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
Madhusmita Misra, MD, MPH

Effects of Anorexia Nervosa on Peak Bone Mass

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Protocol Summary

Anne Klibanski, MD

PROTOCOL TITLE
Effects of Anorexia Nervosa on Peak Bone Mass

FUNDING
NIH, and Global Foundation for Eating Disorders

VERSION DATE
March 29, 2018

SPECIFIC AIMS

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

One of the goals of this proposal is to investigate if co-administration of rhIGF-1 with physiologic estradiol replacement to adolescent girls with AN will increase BMD more than estrogen monotherapy, and bone mass will approach that seen in healthy adolescent girls.

An additional aim for the prospective study is to determine whether co-administration of rhIGF-1 with estradiol to mimic the normal pubertal milieu:

A) Stimulates bone formation through an IGF-1 mediated anabolic effect
B) Increases bone density to a greater extent than estrogen monotherapy
C) Improves bone mass accrual to approach that in healthy controls

Further more we will investigate if co-administration of rhIGF-1 with physiological estradiol replacement will improve bone microarchitecture and bone strength in girls with AN. In a cross-sectional study of adolescent girls with AN and healthy adolescent girls, we will determine whether:

A) Cortical and trabecular bone density and microarchitecture at the ultradistal radius (non-weight bearing bone) and tibia (weight bearing bone) as assessed by HR-pQCT, and bone strength, as assessed by finite element analysis (FEA), are abnormal in AN compared to controls, and are associated positively with nutritional and hormonal predictors of bone mass, including BMI, body fat, fat-free mass, estradiol and IGF-1 levels

In a prospective study of adolescent girls with AN we will determine whether:

B) Co-administration of rhIGF-1 with transdermal estrogen improves cortical and trabecular bone microarchitectural parameters (assessed by HRpQCT) and bone strength (assessed by FEA) more than estrogen alone to approach that in normal adolescent girls. Girls with AN receiving estradiol alone will have an increase in bone microarchitecture and strength versus baseline, but will not normalize compared to healthy controls
C) Changes in IGF-1 levels are associated positively with changes in measures of bone density, microarchitecture and strength.

BACKGROUND AND SIGNIFICANCE

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

Anorexia nervosa (AN) is the third most common chronic illness among adolescent girls (1), with a prevalence of 0.2-1.0% in Western societies (2, 3). The prevalence rate of AN is 269.9 per 100,000 for females, with a linear increase in incidence reported for the 15-24 year age group over the last 50 years (1). Primarily psychiatric in origin, AN results in numerous medical complications, which lead to increased morbidity and mortality in this young population. Although the endocrine and metabolic disturbances of AN typically resolve with weight gain, low bone mineral density (BMD) is a frequent and persistent co-morbid complication of AN. *There are no effective approved therapies to normalize bone mass accrual in this population.*

Why is the low bone mass characteristic of AN of critical health importance? (i) The prevalence, rapidity of development, and severity of low bone mass in adolescents and young women with AN have associated morbidity, (ii) low BMD acquired during adolescence may impose life-long increased fracture risk, (iii) despite the increased prevalence of this disorder, treatment strategies in this population do not exist, and (iv) AN represents a unique model in which to investigate the profound consequence of nutritional deprivation and pubertal disruption on bone mass acquisition. The majority of adolescent girls with AN have low BMD of early onset involving both trabecular and cortical bone (4-6), and clinical fractures occur even in this young age group; lumbar BMD is reduced >2 SD in adolescent AN girls, often after only one year of illness (5). A study in 170 adolescents 10-17 years old with AN found that 44% of girls with ≥ six months of amenorrhea had low lumbar spine bone mass (7). Unfortunately, the time to recovery in adolescents with AN is long (8), and therefore, severe bone mass deficits are common. We have shown that adolescents with AN have a marked reduction in bone mass compared to bone age-matched controls using height-adjusted BMD calculations to eliminate potential bone size confounders (9). A population-based long-term fracture risk cohort study from the Mayo Clinic found that the cumulative incidence of any fracture 40 years after the diagnosis of AN is 57% (10). Low bone density in AN is a consequence of several hormonal alterations including hypogonadism, low IGF-1 levels, high levels of cortisol and alterations in certain hormones such as ghrelin, leptin and PYY that are altered in conditions of low energy availability.

