Effects of Oral vs. Non-oral Contraceptives on the GH/IGF-1 Axis

Primary Mechanisms Underlying the Effects of Oral vs. Non-oral Contraceptives on the GH/IGF-1 Axis and Bone Metabolism in Young Women

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Protocol Title:

Primary Mechanisms Underlying the Effects of Oral vs. Non-oral Contraceptives on the GH/IGF-1 Axis and Bone Metabolism in Young Women

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1.0 Objectives

1.1 Study Objectives

This study will determine whether the negative effects of combined *oral* contraceptive (COC) therapy on bone turnover are dependent on the route of administration such that an attenuation of these effects is observed when a comparable dose of *non-oral* transdermal contraceptive (TDC) therapy and contraceptive vaginal ring (-CVR) therapy are also tested. Millions of women use COC therapy for birth control purposes or regulation of menstrual cycles. TDC and CVR therapies are relatively new FDA-approved contraceptive alternatives to COC. The purpose of the proposed project is to address the potential mechanism(s) by which *oral* ethinyl estradiol (EE) may negatively impair bone via "first pass" effects on the liver and compare these effects to *transdermally*-administered and *vaginally*-administered EE in young women. We will assess mechanistic effects by way of 2-day serial sampling and by an insulin-like growth factor (IGF-1) generation test. The IGF-1 generation test was developed over 20 years ago and is currently used to diagnose growth hormone (GH) insensitivity (Balhara et al., 2012; Buckway et al., 2001). IGF-1 generation tests may also be used to amplify effects not observable by the assessment of fasting or serial concentrations of systemic IGF-1(secreted by the liver) and its associated binding proteins (Balhara et al., 2012; Buckway et al., 2001). This study will be the first study to examine the physiological mechanisms whereby the route of estrogen administration affects the GH/IGF-1 axis and bone turnover in young women.

The **overall purpose** of this study is to explore differences in liver metabolism and bone turnover of oral versus transdermal and vaginal contraceptive therapy. In an effort to expose the route-dependent effects of oral versus transdermal and vaginal contraceptive therapy on liver and bone metabolism, we will examine the effects of ethinyl estradiol on serially-assessed fasting concentrations of the GH/IGF-1 axis and bone turnover and explore physiological mechanisms underlying hepatic responsiveness to oral versus transdermal and vaginal contraceptive therapy using an IGF-1 Generation Test as a probe.

Specific Aim 1: To examine the effects of oral, transdermal, and vaginal contraceptive therapy on serially-assessed fasting concentrations of the GH/IGF-1 axis.

Hypothesis 1A: Significant declines in IGF-1 concentrations will be observed following COC therapy; whereas, TDC and CVR therapy will result in less severe declines or no change in IGF-1 concentrations, similar to Control.
Hypothesis 1B: The most unfavorable alterations in the IGF-1 binding proteins (IGFBP-3, IGFBP-1) and the acid labile subunit (ALS) concentrations will be observed following COC therapy compared to TDC and CVR therapy and compared to Control.

Specific Aim 2: To examine the physiological mechanisms underlying the hepatic responsiveness to oral, transdermal, and vaginal contraceptive therapy by assessing the effect of exogenous GH on the GH/IGF-1 axis using an IGF-1 Generation Test.

Hypothesis 2A: GH-stimulated IGF-1 concentrations will be reduced to a greater extent following COC therapy compared to TDC and CVR therapy and compared to Control.
Hypothesis 2B: GH-stimulated alterations in IGFBP1 and IGFBP3 will be less favorable following COC therapy compared to TDC and CVR therapy and compared to Control.

Specific Aim 3: To examine the effects of oral, transdermal, and vaginal contraceptive therapy on serially-assessed fasting concentrations of markers of bone turnover.

Hypothesis 3A: A significant *reduction* in serum markers of bone formation (osteocalcin, P1NP) will be observed following COC therapy; whereas, TDC and CVR therapy will result in less severe reductions or no change in markers of bone formation, similar to controls.
Hypothesis 3B: A significant *reduction* in serum markers of bone resorption (NTx and CTx) will be observed following COC therapy; whereas, TDC and CVR therapy will result in less severe reductions or no change in markers of bone resorption, similar to controls.

1.2 Primary Study Endpoints

1) Serially-sampled fasting serum concentrations of IGF-1, IGFBP-1, IGFBP-3, and ALS before and after contraceptive therapy
2) Serially-sampled fasting serum concentrations of osteocalcin, P1NP, NTx, and CTx before and after contraceptive therapy
3) The response of IGF-1, IGFBP-1, IGFBP-3,and ALS to exogenously administered GH before and after contraceptive therapy.

1.3 Secondary Study Endpoints

Preadipocyte factor 1 (Pref-1) and sclerostin, two proteins that have recently been observed to play a role in bone health in adolescent girls and women (Fazeli et al., 2010a; Fazeli et al., 2013), may also be assessed.

2.0 Background

2.1 Scientific Background and Gaps

Despite widespread use of combined oral contraceptive (COC) therapy, investigators to date have failed to definitively answer a basic question: Is COC therapy helpful or harmful to bone health in the 18 million premenopausal women who use them (Enewold et al., 2010). Since COC preparations are often adopted for use by millions of women, understanding the negative outcomes on bone are critically important. For physically active women, COC use may pose a unique threat since recent findings suggest that COC in combination with exercise may be particularly detrimental to bone (Weaver et al., 2001; Burr et al., 2000). The physiological mechanism for the harmful effects of COC use on bone likely involves the growth hormone (GH)/ insulin-like growth factor-1 (IGF-1) axis and suppression of IGF-1 production, and these effects may be dependent on the route of contraceptive administration (Leung et al., 2004). Transdermal contraceptive (TDC) and vaginal contraceptive (CVR) therapies may

offer good alternatives to COC therapy because they may *not* suppress hepatically-driven IGF-1 anabolic effects on bone formation in healthy menstruating women. To date, there are no direct comparisons among COC, TDC, and CVR therapies that explore the influence of contraceptives on the GH-IGF-1 axis in healthy young women. The proposed study will address this major gap in the literature.

2.2 Previous Data

1) Prior work by the PI (De Souza) demonstrated the negative impact of COC use on bone turnover in young women (Vescovi et al., 2008).

Premenopausal women with exercise associated menstrual disturbances (EAMD) (n=6) were assigned to receive two weeks of COC therapy (EAMD+OC) or not (EAMD Control) (n=6). COC therapy was one 28-day cycle of norgestimate (NGM) and ethinyl estradiol (EE). After two weeks, serum concentrations of a marker of bone formation, PINP, and a marker of bone resorption, sCTX, were reduced by 30-40% in EAMD+OC when compared to EAMD Controls (+14%). This finding has been reported by others (Grinspoon et al., 2003; Warren et al., 2005) in response to a randomized clinical trial (RCT) (Grinspoon et al,. 2003) over a longer time frame of 10 months (Warren et al., 2005). Reductions in bone turnover could compromise bone remodeling in exercising women.

2) Prior work by a co-investigator (Misra) demonstrated that treatment with transdermal estradiol among anorexic girls resulted in a significant increase in lumbar spine bone mineral density compared to anorexic girls treated with placebo (Misra et al., 2011).

Anorexic girls (n=96) were randomized to undergo an 18-month treatment with either 100 ug 17B estradiol (with progesterone) or placebo transdermally. At the end of the intervention period, girls who received transdermal estradiol demonstrated greater increases in Z-scores at the lumbar spine (p=0.03) compared to those girls who received placebo.

2.3 Study Rationale

COC use is associated with a higher risk of fracture and may impair optimal bone health in adolescents and young women.

An increased risk of fracture has been reported in COC "ever users" (Cooper et al., 1993), "current and recent users" (Vessey et al., 1998), subgroups of women with longer use (> 8 years) (Vessey et al., 1998), and also in younger women (<25 years) and those with a "low dose average" (Vestergaard et al., 2006). In addition, RCTs report decreases in areal and volumetric BMD at the spine, radius, tibia, and in trabecular bone following COC use (Berenson et al., 2004; Hartard et al., 2006).

For Physically Active Women, COC Use May Pose a Unique Threat

Recent studies suggest that low dose COC therapy and, surprisingly, its use in combination with exercise, may be detrimental to bone and cause decreases in bone mineral density (BMD). In the only prospective study that evaluated the impact of COC use in women randomized to an osteogenic exercise program for 24 months, Weaver et al. (2001) reported a *decrease* in spinal bone mineral content (BMC) and BMD among women taking COCs *and* participating in exercise training compared to women who were not taking COC and participating in the same exercise intervention. Moreover, Burr et al. (2000) reported in the same study that the women who neither exercised nor took COC had the greatest improvement in bone strength, total BMD, and fracture index. Hartard et al. (1997) found the highest BMD in women aged 25-35 years who were characterized by long-term exercise (9.4±4.3yr) and short term use of COC (1.6±1.7 yrs). No beneficial effect of exercise on BMD was evident in the group who performed long term exercise (10.4±4.1 yr) and also had a long term intake of COC (8.2±4.1yr). Lastly, women aged 19-23 years who received COC treatment (20µg EE, 150µg desogestrel) for 5 years demonstrated no change in spine BMD during the 5 years of treatment; however, in the women not taking COC, a 7.8% increase in spine BMD was observed by the end of the study, indicating that COC may suppress bone accrual in young women (Polatti et

al., 1995). The negative findings described above involving the association between physical activity, COC use, and bone health support the proposed study's exploration of the effects of different contraceptives on the GH-IGF-1 axis in women of varying activity levels.

The underlying physiological mechanisms for the harmful effects of COC on bone likely involves the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis which are likely dependent on the route of administration.

GH is released by the anterior pituitary and stimulates the liver to produce and secrete IGF-1, a hormone with potent anabolic effects on bone. Specific receptors for IGF-1 are present on fibroblasts and osteoblasts and IGF-1 directly stimulates collagen synthesis, highlighting the importance of IGF-1 on multiple components of skeletal tissue. The negative alterations observed in the GH/IGF-1 axis with hormone therapy, however, appear to depend on the route of administration. When administered orally, estrogen doses greater than what is naturally present in the circulation must be administered due to the reduced bioavailability of the hormone after active metabolism by the liver. The synthesis of hormones, growth factors, and binding proteins by the liver are affected by this so called "first-pass" effect. As such, it is speculated that the "first-pass" effect of oral estrogen suppresses IGF-1 production and upregulates the synthesis of certain binding proteins, such as insulin-like growth factor binding protein-1 (IGFBP-1), which bind to IGF-1 further reducing its bioavailability. Insulin-like growth factor binding protein-3 (IGFBP-3) and the acid labile subunit (ALS) are both bound to IGF-1 as well, forming a ternary complex. Thus, this "first pass" effect of oral estrogens on the GH/IGF-1 axis is proposed to be one of the primary mechanisms explaining why COC therapy has been associated with negative effects on bone health.

On the other hand, transdermal 17β-estradiol (E_2) administration does not appear to impact the GH/IGF-1 axis in the same manner as orally-administered estrogens (EE, conjugated equine estrogens (CEE), or E_2). Estrogens administered transdermally or vaginally are absorbed directly into the systemic circulation, circumventing the portal circulation that follows intestinal absorption, i.e., the "first pass" effect. In fact, GH, IGF-1, IGFBP-1, and IGFBP-3 concentrations during treatment with transdermal E_2 remain unchanged or increased compared to pre-treatment values in postmenopausal women (Kam et al., 2000; Helle et al., 1996; Bellantoni et al., 1996; Paassilta et al., 2000; Weissberger et al., 1991). Thus, transdermally administered EE may likely exert less negative effects on the GH/IGF-1 axis than oral EE. . Because vaginally-adminstered EE also bypasses the first pass effect in the liver, this form of contraception may have less negative effects on the GH/IGF-1 axis as well when compared to orally-administered estrogens. To date, the effects of transdermal and vaginal EE therapy on these factors has yet to be fully described in premenopausal women.

