

**PARTNERS HUMAN RESEARCH COMMITTEE
PROTOCOL SUMMARY**

Answer all questions accurately and completely in order to provide the PHRC with the relevant information to assess the risk-benefit ratio for the study. Do not leave sections blank.

PRINCIPAL/OVERALL INVESTIGATOR

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PROTOCOL TITLE

Fat Mediated Modulation of Reproductive and Endocrine Function in Young Athletes

NCT00946192

FUNDING

NIH

VERSION DATE

December 4, 2014

SPECIFIC AIMS

Concisely state the objectives of the study and the hypothesis being tested.

Specific Aim 1: We will test the hypothesis that in adolescent athletes

- (i) Suboptimal energy availability preferentially lowers fat mass with sparing of lean mass**
- (ii) Lower fat mass leads to altered levels of adipokines (lower leptin, higher adiponectin) and fat regulated hormones (higher ghrelin, PYY), and**
- (iii) Altered levels of adipokines and fat regulated hormones determine hypogonadism and decreased gonadotropin pulsatility.**

In a cross-sectional study, we will specifically investigate whether (i) adolescent athletes in a state of low energy availability have lower fat mass than those with optimal energy availability, (ii) lower fat mass results in alterations in adipokines (decreases in leptin and increases in adiponectin) and fat regulated hormones (increases in ghrelin, PYY), and whether (ii) LH pulsatility patterns are predicted by pulsatility patterns of hormones produced (leptin, adiponectin) or regulated (ghrelin, PYY) by fat.

Specific Aim 2: We will test the hypothesis that in adolescent athletes

- (i) Bone mass accrual rates (assessed by DXA) are markedly decreased in AA compared with EA and controls**
- (ii) Bone microarchitecture at the ultradistal radius and leg (assessed by X-treme CT) is abnormal in AA with decreased trabecular thickness (TbTh) and increased separation (TbSp); deterioration occurs over time in AA compared to EA and controls who demonstrate increases in healthy bone, and**
- (iii) Independent of hypogonadism, adipokines and fat regulated hormones are critical determinants of bone mass accrual rates and bone microarchitecture**

In a prospective observational study, we will compare changes in bone density, trabecular volume, trabecular thickness, number and separation over 12 months in a subset of AA versus EA and controls, and will investigate in a regression model whether altered levels of adipokines (lower leptin, higher adiponectin) and fat regulated hormones (higher ghrelin, PYY) determine lower bone mass accrual rates and altered bone structural parameters independent of gonadal status

Specific Aim 3: In a randomized controlled trial, we will test the hypothesis that in adolescent AA, compared to oral estradiol or no treatment, transdermal estradiol replacement will

- (i) Increase bone mass accrual rates (DXA)**
- (ii) Increase TbTh and bone trabecular volume (X-treme CT), and**
- (iii) Not affect endurance capacity**

In a randomized controlled trial, adolescent AA will be randomized to transdermal estradiol in replacement doses, oral estradiol or no therapy for 12 months. Effects on bone accrual rates and endurance capacity will be assessed in comparison to EA and controls. In a subset of AA, we will examine effects of estradiol replacement on bone structural parameters compared to EA and controls.

Specific Aim 4: We will test the hypothesis that in athletes 14-25 years old

- (i) Bone strength [as assessed using Finite Element Analysis] is lower in adolescent and young adult AA compared with EA and sedentary controls at baseline and decreases prospectively over one-year in AA, whereas bone strength increases in EA over the same duration.**

Specific Aim 5: We will test the hypothesis that in athletes 14-25 years old

- (i) Greater visceral adipose tissue (VAT) and lower subcutaneous adipose tissue (SAT) are associated with lower bone density, impaired microarchitecture and lower bone strength, and that**
- (ii) Greater marrow fat is associated with lower bone density, impaired microarchitecture and lower bone strength.**

Specific Aim 6: We will test the hypothesis that in amenorrheic athletes 14-25 years old

- (i) **Transdermal estradiol, compared to oral estradiol or no treatment will increase bone strength to approximate that in controls, with decreases in marrow fat and no changes in regional body composition.**

Specific Aim 7: We will test the hypothesis that in adolescent and young adult male athletes

- (iv) **Suboptimal energy availability and an energy deficit state preferentially lower fat mass with sparing of lean mass**
- (v) **Lower fat mass leads to altered levels of adipokines (lower leptin, higher adiponectin) and fat regulated hormones (higher ghrelin, PYY, cortisol)**
- (vi) **Altered levels of adipokines and fat regulated hormones determine a relative hypogonadal state**
- (vii) **Hypogonadism and altered levels of adipokines and fat regulated hormones have a deleterious effect on bone density, structure (assessed by HRpQCT) and estimated bone strength (using FEA)**
- (viii) **Higher visceral adipose tissue (assessed using MRI) and marrow fat (assessed using MRS) are associated with impaired bone density, structure and strength**

Specific Aim 8: We will test the hypothesis that in adolescent and young adult male athletes

- (i) **The impact of athletic energy deficit on gonadal status and bone density, structure and strength is less marked than in female athletes of comparable maturity**
- (ii) **The lesser impact of athletic energy deficit on gonadal status compared with female athletes is attributable to greater lean mass, a lower absolute reduction in fat mass than in females (given lower baseline fat in males than in females), and a lesser impact on adipokines and fat regulated hormones**
- (iii) **A lesser impact of athletic energy deficit on testosterone secretion in males (compared with amenorrhea and hypoestrogenism in females) causes greater preservation of lean mass in males and a lesser impact on bone than in females**

BACKGROUND AND SIGNIFICANCE

Provide a brief paragraph summarizing prior experience important for understanding the proposed study and procedures.

Athletic activities are very common in high school and college, and endurance athletes are at high risk for amenorrhea. Athletes with amenorrhea (AA) in turn are at great risk for low bone mineral density (BMD) and thus fractures. However, not all athletes develop amenorrhea, and specific factors that determine why only some athletes develop amenorrhea are not well understood. Our preliminary data indicate lower fat mass in adolescent AA compared with eumenorrheic athletes (EA) and non-athletic controls, despite similar levels of physical activity in AA and EA. Hormones produced or regulated by fat may thus be critical modulators of the hypothalamo-pituitary-gonadal (H-P-G) axis. Understanding determinants of amenorrhea in athletes is critical to developing future therapeutic strategies to treat or prevent amenorrhea, and therefore prevent low BMD. In this prospective study we hypothesize that lower fat mass in athletes determines hypogonadotropic hypogonadism through altered levels of specific adipokines (lower leptin and higher adiponectin) and fat regulated hormones (higher ghrelin, peptide YY (PYY)). Our preliminary data also indicate markedly lower spine BMD in AA than in EA and controls, suggesting that weight-bearing activity cannot protect against deleterious effects of hypogonadism. We also aim to prospectively examine bone mass accrual rates (using DXA) and bone structure (using ultra high resolution digital flat panel volume CT (X-treme CT)) over 12 months in AA versus EA and controls. Because adolescence is critical for bone mass accrual towards attainment of peak bone mass, lower bone accrual rates in AA compared with EA and controls would raise serious concerns for suboptimal peak bone mass and increased fracture risk. Importantly, our data indicate strong associations of fat mass with BMD and microarchitecture (trabecular thickness and separation), and we hypothesize that adipokines and fat regulated hormones are important determinants of BMD and microarchitecture

independent of hypogonadism. Finally, because hypogonadism is an important cause of low BMD, we hypothesize that transdermal estrogen will increase BMD over a year in AA to approximate bone accrual rates observed in EA, and will result in improvement in bone structural parameters. In a **three-arm randomized study**, we will compare transdermal to oral estrogen and no treatment, and anticipate that transdermal estrogen will have the greatest effects on bone mass accrual because of the IGF-1 sparing effects of transdermal estrogen compared with oral estrogen.

