Impact of Individualized Estrogen Therapy on Cardiovascular Disease Risk Parameters in Young Women after Bilateral Oophorectomy: A Randomized Controlled Trial

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Specific aims: Women undergoing bilateral oophorectomy before the natural age of menopause (~51 years) have a higher risk of early mortality in comparison to their age-matched counterparts with intact ovarian function.[1-4] The increase in mortality is primarily related to an increased risk of cardiovascular disease (CVD).[5] The risk can be mitigated, in part, with the use of estrogen-based hormone therapy (ET) given at least until the natural age of menopause, unless there is a contraindication to its use.[1, 5-7] While most professional societies support the use of ET in this group of women, there are no consensus guidelines regarding the optimal estrogen dose required to minimize risk for early mortality, multiple morbidities and accelerated aging, or recommendations regarding how to individualize treatment (tailored to estradiol level) in these young women.[6] The **long-term goal** of this work is to improve and individualize the hormonal treatment of women with bilateral oophorectomy, in order to reduce mortality, CVD risk and other morbidities associated with premature estrogen deprivation. To achieve this goal, we will test the over-arching hypothesis *that an individualized approach to dosing estrogen treatment in young women (<46 years of age) after bilateral oophorectomy would improve parameters of CVD risk*. Current clinical practice is to treat with a dose of estrogen about 2-4 fold higher than that used for vasomotor symptoms in women after natural menopause. While the dose is often increased based on symptoms, this approach is not systematic and the time required to establishing an appropriate dose may be unacceptably prolonged. Furthermore, although symptoms are ameliorated by this approach, it is unclear whether an appropriate dose is reached to mitigate CVD risk and mortality. Therefore, we are proposing to study the impact of hormone therapy on measures of cardiovascular function in young women after bilateral oophorectomy. We will recruit women younger than 46 years of age who undergo bilateral oophorectomy, and are candidates for hormone therapy after the procedure. We will study whether titrating the dose of estradiol to achieve the average premenopausal estradiol level (80-120 pg/ml) is superior to "standard dosing" for improving CVD risk parameters. We will focus on measures of cardiovascular function which show rapid changes to hormonal variation, and are predictors of CVD progression: arterial stiffness, and cardiovascular response to sympathoexcitatory stressors. Our over-arching hypothesis will be tested by the following specific aims:

Aim 1: Compare the cardiovascular function in young women (<46 years) following bilateral oophorectomy who are treated with either the standard dose of estradiol(100 mcg or lower transdermal patch or equivalent oral dose) or titrated estradiol dosing to achieve average premenopausal estradiol level (80-120 pg/ml).

Hypothesis: Women receiving the titrated dosing of estradiol will have better cardiovascular function than women receiving the standard dose of estradiol.

Aim 2: Characterize the relationship between the serum estradiol level and cardiovascular function in young women (<46 years) on estradiol replacement after bilateral oophorectomy.

Hypothesis: There will be a dose response relationship between the serum estradiol level and cardiovascular function.

Aim 3: Compare the body composition in young women following bilateral oophorectomy who are treated with either the standard dose of estradiol (100 mcg or lower transdermal patch or equivalent oral dose) or titrated estradiol dosing to achieve average premenopausal estradiol level (80-120 pg/ml).

Hypothesis: Women receiving the titrated dosing of estradiol will have a body composition closer to their premenopausal state as compared to women receiving the standard dose of estradiol.

Background and Significance: Bilateral oophorectomy performed before the natural age of menopause is an important cause of premature estrogen deprivation, and may not be avoidable in women undergoing surgery for gynecologic cancer or ovarian cancer risk reduction in high risk individuals, such as BRCA mutation carriers.[6] In the absence of estrogen replacement therapy, these women are at increased risk for several adverse health outcomes including CVD, cognitive dysfunction, mood disorders, osteoporosis, accelerated aging and early death. The risk for many of these conditions is higher with younger age at oophorectomy.[6] Many professional societies, therefore, support the use of estrogen therapy for these women, not only for management of menopausal symptoms, but also to mitigate the adverse long-term health consequences of bilateral oophorectomy.[8, 9] The current clinical practice is to use estrogen-based hormone therapy at least until the natural age of menopause unless there is a contraindication to its use.[1] However, in the absence of evidence regarding the impact of the specific estrogen therapy regimens on health outcomes in these women,[10]