Data on the GH/IGF-1 axis in premenopausal women administered EE orally, transdermally, or vaginally are, in fact, quite scarce. In a cross-sectional analysis examining the effects of COC in young women aged 24 years, lower serum IGF-1, higher serum IGFBP-1, and no differences in serum GH and IGFBP-3 concentrations were observed in the women taking oral EE compared to non-users (Hansen et al., 2009). Similarly, treatment with two different COC formulations, one containing EE and dienogest and the other containing EE and levonogestrel, for 21 days resulted in a significant decrease in serum IGF-1 concentrations in both groups of young women compared to baseline values (Balogh et al., 2000). On the other hand, a small prospective study of transdermal EE in five adolescent girls aged 12-21 years demonstrated no change in IGF-1 after 12 months of treatment (Harel et al,. 2010). Prospective studies comparing oral and transdermal therapy have only been conducted in premenopausal women with hypopituitarism and adolescent girls with Turner Syndrome undergoing GH treatment (Nabhan et al,. 2009; Isotton et al., 2012; Janssen et al., 2000). With the exception of the study in adolescents with Turner Syndrome in which no differences in IGF-1 concentrations were observed between the girls taking oral CEE and transdermal E_2 (Nabhan et al., 2009), these prospective studies also demonstrated a suppressive effect of oral E_2 on serum IGF-1 concentrations compared to transdermal E_2 (Isotton et al., 2012; Janssen et al., 2000). To date, there are no direct comparisons among the effects of oral, transdermal, and vaginal contraceptive therapy on the GH/IGF-1 axis in healthy premenopausal women. As such, transdermal contraceptive (TDC) and contraceptive vaginal ring (CVR) therapy may offer a promising alternative to COC therapy because it may *not* suppress key anabolic factors necessary for optimal hepatically-driven anabolic effects on bone. The proposed study will address this major gap in the literature.

Two mechanisms for the suppression of IGF-1 production by oral estrogens have been proposed.

The first mechanism that may be contributing to the suppression of IGF-1 concentrations in women taking COC is that there is reduced GH bioavailability when oral estrogens are administered, thus decreasing the production of IGF-1, IGFBP-3, and ALS. This effect has been observed in postmenopausal women with oral administration of estrogen (EE, CEE, and estradiol valerate) (Weissberger et al., 1991; Kelly et al., 1993); however, no such effect has been described in premenopausal women to date.

A second mechanism that may be contributing to the suppression of IGF-1 concentrations in women taking COC is reduced hepatic responsiveness to GH. Indeed, a novel test, i.e., the IGF-1 Generation Test, can be used to test this effect. Testing the responsiveness of the liver to exogenously administered GH has the potential to amplify subtle differences and abnormalities in the liver that are not otherwise detectable from an examination of fasting concentrations alone. The IGF-1 Generation Test has been used to assess the responsiveness and/or sensitivity of the liver to GH in various disease states such as obesity and GH-deficiency (Gleeson et al,. 2005; Buckway et al., 2001) and in adults during hormone therapy (Lissett and Shalet, 2003; Lieberman et al., 1994). Lieberman et al. (1994), the first investigator to use the IGF-1 Generation Test in adults, reported that postmenopausal women on estrogen therapy demonstrated an attenuated increase in IGF-1 concentrations after GH administration compared to women not on estrogen therapy. Similarly, Lissett and Shalet (2003) used the IGF-1 Generation Test in 9 postmenopausal women treated with three different formulations of estrogen for 6 weeks followed by an 8-week washout period between each treatment phase. The estrogen formulations included oral estradiol valerate, a low dose transdermal E_2 (50 ug/d) and high dose transdermal E_2 (200 ug/d). In response to GH stimulation, serum IGF-1 concentrations were significantly lower after oral estrogen treatment and high dose transdermal treatment than prior to estrogen treatment. However, with low dose transdermal E_2, serum IGF-1 concentrations were no different in response to GH administration than those measured prior to treatment. All three estrogen formulations caused a reduced response of IGFBP-3 to GH stimulation; however, an attenuated response of ALS was only observed during transdermal treatment (both high and low dose). These results indicate that the suppressed IGF-1 concentrations observed in postmenopausal women on estrogen therapy may be due to reduced responsiveness of the liver to GH. In addition, these results also suggest that high doses of estrogen may influence responsiveness to GH regardless of the route of administration. The IGF-1 Generation Test, however, has never been performed in premenopausal women taking COC,TDC, and CVR and will provide valuable information about the impact of exogenous estrogens used for contraceptive purposes on the GH/IGF-1 axis and perhaps help to explain some of the negative effects of COC therapy on the skeleton.

3.0 Inclusion and Exclusion Criteria

Specific inclusion and exclusion criteria are outlined below. We will not be including any members of the following populations: adults unable to consent, individuals below 18 years of age, pregnant women, prisoners, and neonates of uncertain viability or non-viable neonates.

3.1 Inclusion Criteria

1) Female
2) Age 18-30 yrs
3) BMI 18-29 kg/m^2
4) Non-smoking
5) Not using hormonal contraceptives for at least 6 months prior
6) Not currently pregnant nor intending to become pregnant in the next 6 months
7) Not lactating
8) No apparent metabolic, endocrine, musculoskeleletal, or severe psychiatric disease
9) Willing to adhere to maintenance of current exercise training and diet and remain weight stable (±2 kg) during study
10) Variable physical activity acceptable, but mode must be primarily weight bearing
11) At least 9 menses in past 12 months
12) Willing to quit taking any current nutritional supplements and take Calcium and Vitamin D supplements for the duration of the study.
13) If 21 or older, a normal PAP smear must be confirmed.

3.2 Exclusion Criteria

1) Non-weight bearing exercise as primary mode of physical activity
2) Known or suspected metabolic or endocrine disease
3) Pregnant
4) Currently consuming large amounts of soy products
5) Regular consumption of grapefruit juice
6) Current clinical eating disorder or other axis 1 psychiatric or bipolar disorders
7) Oral or hormonal contraceptive use in the last 6 months
8) Currently amenorrheic
9) Hyperparathyroidism
10) Liver or renal disease
11) Evidence of malabsorption or skeletal disorder
12) Thyroid abnormalities (controlled hypothyroidism acceptable)
13) Chronic use of non-steroidal anti-inflammatory drugs (NSAIDS)
14) Taking medications known to have interactions with contraceptive therapy (see section 6.4.6.5)
15) Division I Athlete, on or off season
16) Other Exclusion Criteria proposed by the World Health Organization COC Contraindications (Grossman, 2011)
    For this list of exclusion criteria, please see "WHO Criteria for Contraindication to COC Use" in the Supporting Documents section.

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

Subjects will be removed from the study for the following reasons:

1) Failure of subject to adhere to protocol requirements
2) Subject is no longer interested in participating and decides to withdraw
3) Subject becomes pregnant during the study
4) Identification of a disease or underlying health condition that precludes participation in the study
5) Subject becomes unwilling to adhere to maintenance of current exercise training and diet
6) Subject does not respond well to contraceptive therapy or GH administration, potentially compromising her health

3.3.2 Follow-up for withdrawn subjects

When the study group becomes aware that a subject meets any of the aforementioned criteria resulting in removal of that subject from the study, the subject will be informed of this decision as soon as possible. Upon notification of the decision, the subject will be instructed to immediately cease use of the contraceptive therapy (if in one of the treatment groups) or the growth hormone and return all unused pills (for COC), patches (for TDC), rings (for CVR), or injection device (pen and cartridges)/needles (for subcutaneous GH) to the study group. At that point, the subject will be withdrawn from the study.

For subjects who experienced an adverse event (poor response to contraceptive therapy or GH administration that potentially compromised health), the subject will be contacted days or weeks (as appropriate) after withdrawal from study to follow-up and ensure that they are well.

4.0 Recruitment Methods

4.1 Identification of subjects

Recruitment materials such as flyers will be posted across campus and in the local community and may be placed in student mailboxes. Other recruitment methods may involve listserv emails, classroom and club announcements, the Penn State research website for volunteers (http://www.research.psu.edu/volunteer), and newspaper ads.

4.2 Recruitment process

As previously mentioned, recruitment materials such as flyers will be posted across campus and in the local community and may be placed in student mailboxes. Potential subjects may also be recruited via listserv emails, classroom and club announcements, the Penn State research website for volunteers (http://www.research.psu.edu/volunteer), and newspaper ads.

Interested volunteers will contact the Women's Health and Exercise lab via email or phone. When a volunteer calls/emails the lab to inquire about the study, study personnel will provide the potential participant with information about the study. If the interested volunteer initially contacts the lab via email, she will be requested to complete an initial screening questionnaire through REDCap (https://redcap.ctsi.psu.edu/redcap/surveys/?s=HxDHYhEBVU). Once her responses are received and it has been determined that she meets basic eligibility criteria, she will be called by study personnel and permission will be requested to ask more detailed questions that are relevant to inclusion criteria for the study. The lab member will then ask the volunteer the appropriate questions indicated on the REDCap follow-up phone screen, thereby determining the volunteer's initial eligibility. If the interested volunteer initially contacts the lab via phone, study personnel will verbally ask her the initial and, if necessary, follow-up questions that are on REDCap or will verbally go through the hard copy (paper version) of the phone screen.

4.3 Recruitment materials

The following recruitment materials will be used:

- Flyers/posters will be posted across campus and around the community on public bulletin boards, as well as distributed to mailboxes of students living on campus.
- Websites (http://www.research.psu.edu/volunteer)
- Listserv emails
- Emails to potential participants. We may have contact information for potential participants because they had contacted our lab in the past and agreed to complete a phone screen for a different research project. At the conclusion of the phone screen, the potential participant indicated that it was permissible for us to keep her contact information on file so we may contact her regarding future studies.
- Newspaper Ads
- Classroom/club announcements

4.4 Eligibility/screening of subjects

Screening/eligibility questions will be asked according to the uploaded REDCap initial screening form (sent via email or verbally asked via phone as explained above in section 4.2) to determine the basic eligibility of the subject. If it is determined that the volunteer meets the basic eligibility criteria, more detailed questions to determine eligibility will be asked verbally via phone (uploaded REDCap follow-up phone screen). If a potential volunteer contacts the lab via phone, the hard copy (paper version) of the phone screen may also be used to verbally ask the screening questions and record answers. If a subject meets all criteria that are covered in the screening questionnaires, subjects will be invited to come to the lab for review of the informed consent and lab procedures (after consent is signed) to determine final eligibility.

5.0 Consent Process and Documentation

5.1 Consent Process

5.1.1 Obtaining Informed Consent

5.1.1.1 Timing and Location of Consent

All potential participants will be asked questions pertaining to eligibility during a phone screen. After the phone screen is completed and it has been determined that the volunteer meets all basic inclusion criteria that can be assessed via questions, an appointment will be scheduled for her to come to the lab for the screening visit at which time the study will be explained in greater detail and the volunteer will be given the option to participate in the study and sign the consent form. All participants will be consented in the Women's Health and Exercise Lab located in Noll Lab. At the beginning of the screening visit, a member of the study personnel will explain the information within the consent form to the participant, provide the participant with adequate time to read the consent form, and ensure that the volunteer understands the study and the consent form. When the subject is ready and has no further questions, she will then sign the consent form in the presence of a study group member and receive a copy of the signed consent form.