It is not known whether an equivalent of the female athlete triad of low energy availability, hypogonadism, and low bone density exists in male athletes. In contrast to females, in whom a complex and cyclically changing gonadotropin milieu is necessary for normal occurrence of ovulation and a normal menstrual cycle, monthly cyclical changes in gonadotropin secretion do not occur in males. Whereas alterations in GnRH pulsatility in hyper-exercising females athletes may manifest early as subclinical menstrual dysfunction (luteal phase defects or anovulation) (180) and later as oligomenorrhea (178) depending on the extent of energy deficiency, it is possible that sufficient testosterone secretion persists in male athletes with preservation of bone density, as long as LH secretion is not completely dampened. By better characterizing the consequences of athletic activity on the HPG axis and bone in males, we may also better understand the pathogenesis of athletic energy deficit in females. The proposed study will determine whether the equivalent of the female athlete triad exists in male athletes and the extent of impairment of bone metabolism in male vs. female endurance athletes in an energy deficient state.

RESEARCH DESIGN AND METHODS

Briefly describe study design and anticipated enrollment, i.e., number of subjects to be enrolled by researchers study-wide and by Partners researchers. Provide a brief summary of the eligibility criteria (for example, age range, gender, medical condition). Include any local site restrictions, for example, "Enrollment at Partners will be limited to adults although the sponsor's protocol is open to both children and adults."

Subjects will be 14-25 year old female athletes with AA (n= 145), EA (n=), and female non-athletic controls (n= 42). Because different kinds of physical activity have different effects on bone metabolism, we will enroll only endurance athletes (please see Inclusion Criteria).

For Specific Aims 7-8, 40 adolescent and young adult male athletes 14-21 years old, and 20 adolescent and young adult male non-athletes 14-21 years old will be studied. Eligibility will be assessed over the phone, and the study will include one visit only.

Participation of Children: We will enroll only female adolescents/young adults to optimize power and avoid issues arising from deficiencies of different predominant sex hormones in boys (testosterone) vs. girls (estrogen). Adolescents and young adults will be studied to determine effects on BMD and its predictors in a population that should be actively accruing bone.

Inclusion Criteria

(i) Females 14-25 years old: Our pilot data are reassuring in that young women 14-25 years old with hypothalamic amenorrhea are not adversely affected with estrogen use. In fact, in our prospective study, beneficial effects were observed both in young women 18-25 years old using oral estrogen, and in 14-18 year old adolescent girls using transdermal estrogen. We therefore feel that including girls in the 14-21 year age range will not be hazardous to their bone health. In fact, given the lack of data in this age group, it is important to study younger women and teenagers rather than extrapolate data from studies in adults to this younger population. Hormone dynamics differ in teenagers compared with adults, and bone mass accrual is even more dependent on estrogen and IGF-1 in younger than older women who have already achieved peak bone mass.

(ii) Bone age (BA) \geq 14 years: 98% of adult height is achieved at a BA of 14 because only 2% of growth potential persists at a BA of 14 years. Thus, to avoid potential stunting of growth potential with estrogen replacement, we have chosen to include girls with BA of \geq 14 years.

(iii) BMI between 10th-90th percentiles for age, or BMI of at least 85% of ideal BMI

(iv) Amenorrhea (for AA): absence of menses for \geq three months (74) within a period of oligomenorrhea (cycle length $>$ six weeks) for \geq six months, or absence of menarche at ≥ 16 years

(v) Eumenorrhea (EA and controls): \geq nine menses (cycle length 21-35 days) in preceding year.

(vi) Non-athlete healthy controls will be eligible if weight bearing exercise activity is less than two hours a week and if they are not participating in organized team sports.

(vii) Endurance athletes: severity of low BMD and menstrual dysfunction differ by kind of exercise and activity. For example, runners have a higher prevalence of menstrual irregularity than swimmers and cyclists (131). By limiting enrollment to endurance athletes, we will eliminate variability from the type of activity. Endurance training is defined as ≥ 4 h of aerobic weight-bearing training of the legs or specific endurance training weekly, or ≥ 20 miles of running weekly for a period of ≥ 6 months in the last year. These criteria were developed following discussions with Dr. Gary Skrinar, an expert in exercise physiology and a study consultant, and his interactions with athletic coaches at Boston University. Dr. Skrinar was until recently a Professor in the Department of Health Sciences at the Sargent College of Health & Rehabilitation Sciences, Boston University, a first tier school for competitive athletic activities involving endurance training. Dr. Skrinar has been studying athletes and conducting maximal exercise testing in Sargent College for the past 28 years on populations that include exercising young women. Our criteria differ from those used by a group from Sweden (74) that examined women 16-35 years old. This group specified endurance training as ≥ 6 h of aerobic weight-bearing training of the legs or ≥ 43.75 miles of running weekly for at least 6 months in the last year or 6 h of specific endurance training weekly. These criteria were considered excessive and unrealistic for girls 14-21 years old by Dr. Skrinar and the coaches at Boston University, and the criteria modified as described. Cyclists and swimmers will be excluded because their training does not include true weight-bearing activities. Rowers (but not coxswains) and gymnasts will also be excluded given that these activities have been associated with a preservation of bone density even when associated with amenorrhea likely consequent to differences in the nature of weight bearing and impact. Track and cross-country runners will be included. Dr. Gary Skrinar will review screening information to confirm eligibility.

Subjects and parents will be counseled regarding deleterious effects of excessive exercise on bone at the screening visit, and benefits of behavior modification, namely reduction of activity will be strongly recommended. Counseling will continue through the study. We will counsel subjects with athletic amenorrhea at the screen and at subsequent visits about the importance of reducing energy expenditure or increasing intake. We will also obtain information regarding whether or not they receive such counseling from their health care providers, parents or coaches. Of note, most subjects referred to us for our pilot studies had received significant counseling but did not respond to this by decreasing activity levels. Subjects will also be counseled regarding substance abuse at study visits.

