 Inclusion Criteria for Healthy Adolescent Control Participants

The participant must:
1. Be similar in age and race group to the idiopathic POI group

a. Control participants age must be within one year of age from the POI participant at the time of enrollment. Age may be within one year older or one year younger
b. Race of controls participants will be matched based on race of POI patient participants

2. Have a BMI within 20% of the BMI of the case-matched participant
   a. BMI >32 kg/m^2 cannot be included due to DXA and pQCT measure consistency
3. If postmenarchal, will be regularly menstruating (cycles between 21-35 days)
   a. if POI participant is <12.5yrs (mean age of menarche) will match with a pre-menarchal control participant
4. Be English-speaking

5.2.4 Exclusion Criteria for Healthy Adolescent Control Participants

The participant must not:

1. Have a chronic disease, known to affect bone metabolism (e.g., cystic fibrosis, celiac disease, sickle cell disease, inflammatory bowel disease etc.)
2. Be receiving medications known to affect bone metabolism over previous three months (e.g. anticonvulsants, chronic use of glucocorticoids, Depo-Provera, oral contraceptive pills etc.)
3. Have a learning or developmental disability
4. Be currently taking any SSRIs, antipsychotics or have any documented problems with anxiety or depression
5. Be currently pregnant (as confirmed by pregnancy testing)

5.3 Recruitment Methods

Adolescents with idiopathic POI and healthy adolescent control participants will be recruited through the CCHMC Teen Health Center/Adolescent Medicine, Endocrine Clinic, or Pediatric/Adolescent Gynecology Clinic. We will be notified by the interdisciplinary POI team of upcoming new POI patient appointments that will be within our recruitment age range. We will review the schedules in Adolescent Medicine and Endocrinology for patients that may have a new diagnosis of POI. The study team will notify providers to contact the CRC if the patient does receive a diagnosis of POI. These will be patients who have not yet begun HRT. We will review the primary care THC schedules to recruit control participants. Potential participants will be approached at previously scheduled visits with their healthcare providers. Providers will be told about the study and eligibility criteria. Review of electronic medical record will be performed for both POI and control participants prior to approaching each participant to determine initial eligibility.

Flyers will be posted throughout the medical center to recruit for control participants. Interested participants who contact the study team by phone or email will be asked screening questions regarding exclusion criteria to determine eligibility in addition to review of electronic medical record.

The Office for Clinical and Translational Research (OCTR) healthy controls list will also be utilized to recruit healthy control participants that match POI participants in accordance with the inclusion criteria. The OCTR list consists of patients who have previously agreed to be contacted for research study opportunities.

For POI patients, eligibility criteria must be confirmed. For control participants, we are interested in eligibility criteria and similarity to the POI group. In order to minimize disruptions to clinical care, protected emails will be sent to attending physicians prior to approaching their patients for their potential participation in the study. Patients will be informed about the opportunity to participate in the study during their clinic visit. Verbal permission will be obtained to allow a Clinical Research Coordinator (CRC) to provide further study details. For those who assent, the CRC will take them to a private location to provide more information about the study and invite patient to enroll. Once the patient agrees to participate, a visit to sign consent forms and to complete baseline assessments will be scheduled immediately or via a follow up call. If extra time is needed to confirm study participation, the CRC will obtain verbal permission to follow-up with a phone call within two weeks.

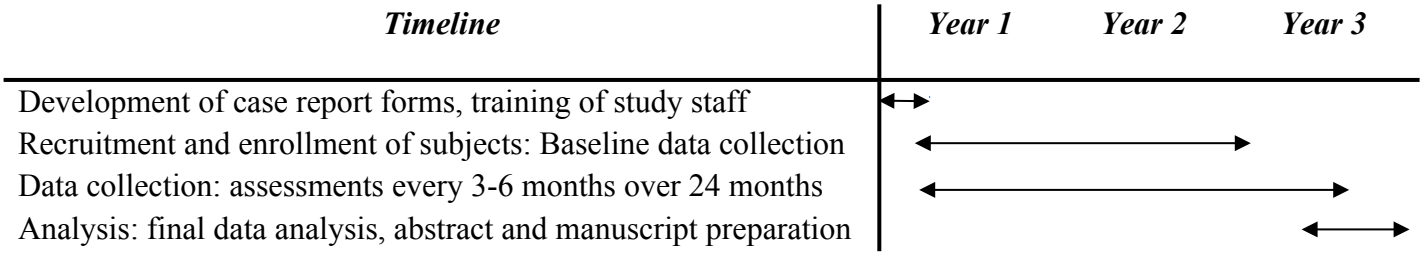
If there is minimal time for study discussion, they will have the option to be contacted by the CRC to be provided with more information. An identified enrollment log will be maintained to track patients who decline to enroll or do not meet study participation criteria. Reasons for declination will be documented on an enrollment log (Appendix I). Other than number of patients approached for the study, results of this log will be used to detect selection or recruitment bias and will not be included in presentations or publications.