5.1.1.2 Coercion or Undue Influence during Consent

During the consent process, potential participants will be made aware that participation in the study is voluntary and that they can take as much time as needed to decide whether or not to participate in the study. Furthermore, subjects will not receive benefits for being the in the study above and beyond what is approved in terms of compensation and providing the subjects with their results (for example, an increase in pay if employees of the PI are in the study or extra credit/change of grades if students of the PI participate in the study).

5.1.2 Waiver or alteration of the informed consent requirement

Not applicable.

5.2 Consent Documentation

5.2.1 Written Documentation of Consent

Subjects will sign and date a printed consent form. The study group member obtaining consent will also sign and date the form. Subjects will then be given a copy of the signed consent form.

5.2.2 Waiver of Documentation of Consent

Not applicable

5.3 Consent – Other Considerations

5.3.1 Non-English Speaking Subjects

Not applicable. All subjects will be able to speak English.

5.3.2 Cognitively Impaired Adults

5.3.2.1 Capability of Providing Consent

Not applicable.

5.3.2.2 Adults Unable To Consent

Not applicable.

5.3.2.3 Assent

Not applicable.

5.3.3 Subjects who are not yet adults (infants, children, teenagers)

5.3.3.1 Parental Permission

Not applicable.

5.3.3.2 Assent

Not applicable.

6.0 Study Design and Procedures

6.1 Study Design

Summary of Study Design: The proposed study is a preclinical trial comparing the short-term effects of oral and non-oral EE on the GH/IGF-1 axis and bone metabolism. Using a prospective repeated measures design, we will test the effects of 2 cycles of COC,TDC, and CVR use on the GH/IGF-1 axis. The primary outcome variables are the key endocrine components of the GH/IGF-1 axis (IGF-1, IGFBP-1, IGFBP-3, ALS) and bone markers (osteocalcin, P1NP, NTx, CTx) in the serially-sampled fasted state (Specific Aim 1 and 3) and following an IGF-1 Generation Test (Specific Aim 2).

There will be a total of 4 groups in the study as described below. There will be three intervention groups: Group 1 will take 2 cycles of COC therapy (56 days),Group 2 will take 2 cycles of TDC therapy (56 days), and Group 3 will take 2 cycles of CVR therapy (56 days). There will be a control group who will be asked not to take hormonal contraceptive therapy. All subjects will be asked to make no change in food intake and physical activity for the study duration.

Primary variables for Specific Aim 1 and 3 include the GH/IGF-1 axis (IGF-1, IGFBP-1, IGFBP-3, ALS) and markers of bone metabolism (osteocalcin, P1NP, NTx, CTx), and will be assessed using fasting blood samples (collected for 2 consecutive days) during the Baseline Period and during a Post Study Period. Study drug will be maintained until post-testing is complete. Primary variables for Specific Aim 2 (IGF-1 Generation Test) will also include the GH/IGF-1 axis as described above and will be assessed at the same intervals (baseline and post-study period). Exogenous GH will be administered for this aim and one repeated session of 5-hour serial blood sampling may be performed at both baseline and post-study to determine concentrations of GH. COC,TDC, and CVR use will be monitored with the use of calendars and blood measures ofsex hormone binding globulin (SHBG). Physical activity will be monitored with the use of physical activity logs.

Detailed Study Design: This is a prospective study with repeated measures, simple random assignment using blocks of 3 *according to preference for contraceptive therapy* (COC,TDC, or CVR). Those interested in contraceptive therapy will be randomized to the COC , TDC , or CVR group. A group not interested in hormonal contraceptive strategies will serve as the control group (Control).

Subjects: Women, age 18-30 yrs, BMI 18-29 kg/m^2

Study Groups Description	Acronym	Intervention
1) Combined Oral Contraceptives	COC	COC Therapy (Apri or a generic equivalent) Days 1-21: 30µg/d EE, 150 µg/d desogestrel Days 22-28: Inactive pills
2) Transdermal Contraceptive	TDC	TDC Therapy (Xulane) 35µg/d EE, 150µg/d norelgestromin

3) Vaginal Contraceptive	CVR	CVR Therapy (NuvaRing) 15µg/d EE/120µg/d etonogestrel
4) Control Group	Control	No COC, TDC, or CVR

The COC we have chosen, Apri, is a monophasic dosing regimen of 21 active pills and 7 placebo pills, which mimics the dosing regimen of the NuvaRing and the Xulane transdermal patch and is included in the dosing regimen most commonly used for oral contraceptives. In addition, Apri contains the same 3rd generation progestin i.e., desogestrel as the NuvaRing which contains etonogestrel, the active metabolite of desogestrel.
The TDC Xulane (35µg/d EE/150µg/d norelgestromin) was chosen because it is the generic version of the original TDC delivery system and is currently the only TDC system available that is approved for use by the FDA as of 2015. The CVR NuvaRing (15µg/d EE/120µg/d etonogestrel) was chosen because it is the only combined CVR delivery system currently available that is approved for use by the FDA as of 2013. We are purposely not randomizing subjects to either take or abstain from contraception hormone therapy, as the request to abstain from contraceptive use would likely be associated with high levels of noncompliance and cross over between those randomized to receive COC,TDC, or CVR therapy and those not. Although this investigation is a short-term preclinical trial, most published RCTs of contraceptive therapy have not randomized on this aspect of the design for similar reasons (Nappi et al., 2003;Berenson et al., 2001; Gargano et al., 2008; Paoletti et al., 2000). The Control group will complete all procedures with the exception of contraceptive therapy.

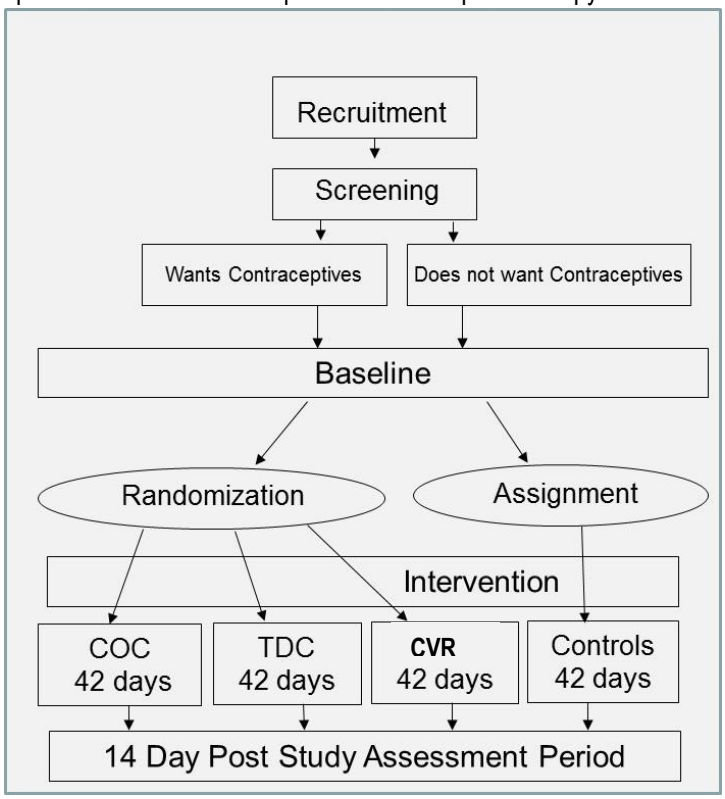

Figure 1. Study Design

Phases of the study: Please see figure 1 for a flow chart of the study procedures. A detailed schematic of study procedures has also been uploaded. The study will involve a screening phase, baseline phase, intervention phase, and post-study phase.

Screening: After subjects are recruited and sign the informed consent, screening will involve collection of anthropometric data; completion of questionnaires to assess calcium intake, health and medical history, exercise history, and menstrual history; a blood sample, physical exam, and urinary pregnancy test.

Baseline (~28 days): The Baseline Period will last for one month and will involve a body weight, whole body DXA scan to assess body composition, pregnancy test (prior to DXA and GH administration), maximal oxygen consumption (VO2max) test to assess aerobic fitness, and completion of a menstrual calendar and exercise training log.. Subjects will also begin taking calcium and Vitamin D supplements. The baseline period will also include collection of 2-day fasting samples to establish baseline concentrations of the GH/IGF-1 axis and bone markers. The IGF-1 generation test will also be conducted during the baseline phase and will involve subcutaneous administration of exogenous GH for 4 days. On the day before the administration of exogenous GH begins, fasting q1hr serial blood sampling for 5 hours may occur at the discretion of the Principal Investigator and with the consent of the research subject. One fasting blood sample will also be collected on day 2, day 4, and day 6 after administration of exogenous GH begins. Finally, one blood sample will be collected during baseline (between days 10-22) to assess concentrations of SHBG.

Intervention (2 cycles): The Baseline Period will be followed by 2 cycles (56 days) of an Intervention Period and a Post Study Period during which study drug is maintained. If randomized to COC, 2 cycles of COC therapy (Apri (or generic), 30µg EE/150 µg desogestrel) will be administered. If randomized to TDC, 2 cycles of TDC therapy (Xulane,

35µg/d EE/150µg/d norelgestromin) will be administered. If randomized to CVR, 2 cycles of CVR therapy (NuvaRing, 15µg/d EE/120µg/d etonogestrel) will be administered. The Control group will receive no intervention. Study procedures during the intervention phase include measurement of body weight and completion of a menstrual and contraceptive therapy log and exercise logs. Supplementation with calcium and vitamin D will continue. A monthly blood sample will be collected during the intervention period for the analysis of SHBG as a measure of compliance to the contraceptive therapy (between days 10-22 of each cycle).

Post-Study Period (~14 days): The post-study period will commence between days 15-17 of contraceptive cycle 2 or days 2-7 of menstrual cycle 2 for the control group, and the study drug will be maintained in the contraceptive groups. During days 15-17/2-7 of cycle 2, collection of 2-day fasting samples to establish concentrations of the GH/IGF-1 axis and bone markers will occur. Fasting q1hr serial blood sampling for 5 hours may be conducted before administration of exogenous GH for 4 days (IGF-1 generation test) (at the discretion of the PI and consent of the participant). If it occurs, it will coincide with the first day of consecutive blood samples. The procedure will mimic the procedure conducted during baseline in that fasting q1hr serial blood sampling for 5 hours will occur the day prior to commencement of subcutaneous administration of GH. One fasting blood sample will also be drawn on day 2, day 4, and day 6 after initiating the GH administration (IGF-1 generation test). During the post-study period, the following procedures will also be completed: measurement of body weight, urinary pregnancy test (prior to GH administration), and completion of menstrual and contraceptive therapy logs and exercise training logs. A monthly blood sample will also be collected for analysis of SHBG to assess compliance to the therapy (between days 10-22 of the second intervention cycle).

We chose to continue therapy during the post-study time period because 1) we wanted a minimal yet adequate treatment period, 2) we wanted to allow ample time to schedule all follow-up testing, particularly when considering that the follow-up procedures require about 12 days to complete, and 3) because it is essential that testing occur while still on therapy.

6.2 Study Procedures (For a detailed schematic of study timeline and procedures, please see uploaded "study timeline" document).

6.2.1 Screening: After study procedures have been explained and the informed consent has been signed, the following screening procedures will occur. The screening procedures may be completed in one study visit or multiple study visits depending on the subject's preference and schedule.