Why is this young population at such high risk for severe and permanent osteopenia and skeletal fragility? The onset of AN most frequently occurs during adolescence, a period of maximal bone accretion, with > 90% of bone mass accrued by 18 years of age (11). Because adolescence is a critical time for attainment of peak skeletal mass, reduction of BMD during this crucial period may lead to long-term morbidity. Indeed, we found that in contrast to normal adolescents who demonstrate marked bone accretion during puberty, adolescents with AN do not increase their bone mass (12). Therefore, bone mass remains low in these girls during a critical period in normal bone development. We have previously shown that adults with onset of AN during adolescence have lower spine BMD than those with adult-onset AN, despite comparable years of amenorrhea (13). Furthermore, low bone mass in adolescents with AN may be permanent despite recovery (14). Approximately half of young women with AN have persistent osteopenia, even after >10 years (15-17). Therefore, there may be a narrow "window-in-time" during adolescence in which maximal accrual of bone mass occurs and during which treatment paradigms to maximize bone accrual must be implemented.

The overall significance of this problem: A large number of adolescent girls suffer from anorexia nervosa resulting in major deficits in bone mass and increased skeletal fragility, which may be permanent. Our proposed study will 1) test the efficacy of replacing a deficiency of an

endogenous bone anabolic hormone, IGF-1, with estradiol as a unique therapy for the low bone formation state in adolescents with AN and 2) enable an understanding of the effects of this disease and its treatment by measuring not only compartment-specific changes in bone density, but microarchitecture and strength at weight bearing and non-weight bearing sites.

Given the increasing prevalence of AN, its profound consequences on bone health, and lack of optimal treatment interventions, these studies will provide critical data needed to identify optimal treatment strategies for this severe co-morbid disease using state- of- the- art endpoints of BMD, bone microarchitecture and strength. Although both low IGF-1 and hypogonadism are associated with increased skeletal fragility in AN, the mechanisms by which these factors interact are incompletely understood. Specifically, the increased skeletal fragility that is associated with AN is poorly reflected by DXA-derived BMD. Furthermore, the magnitude and mechanisms by which IGF-1 deficiency and hypogonadism influence bone microarchitecture are not defined. The growing incidence of eating disorders in adolescent girls and their long-term effects on skeletal health provide strong rationale for studies that will provide a better understanding of these issues and the evaluation of rational therapeutic approaches. The studies described in this proposal utilize both cross-sectional and RCT approaches to achieve this goal. Additionally, our utilization of sophisticated techniques such as high resolution peripheral QCT (HR-pQCT) (described below) will improve our understanding of the relationship between IGF-1, gonadal steroids and bone quality and will aid in the development of effective therapies in the treatment of skeletal fragility in AN.

Furthermore, in recent years, there has been an increasing awareness of the bone fat connection. In the marrow, osteocytes and adipocytes arise from a common progenitor. Recent studies have shown an increase in bone marrow fat in adults with anorexia nervosa which is also correlated inversely with bone mineral density. We will assess the effect of estrogen (bone anti resorptive hormone) and IGF-1 (bone anabolic hormone) on marrow fat in girls with AN and in comparison with healthy controls who receive no intervention. Also bone marrow fat and preadipocyte factor, Pref-1, decrease after recovery from anorexia nervosa. We will determine associations of changes in bone mass following therapy with changes in marrow fat and Pref-1 levels over the same duration. This will help us delineate the physiological connection between bone marrow fat and bone mineral density and structure in AN.

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."

Over the proposed 5 year grant, 100 girls with AN and 36 healthy controls will be enrolled at MGH. We will screen up to 272 subjects. Subjects with AN will be randomly assigned by the MGH Research Pharmacy to one of two arms: (i) IGF-1 with estrogen or (ii) estrogen alone with placebo for 12 months. Healthy controls will be followed prospectively for 12 months. Control subjects will complete the screen and baseline visit only. Study staff will follow-up with control subjects via a telephone call at 12 months after their baseline visit. This telephone call will serve to inform study staff of any medical changes or major life events that have occurred since the baseline study visit. We anticipate a 20% dropout rate based on our previous studies. Only AN subjects will take calcium and vitamin D supplement pills daily; dosage of vitamin D will be based upon each subject's serum levels.