5.4 Subject Availability

Since the POI Clinic was launched in 2016, 18 patients have been seen. We anticipate enrolling one new POI patient per month until we reach our goal of 10 POI patient participants. We will enroll newly diagnosed POI patients who have yet to begin HRT or have only been on HRT for one month. From our patient clinical registry, the CCHMC Teen Health Center clinic saw **10,139 outpatient visits** last year (primary care visits and adolescent consults). We aim to enroll approximate one female control participant per month from this pool until we reach out goal of 10 healthy female control participants. We will begin recruitment with our POI cohort, and then match with healthy controls.

5.5 Study Duration

The chart below shows the timeline of study events. The first month will be devoted to development of study flowchart and Manual of Operations, training of study staff, and creation of a study database. Recruitment will begin shortly after with data collection and follow-up assessments occurring within 2.5 years. Final data analysis and manuscript preparation will occur over 6 months.



5.6 Subject Withdrawal Criteria

If a participant refuses to continue with the study and/or does not show for 2 study visit appointments in a row, attempts will be made to continue to contact by telephone. Records will be reviewed to determine whether outcome events have occurred unless the subject specifically refuses follow-up. Reasons for withdrawal will be documented for all withdrawn subjects. Control participants will be withdrawn from the study if they do not attend study assessment visits or begin a medication that may affect bone metabolism or met other exclusion criteria. POI participants will be withdrawn from the study if they do not attend study assessment visits and/or are deemed by the study physician to be noncompliant with the study intervention (i.e., transdermal estradiol patches). If participants become pregnant during the study course, they must be withdrawn from the study due to radiation exposure with the DXA and pQCT scans.

6. Study Procedures

This study will be conducted in the Schubert Research Clinic. Participants will complete a study visit, at baseline, 3, 6 and 12, 18, and 24 months (Table 1; Appendix II). Participants will complete a follow up phone call at 15 months. If subject is unable to attend 3M visit, we will mail them the questionnaires with a self-addressed envelope and complete a follow up call to assess for adverse events and medication changes. Due to the imaging and/or blood draw, all other study visits will need to take place in the Shubert Research clinic

6.1 Process of Obtaining Consent

Participant consent/assent will be obtained at the baseline visit in language that is understandable. The PI or CRC will verify eligibility and explain the nature of the study and potential risks and benefits to the participant. In addition to obtaining parental consent, assent will be obtained from potential participants less than 18 years. Participants 18 years at study entry will sign an adult consent. Permission to access medical records will be obtained. Medical records will be used to track events leading to participant "lost to follow-up". All consent forms used will include the "AUTHORIZATION FOR USE/DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR A RESEARCH".

Participants who enrolled prior to the study extension will be given the option to extend their participation at their next study visit or contacted via phone to invite them to continue their participation if they have completed the established protocol. Participants will be re-consented/assented if they wish to continue their participation. If a POI case declines to extend their participation, then their matched control will not be invited to be in the extension.

6.2 Measure of Outcomes

Appropriate personal protective equipment will be worn during study visits in compliance with all institutional and public health guidelines.

6.2.1 Anthropometrics and Health History: A medical history and health assessment will be completed. Weight will be obtained using a single calibrated scale (Tronix, Carol Steam, IL) and height by a stadiometer (Detecto, Webb City, MO). Calibration of the scale is monitored daily; recalibration occurs when readings deviate by > 0.1 kg. A urine pregnancy test (SA Scientific, LTD, San Antonio, TX) will be performed prior to the DXA and pQCT scans, per hospital protocol. As used in our previous clinical trials.[12,13]

6.2.2 Nutritional intake and physical activity: Nutritional Intake and physical activity will be evaluated using self-administered, validated surveys [Youth/Adolescent Questionnaire (YAQ) and Youth/Adolescent Activity Questionnaire (YAAQ)], as diet and exercise can have a significant impact on bone accrual during adolescence.[14,15]

6.2.3 Cognitive Measures: Cognitive processing speed and episodic/working memory will be assessed using tools from the NIH Toolbox Cognition Battery. To assess overall intelligence, we will administer the Picture Vocabulary Test (designed for age ≥3yr; assesses receptive word knowledge that is highly associated with overall intelligence). To evaluate cognitive processing speed, Pattern Comparison Processing Speed Tests will be administered (designed for age ≥7yr, which assess the amount of time necessary to process a set amount of information, or, conversely, amount of information that can be processed within a specified unit of time). To assess episodic memory, the Picture Sequence Memory Test will be used (for age ≥ 8yr; assesses cognitive processes involved in the acquisition, storage, and retrieval of new information), and for working memory, the List Sorting Working Memory Test (for children and adolescents, age ≥ 7yr; assesses ability to store information until the amount of information to be stored exceeds one's capacity to hold that information) will be administered.[16] The Children and Adolescent Memory Profile (ChAMP) will be administered.[17] The ChAMP is a norm-referenced test of memory and learning that was designed for use with children, adolescents, and young adults ranging from 5 through 21 years. This test is administered directly to the participant by a trained examiner. The survey is a comprehensive screen that allows both memory screening and in-depth memory evaluation. The ChAMP includes 4 Subtests (Lists, Objects, Instructions, Places), each with immediate and delayed evaluation modules. Composite scores yielded from this measure include: verbal memory index, visual memory index, immediate memory index, delayed memory index, and total memory index. The ChAMP takes approximately 35 minutes to administer.