Exclusion Criteria

- Use of medications affecting bone metabolism including estrogen, progesterone, anabolic steroids, glucocorticoids except local application of glucocorticoid creams (washout period of three months prior to enrollment if medically permissible to discontinue these), phenytoin, phenobarbitone
- Conditions other than endurance training that may cause amenorrhea including PCOS (clinical or preceding laboratory evidence of hyperandrogenism with amenorrhea)
- Individuals who have not had their period for at least 2 years (until an endocrine evaluation has ruled out other causes of amenorrhea)
- Conditions other than endurance training that may cause bone metabolism to be affected
- Abnormal TSH, elevated FSH, hematocrit $< 30\%$

For girls with AA (to be randomized to estrogen and progesterone or no treatment)

- Family history or personal history of conditions that may increase risk of thromboembolism:
 - a. Myocardial infarction or strokes occurring at < 50 years (if not associated with smoking)
 - b. Clotting disorders: normal coagulation profile necessary for enrollment in case of family history
- Current history of smoking
- History of thromboembolism, migraines
- Undiagnosed, abnormal genital bleeding; known, suspected or a history of breast cancer and estrogen-dependent neoplasia; abnormal LFTs with ALT/AST > 2 times the upper limit of normal; known hypersensitivity to product ingredients

Males:

Inclusion Criteria:

- (i) Males 14-21y \geq Tanner stage 4
- (ii) BMI 10th-90th %ile
- (iii) Endurance athletes engaged in sports that involve weight bearing training of the legs. Severity of low BMD and gonadal dys-function may differ by type of exercise (131). By limiting enrollment to weight bearing endurance athletes, we will eliminate variability from type of activity. Endurance training is defined as \geq 4h of aerobic weight-bearing training of the legs or specific endurance training weekly, or \geq 20 miles of running weekly for \geq 6 months in the last year. Cyclists, rowers and swimmers will not be included because these activities do not involve lower extremity weight bearing. Gymnasts will not be included (not an endurance sport),
- (iv) Non-athletes will be eligible if weight bearing exercise activity is $<$ 2h a week and they are not participating in organized team sports.

Exclusion Criteria:

- (i) Use of medications affecting bone metabolism (washout period of 3 months prior to enrollment if medically permissible to discontinue these)
- (ii) Conditions other than endurance training that may cause hypogonadism or low bone density
- (iii) High TSH, FSH or prolactin
- (iv) Standard MRI exclusion criteria such as presence of pacemaker, intracranial metal clips, severe claustrophobia

Briefly describe study procedures. Include any local site restrictions, for example, "Subjects enrolled at Partners will not participate in the pharmacokinetic portion of the study." Describe study endpoints.

Over the proposed 5-year investigation, 145 teenage girls and young adults 14-25 years old with AA, 42 EA and 42 non-athletic controls will be studied. Eligibility will be assessed at the screen, and the study will include a baseline visit and follow up visits at 3, 6, 9 and 12 months. The baseline visit will occur within 8 weeks of the screen. EA and controls will be followed without intervention (except elemental calcium and vitamin D) for 12 months.

For Specific Aim 3 we anticipate screening 2 times the number of athletes that we will eventually enroll in the study to obtain 145 eligible AA adolescents. In addition, for Specific Aims 1 and 2, we anticipate screening 2 times the number of EA (n=42) and non-athletic controls (n=42) required for this section of the study (total subjects to be screened= 458).

For Specific Aims 7 and 8 we anticipate screening (over the phone) 2 times the number of athletes that we will eventually enroll in the study to obtain 40 eligible male endurance athletes. In addition, we anticipate screening (over the phone) 2 times the number of healthy male non-athletes in order to obtain 20 eligible non-athletic controls (total male subjects to be screened = 120).

Screening Visit: Informed consent will be obtained from parents and assent from subjects $<$ 18y old, and informed consent from subjects \geq 18y. Eligibility will be determined at this visit, which will include:

- (a) History (including medications, current and past mental health issues), physical examination, height, weight, BMI, waist, hip circumference
- (b) Screening labs: complete blood count, ALT, AST, TSH, FSH (to rule out premature/primary ovarian failure), 30 cc of blood will be drawn, frozen, and stored for later analysis.
- (c) The Bouchard 3-day activity record (129) (to confirm criteria for endurance training are met).
- (d) Bone age (wrist and hand X-ray) and bone density using DXA: spine, hip and total body.
- (e) Stratification of AA subjects during randomization will be based on Z-scores at the spine (\leq -1 or $>$ -1). Body composition will be assessed by DXA. AA dancers will be randomized using a separate randomization template than AA non-dancers.

(f) Two subtests, Vocabulary and Matrix Reasoning, from the Weschler Abbreviated Scale of Intelligence (WASI), a Color-Word Interference Test subtest from the Delis Kaplan Executive Functioning Scale (DKEFS), and the California Verbal Learning Test (CVLT).

The DXA, bone age and cognitive tests will be completed at the screening visit, in between the screening and baseline visits or at the baseline visit.

Baseline Study Visit: This visit will occur within eight weeks of the screen and will include:

- (a) History and physical examination with anthropometric measurements
- (b) Eating disorder questionnaires (EDI-2), Three-Factor Eating Questionnaire-R18 (TFEQ), and a 4-day food diary (to assess caloric intake)
- (c) Resting energy expenditure (REE) using indirect calorimetry
- (d) Salivary cortisol at 11 p.m.
- (e) Fasting (or 2 hour post-prandial) blood samples for leptin, adiponectin, ghrelin, PYY, IGF-1, insulin, estradiol, testosterone, SHBG, FSH and LH, bone turnover markers (PINP and N-telopeptide).
- (f) Fasting (or 2 hour post-prandial) calcium, phosphorus and 25(OH) vitamin D
- (g) Indirect calorimetry and handgrip strength. This exam will be performed at the Clinical Research Center at Massachusetts General Hospital or at the Charlestown Navy Yard site. This exam will be completed sometime in between screening and baseline visits or at the baseline visit.
- (h) Bone microarchitecture at the ultradistal radius and leg (non-dominant) will be assessed using X-treme CT in a subset of 100 AA (first ~33 in each randomization group), 36 EA, 36 controls sometime between the screening and baseline visits or at the baseline visit
- (i) MRI (regional fat and muscle) assessment
- (j) Finite element analysis (FEA) will also be performed.
- (k) Optional fMRI and evaluation through the State Trait Anxiety Inventory (STAI) scores and addictive behaviors using the Alcohol Use Disorder Identification Test (AUDIT) and Drug Abuse Screening Test-10 (DAST-10) in subset of 6 AAs randomized to treatment, 6 EAs, and 6 HC 18-25 years.