Body Weight/BMI: Participants will be weighed to the nearest 0.01kg on a digital scale in the laboratory with a standard outfit of tee shirt and gym shorts. At the first visit, body height will also be measured. Body mass index (BMI) will be calculated from weight (kg) and height (m^2).

Questionnaires: Participants will be asked to fill out: 1) a health, exercise and nutrition survey (demographic, menstrual, and medical history questionnaires which include prescription medications, NSAID use, history of weight, COC use, exercise patterns, dietary practices, use of supplements, and a physical activity survey); 2) the bone-specific physical activity questionnaire (BPAQ), 3) the brief calcium assessment tool (BCAT), and 4) a medical history questionnaire for the clinical research center (CRC).

Physical Exam: Participants will undergo a physical exam conducted by a physician or nurse practitioner at the CRC to assess general health and to assess if there are any contraindications to a hormonal contraceptive strategy. Participants age 21 or older must present evidence of a normal pap smear within the past 36 months; otherwise, they will be excluded from the study.

Pregnancy Test: Pregnancy tests will be performed using urinary human chorionic gonadotropin during Screening. If a subject is pregnant, she will be informed immediately and excluded from the study.

Blood Sample: A fasting (no food or drink, except water, 12 hours prior to the scheduled test) blood sample will be taken for analysis of 1) complete blood count and chemistry panel, and 2) selected endocrine hormones. To control

for normal changes in blood hormone levels that occur throughout the day and/or with changes in posture, samples will be drawn while in a fasted state, supine position, between 7-10:00 a.m. and before exercise.

6.2.2 Baseline: After subjects complete screening, the Baseline Period will begin on the first day of their next menstrual period and continue until the first day of their next menstrual period. During this time the following procedures for Baseline will occur. The number of visits and the length of each visit will be determined by the subject's preference and schedule.

2-day Fasting Blood Sampling to Establish Fasting Concentrations of GH/IGF-1 Axis and Bone Metabolism: During days 2-7 of the baseline period, blood will be obtained on 2 consecutive days for the IGF-1-related measures of IGF-1, IGFBP-1, IGFBP-3, and ALS, and for markers of bone turnover. Subjects will report to the CRC in the fasted state (no food for 8 hours prior the appointment) in the morning on 2 consecutive days between 0700-0800 hours. Subjects will remain in supine position for 15 minutes prior to the collection of each blood sample.

IGF-1 Generation Test: To assess the impact of COC/TDC/CVR therapy on responsiveness to GH, the IGF-1 Generation Test will be conducted during the first two weeks of baseline.

On the second day of the 2-day fasting blood samples, study participants will be injected subcutaneously with recombinant human GH (rhGH) (Omnitrope) in the morning by a nurse at the CRC at a dose of 0.033 mg/kg/d subcutaneously (Coutant et al., 2012). This dose has previously been used in adults without side effects and is comparable to that administered in the laboratory of our co-investigator, Dr. Misra (Fazeli, et al., 2010b). These injections will occur each morning for the following 3 days. A urinary pregnancy test will be performed prior to initiation of GH administration.

On day 2, day 4, and day 6 *after* subcutaneous administration of GH begins, study participants will again report to the CRC in the morning between 0700-0800 hours in a fasted state for a single blood draw for analysis of IGF-1, IGFBP-1, IGFBP-3, and ALS. Subjects will remain in supine position for 15 minutes prior to the collection of each blood sample.

Subjects will be asked to refrain from resistance training for the duration of the IGF-1 generation test.

At the discretion of the PI and with the consent of research participants, the following procedure may be performed in conjunction with the IGF-1 generation test: On day 1 of the 2-day fasting blood sampling (explained above and occurring between days 2-7 of baseline), an IV will be inserted and blood will be collected each hour for 5 hours in the fasted state (q1hr serial blood sampling for 5 hours). The first draw will be collected 15 minutes after the subject assumes a supine position and the IV is inserted. Subsequent samples will be collected each hour thereafter. The first draw will be assayed for GH, IGF-1, IGFBP-1, IGFBP-3, and ALS; whereas, the remaining 5 draws will only be assayed for GH due to its diurnal rhythm.

Blood Sample for SHBG: In order to establish estrogen exposure in the participants, a blood sample will be drawn between days 10-22 of the baseline period for measurement of SHBG. Subjects will remain in supine position for 15 minutes prior to the collection of the blood sample. If possible, we will try to coincide this blood sample with the blood samples already being taken for the IGF-1 generation test.

Body Weight/BMI: Participants will be weighed weekly during the baseline phase to the nearest 0.01kg on a digital scale in the laboratory with a standard outfit of tee shirt and gym shorts. Body mass index (BMI) will be calculated from weight (kg) and height (m^2) (height measured during screening).

Body Composition Test: Body composition will be assessed using a total body scan on a GE Lunar iDXA (dual-energy x-ray absorptiometry or "DXA"). All scans will be performed by a technician certified by the International Society of Clinical Densitometry. Prior to this test, a urinary pregnancy test will be performed as a precaution.

VO_2max: A maximal oxygen consumption test (VO2max) will be performed to determine fitness level. After a 2-5 minute warm up, the test requires the participant to jog or run on a treadmill while the treadmill increases in speed

and/or slope. The test usually lasts about 7-12 minutes. Blood pressure will be monitored before and after the test, heart rate will be monitored throughout the test, and the level of fatigue experienced by the participants will be recorded. Indirect calorimetry will be used to determine the maximal oxygen consumption.

Physical Activity: Subjects will record any exercise they perform over 7 days using physical activity logs for one week during the Baseline Period. Physical activity will be quantified by min/week, average MET level, mode, frequency, intensity, and total exercise volume. Subjects will be instructed to maintain their typical training regimen throughout the study.

Menstrual Calendar: Subjects will complete a menstrual calendar each day to document menstrual symptoms (degree of bleeding, cramps, spotting, etc.).

Calcium and Vitamin D Supplementation: Participants will begin taking 800 IU of Vitamin D at the beginning of the baseline period. Based on the estimate of daily calcium intake determined at screening using the Brief Calcium Assessment Tool (BCAT), subjects will be supplemented with calcium such that their daily intake approximates 1000 mg (current recommended daily intake for this age group).

6.2.3 Intervention: **After baseline procedures are conducted, subjects will be randomly assigned to groups depending on whether they wished to be considered for COC/TDC/CVR therapy or not. After they are randomized, they will begin the Intervention on the first day of their next menstrual period. Thereafter, they will undergo 2 cycles of the Intervention. A Post Study Period will begin mid-way through the second cycle, and the study drug will be continued during the entire Post Study Period.**

Contraceptive Therapy Administration Plan for COC,TDC, and CVR Groups: On the first day of the Intervention, the participants randomized to the COC group will begin taking the COC pill (Apri (or generic equivalent)). One pill will be ingested orally at the same time each day for the duration of the Intervention, with the exception of one pill-free week. Each pill pack contains a total of 28 pills. Each participant in the COC group will ingest active pills containing 30 µg EE and 150 µg desogestrel from the first pack each day for the first 21 days. To allow for withdrawal bleeding, pills ingested on days 22 and 28 will not contain active ingredients, The participants will begin the second pill pack on day 29 and will ingest a pill with active ingredients (30 µg EE and 150 µg desogestrel) from the second pack each day for days 29-49. On day 50, the participants will immediately begin a 3rd pill pack (no inactive pills will be used from the 2nd pack in order to maintain concentrations of EE during the post-study testing), and will ingest a pill with active ingredients (30 µg EE and 150 µg desogestrel) from the third pack for days 50-56. Thus, a pill with active ingredients (30 µg EE and 150 µg desogestrel) will be ingested days 1-21 and 29-56 of the study period (including intervention and post-study period). Participants in the TDC group will apply a 14 cm^2 patch (Xulane: 35µg/d EE,150µg/d norelgestromin) to the abdomen, upper arm or buttock. The patch will be changed once weekly on the same day each week for weeks 1-3 (days 1-21, removed on day 22) and weeks 5-8 (days 29-56). Week 4 (days 22-28) will be a patch-free week. Participants in the CVR group will insert a vaginal ring into the vagina on Day 1 of the intervention. The vaginal ring will be removed and discarded after 3 weeks of continuous use (days 1-21 of continuous use and removed on day 22). There will be one week (days 22-28) that will be ring-free. A new ring will be inserted for days 29-49. On day 50, the second ring will be removed, and a third ring will be immediately inserted into the vagina. The third ring will remain in the vagina for the last week of the post-study period (days 50-56).

Body Weight/BMI: Participants will be weighed weekly during the intervention to the nearest 0.01kg on a digital scale in the laboratory with a standard outfit of tee shirt and gym shorts. Body mass index (BMI) will be calculated from weight (kg) and height (m^2) (height measured during screening).

Monthly Blood and Compliance Sample for SHBG: In order to test compliance with therapy (COC/TDC/CVR) or no therapy (control group), , a blood sample will be drawn once during each of the 28 days of the Intervention for measurement of SHBG. The blood draws will occur between days 10-22 and between days 38-50 of the Intervention period/Post Study period. Study participants will report to the lab between 7:00 and 10:00 AM in the fasted state and will be asked to remain in the supine position for 15 minutes prior to the blood draw.

Physical Activity: Subjects will record any exercise they perform over 7 days using physical activity logs for one week during the intervention period. Physical activity will be quantified by min/week, average MET level, mode, frequency, intensity, and total exercise volume. Subjects will be instructed to maintain their typical training regimen throughout the study.

Menstrual Calendar and COC/TDC/CVR Log Diary: Subjects will complete a menstrual calendar and COC/TDC/CVR log each day to document menstrual symptoms (degree of bleeding, cramps, spotting, etc.) and therapy use.

Calcium and Vitamin D Supplementation: The supplementation as explained in the baseline phase will continue during the intervention.

6.2.4 Post-Study Period: **Subjects will complete a 2-week Post Study Period during which the study drug will be continued. This phase of the study will begin between days 15-17 of the 2nd intervention cycle. For the Control group, the Post Study Period will begin between days 2-7 of the 2nd menstrual cycle during the intervention.**

Contraceptive Therapy Administration Plan for COC, TDC, and CVR Groups: Administration of contraceptive therapy will continue for the COC, TDC, and CVR groups during the post-study period as described in the intervention section. The COC group will continue to take active pills each day from the second and third pill pack (as described above), the TDC group will continue to change the patch weekly, and the CVR ring will remain inserted in the vagina for the CVR group (but the ring will be replaced halfway through the post-study period). Administration of contraceptive therapy will be done when the post-study testing is complete. Therefore, if the post-study testing is completed a few days prior to day 56 of contraceptive therapy (~day 14 of post-study), then the subject will be instructed to discontinue contraceptive administration. If the post-study testing is completed a few days after day 56 of contraceptive therapy (day 14 of post-study) due to scheduling difficulties, the participants will be asked to continue taking contraceptive therapy as directed (following appropriate schedules for taking the pill or changing the patch or ring) until the post-study testing is complete.

2-day Fasting Blood Sampling to Establish Fasting Concentrations of GH/IGF-1 Axis and Bone Metabolism: During days 1-3 of the post-study period (days 15-17 of the second intervention contraceptive cycle/days 2-7 of the second intervention menstrual cycle), blood will be obtained on 2 consecutive days for the IGF-1-related measures of IGF-1, IGFBP-1, IGFBP-3, and ALS and for markers of bone turnover. Subjects will report to the CRC in the fasted state (no food for 8 hours prior the appointment) in the morning on 2 consecutive days between 0700-0800 hours. Subjects will remain in supine position for 15 minutes prior to the collection of each blood sample.