During the ChAMP and NIH toolbox assessments certain adjustments can be made to limit the amount of time the CRC and participant are within 6 feet of one another, and to limit the touching of shared objects. Adjustments can include sitting at least 6 feet away from participants for all verbal assessments, instructing the participant to follow prompts on the iPad (rather than the study staff touching the iPad), and ensuring frequent hand sanitization throughout the assessments. These precautions should be followed anytime it is recommended by the institution or public health officials.

6.2.4 Quality of Life, Mood, and Sexual Health Measures: The Child Health Questionnaire–Child Self-Report Form (CHQ-CF87) is an 87-item self-report validated questionnaire designed for children/adolescents age 10-18 years[18,19] The tool consists of 12 summated subscales and is designed to measure the physical and psychosocial health of adolescents. The subscales include change in health in the last year, bodily pain, behavior, mental health, among others. Items are scored from 0-100, except for the change in health during the last year, and family cohesion variables, which are scored from 1-5. Higher scores on all other variables indicate better quality of life. This instrument has a record of reliability and validity for evaluating aspects of health that are pertinent across age, gender, health condition, and socioeconomic status in adolescents.[18] The Menopause Rating Scale (MRS) is a 11-item self-report validated measure to assess symptoms associated with menopause, which these patients often experience.[20] Anxiety will be assessed using the self-report Screen for Child Anxiety Disorders (SCARED) assessment.[21] Participants will complete the Child Depression Inventory (CDI-II) to assess for depression.[22] Participants age 18 and over will also complete the Beck Depression Inventory II (BDI-II) to measure levels and patterns of depression.[23] Necessary protocols are developed for handling cases in which participants may present with elevated depression and/or suicidal ideation to ensure their safety.

6.2.5 Study Medications: The intervention in this trial represents the standard of care for patients with POI. In an open-label fashion, participants with POI will receive transdermal estradiol (beginning at a dose of 25 µg/patch applied weekly), with the dose increased at 3, 6 and 12, and 18 months (to 37.5, 50, 75, and 100 µg/patch). There will be the most clinical improvement during the treatment phase from baseline to 12 months, as transdermal estradiol dosing is incrementally increased throughout the trial. This study design will provide data to monitor trends as POI patients initiate treatment. POI patients will be eligible to participate if they have not yet initiated transdermal estradiol or have only been using transdermal estradiol for one month or less. The study medication will be administered to participants with POI at study visits or mailed to their home if additional supply is needed. The transdermal estradiol patches will be provided by Investigational Pharmacy utilizing the Investigational Drug Service.

Months:	0	3	6	12	18	24
Transdermal Estradiol dose	25 µg	37.5 µg	50 µg	75 µg	100 µg	100 µg

Oral medroxyprogesterone acetate will be added (10 mg/day for 12 days/month) in the event of spontaneous vaginal bleeding or spotting over the 24-month protocol. This regimen has been used previously in women with POI[24] and represents the current standard of care for a hypogonadal adolescent girl.[25] The appropriate dose and regimen for optimal bone development remains unknown. However, transdermal estrogen appears to be safer as the hepatic first-pass effect is bypassed, with less effects on thrombosis and minimal IGF-I suppression (compared to oral estrogen).[24-26] If participants are found to be vitamin D deficient [25-hydroxyvitamin D, 25OHD < 20 ng/mL], they will receive vitamin D3 treatment, 2000 IU daily for 6 weeks, per recommended guidelines for adolescents,[27] with a follow-up 25OHD obtained at the next study visit. Adherence will be assessed via patch/pill counts at follow-up study visits, as well as monitoring of serum estradiol concentrations.

6.2.6 DXA Measures of Bone Mineral Density and Body Composition:

To assess the axial (central) skeleton, we will obtain DXA measures of the lumbar spine, hip and whole body at baseline, 6, 12, 18, and 24 months. If baseline DXA measures were obtained as part of clinical care within 6 months prior to study enrollment, these results will be collected from the EMR in order to not expose the participant to unnecessary radiation. Along with the spine, the whole body is a site recommended for BMD monitoring in children and adolescents. The hip is also recommended in mid-adolescence as this site is the one most commonly monitored as part of adult bone density screening. DXA scans will be acquired on a Hologic (Bedford, MA) Horizon densitometer and analyzed with Apex 5.5 software. Age- and race-specific Z-scores will be calculated for all measures.[28] Body composition from the whole body will represent a secondary outcome.