Follow-up Outpatient Study Visits at 6 and 12 months: These will include:

- (a) History and physical examination with anthropometric measurements, adverse event review, changes to health status (including mood disorders)
- (b) Bouchard 3-day activity record (daily energy expenditure), 4-day food diary (caloric intake), EDI-2, TFEQ, REE (indirect calorimetry)
- (c) Handgrip strength in 60 AA (20 in each treatment group) (after drop-outs), 20 EA, controls
- (d) BMD and body composition using DXA (lumbar spine, hip, total body)
- (l) 12 m only: Bone structure at the ultradistal radius and leg (X-treme CT) in the 90 AA, 30 EA and 30 controls (after dropouts); MRI (regional fat and muscle) assessment. Finite element analysis (FEA) will also be performed.
- (e) Salivary cortisol at 11 p.m.
- (f) Fasting (or 2 hour post-prandial) blood samples for leptin, adiponectin, ghrelin, PYY, IGF-1, insulin, estradiol, testosterone, SHBG, FSH and LH, bone turnover markers (PINP and N-telopeptide).
- (g) Color-Word Interference Test subtest from DKEFS and the CVLT.
- (h) Follow up of fMRI and evaluation through the State Trait Anxiety Inventory (STAI) scores and addictive behaviors using the Alcohol Use Disorder Identification Test (AUDIT) and Drug Abuse Screening Test-10 (DAST-10) at 6 month visit only in the 6AAs scanned at baseline.

Follow-up Outpatient Study Visits at 3 and 9 months (abbreviated safety evaluation visits):

- (a) History and physical examination with anthropometric measurements, adverse event review
- (b) Fasting (or 2 hour post-prandial) blood samples for IGF-1, estradiol, testosterone, SHBG and LH, bone turnover markers (PINP and N-telopeptide).

Male Study Visit:

- (a) History and physical exam (H&P)
- (b) 7-8 AM Hormones: TSH, FSH, LH, testosterone, SHBG, estradiol, IGF-1, leptin, ghrelin (total), PYY (total),
- (c) 11 PM salivary cortisol (to be mailed back);

- (d) Calcitropic Measures and Markers of Bone Turnover: calcium, phosphorus, 25(OH) vitamin D, 1,25(OH)₂ vitamin D, PTH; PINP and CTX.
- (e) Activity Assessment: Bouchard 3-day activity record (129). Habitual activity will be further assessed using a standardized exercise questionnaire, which provides a good estimate of past year physical activity (173, 199),
- (f) 4-day food diary to assess caloric intake (NDS, version 4, Minneapolis, MN) and determine standard macronutrient intake,
- (g) Eating disorder questionnaire (EDI-2) (186), Three-Factor Eating Questionnaire-R18 (TFEQ),
- (h) Resting energy expenditure (REE) using indirect calorimetry,
- (i) VO₂ max and handgrip strength,
- (j) BMD: Lumbar spine, hip, radius, whole body areal BMD will be measured by DXA,
- (k) Body Composition: Whole body DXA will be used to assess fat and lean mass. In a subset of 10 athletes and 10 non-athletes, we will use MRI to assess SAT, VAT, thigh muscle area and marrow fat, (198) Bone microarchitecture and strength will be determined at the ultradistal radius and distal tibia using HR-pQCT (Xtreme CT, Scanco Medical AG). Bone strength will be determined using FEA of HR-pQCT scan data (187).

DNA testing:

We may ask subjects to participate in DNA testing via a saliva collection and/or blood collection at some point during this study in order to determine if there is a genetic basis for the hormonal changes seen in non-menstruating athletes. During one of the 6 visits for the “Fat Mediated Modulation of Reproductive and Endocrine Function in Young Athletes” study, we may draw 50cc (slightly more than 3 tablespoons) of blood for DNA testing, and may also ask for a sample of saliva for DNA testing. This will be an optional portion of the study, with a separate section of the consent forms to address this.

MRI:

We would like to assess SAT, VAT, thigh muscle area and marrow fat of AA prior to treatment and EA and non-athlete controls at some point during their 1 year study enrollment using MRI.

Finite Element Analysis:

FEA is an established analytical engineering technique to estimate stresses and strains induced by mechanical loading of complex structures, and is used to study bone strength in humans. Micro-finite element techniques applied to data from HR-pQCT strongly predict *in vitro* femoral and vertebral breaking strength independent of DXA-derived BMD (179, 180). Spine FEA differentiates patients with and without vertebral fractures (181) and hip FEA predicts new hip fractures independent of DXA-derived BMD (182). HR-pQCT is used for FEA using micro-finite element (μFE) techniques (183-185). In this context, FEA parameters correlate more strongly with failure load of the distal radius than either bone mass or microstructure (161) and clarify the structural mechanisms underlying fragility in subjects with fractures (159), including those occurring during the adolescent growth spurt (160). Of note, FEA estimates of bone strength have not been reported in female athletes, nor has FEA been utilized to monitor response to therapeutic intervention in this population. FEA will be assessed in 66 AA, 22 EA and 22 controls at baseline and 60 AA, 20 EA and 20 controls (after anticipated drop-outs) at 12m.

fMRI and Evaluation of Cognition, Mood, and Addictive Behaviors:

Exercise can impact neural functions on many fronts, including decreasing anxiety and addictive behavior, and improving cognition. We would like to assess a subset of 6 AAs, 6 EAs, and 6 HCs ages 18-25 years. This will be an optional portion of the study, with a separate section of the consent form to address this. fMRI scanning will be performed using (i) the stop-signal task for assessing cognition and (ii) estroop task for assessing anxiety. The EAs and AAs will receive one fMRI scan at baseline. The AAs will receive one scan at baseline and a follow-up scan at the 6 month visit.

Stop signal task: In this task, participants will categorize visual stimuli as fast as possible but are instructed to withhold a response when a stop signal appears on a randomly selected 25% of all trials. The time delay between the onset of the imperative stimulus and the stop signal will be adapted to the participants' individual response times using a staircase tracking algorithm. A mathematical framework allows for estimating the duration of the stop process ("stop signal reaction time") based on the distribution in the go trials as measure of duration of the inhibition process and therefore inhibition ability

Estroop Task: For this task participants will view human faces with happy or fearful expression labeled as either 'happy' or 'fear'. Subjects then indicate the emotional expression on each face ignoring the words written across the faces. Reaction time and accuracy of response will be calculated.

In addition, anxiety will be evaluated using State Trait Anxiety Inventory (STAI) scores and addictive behaviors will be assessed using Alcohol Use Disorder Identification Test (AUDIT) and Drug Abuse Screening Test-10 (DAST-10).