IGF-1 Generation Test: To assess the impact of COC/TDC/CVR therapy on responsiveness to GH, the IGF-1 Generation Test will be conducted again during the post-study period. It will begin during days 1-7 of the post-study period (days 15-21 of the second cycle).

Similar to the baseline period, study participants will receive an injection from CRC nursing staff of recombinant human GH (rhGH) (Omnitrope) at a dose of 0.033 mg/kg/d subcutaneously in the morning for four days (Coutant et al., 2012). A urinary pregnancy test will be performed prior to initiation of GH administration.

On day 2, day 4, and day 6 *after* subcutaneous administration of GH begins, study participants will again report to the CRC in the morning between 0700-0800 hours in a fasted state for a single blood draw for analysis of IGF-1, IGFBP-1, IGFBP-3, and ALS. Subjects will remain in supine position for 15 minutes prior to the collection of the blood sample.

Subjects will be asked to refrain from resistance training for the duration of the IGF-1 generation test.

At the discretion of the PI and with the consent of research participants, the following procedure may be performed in conjunction with the IGF-1 generation test: On day 1 of the 2-day fasting blood sampling (explained above and

occurring between days 1-3 of the post-study period), an IV will be inserted and blood will be collected each hour for 5 hours in the fasted state (q1hr serial blood sampling for 5 hours). The first draw will be collected 15 minutes after the subject assumes a supine position and the IV is inserted. Subsequent samples will be collected each hour thereafter. The first draw will be assayed for GH, IGF-1, IGFBP-1, IGFBP-3, and ALS; whereas, the remaining 5 draws will only be assayed for GH due to its diurnal rhythm.

Blood and Compliance Sample for SHBG: In order to test compliance with therapy (COC/TDC/CVR) or no therapy (control group), a blood sample will be drawn during the 14 days of the post-study period for measurement of SHBG (if not already completed in the second cycle of the intervention period prior to the start of the post-study period). Study participants will report to the lab between 7:00 and 10:00 AM in the fasted state and will be asked to remain in the supine position for 15 minutes prior to the blood draw. We will try to coincide this blood sample with the blood samples already being taken for the IGF-1 generation test. CVR

Body Weight/BMI: Participants will be weighed weekly during the post-study period to the nearest 0.01kg on a digital scale in the laboratory with a standard outfit of tee shirt and gym shorts. Body mass index (BMI) will be calculated from weight (kg) and height (m^2) (height measured during screening).

Physical Activity: Subjects will record any exercise they perform over 7 days using physical activity logs for one week during the post-study period. Physical activity will be quantified by min/week, average MET level, mode, frequency, intensity, and total exercise volume. Subjects will be instructed to maintain their typical training regimen throughout the study.

Menstrual Calendar and COC/TDC/CVR Log Diary: Subjects will complete a menstrual calendar and COC/TDC/CVR log each day to document menstrual symptoms (degree of bleeding, cramps, spotting, etc.) and therapy use.

Calcium and Vitamin D Supplementation: The supplementation as explained in the baseline phase will continue during the post-study period.

6.2.1 EXAMPLE: Visit 1 or Day 1 or Pre-test, etc. (format accordingly)

Provided above and formatted according to study phases.

6.2.2 EXAMPLE: Visit 2 or Day 2 or Post-test, etc. (format accordingly)

Provided above and formatted according to study phases.

6.3 Duration of Participation

Duration of participation in the study will be approximately 4 months to include a ~2-week screening phase, ~28-day baseline period, 2 cycle (56 days) intervention period, and ~14-day post-study period (which will coincide with days 43-56 of the intervention period).

6.4 Test Article(s) (Study Drug(s) and/or Study Device(s))

6.4.1 Description

Combined Oral Contraceptive:Apri (or generic equivalent). Apri (or generic equivalent) is an FDA-approved combined hormonal contraceptive indicated for the prevention of pregnancy. It is in the form of a pill that is ingested orally each day.

Please note that we may be using other generic forms of Apri, which is sold under various names, including Desogen, Emoquette, Ortho-CEPT, Reclipsen, and Solia.

Transdermal Contraceptive: Xulane. Xulane is an FDA-approved combined hormonal contraceptive indicated for the prevention of pregnancy. It is in the form of a transdermal patch that can be worn on the upper outer arm, abdomen, buttock, or back.

http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=f7848550-086a-43d8-8ae5-047f4b9e4382&audience=consumer

Vaginal Contraceptive: NuvaRing. NuvaRing is an FDA-approved combined hormonal contraceptive indicated for the prevention of pregnancy. It is in the form of a polymeric vaginal ring that is inserted into the vagina. It remains in the vagina continuously for three consecutive weeks.

Recombinant Human Growth Hormone: Omnitrope is an FDA-approved human growth hormone that is prescribed to children or adolescents who have short stature without definable cause or are growing slowly due to various pathological conditions (inadequate GH production, Prader Willi Syndrome, Turner syndrome) or idiopathic reasons. It is also prescribed to adults with GH disorders.

6.4.2 Treatment Regimen

COC Therapy (Apri or one of its generic equivalents): 30µg/d ethinyl estradiol, 150 µg/d desogestrel. The drug is administered orally during all days of the intervention and post-study. Pills ingested during days 1-21 and days 29-42 of the intervention and during days 1-14 of the post study period (days 43-56 when continuing from the intervention) will be active tablets containing 30 µg EE and 150 µg desogestrel. To allow for withdrawal bleeding, pills ingested on days 22 to 28 will be placebo tablets without active ingredients. Duration of administration may vary slightly during the post-study period such that as soon as post-study testing is complete, the ingestion of the pill will be discontinued.

TDC Therapy (Xulane): 35µg/d EE, 150µg/d norelgestromin (entire 14 cm^2 patch contains 0.53 mg EE and 4.86 mg norelgestromin which is delivered over the 7 days). The drug is administered transdermally for a total of ~ 56 days, with the exception of one patch-free week. The patch is changed once per week for weeks 1-3 and weeks 5-8 after baseline (during intervention and post-study). Duration of administration may vary slightly during the post-study period such that as soon as post-study testing is complete, usage of the patch will be discontinued.

CVR Therapy (NuvaRing): 15µg/d EE, 120µg/d etonogestrel (rin contains 2.7 mg EE and 11.7 mg etonogestrel which is delivered over 21 days). The drug is administered vaginally for ~56 days, with the exception of one ring-free week. The ring is changed twice during the study. It will be inserted on day 1 and removed on day 22 of the intervention period. A new ring will be inserted on day 29 and will be removed on day 50. A new ring will be inserted on day 50 and will be removed on day 57, or whenever the post-study testing is complete.

Recombinant human GH (Omnitrope): 0.033 mg/kg/d subcutaneously in the morning for four days during baseline and during post-study period (for a total of 8 days).

No dose adjustments will be made.

6.4.3 Method for Assigning Subject to Treatment Groups

Simple random assignment using blocks of 3 *according to preference for contraceptive therapy* (COC or TDC or CVR) will be used. In other words, those interested in contraceptive therapy will be randomized

using blocks of 3 to the COC group,TDC group, or CVR group. A group not interested in hormonal contraceptive strategies will serve as the control group.

6.4.4 Subject Compliance Monitoring

During the intervention and post-study period, subjects will be asked to record when they take their pill each day (for COC group), when they change the patch each week (for TDC group), or when they change the ring (for the CVR group) on the contraceptive calendar that they are provided. Subjects will also be asked to return pill containers and patch and ring packages (both used and unused). Finally, each month during the intervention and post-study periods, a blood sample will be collected for determination of SHBG concentrations.

Compliance to GH administration will be assessed via the growth hormone log on which the subjects will be asked to record the time of day corresponding to the appropriate dates that the growth hormone was injected. Because this procedure will be completed in the CRC, the nurses and study personnel will also have documentation that the injections were completed.

6.4.5 Blinding of the Test Article

Not applicable. Test article is not blinded.

6.4.6 Receiving, Storage, Dispensing and Return

6.4.6.1 Receipt of Test Article

The contraceptives, Apri (or one of its generic equivalents), Xulane, and NuvaRing, will be purchased from a pharmacy and the labeling will be as follows:

Apri Packaging: **From the packaging:** "Apri 28 Day Regimen blister cards for desogestrel and ethinyl estradiol tablets provide an oral contraceptive regimen of 21 round rose-colored tablets. Each rose-colored "active" desogestrel and ethinyl estradiol tablet for oral administration contains 0.15 mg desogestrel (13-ethyl-11- methylene-18,19-dinor-17 alpha-pregn-4-en- 20-yn-17-ol) and 0.03 mg ethinyl estradiol (19-nor-17 alpha-pregna-1,3,5 (10)-trien-20-yne-3,17-diol). Inactive ingredients include colloidal silicon dioxide, FD&C Blue No. 2 Aluminum Lake, FD&C Red No. 40 Aluminum Lake, hydroxypropyl methylcellulose, lactose monohydrate, polyethylene glycol, polysorbate 80, povidone, pregelatinized starch, stearic acid, titanium dioxide, and vitamin E. Apri 28 Day Regimen blister cards also contain 7 white "inactive" tablets for oral administration, containing the following inactive ingredients: lactose anhydrous, magnesium stearate, microcrystallinecellulose and pregelatinized starch."

For the purposes of this study, each subject will receive three pill packs. The subjects will be instructed to take one pill daily at the same time each day for days 1-56 (including the 14 day post-study period). COC administration will end when the post-study testing is complete (may be a few days before or after day 14 of the post-study period (day 56 when continuing from intervention). The first pill will be taken during the first 24 hours of her menstrual period (day 1).

Xulane Packaging: The patch has a contact surface area of 14 cm^2, contains 4.86 mg norelgestromin and 0.53 mg ethinyl estradiol released over 7 days, or (0.035mg/0.15mg)/24 hours.

The patch itself is a thin, matrix-type transdermal contraceptive patch consisting of three layers. The backing layer is composed of a peach flexible film consisting of a pigmented polyethylene outer layer and a polyester inner layer. It provides structural support and protects the middle adhesive layer from the environment. The middle layer contains polyisobutylene/polybutene adhesive, crospovidone, mineral oil, non-woven polyester fabric, oleyl alcohol and dipropylene glycol as inactive components. The active components in this layer are the hormones, norelgestromin and ethinyl estradiol. The third layer is the release liner, which protects the adhesive layer during storage and is removed just prior to application. It is a transparent polyester film with a fluoropolymer coating on the side that is in contact with the middle adhesive layer.

The Xulane transdermal system uses a 28-day (four-week) cycle. Typically, a new patch is applied each week for three weeks (21 total days). Week Four is patch-free. For the purposes of this study, the week four will be a patch-free week as is typical with this type of contraceptive administration. However, after this patch-free week, a new patch will be administered each week for the next 4 weeks (weeks 5-8). If necessary, a new patch will be administered on day 57 until the completion of the post-study testing at which time it can be removed. Every new patch will be applied on the same day of the week. This day is known as the "Patch Change Day." For example, if the first patch is applied on a Monday, all subsequent patches should be applied on a Monday. Only one patch will be worn at a time. Subjects will be instructed not to cut, damage or alter the Xulane patch in any way. The woman will be instructed to apply her first patch during the first 24 hours of her menstrual period (day 1).