6.2.7 Peripheral Quantitative Computed Tomography:
To assess the appendicular (peripheral) skeleton, bone and muscle measures of the non-dominant radius and left tibia will be obtained by pQCT (Stratec XCT 2000 12-detector device, Orthometrix, Inc., White Plains, NY) at baseline, 6, 12, 18 and 24 months. Scans will be acquired with a voxel size of 0.4 mm, slide thickness of 2.3 mm, and scan speed of 25 mm/sec, and analyzed with manufacturer software version 6. Measurement of the radius and tibia will allow for assessment of both weight-bearing and non-weight-bearing skeletal sites. A scout view will be obtained to place the reference line at the proximal border of the distal (tibial and radial) growth plate. Subsequent scans will be obtained at the 3%, 38% and 66% of tibial length and 3% and 66% of radial length, proximal to the reference line to assess trabecular and cortical BMD (mg/cm^3). Scans at the 66% site will be analyzed for muscle cross-sectional area. The coefficient of variation for short-term precision in pQCT outcomes in our laboratory ranges from 0.5-1.6%.

6.2.8 Laboratory Measures: At baseline and every 6 months thereafter (Table 1), adolescents with POI will have blood drawn via venipuncture using established assays in the main CCHMC Laboratory. We will measure serum bone biomarkers to obtain data on bone formation (osteocalcin + bone specific alkaline phosphatase) and bone resorption (C-telopeptides, CTx), and note responses to HRT. Serum osteocalcin will be measured using an electro-chemiluminescent immunoassay (ECLIA). Serum bone specific alkaline phosphatase will be measured by ELISA. Serum CTx will be measured by enzyme-linked immunosorbent assay (ELISA). Serum 25OHD will be measured by chemiluminescent assay. Serum calcium and phosphorus will be measured using colorimetric assays. Serum estradiol will be measured using a chemiluminescent immunoassay. Serum FSH will be measured by ECLIA and anti-Mullerian hormone (AMH) by quantitative enzyme linked immunosorbent assay. Given known interactions between insulin-like growth factors (IGFs) and bone acquisition, IGF-I and IGF binding protein 3 will be measured by ELISA. As lipid parameters and glucose tolerance could potentially be altered by HRT, total cholesterol; triglycerides; high density lipoprotein; and low density lipoprotein will be assessed. Fasting glucose will be measured using a Hitachi glucose analyzer and fasting insulin by radioimmunoassay. If baseline assays were collected as part of clinical care within three months of study enrollment, they may also be gleaned from the EMR instead of an additional blood draw. In total, the amount of whole blood obtained at each blood draw will not exceed 30 mL or 1 ounce.

6.2.9 Radiograph: Adolescents with POI will undergo a radiograph of the left wrist to assess bone age at baseline, 12-month, and 24- month study visit to monitor skeletal maturity. The follow up radiograph at 12 month and then 24 month will only be completed if the growth plates were not fused. The bone age radiographs is safe as the effective dose of radiation received during each exposure is only 0.2 microSv. If baseline radiograph was obtained as part of clinical care within 6 months prior to study enrollment, these results will be collected from the EMR in order to not expose the participant to unnecessary radiation.

6.3 Other Assessments
Case Report forms (CRFs) will be used to collect data on the past medical and health history of each participant. We have also created unique CRFs to capture data on exercise and diet, obtained from the YAQ. A follow up

phone call will occur at 15 months to collect data on continued health and POI participants estrogen patch use. The CRC will be responsible for assuring completeness of all sections of CRFs.

6.4 Study Windows

Participants will complete 6 study visits total, over 24 months. The ideal study visit window is +/- 1 month from target date. Any visit other than the 3-month visit can be completed up to 3 months after the target date in the event that completing a visit during the ideal window is not possible. If an in-person study visit is not possible, an abbreviated remote visit may be done via phone or mail. The measures that can be completed remotely have been marked with an asterisk in tables 1 and 2.

Tables 1 and 2 show the schedule of procedures for POI participants and healthy female control participants.

Table 1: POI Participant Assessments Over 24-month

Months:	0	3	6	12	15	18	24
Anthropometrics							
Height/Weight	■	■	■	■		■	■
Vital signs	■	■	■	■		■	■
Physical examination	■	■	■	■		■	■
Imaging							
DXA (spine, hip, whole body)	■		■	■		■	■
pQCT (radius and tibia)	■		■	■		■	■
Bone age radiograph (left wrist)	■			■			■
Safety outcomes							
AST, ALT, GGT	■			■			■
Total cholesterol, HDL, LDL, triglycerides	■			■			■
Glucose, insulin	■			■			■
Hormonal and Bone Biomarkers							
Serum BSAP, osteocalcin, C-telopeptides	■		■	■		■	■
25-hydroxyvitamin D	■			■			■
Calcium, phosphorus	■			■			■
FSH	■						
Anti-Mullerian Hormone (AMH)	■						
Estradiol	■		■	■		■	■
IGF-I,	■		■	■		■	■
IGFBP-3	■		■	■			
Prolactin	■						
T4, TSH	■						
Thyroid antibody panel (thyroid peroxidase and thyroglobulin)	■						
21-hydroxylase antibodies	■						
Surveys							
Nutrition survey (YAQ)*	■			■			■
Activity assessment (YAAQ)*	■	■	■	■		■	■
Cognitive processing speed	■			■			■
Memory- episodic and working	■			■			■
Child Health Questionnaire–Child Self-Report Form (CHQ-CF87)*	■	■	■	■		■	■
Menopause Rating Scale (MRS)*	■	■	■	■		■	■
Child Depression Inventory-II (CDI-II)*/	■	■	■	■		■	■