treatment is usually tailored to symptom control. The general practice is to **use a dose of estrogen higher than that used for vasomotor symptom treatment in women after natural menopause** in order to approximate the physiologic estradiol levels in premenopausal women.[11] A transdermal estradiol patch delivering 100 mcg per day or lower, or its equivalent oral dose (2 mg per day), is commonly recommended for these young women.[12] However, this choice is not based on rigorous scientific evidence. While this approach serves to alleviate vasomotor symptoms in most cases, it is currently unknown if this dose is an effective strategy to prevent long-term adverse health consequences of early estrogen deprivation, and if an individualized treatment approach may result in better long-term health outcomes. Additionally, estradiol absorption and metabolism can be highly variable, accounting for differences in estradiol levels among women on identical estradiol dosing. **The current clinical practice is to titrate the estradiol dose to symptom control, and not to achieve the average premenopausal estradiol level of 80-120 pg/ml. It is reasonable to speculate that ET in young women with premature estrogen deprivation should be individualized and titrated to estradiol level in order to optimize their long-term health outcomes.**

This novel study has been designed to test hypotheses relevant to the health of a group of **understudied young women** at risk for increased mortality and numerous adverse long-term health outcomes. We are proposing to study the trends CVD risk markers between young women with bilateral oophorectomy who are treated with standard-dose estradiol therapy versus those whose estradiol dose is titrated to achieve the average premenopausal estradiol level (80-120 pg/ml). The CVD risk parameters that will be studied in the current protocol include arterial stiffness and cardiovascular response to sympathoexcitatory stressors.

Previous studies have also reported that menopause accelerates epigenetic aging of blood. There is preliminary data to suggest significant change in steroid metabolome after menopause. Further, abnormal steroid metabolome in menopausal women is associated with decline in executive function, processing speed, and working memory. Our overall hypothesis is that adrenal aging is accelerated after bilateral ooophorectomy, as characterized by abnormal 24h urine steroid metabolome. We are therefore proposing to study the trends in 24-hour urine steroid metabolome after bilateral oophorectomy, and the impact of individualized estradiol dosing on the same.