NuvaRing Packaging: NuvaRing (etonogestrel/ethinyl estradiol vaginal ring) is a non-biodegradable, flexible, transparent, colorless to almost colorless, combination contraceptive vaginal ring containing two active components, a progestin, etonogestrel (13-ethyl-17-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3-one) and an estrogen, ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol). When placed in the vagina, each ring releases on average 0.120 mg/day of etonogestrel and 0.015 mg/day of ethinyl estradiol over a three-week period of use. NuvaRing is made of ethylene vinylacetate copolymers (28% and 9% vinylacetate) and magnesium stearate and contains 11.7 mg etonogestrel and 2.7 mg ethinyl estradiol. NuvaRing is not made with natural rubber latex. NuvaRing has an outer diameter of 54 mm and a cross-sectional diameter of 4 mm. The molecular weights for etonogestrel and ethinyl estradiol are 324.46 and 296.40, respectively.

For the purposes of this study, each subject will receive three rings. The subjects will be instructed to insert the first ring during the first 24 hours of her menstrual period (day 1) at the start of the intervention. The ring will be in place continuously for 3 weeks and will be removed on day 22, after 21 days of continuous use. Day 22-28 will be a ring-free week. A second ring will be used for days 29-49 (removed on day 50 after 21 days of continuous use), and a third ring will be used for the remainder of the study (day 50 until the end of post-study period).

Recombinant Human GH (rhGH, Omnitrope) will be purchased from a pharmacy. It is a somatropin (rDNA origin) injection. We will be using 5.8 mg vials of Omnitrope. The vials contain a lypholized powder which is reconstituted with bacteriostatic water (contains benzyl alcohol as a preservative).

The lypholized powder will be reconstituted with the bacteriostatic water by nurses in the CRC. The Omnitrope vial with the diluent can be stored for 3 weeks. For each subject and for each injection, the nurses will draw up into a syringe the exact amount that is needed for that subject on that day. The exact dose to be injected each day depends on the subject's body weight, which will be supplied to the CRC.

For each research participant, nurses in the CRC will inject the human GH subcutaneously each morning for 4 days during the baseline period and the post-study period as the study design indicates. The appropriate dose based on each subject's body weight will be explicitly provided to the CRC.

GH Administration will follow the directions clearly outlined in the Instructions for Use (see attached supplementary information in the drugs section of the application on CATS).

6.4.6.2 Storage

All study drugs will be stored in the Clinical Research Center following the storage requirements by the manufacturer.

Apri oral pills and Xulane transdermal patches will be stored at room temperature (25 degrees Celsius) in a locked cabinet in the CRC. Unused transdermal patches will be kept in their protective pouches. The subjects will be instructed to fold the sticky sides of the used patch together and put back in the protective pouch to be returned to the study group.

The pouches containing the NuvaRing will be stored in a research-designated, locked refrigerator (2-8 degrees Celsius) in the CRC. After being dispensed to the subjects, the NuvaRing can be stored at room temperature (25 degrees Celsius). The subjects will be instructed to keep the pouch with the ring out of direct sunlight and temperatures exceeding 30 degrees Celsius.

The rhGH (Omnitrope) will be stored protected from light in a research-designated locked refrigerator in the CRC at a temperature of 2 to 8 degrees Celsius. This locked refrigerator is only used for study drugs and the temperature is monitored daily. The powder form will be stored until the expiration date, and the reconstituted form that is used for injection will be stored for 3 weeks then discarded.

6.4.6.3 Preparation and Dispensing

The pharmaceuticals in this study (contraceptive therapy and growth hormone) will be prescribed by Dr. Richard Legro or a CRC physician (Dr. Roberta Millard), who are both members of the study team for this research.

Study drug will be assigned to each subject indicating preference for contraceptive therapy by a block of 3 random assignment to oral, transdermal, or vaginal therapy. Each subject will be supplied with three oral pill packets, seven or eight transdermal patches (more will be provided as necessary if a patch falls off during the week), or three vaginal ring pouches. Once assigned to the study group, a CRC nursing staff member and a research WHEL staff member will dispense the study drug to the subject and provide detailed instructions regarding how to take the study drug.
For the rhGH, nurses in the CRC will reconstitute the Omnitrope vial of powder using a vial of diluent (bacteriostatic water with benzyl alcohol). The appropriate dose for each subject will be calculated by CRC and WHEL study staff (0.033 mg/kg/d). Uploaded on the CATS system is a detailed instruction sheet from the manufacturer on how to prepare and administer the drug. The injections will be given by the CRC nursing staff.

In addition to detailed instructions from the study team, subjects will receive the patient information for each drug (when available) describing how to take the drug.

6.4.6.4 Return or Destruction of the Test Article

All empty pill, patch, and ring packets will be returned weekly and properly disposed of on-site.

As stated in the prescribing information, "each NuvaRing (etonogestrel/ethinyl estradiol vaginal ring) is individually packaged in a reclosable aluminum laminate sachet consisting of three layers, from outside to inside: polyester, aluminum foil, and low-density polyethylene. The ring should be replaced in this reclosable sachet after use and discarded in a waste receptacle out of the reach of children and pets. It should not be flushed down the toilet."

The injection device/needles/vials will be properly disposed of on-site (i.e. the needles will be put in a biohazard sharps container).

6.4.6.5 Prior and Concomitant Therapy

No prior and/or concomitant medical therapy will be administered.

Concomitant medications/products within medications that are not permitted during the study include lamotrigine (an anticonvulsant used for epilepsy), phenytoin, barbiturates, carbamazepine, bosentan, felbamate, griseofulvin, oxcarbazepine, rifampicin (rifampin), topiramate, rifabutin, rufinamide, aprepitant, phenylbutazone, ampicillin, tetracyclines, and products containing St. John's wort. Atorvastatin, rosuvastatin, ascorbic acid, acetaminophen, itraconazole, voriconazole, fluconazole, grapefruit juice, ketoconazole, and vaginal miconazale nitrate should also be avoided. Women also cannot be on thyroid hormone replacement therapy or taking HIV (human immunodeficiency virus) or HCV (hepatitis C virus) protease inhibitors.

7.0 Data and Specimen Banking

7.1 Data and/or specimens being stored

All serum that is collected during the study with the exception of the screening blood sample will be stored. The serum will be labeled with the subject ID, time point of the study and date, and the specific draw.

All data collected for each participant as outlined in the study procedures will also be stored. The data will be labeled with subject ID, time point of the study, and date, and may also include height, weight, age, and BMI.

7.2 Location of storage

Serum samples will be stored in a -80 freezer in Noll Laboratory. Specific locations of these freezers include Room 117, Room 121, Room 2 and the Noll Laboratory hallway and basement.

Data will be stored in locked offices and lab spaces in Noll Laboratory to include Rooms 109, 110, 116, 117, 122, 123, 124, 131, 206, and 207).

7.3 Duration of storage

The data and serum samples will be stored indefinitely unless the participant does not provide permission for the samples to be kept indefinitely for future research and requests that the data and specimens be destroyed after use for this particular study. In this case, the data and serum samples will be appropriately destroyed/discarded when all data analysis and manuscripts for the study are complete. Otherwise, the data and serum will be stored indefinitely until the principal investigator decides that it is no longer needed.

7.4 Access to data and/or specimens

Study team members and lab personnel will have access to the data and specimens.

7.5 Procedures to release data or specimens

The data and specimens will only be stored indefinitely for use in our laboratory and that of the co-investigators who are part of the study team; therefore, no procedure for release of data or samples is necessary.

7.6 Process for returning results

The data and specimens will only be stored indefinitely for use in our laboratory and that of the co-investigators who are part of the study team; therefore, no procedure for returning results about the use of data and samples is necessary.

8.0 Statistical Plan

8.1 Sample size determination

Because this is a pilot study to obtain preliminary data for submission of an NIH R01 proposal, we aim to complete 12 subjects in each of 4 groups (COC group, TDC group, CVR group, and control), totaling 48 subjects.

Power analysis is based on the primary endpoint, i.e., average change in IGF-1 levels with the IGF-1 generation test over 28 days in the groups receiving TDC vs. COC. A total of 12 participants (6 each in the COC and TDC estradiol arms) will be required to detect a difference of 1.348*SD in mean change in IGF-1 levels in groups receiving TDC vs. COC, with a power of 86%, using a two sided p=0.05 level test (Weissberger et al., 1991). Because we are adding a group and to ensure that we see a difference among the groups, we aim to complete 12 subjects per group.

To account for a potential screening failure rate of 33% and potential drop-out rate after randomization of 30%, we plan to enroll a total of 100 subjects (25 per group).

8.2 Statistical methods

To determine baseline differences among groups, we will use a one-way analysis of variance (ANOVA). For longitudinal analyses, repeated measures ANOVA as well as mixed linear models will be used to determine if response of the GH/IGF-1 axis to 1) route of administration of contraceptive therapy and 2) exogenous GH differs among groups before and after contraceptive therapy.

9.0 Confidentiality, Privacy and Data Management

9.1 Confidentiality

9.1.1 Identifiers associated with data and/or specimens

The identifiers that will be used to label the study data and/or specimens may include some or all of the following: age, date of birth, height, weight, body mass index, and the assigned subject ID. Samples that are sent to Quest Diagnostics for screening assays will contain the name. However, name and subject ID will not both be present on any data or specimens.

9.1.1.1 Use of Codes, Master List

The list linking the code numbers (subject ID) to the participant's name and contact information will be stored in a password-protected file on password-protected computers. This file will also be stored on a server that only lab personnel can access after they have logged into a password-protected computer using their personal HHD username and password. Access to this list will be limited to lab personnel. After all of the data for the study is entered and analyzed, manuscripts have been published, the study is completed, and it has been determined that the list is no longer needed for data analysis and publication reasons, the list will be destroyed.

9.1.2 Storage of Data and/or Specimens

Both hardcopy and electronic data will be stored in the Women's Health and Exercise Lab in Noll Laboratory. The hardcopy data will be stored in locked offices or labs of the Women's Health and Exercise Lab (Noll Lab Rooms 109,110,122,123,124,116,117,206, 207). Electronic data will be stored on password-protected computers in our laboratory. Only lab personnel can access the data after they log into the computer using their personal HHD username and password. Furthermore, privileges to view the research drives with the lab files is under the administration of the lab supervisors and the postdoctoral scholar. Therefore, only those given permission to view the research drives are able to do so after logging in to the computer.

Specimens will be stored in -20C or -80C freezers that are located in Noll Lab (Noll basement and hallway, Noll rooms 116,117,121,124).

Data and samples will be stored indefinitely until it is determined that they are no longer needed. The exception to this is if subjects did <u>not</u> provide permission for us to keep their samples indefinitely for future undetermined research. In this case, the samples will be destroyed when it has been determined that the samples are no longer needed for this research study (all data has been analyzed and manuscripts published).

The informed consent contains a section specific for sample donation for future research. If permission is granted to keep the samples for future research, participants will initial and sign the consent form in the correct spaces as shown below:

You should initial below to indicate what you want regarding the storage of your leftover blood for future research studies.
a. Your samples may be stored and used for future research studies to learn about metabolic- or endocrine-related conditions (conditions involving hormones).
_____ Yes _____ No
b. Your samples may be shared with other investigators/groups without any identifying information.
_____ Yes _____ No

9.1.3 Access to Data and/or Specimens

Only lab personnel who have completed the appropriate trainings will have access to the data or specimens.