Beck Depression Inventory II (BDI-II)*							
Screen for Child Anxiety Related Disorders (SCARED)*	■	■	■	■		■	■
Children and Adolescent Memory Profile (CHAMP)	■			■			■
Follow Up Phone Call*					■		

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Table 2: Control Participant Assessments Over 24-

Months:	0	3	6	12	15	18	24
Anthropometrics							
Height/Weight	■	■	■	■		■	■
Vital signs	■	■	■	■		■	■
Physical examination	■	■	■	■		■	■
Imaging							

DXA (spine, hip, whole body)	■		■	■		■	■
pQCT (radius and tibia)	■		■	■		■	■
Surveys							
Nutrition survey (YAQ)*	■			■			■
Activity assessment (YAAQ)*	■	■	■	■		■	■
Cognitive processing speed	■			■			■
Memory- episodic and working	■			■			■
Child Health Questionnaire–Child Self-Report Form (CHQ-CF87)*	■	■	■	■		■	■
Menopause Rating Scale (MRS)*	■	■	■	■		■	■
Child Depression Inventory-II (CDI-II)*/ Beck Depression Inventory II (BDI-II)*	■	■	■	■		■	■
Screen for Child Anxiety Related Disorders (SCARED) *	■	■	■	■		■	■
Children and Adolescent Memory Profile (CHAMP)	■			■			■
Follow Up Phone Call*					■		

6.5 Participant Retention and Compliance

To ensure that subjects complete the proposed study, we will continue to employ strategies that have been successful with our team:

Enrollment packet: Once a baseline visit is scheduled, we will send a packet of information including the consent/assent documents for their review, directions to the SRC, a welcome letter, study team contact information, and appointment reminder.

***Flexible scheduling of study visits*:** We will make every effort to schedule all study visits at a convenient day and time for the participant.

***Telephone/Email reminders for each appointment*:** Study visits will typically be scheduled two weeks-one month in advance and participants will be called and/or emailed with a reminder prior to the visit.

***Contact with study team*:** Participants will be provided with all contact information to the PI and CRC and will be encouraged to contact the study team with any questions or concerns.

Birthday Cards: Participants will receive birthday cards from the study team.

7. Special Considerations

DXA: A clinical report with BMD Z-scores will be provided for each participant as is the case for clinical studies. A copy of the DXA report will be included in the medical record for POI participants as this information can be used to guide clinical care. For control participants, a study physician will review the DXA scans. Any control participant with a DXA scan Z-score less than -2.0 SD will be referred for further clinical assessment.

pQCT: As pQCT is a research tool at the present time, a report with the participant's vBMD and other pQCT parameters will be provided to the investigators for the patient's research file. This information will not be included in the medical record as this modality is not used for clinical care at the present time.

***Laboratory Testing*:** The CRC will review results obtained at the study visit, obtaining the data from Epic and reviewing with the PI.

8. Data Management and Analysis/Methods

8.1 Study Organization

All pertinent information including all correspondence with IRB will be kept electronically on the department's shared drive. Logs of recruited patient, including refusals, enrollment will be kept in a confidential file on the shared drive. Confidentiality will be maintained by storing all scan data on a secure, password-protected server, which is located behind an electronic "firewall" in the CCHMC data center. All hard copies of questionnaires, consents, and contact information will be stored in a locked cabinet.

8.2 Data Entry

Biostatistician, Mekibib Altaye, PhD, will advise the PI and CRC on data management activities including data entry of completed study forms, data cleaning, preparation of data sets, and conducting descriptive statistical analyses. A REDCap (Research Electronic Data Capture) database will be constructed. REDCap transmission is encrypted and was developed specifically around HIPAA-Security guidelines. The study coordinator will be responsible for entering the data into the database. All data will be entered or electronically imported and checked using quality assurance procedures for double verification, range, and error checks.