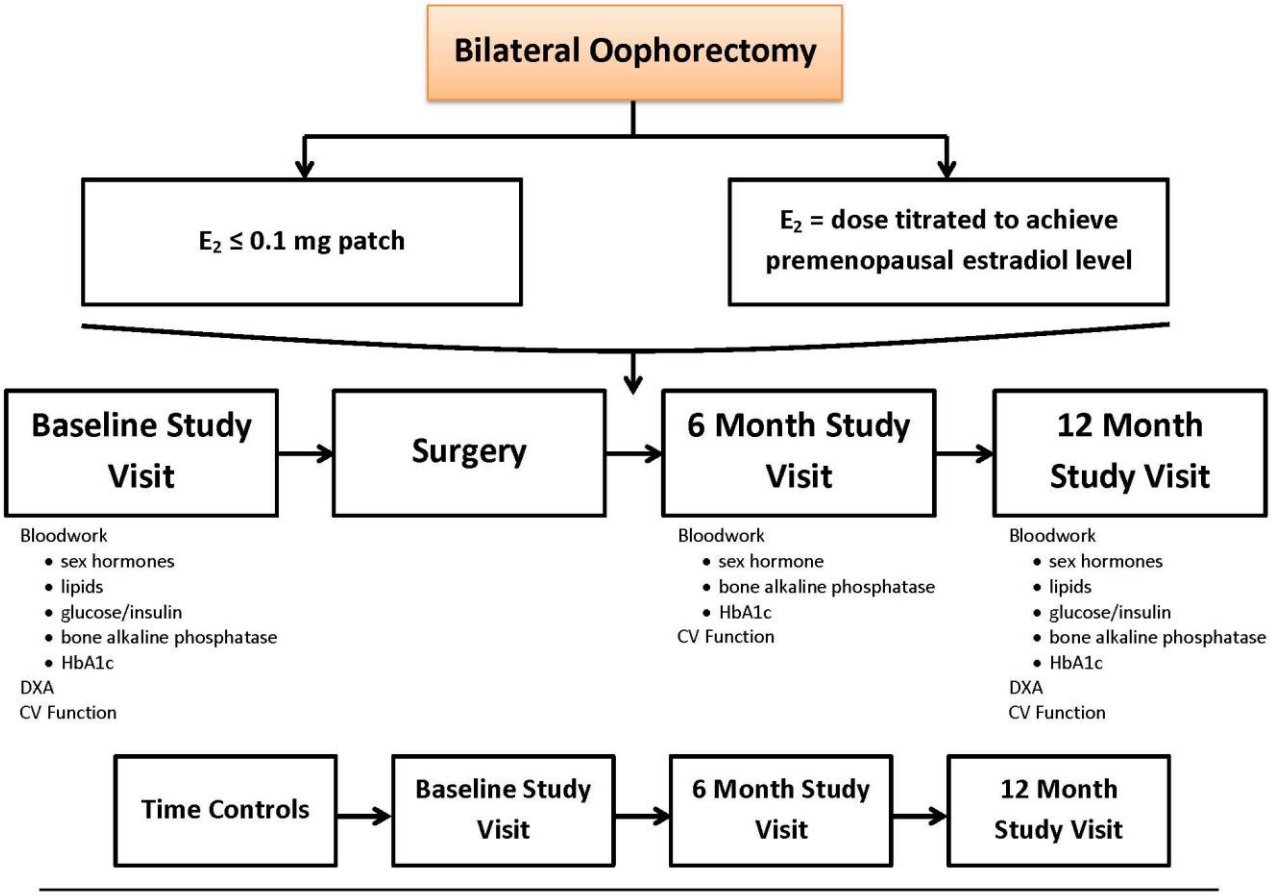


Figure 1: Study Protocol
CV: Cardiovascular

Methods: The overall study protocol is summarized in Figure 1.

Subject Information:

Subject population: Patients scheduled for bilateral oophorectomy or completion oophorectomy (in cases with a prior history of unilateral oophorectomy) in Mayo Clinic Gynecology Department will be considered for participation in the study. We plan to screen about 100 women between the ages of 21- 45 years, over the next three years, in order to accrue 50 research participants. The study members from Gynecology will help maintain a list of patients scheduled for Gynecology consultation for oophorectomy. We will screen these potential participants on the phone using a screening questionnaire prior to their visit in Gynecology. Those patients who meet study criteria will be invited to participate.

Inclusion Criteria:

1. Premenopausal women undergoing (or completion of) bilateral oophorectomy and starting menopausal hormone therapy at Mayo Clinic, Rochester; or premenopausal women not undergoing the procedure for the time control group
2. Currently between the ages of 21- 45 years
3. Able to participate fully in all aspects of the study
4. Able to understand and sign the informed consent.

Exclusion Criteria:

1. Contra indication to hormone therapy
2. Chemotherapy or radiation therapy in the preceding 3 months (only applicable to those participating in the cardiovascular testing)
3. Currently using tobacco, or past use in preceding 12 months (only applicable to those participating in the cardiovascular testing)
4. Current use of medication that alters autonomic or vascular function (e.g. α-blockers, β-blockers, etc.) (only applicable to those participating in the cardiovascular testing)
5. For Time Controls: Are currently pregnant or lactating, or are of child-bearing potential or are likely to become pregnant during the study and unwilling to use contraception; Acceptable forms include:
    a. Barrier methods (such as a condom or diaphragm) used with a spermicide (a foam, cream, or gel that kills sperm)
    b. Copper IUD
    c. Hysterectomy
    d. Tubal ligation
    e. Abstinence (no sex)
6. Any condition or factor judged by the investigator to preclude participation in the study

Study Design and Procedures: This will be a randomized open-label trial. Participants will be randomized 1:1 to one of 2 groups, distinguished by the **estradiol replacement therapy regimen**. One group will receive the standard 100 mcg or lower transdermal estradiol patch as clinically indicated (or an equivalent oral dose; n=up to 25). The other group will be on transdermal (or oral) estradiol therapy titrated to achieve a serum estradiol level of 80-120 pg/ml (n=up to 25). Participants with an intact uterus will also be prescribed a progestogen for endometrial protection. A third group of healthy age-matched premenopausal women, not on any hormonal contraception will be recruited via an advertisement, and will be studied as time controls (n=up to10).