Combined Screening and Baseline Visit

For subjects who live or attend school far from Boston, we may offer to combine the first two visits, the screen and baseline. Because these visits occur within 8 weeks of each other, some subjects have difficulty with the time and expense necessary to come to Boston twice within this period. In the case of the combined visit, subjects will be phone-screened by a study co-investigator prior to scheduling the visit to make sure that inclusion criteria are met and exclusion criteria ruled out. Some information regarding exclusion criteria that we will not be able to gather prior to the combined visit would be bone age < 14 years, hematocrit < 30% and an abnormal TSH level. Subjects with any history of thyroid disorder will not be eligible for the combined visit. Because these subjects will not be completing the overnight portion of the baseline visit involving frequent sampling, the primary reason for obtaining the CBC prior to this visit is no longer applicable. To ensure that bone age of subjects completing the combined visit is mature enough to continue study participation, we will restrict the combined visit to subjects who are of a chronological age of 16 years or older at the time of the visit, making it very likely that the bone age is > 14 years. Additionally, we will not proceed with the baseline visit if the x-ray of the wrist and hand at the combined visit indicates a bone age of < 14 years. Study drug will be started only after inclusion/exclusion criteria have been confirmed at the combined visit.

Subjects will complete all screening and baseline procedures at this visit. The subject will be consented and a complete medical history and physical exam will be performed before continuing with further study procedures. If subject is found to have any health concern or is found to meet any exclusion criterion during the history and physical, further study components will be canceled. Following this portion of the visit, the subject will complete the rest of the study procedures occurring at the screening and baseline visit, including:

- (a) Screening labs: complete blood count, K, ALT, AST, TSH, FSH (to rule out premature/primary ovarian failure), 30 cc of blood will be drawn, frozen and stored for later analysis
- (b) Fasting (or 2-hr post prandial) calcium, phosphorus and 25(OH) vitamin D
- (c) Bone age (wrist and hand X-ray) and bone density using DXA: spine, hip and total body.
- (d) The Bouchard 3-day activity record (129) (to confirm criteria for endurance training are met).
- (e) Eating disorder questionnaires (EDI-2), Three-Factor Eating Questionnaire-R18 (TFEQ), and a 4-day food diary (to assess caloric intake)
- (f) Two subtests, Vocabulary and Matrix Reasoning, from the Weschler Abbreviated Scale of Intelligence (WASI), a Color-Word Interference Test subtest from the Delis Kaplan Executive Functioning Scale (DKEFS), and the California Verbal Learning Test (CVLT).
- (g) Resting energy expenditure (REE) using indirect calorimetry
- (h) Indirect calorimetry and handgrip strength. This exam will be performed at the Clinical Research Center at Massachusetts General Hospital or at the Charlestown Navy Yard site.
- (i) Bone microarchitecture at the ultradistal radius and leg (non-dominant) will be assessed using X-treme CT in a subset of 100 (~33 in each randomization group) AA, 36 EA, 36 controls

- sometime between the screening and baseline visits or at the baseline visit. Finite element analysis (FEA) will also be performed.
- (j) MRI (regional fat and muscle) assessment.
 - (k) Salivary cortisol at 11 p.m. to be sent back to MGH

For the amenorrheic athletes, subjects will be randomized upon receiving results from the DXA scan, and verifying eligibility based upon the lab results and bone age x-ray. Subjects will start study medication as soon as possible.

Early Termination Visit: This will include procedures performed at the 12 month visit. Subjects will be asked to return unused medications and all questionnaires.

For studies involving treatment or diagnosis, provide information about standard of care at Partners (e.g., BWH, MGH) and indicate how the study procedures differ from standard care. Provide information on available alternative treatments, procedures, or methods of diagnosis.

No treatment thus far has been successful in increasing bone density in adolescent athletes with amenorrhea. A recent review of the literature for data regarding efficacy of OCPs and hormone replacement in improving BMD in pre- and perimenopausal women (65) found only two randomized controlled trials (RCTs) that demonstrated a beneficial role of estrogen replacement on BMD in oligo/amenorrheic premenopausal women (66, 67), and one that reported no change (68). However, these studies did not target AA in normal weight adolescents/young adults, the population we plan to study, and the population where these data are needed.

In the study by Hergenroeder et al that showed a beneficial effect of estrogen on BMD, only five amenorrheic women were randomized to estrogen replacement, and their mean weight was 79% of ideal (67), indicating that they were very low weight and likely had anorexia nervosa rather than AA. The authors noted an increase in BMD but did not adjust for weight changes over the study duration. Larger studies have not demonstrated significant changes in BMD with oral estrogen in low weight amenorrheic women with anorexia nervosa (69, 70), particularly after controlling for weight recovery. Therefore, results from the study by Hergenroeder et al cannot be extrapolated to teenagers and young women with AA **without** low weight, which is the population we plan to study, and for which there is a real dearth of data regarding efficacy of estrogen despite its widespread use. The study by Castelo-Branco et al was larger and showed a beneficial effect of estrogen replacement in women with hypothalamic oligomenorrhea, but women were self selected into the control arm not receiving estrogen, and the authors did not control for weight changes (66). The study by Gibson et al was a pilot study that showed small non-significant increases in BMD (68). Most retrospective studies in adult AA suggest a beneficial effect of estrogen replacement on BMD (71, 72), and a sparing effect of estrogen replacement on stress fractures (72, 73). Other reported studies are either not controlled, have small numbers of subjects, do not specifically address AA, or adjust for confounders such as weight changes (10, 71, 74-77). Of importance, estrogen effects in an adolescent and young adult population that should be actively accruing bone are likely very different from those in adult AA who have achieved peak bone mass. Despite the importance of estrogen in bone accrual in the teenage years, no studies have investigated effects of estrogen replacement in estrogen deficient adolescent athletes.

Describe how risks to subjects are minimized, for example, by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk or by using procedures already being performed on the subject for diagnostic or treatment purposes.

(i) Risks from procedures:

There is a risk of superficial bruising and discomfort at the venepuncture site. DXA and bone age evaluation involve minimal radiation (~0.02 mSv for entire study) - less than 1% of the background radiation received/person/year in the US. The effective radiation dose with X-treme CT is well within

safety limits of radiation exposure in children (0.027 mSv per measurement compared with 0.1 mSv with a chest X-ray). The total effective dose from all exams for the entire study is about 0.3 mSv which is about 3% of the effective dose (3mSv) everyone gets from the background radiation per year.

A pregnancy test will be performed at all visits.

(ii) Risks from medications:

Estrogen administration is associated with minimal possible side effects, especially when given transdermally. Occasional side effects may occur, the commonest of which are mild headaches, and very rarely some irritation or redness at the application site. Adverse reactions that have been reported with estrogen therapy include abnormal bleeding patterns, changes in nature and quantity of vaginal discharge, tenderness and enlargement of breasts, nausea, vomiting, abdominal pain, bloating, gall bladder disease, rashes, intolerance to contact lenses, migraines, dizziness, depression, abnormal movements, and changes in weight. These adverse effects are, however, rare, and seen more commonly with the estrogen taken orally rather than transdermally. More serious side effects include hypertension, lipid and clotting disorders and a higher risk of breast cancer. These are more commonly seen in older women and in women with a family history of clotting disorders and cancer. In addition, we plan to administer estrogen to hypogonadal adolescents who are at an age when estrogen levels are steadily rising in healthy adolescents, a very different physiological state compared to post-menopausal women, a time of life when estrogen levels are expected to be low in these women. Continuous estrogen administration without cyclic progesterone can predispose the uterus to cancer. However, our subjects with AA who receive transdermal estrogen will also receive progesterone (Prometrium) 200 mg pills orally daily for the first 12 days of every month, and subjects with AA receiving oral estrogen (0.030 mg ethinyl estradiol) will also receive concomitant progesterone (0.15 mg of desogestrel, a synthetic progesterone) daily for 21/28 days. We will monitor the subjects' blood pressure at each visit and will determine through careful questioning and examination the appearance of any side-effect.