8.3 Quality Control Methods

Standard procedures to ensure accurate and reliable collection of data will include designing data forms with clear instructions on all data collection activities. A study ***Manual of Operations*** will be developed to describe the study's purpose, eligibility criteria, and all study procedures. Manuals will be available to study staff to ensure standard and accurate implementation of the protocol and be used for staff training purposes. The CRC is trained to obtain informed consent and assent, conduct research interviews, complete case report forms, and administer questionnaires. Quality control for DXA and pQCT measurements focuses on three elements: machine performance and calibration, scan acquisition, and scan analysis. Monitoring of DXA performance involves daily scanning of an anthropomorphic spine phantom, scanning of a total body phantom 3 times per week to assess calibration for total body BMC and soft tissue masses; and an "air scan" weekly to assess alignment of the tabletop. Calibration of the pQCT scanner is checked before use each day by scanning a phantom with known density. DXA and pQCT technicians receive frequent feedback regarding scan quality and scan analysis. In the event that a participant moves during a DXA or pQCT scan, the scan will be repeated up to a maximum of one attempt total per skeletal site.

8.4 Data Analysis Plan

All statistical analyses will be performed using SAS statistical software (Cary, NC). First, univariate descriptive statistics (e.g., means, medians, frequencies and percentages) will be examined for each variable at each study time point to examine the data distribution, identify outliers and/or erroneous values, and the variability of the measures. We will also examine the continuous variables for deviation from normality, and transform data for normality where appropriate. We will also describe and characterize variables by patient group using the summary measures described above.

<u>Specific Aim 1</u>

We will conduct bivariate analyses to examine the association among demographic and other potential confounders or covariates with outcome measures. We will use Chi-square, two sample t-tests or Wilcoxon rank sum tests, as appropriate, to examine these associations. We will compare the BMD and BMD Z-score between each patient group and healthy control subjects using a two sample t-test or Wilcoxon rank sum test as appropriate. As a secondary analysis we will follow this by modeling the BMD Z-score at presentation as a function of group and potential confounders or covariates (e.g., pubertal status, duration of amenorrhea, height and height for age Z-score), each will be included in a subsequent multivariate model, if found to be significant in the bivariate analysis. This will be accomplished by using a general linear model with an identity link

function. Given the small sample size and the pilot nature of the study the multivariate analysis is considered a secondary analysis but should provide important information for a future larger study.

Specific Aim 2

To examine the impact of the HRT treatment over time, we will initially estimate the trajectory across time points for each group along a 95% confidence band separately. We will then compare the rate of change between the two groups by examining the trajectories and their corresponding 95% confidence interval for overlap. This will be followed by examining the change in BMD or BMD-Z score from baseline (time 0) to 12 months for the idiopathic POI group using a paired t-test. As a secondary analysis we will conduct a linear mixed model analysis where the BMD or BMD Z-score is modeled as a function of time (0, 3, 6, 12, 18, and 24 month) while adjusting for potential covariates or confounders. In this model, the intercept and slope will be treated as random variables to allow them to vary between subjects. The parameter of interest in this analysis will be the coefficient of the time variable that can be used to estimate the rate of change in BMD or BMD Z-score across time. The comparison of the rate of change in BMD Z-score between the idiopathic POI cases and controls will be examined by fitting a mixed model where the BMD Z-score is modeled as a function of group (POI vs Control), time and the time by group interaction (Figure 5). In this case, the parameter of interest would be the coefficient of the interaction term.

For this pilot study, our sample size is restricted by POI patient availability (given the rarity of the diagnosis) and is estimated to be 10 subjects per group.

9. Facilities and Performance Sites

A. **Recruiting Sites:**

Providers at the following recruitment sites will be made aware of the inclusion/exclusion criteria in order to screen for potential subjects:

i. CCHMC Teen Health Center (Burnet Campus; Location C, 2nd floor, Suite 2.622)

ii. CCHMC Pediatric Endocrine Clinic (Burnet Campus; Location E, 2nd floor)

iii. CCHMC Pediatric/Adolescent Gynecology Clinic (Burnet Campus; Location C, 2nd Floor, Suite 2.622; Location C, 2nd Floor, Surgery Clinic space; Location A, Floor 1 Nephrology/Urology/Gynecology Clinic Space)

Participants may be recruited using IRB-approved material including advertising and/or print, electronic, social and digital media, news/broadcast, face-to-face marketing and flyers at Cincinnati Children's and in the community.

B. Study Visits: Study visits will occur at the Schubert Research Clinic (SRC) in Location T at CCHMC. Participants will also be escorted to the CCHMC Dept of Radiology by the CRC to have a bone age radiograph obtained.

10. Potential Benefits

Data from the proposed trial will guide the design of future prospective studies that evaluate the effects of traditional treatments (e.g., HRT), including a longer study to monitor HRT therapy, as well as more experimental treatments (e.g., skeletal agents) that may benefit young women with this rare condition. In addition, findings are expected to open avenues of research for adolescents and women with estrogen deficiency in other clinical settings. For participants, results from the study procedures may help to guide clinical care for both healthy control participants and POI participants, and we expect medication provided to POI patients will be beneficial to both physical and psychology health.