9.1.4 Transferring Data and/or Specimens

Data and/or specimens may be transferred to the co-investigator, Dr. Madhusmita Misra. She is located in Boston, Massachusetts and is associated with the Neuroendocrine Unit of Massachusetts General Hospital and with Harvard Medical School. The transfer of specimens will occur according to guidelines established by Penn State Environmental Health and Safety (EHS) and will occur under the supervision of Penn State EHS. The transfer of data – either paper or electronic – will occur via postal service or e-mail; however, the names of subjects will not be attached to this data to ensure privacy and confidentiality.

9.2 Privacy

The study team that has been approved to work on the protocol will have access to hardcopy data, electronic data, subject contact information, and the list linking the subject name to the subject ID. Study team members will have access to the locked offices as well as their personal HHD login username and password. Access to the files stored on the research drive will be permitted at the discretion of those serving as administrators of the research drive (i.e. the lab supervisors and/or investigators of the study). Information will be stored in locked offices in our laboratory and on password-protected computers. We will never identify the participants' data in publications or presentations resulting from the research.

Interactions with the subjects and collection of personal information will be conducted in a professional manner by trained lab personnel in a laboratory space (office or testing room). The identity and personal information of subjects will be treated as confidential information and remain among the study group.

Only information that is pertinent to completing the study protocol will be asked of the participants.
Research procedures will be explained in detail to subjects, and subjects will not be pressured, coerced, or forced to answer any questions or complete any procedures that they refuse to answer/complete. Subjects who did not wish to answer certain questions or complete certain procedures will be reminded that study participation is voluntary and can be stopped at any time.

10.0 Data and Safety Monitoring Plan

This study does not involve more than minimal risk to study participants.

10.1 Periodic evaluation of data
Not applicable

10.2 Data that are reviewed
Not applicable

10.3 Method of collection of safety information
Not applicable

10.4 Frequency of data collection
Not applicable

10.5 Individual's reviewing the data
Not applicable

10.6 Frequency of review of cumulative data
Not applicable

10.7 Statistical tests
Not applicable

10.8 Suspension of research
Not applicable

11.0 Risks

COC Therapy (for COC group): There are several rare risks associated with using birth control pills including, but not limited to, blood clots, heart attack, stroke, bleeding in the brain, gall bladder disease, formation of liver tumors, breast cancer, high

blood pressure, vision problems, and unfavorable cholesterol levels. It is unlikely that a complication will occur because the risk of serious disease or death is very small in healthy women without underlying risk factors. Further, we are excluding women who have contraindications to COC therapy (i.e., smokers; women with a history of blood clots, heart disease, or stroke; women with hypertension, liver or gallbladder disease, cancer, or diabetes; women who have recently had major surgery with prolonged immobilization; women who are breastfeeding or recently postpartum). Additionally, symptoms such as nausea, vomiting, abdominal cramps and bloating, breakthrough bleeding, migraine headaches, breast tenderness, edema (swelling), melasma (spotty darkening of skin), yellowing of the skin, weight and appetite changes, rash, intolerance to contact lenses, depression and/or anxiety, loss of scalp hair, and vaginal infections may be associated with taking the birth control pills. In general, the most common complaint while taking birth control pills is breakthrough bleeding. Because this study is a short duration (6 weeks), this will resolve quickly after study completion.

TDC Therapy (for TDC group): Potential side effects of TDC use include nausea, breast discomfort, headache, skin irritation, breakthrough bleeding, edema, melasma (spotty darkening of skin), dizziness, and gastrointestinal problems. In the case of skin irritation, the patch may be removed and moved to a different location to reduce irritation. For other side effects, the subjects will be referred to a physician in the CRC or will be encouraged to contact their own health care provider. Serious risks associated with TDC therapy include blood clots, heart attack, stroke, and gallbladder disease. In rare cases, liver tumors or cancer of the reproductive organs/breasts may occur. However, these risks will be minimized by only including healthy women who do not present with contraindications for TDC therapy (for example: smokers; women with a history of blood clots, heart disease, or stroke; women with hypertension, liver or gallbladder disease, cancer, or diabetes; women who have recently had major surgery with prolonged immobilization; women who are breastfeeding or recently postpartum).

For a detailed list of the risks/side effects associated with TDC (Xulane), please consult the following website: http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=f7848550-086a-43d8-8ae5-047f4b9e4382

CVR Therapy (for CVR group): Potential common side effects of CVR include vaginitis, headaches, mood changes, device-related events (expulsion/discomfort/foreign body sensation), nausea and vomiting, vaginal discharge, increased body weight, vaginal discomfort, breast pain or tenderness, dysmenorrhea, abdominal pain, acne, decreased libido, and chloasma. Serious risks associated with CVR use are deep vein thrombosis, anxiety, cholelithiasis, vomiting, strokes and myocardial infarction, toxic shock syndrome, impaired liver function and liver tumors, increased blood pressure, and gallbladder disease. These risks will be minimized by only including healthy women who do not present with contraindications for hormonal contraceptive therapy.

Resting Blood Samples: The risks associated with single blood samples obtained with a needle and syringe or intravenous catheter may include one or all of the following: local discomfort, occasional dizziness and nausea, and black and blue marks. Blood clots attached to the walls of a blood vessel, and infections are very rare but are also potential risks. These risks will be minimized or eliminated by having only trained individuals who use sterile techniques draw blood. A trained assistant will closely monitor the subject while blood is being obtained in a supine position.

Subcutaneous GH administration: Growth hormone can sometimes cause side effects. The study participant may immediately experience pain, bleeding or swelling at the site of the injection. Other side effects may develop more slowly. Common side effects or most frequently observed adverse reactions include 1) swelling of the hands and feet, 2) carpal tunnel syndrome, 3) joint and back pain, 4) burning or tingling of the skin, 5) stuffy nose, 6) headaches, and 7) temporary high blood sugar (if the subject did not have high blood sugar when she started taking the study drug, then this condition should go away when the injections are stopped). Uncommon but potentially severe side effects include 1) high blood pressure, 2) pain and burning at the site of injection, 3) increased liver enzymes, 4) a dimple at the site of injection (this may happen if the study drug is always injected in the same place; to prevent this, the place where the injection is performed should be changed often), 5 previously present nevi (black birth marks) may increase in size (rare), 6) allergic reactions to a chemical called *m*-cresol, which is part of the study drug injection, 7) feeling tired or cold due to production of less thyroid hormone, resulting in puffy boggy swelling around the eyes, on the backs of the hands and feet, above the collarbone, 8) increase in pressure inside the brain causing severe headache (rarely occurs and should go away if the study drug dose is lowered or stopped), 9) diabetic retinopathy, 10) pancreatitis. Most of these symptoms disappear by themselves after a period of time or with a decrease in dosage or stopping of the drug. If symptoms do not go away, then the subject will need to stop taking the study drug. A higher risk for leukemia (a type of blood cancer) from growth hormone was noted in one small study. However experts now agree that the risk for

leukemia in people receiving growth hormone is not greater than that for anybody else. Lastly, giving growth hormone in pregnancy can be harmful to the unborn baby; however, we will do a pregnancy test during baseline to ensure that the subject is not pregnant.

Overnight Fast: After the overnight fast, subjects may feel faint or dizzy. They are encouraged to drink water the morning of the blood draw and will be provided with a snack after all blood draws required for the study visit are completed.

Blood Loss: There may be a risk of reduced amount of blood (plasma volume), red blood cell count or the amount of iron-containing substances that carry oxygen on red blood cells (hemoglobin levels) as a result of the blood lost over the course of the study. To minimize risks, we intend to screen individuals who may have low levels of hemoglobin and hematocrit. If these parameters are out of the normal range during the screening tests, these volunteers may be discontinued from the study or be required to wait several weeks prior to beginning the study after it has been determined that hemoglobin and hematocrit are in the normal range.

DXA Body Composition Assessment: The Dual Energy X-ray Absorptiometry (DXA) body composition procedure exposes an individual to a small amount of radiation where the X-ray beam crosses the body. This protocol calls for one total body scan. On occasion a scan may need to be repeated for quality control purposes. This radiation exposure is not necessary for medical care and is for research purposes only. There is minimal risk from this exposure. To ensure that a fetus is not exposed to radiation, a urinary pregnancy test will be conducted prior to the DXA scan.

Urine Sample for Pregnancy Tests: There are no known risks associated with the self-collection of one's urine. Volunteers will be given screw top and airtight containers to collect the urine.

Exercise Testing (VO2max): During exercise testing, volunteers may occasionally experience lightheadedness, chest discomfort, and cramping in the legs. Irregular heart beats and irregular blood pressure responses may occur. The risk of death during an exercise test is minor (0.5 per 10,000 tests), but does exist. These risks are usually found in individuals with existing heart conditions. Other potential risks, including fainting, nausea, muscle strain, and muscle soreness, will be minimized by proper warm-up procedures. Proper procedures for stopping the tests will be observed should a participant have abnormal symptoms associated with this test. Should an emergency situation occur, research personnel will call 911. During the exercise test, heart rate will be monitored continuously via a Polar heart rate monitor and rating of perceived exertion (RPE); blood pressure will be monitored before and after the test. Expired air will be collected using standard equipment in order to assess maximal oxygen uptake, which is a marker of aerobic fitness. A medical history will be obtained in order to identify absolute and relative contraindications to exercise testing. Trained personnel will administer the exercise tests and follow the proper procedures according to the guidelines of the American College of Sports Medicine (ACSM).

Physical Activity Assessments: There are no known risks associated with the collection of information about one's exercise practices.

Additional Costs Associated with Participation in Study: Study participants may spend additional money (that is not included in the compensation) on transportation to and from the laboratory.

Inconveniences: The following aspects of the study may represent inconveniences: frequent lab visits, fasting prior to and during blood sampling, repeated blood sampling, long study visits, two 4-day periods of subcutaneous injections, pregnancy tests, daily food logs, daily activity logs, daily menstrual logs, questionnaires, tests which are physically invasive and involve wearing cumbersome equipment.

12.0 Potential Benefits to Subjects and Others

12.1 Potential Benefits to Subjects

Individual subjects will receive the results and explanation of results of certain procedures completed, to include 1) body composition measurements, 2) maximal oxygen consumption measurement as an indicator of aerobic fitness,

and 3) serum concentration values of selected hormones. This information will provide the subjects with valuable information about their health and fitness.

In addition, the study will provide individual subjects with exposure to different types of hormonal contraceptive therapy which may be informative to them if they are eventually seeking to begin hormonal contraception.

12.2 Potential Benefits to Others

The benefits to society include increasing our knowledge of how hormonal contraceptive therapy, in particular oral, transdermal, and vaginal contraceptive therapy, affects bone health of young, exercising women via its effects on the GH/IGF-1 axis. This study will also help us determine whether or not a newer, alternative form of hormonal contraceptive therapy, i.e., transdermal or vaginal contraceptive therapy, may preserve bone health among young, exercising women by not disrupting the GH/IGF-1 axis. Osteoporosis is a major concern for women later in life, and hormonal contraceptive therapy, especially oral contraceptive therapy, is extremely common among young women. As such, this study may help to provide valuable information about the safety profile of three forms of hormonal contraceptive therapy in terms of the GH/IGF-1 axis and bone health.

13.0 Sharing Results with Subjects

Study participants will receive their DXA scan and body composition information, results from the screening blood draw (complete blood count and chemistry panel and selected endocrine hormone concentrations), and their maximal oxygen consumption value.

Study results will not be shared with members outside the study team, including physicians. If the study participant wishes to share her results with her physician, we will give her the results to share with her physician but we will not directly provide the physician with the results.