The women deemed eligible based on the phone screen will be scheduled for a baseline medical assessment by an MD in the Mayo Clinic Menopause and Women's Sexual Health Clinic (MWSH). This is a normal part of clinical care for these women prior to oophorectomy. This visit will occur after they have been evaluated in the Department of Gynecology, and a decision to proceed with bilateral (or completion) oophorectomy has been made. Relevant past history including history of pregnancy induced hypertension and gestational diabetes will be obtained. If the patient is eligible and interested in study participation, they will meet with a study team member who will review the informed consent and answer all of the patients' questions.

After they agree to participate and sign the consent form, they will complete questionnaires to assess the following (these questionnaires will also be assessed at 6 and 12 month visits):

a. *Menopausal symptoms severity: Menopause Rating Scale (MRS)*. This self-reported questionnaire consists of 11 items covering psychological, somatic, and urogenital subscales. It can be easily administered and scored, and completed in less than 5 minutes.

b. *Exercise/physical activity: International Physical Activity Questionnaire (IPAQ).* This is a Godin physical activity questionnaire, and a detailed 7-day exercise training record.

c. *Sleep: Pittsburgh Sleep Quality Assessment (PSQI).* This is a self-reported assessment of sleep quality and disturbances over a 1-month period. It consists of 19 items covering sleep quality, latency, duration, efficiency, disturbances, medications, and daytime dysfunction. It can be easily administered and scored in less than 10 minutes.

d. *Mood: Patient Health Questionnaire-9.* The PHQ-9 is a 9-question screen to assess the presence and severity of depression. The results of the PHQ-9 may be used to make a depression diagnosis according to DSM-IV criteria. It can be completed in less than 3 minutes.

e. *Anxiety: Generalized Anxiety Disorder 7 (GAD-7).* The GAD-7 is a self-reported questionnaire to assess the presence and severity of anxiety. It has 7 items and the final assessment regarding the presence and severity of anxiety is based on the sum of the score on all 7 items.

f. *Sexual function (Female Sexual Function Index, FSFI and Female Sexual Distress Scale-Revised, FSDS-R)*: The FSFI, a validated 19-item questionnaire with scores ranging from 2.0-36.0, is designed to assess female sexual function with a lower score indicating greater sexual dysfunction. The FSFI is divided into six domains: desire, arousal, lubrication, orgasm,

satisfaction, and pain. A total FSFI score of less than or equal to 26.55 identifies women with sexual dysfunction. The FSDS-R, a 13-item scale, measures sexually-related personal distress in women with sexual complaints with high test-retest reliability. Scores range from 0-52. A score greater than or equal to 11 indicates clinically significant sexual distress.

g. *Quality of life: Utian Quality of Life Scale (UQOL).* This is a self-reported assessment of perceived well-being distinct from menopausal symptoms. It consists of 23 items covering occupational, health, sexual, and emotional domains. It can be easily administered and scored in a few minutes.

We will ask participants to collect 24h urine sample. A mail-in kit will be sent to the participant, and clear instructions for collection will be provided. Participants on any kind of exogenous glucocorticoid therapy will be excluded from this part of the study. Urine samples will be aliquoted and stored as a part of the IRB # 13-005838 biobank. Urine steroid profiling will be performed in batches in the ICL lab.

The participants will then be asked to report to the clinical research and trials unit (CRTU) for the baseline study visit. When they report to the CRTU for the baseline visit, they will be in the fasting state (8 hours) to complete the tests listed below. Participants will also refrain from vigorous exercise, caffeine, and alcohol for 24 hours prior to the study days.