11. Potential Risks, Discomforts, and Inconveniences

Adverse event information will be collected from start through study completion. Potential discomforts involved in the study include:

A. Discomfort completing questionnaires about health history, eating habits, mood, and cognitive processing

B. Inadvertent release of information from health and study records.

C. *DXA, pQCT, and Radiograph*: Every person is exposed on a daily basis to a certain amount of background radiation originating from soil, rocks, outer space and within the body itself. Background radiation exposure in Cincinnati is about 3000 micro Sieverts a year or about 8 micro Sieverts a day. Proposed DXA scans would expose each participant to 10.9 micro Sieverts and pQCT scans would expose participants to total of 4.8 micro Sieverts. The total radiation exposure from the DXA and pQCT study is expected to be 15.7 micro Sieverts. However, in the case that a scan is repeated the maximum radiation exposure at that study visit will be 31.4 micro Sieverts which is less than the amount an individual receives over <5 days from background radiation. [29] The bone age radiographs expected radiation received during each exposure is only between 0.0001-0.1 mSV.[30] Over the entire two year duration of the study, the maximum radiation exposure is 180 micro Sieverts or less than the amount exposed from background radiation for 4 weeks.

12. Data Safety & Monitoring

A data safety and monitoring board will be utilized. In agreement with the grant dispersed from the Patty Brisben Foundation, an audit of records may be conducted. All adverse events will be reported to the CCHMC IRB. The following will be carried out to ensure safety:

Frequency of Data Review*:* A report will be generated by research study team during monthly meetings. Recommendations will be made following meeting and the team will respond appropriately to these recommendations.

Subject Recruitment, Accrual and Retention*:* The rate of subject recruitment and accrual, and adherence to inclusion/exclusion criteria will be reviewed monthly during the recruitment period. During the review process, we will evaluate racial/ethnic distribution of enrolled subjects to ensure diversity. Retention rate will be monitored on a monthly basis throughout the study.

Compliance with Data Collection*:* The PI will review data collection and case report forms to assess accuracy of data collection. This will be done monthly during the follow-up phase of the study. Missing data will be noted, along with a reason for any protocol deviation.

Adverse Event*:* If an adverse event should occur (whether related or unrelated to study participation), it will be documented using an Adverse Event Form (Appendix III). Any serious adverse event (hospitalization, serious illness) will be reported to the PI by the study coordinator within 48 hours of recognition. If a subject were to experience a serious adverse event that is thought to be related to study participation, the subject will be withdrawn from the study protocol. Any non-serious adverse event will be reported to the PI and reviewed.

13. Privacy and Confidentiality Provisions

A. Recruitment for the study, administration of medical history questionnaires, and data collection will be done in private, secluded locations to protect the privacy of the patient. Patients will only be recruited by study

personnel after they have expressed interest in participation in the study to their provider. In this way, potential subjects will never be approached unexpectedly by someone they do not know for research purposes.

B. To protect further confidentiality, a copy of the informed consent will NOT be placed in the participant's medical record. Each participant will be assigned an independent study number and all questionnaires and study data will be tracked and maintained using this number. Please note that many of these results will also be in Epic and can help guide clinical care. For the purposes of scheduling study visits, we will collect name, address, day and evening telephone numbers, and alternate contact information. Only the PI and CRC will have access to the REDCap database which links patient names and study number. Others involved in the project will have access to the REDCap database that only identifies the participants by study number.

C. Chance of Discovering Suicidal Risk: If during the course of the study participants present a reason to believe they are at risk for suicide or otherwise harming themselves, necessary actions will be taken. This may include notifying parents, doctors, and therapist. If this were to occur, we would not be able to ensure confidentiality of study participation.

14. Cost of Participation

Participant and/or their insurance will not be billed for tests or procedures performed for the purposes of this study.

15. Payment for Participation

Each participant will receive $50 per visit for a potential total compensation $300 for completing all study visits. The Clincard system will be used for all monetary compensation.