We have carefully considered the possibility that estrogen administration may decrease bone density and may thus have negative effects on bone health, based on papers by Hartard et al (88) and Burr et al (89). The study by Hartard et al was retrospective (88), and it is possible that girls enrolled in this study were more likely to be prescribed oral contraceptives by their providers if they had a longer duration of amenorrhea or lower weight, and low weight is an important predictor of low bone density. We are planning to study young women and teenagers with normal weight who are amenorrheic, and we will monitor weight over the study duration. Burr et al studied healthy women who were not estrogen deficient, either exercisers or not exercisers, and examined the effects of exercise and estrogen use on bone density in a non-randomized controlled trial (89). Therefore, these data are not applicable to an estrogen deficient population. Of note, women receiving estrogen did better than those who exercised but did not get estrogen. It is reassuring that other studies have demonstrated either some or no increase in BMD with use of oral estrogen, and none suggest that BMD decreases with estrogen use (12, 66, 72-78). In addition, our **pilot preliminary data** show that in six young women followed prospectively over 12 months, estrogen use was associated with a significant increase in BMD.

Bone density information will be presented to the DSMB at each meeting, and study termination will be considered for subjects demonstrating a greater than 10% decrease in lumbar spine bone density from baseline, if randomized to the estrogen arm.

Estrogen administration in pregnancy may be teratogenic. We will thus perform a pregnancy test prior to administering estrogen and every three months. We will not administer estrogen to subjects with contraindications to estrogen therapy such as:

- Known or suspected pregnancy
- Undiagnosed abnormal uterine bleeding
- History of clotting disorders such as thrombophlebitis and thromboembolic disorders
- Known or suspected cancer of the breasts, uterus or ovaries
- History of smoking (please see details described under Exclusion Criteria)

All study subjects will be counseled regarding risks of pregnancy, and birth control measures will be discussed at each study visit (see handout to be given to subjects at study visits regarding contraceptive methods that are possible to use). Girls randomized to the arm receiving oral estrogen and progesterone (oral contraceptive pill) will also be cautioned about the risks of pregnancy with missed pills. Girls randomized to the estrogen patch arm will be cautioned that estrogen patches used in this study do not have contraceptive efficacy. We will also discuss with our subjects that study medications will not protect

against sexually transmitted diseases and that appropriate measures need to be adopted (use of barrier contraception) to prevent these diseases.

Oral progesterone has been associated with clotting abnormalities, dizziness, nausea, abdominal pain, fatigue, headaches, insomnia, nervousness, sleepiness and breast tenderness and secretion. Rare cases of breast cancer have been reported in women taking combined estrogen and progesterone. Rare instances of abnormal liver function have also been reported. The doses of progesterone that will be given are small and are less likely to cause these side effects. Particularly micronized progesterone (Prometrium) is associated with few adverse effects. The oral estrogen-progesterone preparation we plan to use is the generic form used for birth control, and most commonly used amongst adolescents.

Oral calcium can cause constipation and a metallic taste in the mouth.

Vitamin D in the doses being administered does not cause any side effects.

Subjects will be instructed to call us if they develop any side-effects of the medication.

Subsequent management will be decided based on the severity of the side-effect. Serious side effects such as thrombosis will necessitate protocol discontinuation (described under 'Criteria for discontinuing the protocol'). If a subject develops a minor side effect like breakthrough bleeding, she will be asked to continue medications as per the protocol. Worsening of symptoms may necessitate temporarily withholding medication until other potential causes of symptoms are ruled out. Withholding medication for longer than two months will necessitate discontinuation of the study.

A physician will be available by pager at all times during the study to answer any questions or address any concerns that a subject may have. All efforts will be made to protect the confidentiality of study subjects who will be referred to by an enrollment number only.

Describe explicitly the methods for ensuring the safety of subjects. Provide objective criteria for removing a subject from the study, for example, objective criteria for worsening disease/lack of improvement and/or unacceptable adverse events. The inclusion of objective drop criteria is especially important in studies designed with placebo control groups.

One central Data Safety Monitoring Board (DSMB) will be convened at MGH every six months to review all adverse events. Moreover, additional meetings may be called to consider urgent issues. Bone density information will be presented to the DSMB at each meeting, and study termination will be considered for subjects demonstrating a greater than 10% decrease in lumbar spine bone density from baseline, if randomized to the estrogen arm.

1. A serious adverse event related to use of estrogen, e.g. thrombosis. Details will be reported to the DSMB and to the primary care provider of the subject. The safety of estrogen use in this population will be reassessed.
2. Pregnancy
3. Development of other disorders that may affect bone metabolism during the study
4. Therapeutic use of medications that may affect bone metabolism during the study period
5. > 10% decrease in BMD in subjects randomized to estrogen as determined by the DSMB

FORESEEABLE RISKS AND DISCOMFORTS

Provide a brief description of any foreseeable risks and discomforts to subjects. Include those related to drugs/devices/procedures being studied and/or administered/performed solely for research purposes. In addition, include psychosocial risks, and risks related to privacy and confidentiality. When applicable, describe risks to a developing fetus or nursing infant.

Please see page 6

EXPECTED BENEFITS

Describe both the expected benefits to individual subjects participating in the research and the importance of the knowledge that may reasonably be expected to result from the study. Provide

a brief, realistic summary of potential benefits to subjects, for example, “It is hoped that the treatment will result in a partial reduction in tumor size in at least 25% of the enrolled subjects.” Indicate how the results of the study will benefit future patients with the disease/condition being studied and/or society, e.g., through increased knowledge of human physiology or behavior, improved safety, or technological advances.

Potential Benefits include an increase in BMD in AA girls who are randomized to estrogen and a better understanding of the risk for low BMD from prolonged amenorrhea in other AA girls. In addition, use of calcium and vitamin D may cause an increase in BMD in all subjects.

Given that (i) there is no available treatment for low BMD in AA, (ii) oral estrogen is not effective in increasing BMD in adult with AA, and (iii) no studies have examined the effect of transdermal estrogen (not IGF-I suppressive) on BMD in AA, we believe that equipoise does exist in this study.

EQUITABLE SELECTION OF SUBJECTS

The risks and benefits of the research must be fairly distributed among the populations that stand to benefit from it. No group of persons, for example, men, women, pregnant women, children, and minorities, should be categorically excluded from the research without a good scientific or ethical reason to do so. Please provide the basis for concluding that the study population is representative of the population that stands to potentially benefit from this research.