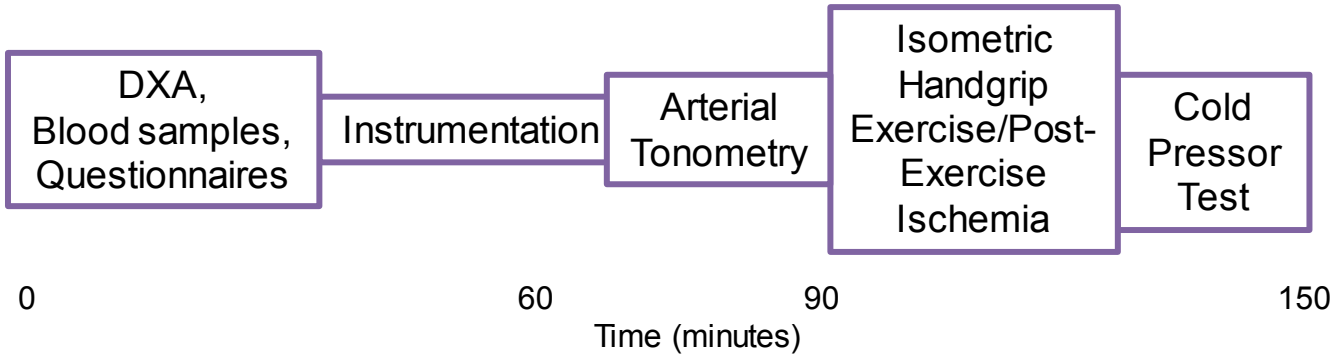
Baseline Study Day Protocol:

1. ***Urine Pregnancy Test:*** Urine Pregnancy tests will be performed on subjects determined to be of child-bearing potential. Nurses in the CRTU will assess the need for this, and confirm through a pregnancy test their status before beginning physiological testing.
2. ***Blood sampling****:* Baseline blood samples will be collected for estradiol, estrone, testosterone (total and free), glucose, insulin, lipid panel, norepinepherine, a bone alkaline phosphatase and HbA1c. We will also plan to store 5 ml each of serum and plasma for future testing, including genomic studies.. The norepinepherine test will be omitted for the limited participation subjects.
3. ***Dual energy x-ray absorptiometry (DXA)****:* Body composition will be measured prior to the study visit in the CTSA body composition core using DXA. All subjects will have whole-body and regional fat and fat-free mass measured by regional analysis of whole-body DXA scans with Lunar software. (Baseline DXA scan for the limited participation subjects can be done within a month after oophorectomy.)
4. ***Cardiovascular monitoring***: Electrocardiogram (ECG) and continuous blood pressure will be monitored and recorded throughout the protocol. We will use a Nexfin to record continuous blood pressure. The pneumatic cuff will be placed around the middle finger of the non-dominant hand for continuous, non-invasive monitoring of blood pressure. The Nexfin has previously been reported to accurately record changes in mean arterial pressure (MAP) during both exercise and apnea, and has been used extensively in Dr. Joyner's experiments. The Nexfin cuff will be positioned at heart level and kept at the same level throughout the duration of the study visit. Cardiovascular activity will be recorded using a 3-lead electrocardiogram (Cardiocap) placed in the Lead II configuration.
5. ***Arterial Tonometry***: Arterial tonometry estimates central blood pressure which is an important determinant of flow through the carotid and cerebral vessels. Additionally, indices of arterial stiffness can be calculated from non-invasive applanation tonometry. Participants will be studied in the supine position after approximately 15 minutes of relaxation. Tonometry transit distances from the supra-sternal notch to the femoral, carotid, and radial recording sites will be measured with a tape measure. High-fidelity pressure waveforms will be recorded non-invasively using a pencil-type Millar Micro-tip pressure transducer from the radial, femoral and carotid arteries in that order in rapid succession. An aortic pressure waveform is derived from the radial pulse using the application of a generalized transfer function (SphygmoCor). Arterial applanation tonometry will then be performed on the radial, femoral, and carotid arteries in rapid succession over a 15 min period. Optimal recording of the pressure wave will be obtained when the hold-down force of the transducer on the artery is such that the resulting waveform has a stable baseline for at least 10 beats.
6. ***Isometric Handgrip Exercise and Post-Exercise Ischemia:*** Maximal Voluntary Contraction (MVC, kg) of the non-dominant arm will be determined as the average of the two highest measurements from 5 trials using a hand dynamometer. Static exercise will consist of squeezing a transducer forcefully enough to consistently maintain a moderate workload (30% MVC; a combination of central command, mechanoreflex, and metaboreflex stimulation) [17, 18]. Visual feedback of force production is provided to the subject. Following