REFERENCES

1. Gordon CM, Kanaoka T, Nelson LM. Update on primary ovarian insufficiency in adolescents. *Curr Opin Pediatr.* 2015;26(4):511-519.
2. Nelson LM. Primary ovarian insufficiency. *N Engl J Med.* 2009;360(6):606-614.
3. Kalkwarf HJ, Abrams SA, DiMeglio LA, Koo WW, Specker BL, Weiler H. Bone densitometry in infants and young children: the 2013 ISCD Pediatric Official Positions. *J Clin Densitom.* 2014;17(2):243-257.
4. Sadeghi MR. New hopes for the treatment of primary ovarian insufficiency/premature ovarian failure. *J Reprod Infertil.* 2012;14(1):1-2.
5. Gordon CM, Zemel BS, Wren TA, et al. The determinants of peak bone mass. *J Pediatr.* 2017;180:261-269.
6. Bakhsh H, Dei M, Bucciantini S, Balzi D, Bruni V. Premature ovarian insufficiency in young girls: repercussions on uterine volume and bone mineral density. *Gynecol Endocrinol.* 2015;31(1):65-69.
7. Popat VB, Calis KA, Vanderhoof VH, et al. Bone mineral density in estrogen-deficient young women. *J Clin Endocrinol Metab.* 2009;94(7):2277-2283.
8. Gold EB, Crawford SL, Avis NE, et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol.* 2013:70-83.
9. Hubayter ZR, Popat V, Vanderhoof VH, et al. A prospective evaluation of antral follicle function in women with 46, XX spontaneous primary ovarian insufficiency. *Fertil Steril.* 2010;94(5):1769-1774.
10. Covington SN, Hillard PJ, Sterling EW, Nelson LM, Group POIR. A family systems approach to primary ovarian insufficiency. *J Pediatr Adolesc Gynecol.* 2011;24(3):137-141.
11. Pederson J, Kumar RB, Hillard PJA, Bachrach LK. Primary ovarian insufficiency in adolescents: a case series. *Int J Pediatr Endocrinol.* 2015;2015(1):13.
12. Gordon CM, Grace E, Emans SJ, et al. Effects of oral dehydroepiandrosterone on bone density in young women with anorexia nervosa: a randomized trial. *J Clin Endocrinol Metab.* 2002;87(11):4935-4941.
13. DiVasta AD, Feldman HA, Giancaterino C, Rosen CJ, LeBoff MS, Gordon CM. The effect of gonadal and adrenal steroid therapy on skeletal health in adolescents and young women with anorexia nervosa. *Metabolism.* 2012;61(7):1010-1020.
14. Rockett HR, Wolf AM, Colditz GA. Development and reproducibility of a food frequency questionnaire to assess diets of older children and adolescents. *J Am Diet Assoc.* 1995;95(3):336-340.
15. Berkey CS, Rockett HR, Field AE, et al. Activity, dietary intake, and weight changes in a longitudinal study of preadolescent and adolescent boys and girls. *Pediatrics.* 2000;105(4):E56.
16. Beaumont JL, Havlik R, Cook KF, et al. Norming plans for the NIH Toolbox. *Neurology.* 2013;80(11 Supplement 3):S87-S92.
17. Sherman E, Brooks B. *Child and Adolescent Memory Profile.* Lutz, FL: Psychological Assessment Resources, Inc.; 2015.
18. Landgraf J, Maunsell E, Speechley KN, et al. Canadian-French, German and UK versions of the Child Health Questionnaire: methodology and preliminary item scaling results. *Qual Life Res.* 1998;7(5):433-445.
19. Raat H, Landgraf J, Bonsel G, Gemke R, Essink-Bot ML. Reliability and validity of the child health questionnaire-child form (CHQ-CF87) in a Dutch adolescent population. *Qual Life Res.* 2002;11(6):575-581.
20. Heinemann K, Ruebig A, Potthoff P, et al. The Menopause Rating Scale (MRS) scale: A methodological review. *Health Qual Life Outcomes.* 2004;2:45.
21. Birmaher B, Brent D, Chiappetta L, Bridge J, Monga S, Baugher M. Psychometric properties of the Screen for Child Anxiety Related Emotional Disorders (SCARED): A replication study. *J Am Acad Child Adoles Psychiatry.* 1999;38(10):1230-1236.
22. Kovacs M. *The Children's Depression Inventory.* Toronto,ON: Multi-Health Systems, Inc.; 2011.
23. Richter P, Werner J, Heerlein A, Kraus A, Sauer H. On the validity of the Beck Depression Inventory. A review. *Psychopathology.* 1998;31(3):160-168.
24. Popat VB, Calis KA, Kalantaridou SN, et al. Bone mineral density in young women with primary ovarian insufficiency: results of a three-year randomized controlled trial of physiological transdermal estradiol and testosterone replacement. *J Clin Endocrinol Metab.* 2014;99(9):3418-3426.

25. Cintron D, Rodriguez-Gutierrez R, Serrano V, Latortue-Albino P, Erwin PJ, Murad MH. Effect of estrogen replacement therapy on bone and cardiovascular outcomes in women with Turner syndrome: a systematic review and meta-analysis. 2016:[Epub ahead of print].
26. Jospe N, Orlowski CC, Furlanetto RW. Comparison of transdermal and oral estrogen therapy in girls with Turner's syndrome. *J Pediatr Endocrinol Metab.* 1995;8(2):111-116.
27. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. *J Clin Endocrinol Metab.* 2012;97(4):1153-1158.
28. Zemel BS, Kalkwarf HJ, Gilsanz V, et al. Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the bone mineral density in childhood study. *J Clin Endocrinol Metab.* 2011;96(10):3160-3169.
29. SR T, HJ K, DD B, JE H. Effective dose of dual-energy X-ray absorptiometry scans in children as a function of age. *J Clin Densitom.* Winter 2005;8(4):415.
30. Mettler FA, Huda W, Yoshizumi TT, Mahesh M. Effective Doses in Radiology and Diagnostic Nuclear Medicine: A Catalog 1. *Radiology.* 2008;248(1):254-263.