Inclusion of Women and Minorities: We will enroll only female adolescents and young adult women to optimize power and to avoid issues rising from deficiencies of different predominant sex hormones in boys (testosterone) vs. girls (estrogen). Adolescents will be studied in order to determine effects on bone density and its predictors in a population that should be actively accruing bone.

Inclusion of Children: During adolescence and the young adult years, more than 90% of bone mass is formed, making the study of bone accretion and factors which interfere with normal bone accretion during this time particularly important. We are therefore investigating changes in bone and whole body metabolism in normal girls, EA and girls with AA ages 14-25 years, and the effects of estrogen administration on bone in AA. Because 99% of adult height has been achieved by the time the bone age is 15 years or more, estrogen administration will not compromise adult height in the group receiving this. Our preliminary data indicate that estrogen use is not harmful in young adult women 14-25 years old. All subjects will be included in the study only after consent forms have been signed (further details are described under ‘Study Population’).

When people who do not speak English are excluded from participation in the research, provide the scientific rationale for doing so. Individuals who do not speak English should not be denied participation in research simply because it is inconvenient to translate the consent form in different languages and to have an interpreter present.

People who do not speak English will not be excluded from study participation. If a patient who does not speak English should wish to participate in our studies, a consent form in the patient’s language will be prepared and an interpreter will be made available at the study visits.

For guidance, refer to the following Partners policy:

Obtaining and Documenting Informed Consent of Subjects who do not Speak English
<http://healthcare.partners.org/phsirb/nonengco.htm>

RECRUITMENT PROCEDURES

Explain in detail the specific methodology that will be used to recruit subjects. Specifically address how, when, where and by whom subjects will be identified and approached about participation. Include any specific recruitment methods used to enhance recruitment of women and minorities.

Recruitment for the study will be carried out through mailings to pediatricians, nutritionists, therapists, sports medicine specialists, high schools and colleges in the New England area, and through advertisements in local newspapers and magazines.

Subjects will be recruited over a four year period, thus at the rate of about ~ 40 subjects per year (27 AA, 12 EA and healthy adolescents). The accrual goal is reasonable given systems of referral already in place from our studies in girls with anorexia nervosa. We have collaborations ongoing with primary care providers, psychiatrists, therapists, dieticians and adolescent medicine physicians in the New England area for patients with eating disorders and the female athlete triad. Our group informs these providers about new studies, and providers then present studies to their patients who contact our group if interested in study participation. We have been very successful in our recruitment efforts in girls with anorexia nervosa, and expect recruitment of AA to be easier given that prevalence of female athletes is higher than that of anorexia nervosa, and because AA girls have fewer psychiatric co-morbidities compared with girls with anorexia nervosa. In addition, a co-investigator in this grant is Dr. Gary Skrinar, who is a very well known for his work with athletes. Dr. Skrinar was until recently a Professor in the Department of Health Sciences at the Sargent College of Health & Rehabilitation Sciences, Boston University, a member and Fellow of the American College of Sports Medicine and a Certified ACSM program Director. Given Dr. Skrinar's expertise in this field and access to providers looking after athletes, we anticipate recruitment to be steady and to be completed in the specified period. Mailings will also be sent out to our regional high schools. In addition to mailing lists already in place, we are preparing mailing lists of area Sports Medicine specialists who are most likely to see young athletes. We anticipate completing enrollment in four years; such that the last subject will complete the one-year follow up by the end of the fifth year. We anticipate another month will be required by the Core Laboratory to run samples for secondary endpoints, and two months for data analysis. Three months will be necessary to write up results of the study. Study duration is thus anticipated to be 5.5 years.

Recruitment of males will be carried out through mailings to pediatricians, therapists, dieticians, sports and adolescent medicine specialists, high schools and colleges in New England, and advertisements in local newspapers and magazines. Subjects will be recruited over 11 months at the rate of 5-6 subjects/month. The accrual goal is reasonable given referral systems in place from our studies in young athletes. We have collaborations ongoing with primary care providers, psychiatrists, therapists, dieticians and sports and adolescent medicine physicians in New England for patients with eating disorders and athletes. Our group informs providers about new studies, and providers then present the studies to patients who contact our group if interested in participation. We will inform our referral pool of this addition to the original study. We will also send information to local teams and coaches. We anticipate that a month will be required to run blood samples for secondary endpoints, and for data analysis. Study duration is anticipated to be 1 year.

Provide details of remuneration, when applicable. Even when subjects may derive medical benefit from participation, it is often the case that extra hospital visits, meals at the hospital, parking fees or other inconveniences will result in additional out-of-pocket expenses related to study participation. Investigators may wish to consider providing reimbursement for such expenses when funding is available

Subjects will receive a \$125 stipend for the baseline, 6 month, and 12 month visits and a \$75 stipend for the 3 month and 9 month visits, \$50 for baseline and 12 month MRI (\$100 total), and \$100 for optional fMRI visit (\$100 for EAs and HCs, \$200 for AAs completing both scans). Parking at MGH

will be offered at no cost; transportation costs to Massachusetts General Hospital will be reimbursed up to \$25.

For guidance, refer to the following Partners policies:

Recruitment of Research Subjects

<http://healthcare.partners.org/phsirb/recruit.htm>

Guidelines for Advertisements for Recruiting Subjects

<http://healthcare.partners.org/phsirb/advert.htm>

Remuneration for Research Subjects

<http://healthcare.partners.org/phsirb/remun.htm>

CONSENT PROCEDURES

Explain in detail how, when, where, and by whom consent is obtained, and the timing of consent (i.e., how long subjects will be given to consider participation). For most studies involving more than minimal risk and all studies involving investigational drugs/devices, a licensed physician investigator must obtain informed consent. When subjects are to be enrolled from among the investigators' own patients, describe how the potential for coercion will be avoided.

Written, informed consent will be obtained from subjects \geq 18 years old. For subjects 14-17 years old, consent will be obtained from one parent, and assent from the subject. Consent forms, data safety monitoring plan and protocol will be per Institutional Review Board guidelines.

NOTE: When subjects are unable to give consent due to age (minors) or impaired decision-making capacity, complete the forms for Research Involving Children as Subjects of Research and/or Research Involving Individuals with Impaired Decision-making Capacity, available on the New Submissions page on the PHRC website:

<http://healthcare.partners.org/phsirb/newapp.htm#Newapp>

For guidance, refer to the following Partners policy:

Informed Consent of Research Subjects

<http://healthcare.partners.org/phsirb/infcons.htm>

DATA AND SAFETY MONITORING

Describe the plan for monitoring the data to ensure the safety of subjects. The plan should include a brief description of (1) the safety and/or efficacy data that will be reviewed; (2) the planned frequency of review; and (3) who will be responsible for this review and for determining whether the research should be altered or stopped. Include a brief description of any stopping rules for the study, when appropriate. Depending upon the risk, size and complexity of the study, the investigator, an expert group, an independent Data and Safety Monitoring Board (DSMB) or others might be assigned primary responsibility for this monitoring activity.