2 minutes of quiet rest, subjects will complete 2 minutes of static handgrip exercise at 30% MVC, followed by 2 minutes of post exercise ischemia (PEI). PEI will begin at the 2-minute mark of exercise prior to the end of contraction and consists of rapid inflation of an occlusion cuff on the exercising arm proximal to the antecubital fossa to suprasystolic pressure (~220 mmHg); the cuff will remain inflated for 2 minutes. PEI traps local metabolites and preserves activation of metabosensitive afferent nerve endings, thus isolating the metaboreflex from central command and the mechanoreflex [19,20,21,22,23]. Participants will be asked to rate their perceived exertion (RPE) at the end of the exercise (Borg scale; range 6 to 20).

7. ***Cold Pressor Test:*** The subject's hand will be immersed up to the wrist in ice water (0-4 degrees C) for 3 minutes while changes in blood pressure and heart rate are monitored. The Cold Pressor Test will be used to measure non-specific sympathetic reactivity. In addition, expected large fluctuations in blood pressure will be used to obtain an index of baroreflex sensitivity. If a participant has a history of, or suspected to have Raynaud's phenomenon this test will not be completed.

Figure 2. Approximate Timeline for Study Day Visits



In addition to the groups above**,** in order to keep an optimum recruitment, we will recruit the women who refuse the cardiovascular testing, for blood-work, questionnaires and DXA scan only.

Surgery and dismissal: Participants will be randomized 1:1 to one of the 2 groups upon dismissal from the hospital after bilateral oophorectomy: standard dose estradiol, (including dosing dictated by personal medical history) or titrated dose estradiol therapy. Women with an intact uterus will also be prescribed a progestogen for endometrial protection. The preferred progestogen will be oral micronized progesterone, given as oral dose100 mg daily or 200 mg cyclically during the first 12 days of month. Women belonging to the titrated dose ET group will initially be dismissed on the standard 100 mcg transdermal estradiol patch. They will have their estradiol levels checked once every 3-4 weeks (clinical visits overseen by our MD investigators in the Menopause and Women's Sexual Health Clinic) and the estradiol dose accordingly titrated until they reach the desired goal of an estradiol level of 80-120 pg/ml. if women in the standard dose group report residual symptoms despite treatment, we will offer titration to a higher dose of ET. Women intolerant of the estradiol patch will be treated with oral estradiol at an equivalent dose. Women with intolerance/side-effects/abnormal uterine bleeding with progesterone will be offered an alternative progestogen for uterine protection. Clinical care of the subjects will be overseen by clinical care providers in the Menopause and Women's Sexual Health Clinic.

The study is designed to have active study participants on varying hormone replacement after oophorectomy as dictated by their clinical needs.

Post-operative Study Visit at 6 Months: The same protocol will be followed as the baseline study day protocol above except we will not check glucose, lipids and insulin levels, and DXA scan will not be performed, as it will take longer than 6 months to see changes in these measures. Blood samples drawn for levels of estradiol and estrone will ideally be drawn on the second day after application of a new patch.

Post-operative Study Visit at 12 Months: Subjects will report to the clinical research and trials unit (CRTU), and the same protocol will be followed as that of the baseline study day visit. All blood samples will be drawn in

the same way as done for the baseline visit. A 24-hour urine sample will be collected again in a mail-in kit as described above. Participants on any kind of exogenous glucocorticoid therapy will be excluded from this part of the study. Urine samples will be aliquoted and stored as a part of the IRB # 13-005838 biobank. Urine steroid profiling will be performed in batches in the ICL lab.

Time controls will complete baseline, 6 month, and 12 month study visits.

Participant Retention: All participants will be remunerated up to $300. Participants will be remunerated $100 for each of the following visits: baseline, six months and twelve months. If a participant refuse the cardiovascular testing, and chooses the option to complete the blood-work, questionnaires and DXA scan only, they will be remunerated $50 for each of the following visits: baseline, six months and twelve months. Amounts will be prorated according to the visits the participant completes. In addition, participants will be provided with Mayo parking vouchers for the time they are onsite for study visits. For CRTU visits, participants will be given six-hour vouchers, and for other visits participants would be given 2 hour parking vouchers. After the long visits in the CRTU, which requires fasting for 8 hours, participants will be provided a meal.