14.0 Economic Burden to Subjects

14.1 Costs

Study participants may spend additional money on transportation to and from the laboratory; otherwise, there are no additional costs related to study participation. There is the possibility that study participants may have to pay for all or part of the cost of a pap smear to be eligible for the study, depending on their health insurance.

14.2 Compensation for research-related injury

Compensation is not available for research-related injury.

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

15.0 Number of Subjects

Because this is a pilot study to obtain preliminary data for submission of an NIH R01 proposal, we aim to complete 12 subjects in each of 4 groups (COC group, TDC group, CVR group, and control), totaling 48 subjects.

To account for a potential screening failure rate of 33% and potential drop-out rate after randomization of 30%, we plan to enroll a total of 100 subjects (25 per group).

16.0 Resources Available

16.1 Facilities and locations

Recruitment will occur on the Penn State University Park campus and in the surrounding State College community. Study procedures will be completed on the Penn State University Park campus in Noll Laboratory in the Women's Health and Exercise Laboratory and the Clinical Research Center.

The Women's Health and Exercise Laboratory (WHEL), co-directed by Dr. Nancy I. Williams and Dr. Mary Jane De Souza, is located in the Department of Kinesiology at the Penn State University Park campus in the Noll Laboratory down the hall from the Clinical Research Center (CRC). The WHEL is comprised of two offices (Rooms 109 and 123 Noll) and seven research laboratories (Rooms 110, 116, 117, 122, 124, 206, and 207 Noll). The space includes thirteen computer workstations for graduate students, research fellows and research assistants. The WHEL is comprised of seven state-of-the-art research laboratories.

1. Bone, Body Composition Laboratory: This is located in Room 207 Noll and is equipped with a GE Lunar iDXA dual energy x-ray absorptiometer for the assessment of bone mineral density and body composition by a technician certified by the International Society for Clinical Densitometry. This space also houses the Stratec XCT3000 pQCT which estimates bone strength, and assesses cortical and trabecular volumetric bone density and bone area as well as cortical thickness. An independent computer operating system is dedicated to the pQCT.

2-3. Biochemistry Lab 1 and Biochemistry Lab 2: Biochemistry Lab 1 (Room 116; 595 sq ft.) and Biochemistry Lab 2 (Room 117; 198 sq ft.) are fully equipped laboratories supporting a wide array of assay platforms necessary for hormone assays. Biochemistry Lab 2 is for conducting radioimmunoassays. These labs include: a PerkinElmer Precisely 2470 Automatic gamma counter running Wallac Wizard2 software, a Dynex Magella Biosciences ultra wash plus plate washer, 1 Dynex Tech MRXII plate reader, 2 Lab-Line MicroTiter plate shakers, 1 VWR Scientific pH meter, 1 Mettler Toledo analytical scale, 2 Corning hot plates, 1 Precision Systems micro osmometer, 3 Thermo Scientific ultra low -80 freezers (stored in room 117 and Noll hallway), 1 VWR -20 freezer, 2 refrigerator/freezers (Frigidaire, and Revco).

4. Metabolic Testing Laboratory: This lab is located in Room 122, has a metabolic cart (Viasys Healthcare, Vmax Encore Metabolic Cart) and a hospital bed for indirect calorimetry assessment of resting metabolic rate. The WHEL also houses a research grade treadmill (Trackmaster® Model# TMX425CP) and metabolic cart (Viasys Healthcare, Vmax Encore Metabolic Cart) for assessment of oxygen uptake and energy expenditure during exercise which are stored in Room 131.

5. Biological Specimen Processing and Storage Laboratory: This lab is located in Room 124 and is divided into several workstations for processing and long-term storage of urine and blood samples and includes: six -20 freezers, 1 refrigerator, 1 Forma Scientific Centrifuge, 1 IEC Centra CL2 centrifuge, and a temperature controlled Eppendorf 5804-R centrifuge.

6-7. Clinical Data Management Laboratories: These labs are located in Rooms 110 and 206. Room 206 is our computer database repository, housing several dedicated computers for performing data analysis.

Clinical Research Center:

The CRC is located in the CRC wing (The Elmore Clinical Research Wing) of Noll Lab. The CRC is a 3-floor 14,000 sq.ft. continuous expansion with the existing Noll Physiological Research Center building. It is the University Park satellite site of the Penn State General Clinical Research Center. The CRC provides staff and a variety of services including but not limited to: planning meetings with investigators to facilitate inclusion of desired ancillary testing, nursing interventions and documentation, medication administration, blood sampling via venipuncture or vascular access devices, anthropometric measurements, metabolic testing (resting metabolic rate) and pulmonary function testing, insertion and maintenance of intravenous access, exercise stress testing (treadmill), and bone densitometry and body composition testing (DXA).

The CRC Nursing Department is on the second floor of the Elmore Clinical Research Wing and includes the following facilities: A nurse's station with clinical records files and clinician office. The following nursing department facilities are shared by CRC investigators: exam room and two invasive procedure rooms, procedure room for testing exercise and pulmonary function, small specimen processing room with refrigerated and unrefrigerated centrifuges, five hospital-style bedrooms with bathrooms, and overnight beds.

16.2 Feasibility of recruiting the required number of subjects

We are recruiting women between the ages of 18-30 years. This age range encompasses both undergraduate and graduate students. The student body at PSU University Park is comprised of approximately 46,184 students which includes approximately 35,000 undergraduate students and 10,000 graduate students.

http://admissions.psu.edu/pennstate/campuses/?campusCode=UP

Among undergraduate students, 54% of the student body is men and 46% is women. If we are just considering undergraduate students, we would ideally have access to 16,100 potential subjects. The number that we need to recruit for this small pilot study is only 0.6% of the ideal number of potential subjects.

http://admissions.psu.edu/apply/statistics/

Of course, we have additional inclusion/exclusion criteria so the potential number of subjects that we have access to is smaller than that listed above. However, because we are conducting a small pilot study on a large university campus where almost half of the student population is female and within the desired age range, it is very feasible to recruit the required number of subjects for this study.

16.3 PI Time devoted to conducting the research

The PI has successfully conducted (and continues to conduct) many research studies to include a large randomized controlled trial. Recruitment has ended, however, for the large RCT, and one small study underway in the Fall Semester 2013 just finished. The PI will also receive one course buy out with the ASBMR grant if awarded.

Further, beginning in Spring 2014, the WHEL will have 3 full-time graduate students – two of whom are experienced with conducting research studies in the lab. There is also the potential for 2 more graduate students to be joining the lab in Fall 2014. There are also 2 full-time postdoctoral scholars in the lab, and 1 full-time laboratory technician. These members of the lab in addition to the several undergraduate students who volunteer in the lab will help to ensure that the research is completed.

16.4 Availability of medical or psychological resources

On the University Park campus, medical resources are available at University Health Services and psychological resources are available at CAPS (Counseling and Psychological Services). Within Noll Laboratory where all study procedures will occur there is also a Clinical Research Center with onsite nurses and physicians if needed.

16.5 Process for informing Study Team

The Women's Health and Exercise Lab has weekly lab meetings that are conducted by the lab supervisor (and principal investigator). All graduate students, postdocs, lab techs, project coordinators, and honors undergraduate students who are part of the study team and work in the lab are required to attend the meetings. Discussion about the protocol and study duties will occur at these meetings. The PI will also meet individually with study team members as necessary.

Appropriate training of study team members according to their assigned duties will occur prior to solo execution of the study procedures by study team members.

17.0 Other Approvals

Prior to commencing research, we will obtain approval from the Institutional Biosafety Committee at Penn State for the use of biohazardous materials.

18.0 Subject Stipend and/or Travel Reimbursements

Study participants will receive $400.00 upon completion of the study. The subjects will receive this money in three payments as follows:

1) After completion of baseline, subjects will receive $100.
2) After completion of the first 6 weeks of the intervention, subjects will receive $50.
3) After completion of the post-study period, subjects will receive $250.

Neither travel reimbursement nor extra credit will be offered to subjects/students.

19.0 Multi-Site Research

This is not a multi-site study.

19.1 Communication Plans

Not applicable.

19.2 Data Submission and Security Plan

Not applicable.

19.3 Subject Enrollment

Not applicable.

19.4 Reporting of Adverse Events and New Information

Not applicable.

19.5 Audit and Monitoring Plans

Not applicable.

20.0 Adverse Event Reporting

20.1 Adverse Event Definitions

For drug studies, incorporate the following definitions into the below responses, as written:	
Adverse event	Any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related
Adverse reaction	Any adverse event caused by a drug
Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction". • *Reasonable possibility.* For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
Serious adverse event or Serious	Serious adverse event or Serious suspected adverse reaction: An adverse event or suspected adverse reaction that in the view of either the investigator or

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

For device studies, incorporate the following definitions into the below responses, as written:	
Unanticipated adverse device effect	Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

20.2 Recording of Adverse Events

During study, participants will be routinely questioned about how they are feeling, particularly after initiating administration of a drug. In addition, when the drug is dispensed, study participants will be instructed to inform the study team if they do not feel well after initiating the study drug or if they experience any of the side effects/risks that may be associated with taking the drugs.

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

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

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

20.3 Causality and Severity Assessments

The investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse

event was caused by the study drug(s) or device(s); and 3) if the adverse event meets the criteria for a *serious adverse event*.

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

20.4 Reporting of Adverse Reactions and Unanticipated Problems to the FDA

20.4.1 Written IND Safety Reports

Not applicable. All study drugs are FDA-approved.

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

Not applicable. All study drugs are FDA-approved

20.5 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

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

20.6 Unblinding Procedures

Not applicable. This study is not blinded.

20.7 Stopping Rules

This study does not involve high risk to study subjects; therefore, it is unlikely that the entire study would need to be interrupted or stopped.

In the event that multiple subjects report adverse events or adverse reactions during the study, the study team and clinical staff will consult together to determine if the adverse events that have occurred warrant interruption of the study.

21.0 Study Monitoring, Auditing and Inspecting

21.1 Study Monitoring Plan

21.1.1 Quality Assurance and Quality Control

It is currently unclear if FDA regulations apply to this study; however, the principal investigator, Dr. De Souza, will monitor the conduct of the study via regular meetings with study team members who will be collecting the data. Also, a project coordinator will be designated to ensure that the data are collected, recorded, and stored in a manner that is in compliance with the protocol and appropriate policies.

Also, Dr. Robert Mooney from the CRC will be the Medical Monitor and will assess any adverse events that may arise.

21.1.2 Safety Monitoring

If an event occurs to a study participant that is determined to fit the criteria of a reportable adverse event, adverse reaction, etc. (at the discretion of the principal investigator), a description of the adverse event will be written and the adverse event will be reported to the IRB following IRB guidelines for "reportable new information."

The **Principal Investigator** will confirm that all adverse events (AE) are correctly entered into the AE case report forms by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; and will notify the IRB, FDA, sponsor and/or DSMB of all applicable AEs as appropriate. All assessments of AEs will be made by a licensed medical professional, Dr. Madhusmita Misra or Dr. Richard Legro, who are investigators on the research.

The **research coordinator** (with the help of the study team member who may have observed or first heard about the adverse event (if applicable)) will complete the appropriate report form and logs; assist the PI to prepare reports and notify the IRB, FDA, and/or DSMB of all Unanticipated Problems/SAE's.

The **Monitor** will confirm that the AEs are correctly entered into the case report forms. The Monitor will confirm that the adverse events are consistent with the source documents and are reported to the appropriate regulatory bodies, as required.

21.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the Penn State quality assurance program office(s), IRB, the sponsor, and government regulatory bodies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

22.0 References

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23.0 Appendix