NOTE: Regardless of data and safety monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for protecting the rights, safety, and welfare of subjects under his/her care.

One central Data Safety Monitoring Board (DSMB) will be convened at MGH every six months to review all adverse events. Moreover, additional meetings may be called to consider urgent issues. Bone density information will be presented to the DSMB at each meeting, and study termination will be considered for subjects demonstrating a greater than 10% decrease in lumbar spine bone density from baseline, if randomized to the estrogen arm.

- Ellen O'Donnell, Ph.D., Licensed Clinical Psychologist, Massachusetts General Hospital, Instructor, Harvard Medical School
- Soja Park-Bennett, M.D., Pediatric Endocrine Unit, Massachusetts General Hospital
- Mariette Murphy, MD, Adolescent Medicine physician, Massachusetts General Hospital
- Jean Mulder, MD, (expert on metabolic bone disease), Division of Endocrinology, Brigham and Women's Hospital
- Brian Healy, PhD, Biostatistician, Massachusetts General Hospital

All data will be recorded on specified Case Report Forms.

Adverse events or other unanticipated problems will be reported to the PHRC as described in the PHRC policy on 'Adverse Event Reporting and Unanticipated Problems Involving Risks to Subjects or Others.

In addition, semi-annual data review and evaluation of side effects will be conducted. Results of the semi-annual review will be forwarded to the Partners IRB-

Describe the plan to be followed by the Principal Investigator/study staff for review of adverse events experienced by subjects under his/her care, and when applicable, for review of sponsor safety reports and DSMB reports. Describe the plan for reporting adverse events to the sponsor and the Partners' IRB and, when applicable, for submitting sponsor safety reports and DSMB reports to the Partners' IRBs. When the investigator is also the sponsor of the IND/IDE, include the plan for reporting of adverse events to the FDA and, when applicable, to investigators at other sites.

NOTE: In addition to the adverse event reporting requirements of the sponsor, the principal investigator must follow the Partners Human Research Committee guidelines for Adverse Event Reporting

Adverse events or other unanticipated problems will be reported to the PHRC as described in the PHRC policy on 'Adverse Event Reporting and Unanticipated Problems Involving Risks to Subjects or Others.

MONITORING AND QUALITY ASSURANCE

Describe the plan to be followed by the principal investigator/study staff to monitor and assure the validity and integrity of the data and adherence to the IRB-approved protocol. Specify who will be responsible for monitoring, and the planned frequency of monitoring. For example, specify who will review the accuracy and completeness of case report form entries, source documents, and informed consent.

NOTE: Regardless of monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for ensuring that the study is conducted at his/her investigative site in

accordance with the IRB-approved protocol, and applicable regulations and requirements of the IRB.

Specimens: Blood specimens and all other testing will be obtained from subjects for research purposes only. From the study visits, most blood samples will be frozen and stored in freezers in the Neuroendocrine Unit of Massachusetts General Hospital. These freezers are accessible to no one but the Neuroendocrine staff. Some blood samples will be sent to the chemistry laboratories of MGH for analysis, and left over samples will be disposed of as per institutional guidelines.

The privacy of subjects and confidentiality of data obtained from subjects will be maintained strictly. In any reports or publications resulting from the study, the privacy and anonymity of individuals in the report will be protected. The subject's name will be removed as markers on any clinical reports submitted as attachments to an adverse event report. Subjects will be assigned a code, which will be used in all data used for analysis.

Adverse Events: Adverse events or other unanticipated problems will be reported to the PHRC as described in the PHRC policy on 'Adverse Event Reporting and Unanticipated Problems Involving Risks to Subjects or Others

Data Quality Control: Accuracy of data entry will be checked using different methods at the beginning of the study and later when site monitoring has started. At the start of the study, before the first site monitoring data is returned, the coordinator will check 25 random data items on the database against the case records each month. If the error rate of data entry is worse than 2.5% overall or appears in crucial variables we will consider that a "data entry problem" exists and initiate whatever corrective action is necessary. One corrective action would be to initiate double entry of all data.

For guidance, refer to the following Partners policies:

Data and Safety Monitoring Plans and Quality Assurance

<http://healthcare.partners.org/phsirb/datasafe.htm>

Adverse Event Reporting Guidelines

<http://healthcare.partners.org/phsirb/adverse.htm>

PRIVACY AND CONFIDENTIALITY

Describe methods used to protect the privacy of subjects and maintain confidentiality of data collected. This typically includes such practices as substituting codes for names and/or medical record numbers; removing face sheets or other identifiers from completed surveys/questionnaires; proper disposal of printed computer data; limited access to study data; use of password-protected computer databases; training for research staff on the importance of confidentiality of data, and storing research records in a secure location.

NOTE: Additional measures, such as obtaining a Certificate of Confidentiality, should be considered and are strongly encouraged when the research involves the collection of sensitive data, such as sexual, criminal or illegal behaviors.

Most blood samples will be frozen and stored in freezers in the Neuroendocrine Unit of Massachusetts General Hospital. These freezers are accessible to no one but the Neuroendocrine staff. Some blood

samples will be sent to the chemistry and hematology laboratories of MGH for analysis, and left over samples will be disposed of as per institutional guidelines.

The privacy of subjects and confidentiality of data obtained from subjects will be maintained strictly. In any reports or publications resulting from the study, the privacy and anonymity of individuals in the report will be protected. The subject's name will be removed as markers on any clinical reports submitted as attachments to an adverse event report. Subjects will be assigned a code, which will be used in all data used for analysis.

SENDING SPECIMENS/DATA TO RESEARCH COLLABORATORS OUTSIDE PARTNERS

Specimens or data collected by Partners investigators will be sent to research collaborators outside Partners, indicate to whom specimens/data will be sent, what information will be sent, and whether the specimens/data will contain identifiers that could be used by the outside collaborators to link the specimens/data to individual subjects.

Not applicable

Specifically address whether specimens/data will be stored at collaborating sites outside Partners for future use not described in the protocol. Include whether subjects can withdraw their specimens/data, and how they would do so. When appropriate, submit documentation of IRB approval from the recipient institution.

Specimens and data will not be stored at collaborating sites outside Partners.

RECEIVING SPECIMENS/DATA FROM RESEARCH COLLABORATORS OUTSIDE PARTNERS

When specimens or data collected by research collaborators outside Partners will be sent to Partners investigators, indicate from where the specimens/data will be obtained and whether the specimens/data will contain identifiers that could be used by Partners investigators to link the specimens/data to individual subjects. When appropriate, submit documentation of IRB approval and a copy of the IRB-approved consent form from the institution where the specimens/data were collected.

No specimens or data will be collected by research collaborators outside Partners.