Statistics (prepared in collaboration with Mariza de Andrade, Ph.D.): For the proposed investigation, women 21-45 years of age undergoing bilateral oophorectomy will be randomized to one of two groups, based on the regimen of estradiol replacement therapy (standard versus titrated). In addition to estradiol, patients who do not undergo hysterectomy will receive a progestogen as clinically indicated. A randomization schedule will be generated by the Section of Biostatistics using varied blocked randomization to attain an equal number of women assigned to each treatment group. Covariates not balanced through randomization, such as additional hormone use in patients with hysterectomy, will be accounted for in analysis. For all aims, the primary analyses will be performed using intention to treat with **estradiol dosing regimen** as the *treatment group variable* and secondary analyses will be performed using the **serum estradiol concentration (E2)** as a *continuous explanatory variable*.

Statistical Analysis:
Aims 1 and 3: We will first perform cluster analysis among the variables to identify set of variables that are highly correlated, and regression analysis to identify the dependency between the outcome variable and its covariate. We will also perform a multivariable regression analysis taking into account all the covariates and applying penalized methods such as least absolute shrinkage selection operator (lasso) [17] for covariate selection.
Aim 2: We will plot the data to identify the patterns between the outcome variable and the covariates. We will then fit a mixed-effects model with two fixed effects parameters, the intercept and the slope of the linear trend for the population, and two random effects for each participant, i.e., the deviations in intercept and slope of that participant's time trend from the population values.
Power calculation: The analysis of the 12 month outcomes will be considered the primary analysis, with analyses of the earlier time point used to supplement the primary findings. In all cases, distributional assumptions will be assessed with transformations or non-parametric methods used as appropriate. When the direction of a difference is hypothesized a priori (e.g. women receiving the lower dose of estradiol will have poorer endothelial vasodilator function) a one-tailed test will be used; if the direction is not stated a two-tailed test will be used.
Sample-size/statistical power considerations: In general, a sample-size of N=25 per group will provide statistical power (one-tailed, alpha=0.05) of 82% to detect a difference between groups of 0.75 standard deviation units. Therefore, although preliminary data are not available for each aim and the statistical power will differ for the multiple facets of each aim, we believe that a sample-size of N=25 per group will provide adequate statistical power to test both our primary and secondary hypotheses.

Investigative Sequence and Timeline:

Year	*Aims*	*Number of Studies (Total = 150)*
1	*1 and 2*	*~50 (~15-18 participants and 3 visits each)*
2	*1 and 2*	*~50 (~15-18 participants and 3 visits each)*
3	*1 and 2*	*~50 (~15-18 participants and 3 visits each)*

Summary and Significance: This novel clinical study proposes an individualized approach to hormonal management of women with premature estrogen deprivation. We hypothesize that an individualized approach, tailored to a target estradiol level is more likely to effectively mitigate CVD risk in the young women under study. The results from the current study have the potential to significantly impact the current clinical practice related to the care of young women undergoing bilateral oophorectomy. Furthermore, there is potential for extrapolating these principles to the care of women experiencing premature menopause due to non-surgical causes (primary ovarian insufficiency).

Limitations: Women with an intact uterus after oophorectomy will be on a progestogen in addition to ET for endometrial protection. It is conceivable that the progestogen may impact some of the parameters under study. We are hypothesizing that each woman will serve as her own control and the baseline measurements will be compared to the measurements obtained on estrogen therapy.

Future plans: The current study will provide pilot data regarding the impact of individualized estradiol therapy on CVD risk parameters in young women after bilateral oophorectomy. Our next step would be to obtain extramural funding in order to pursue a larger-scale study to evaluate the effect of individualized estradiol therapy on other long-term health consequences in young women with bilateral oophorectomy, including blood pressure control, changes in metabolic profile (including glycemic control and lipid profile), cognitive function, mood, quality of life and sexual function. We also intend to expand our study population to include women with premature estrogen deprivation due to non-surgical causes, i.e., those with primary ovarian insufficiency, or ovarian failure due to chemotherapy or radiation. It is conceivable individualized hormone therapy, targeted to estradiol levels, will become the standard of care in women with premature estrogen deprivation.

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