AN Subjects: Subjects with AN will be 14-22 years old- an age span before peak bone mass is achieved, with a bone age (BA) of ≥14years and meet DSM V criteria for AN. Our inclusion criteria for AN refers to the diagnosis of (a) Anorexia Nervosa or (b) Atypical Anorexia Nervosa with significant weight loss per DSM V. We will use a BA of 14years as the cut-off because all epiphyses are near fusion and 98% of adult height has been attained at this skeletal age (19).
Control Subjects: (i) Healthy adolescent girls 14-22 years with a BA of ≥14years; (ii) BMI between the 10th-90th percentiles for age, (iii) regular menstrual periods every 28-35 days and/or >9 menses within the last 12 months for subjects ≥ 2 years post-menarche. Control subjects will be studied during the early follicular phase of their cycles (days1-10).

Inclusion Criteria: AN:

- Subjects with AN will be 14-22 years old
- Bone age (BA) of ≥14 years
- Meet DSM V criteria for AN or Atypical AN with significant weight loss
- Subjects at MGH will be evaluated by an MGH study psychiatrist or psychologist before enrollment.

Inclusion Criteria: Controls:

- Healthy adolescent girls 14-22 years
- BA of ≥14 years
- BMI between the 10th-90th percentiles for age
- Regular menstrual periods every 28-35 days and/or >9 menses within the last 12 months for subjects ≥ 2 years post-menarche.

Exclusion Criteria:

- Diseases known to affect bone metabolism including untreated thyroid disease, Cushing's syndrome, diabetes, pituitary disease, renal failure and prior bone fracture within six months of the study.
- Medications known to affect bone metabolism, including gonadal steroids and excluding oral contraceptives or estrogen/progestin containing compounds within two months.
- Evidence of suicidality, psychosis, or substance abuse based on an interview with the study psychiatrist or psychologist and recommendations.
    - In the event that evidence of suicidality, psychosis or substance abuse is uncovered in the interview, the current treatment team for the subject will be contacted and informed by the current study psychologist or psychiatrist. In the event a subject does not have a treatment team in place, referrals will be made by the current study psychologist or psychiatrist. If danger is imminent and safety can not be guaranteed, 911 can be called.
- Premature ovarian failure, as demonstrated by an elevated FSH.
- Abnormal TSH.
- Hematocrit <30%, Potassium <3.0 mmol/L, Glucose <50 mg/dl
- Pregnancy
- History of malignancy
- Absolute contraindications to estrogen therapy (for girls with AN)

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.

Screening Visit: Eligibility for participation in the protocol will be determined at a screening visit. Evaluations on this visit will include:

(a) History and physical exam including Tanner stage, height and weight.
(b) Blood laboratory tests for glucose, TSH, LH, FSH, potassium and hematocrit, IGF-1, urine or serum pregnancy test and Bone age estimated using an x-ray of the left hand and wrist by standards of Greulich and Pyle (19).
    i. In cases where the family can provide records of the lab work required for screening performed within two months of the screening visit, we will not repeat those labs.
    ii. Blood laboratory tests can be done at baseline visit if unable to obtain at screening visit. If blood laboratory tests are done at baseline, medications will be withheld until laboratory results are received and assessed.

Baseline Visit:

(a) Bone age estimated using an x-ray of the left hand and wrist by standards of Greulich and Pyle (19) if not completed at the screening visit.
(b) Complete History and Physical: Including growth, menstrual history, Tanner staging staging and fundoscopic exam.
(c) Anthropometric Measurements: Height will be measured in triplicate using a stadiometer, and weight on a single electronic scale in the Clinical Research Center (CRC).
(d) Nutritional Assessment: CRC dieticians at MGH will evaluate nutritional status using 4-day food records for macro- and micronutrient intake (Nutrition Data Systems, version 4, Minneapolis, MN) and indirect calorimetry (metabolic cart) to determine respiratory quotient (RQ) and resting energy expenditure. (All subjects consent to the metabolic cart, although it is treated as optional during the study. A food diary log may be provided at the screening visit or baseline visit. Although all subjects consent to the food diary log, its completion is treated as optional during the study.)
(e) EDI2:Eating Disorders Inventory-2 questionnaire, which has been validated in adolescent patients with eating disorders (25) and includes assessment of body image. This is a 91-item, 11 scale, six-point forced-choice self-report inventory assessing behavioral and psychological traits common in AN. (This can be completed at the screen visit)
(f) BDI-II: Beck Depression Inventory-2 questionnaire is a self-report inventory assessing depression and mood traits common in AN (All subjects consent to standardized psychiatric tests, although they are treated as optional during the study. This component can also be completed at the screen visit, instead of the baseline visit.). For safety concerns related to suicidality, after every administration of the BDI-II, a research assistant or study nurse will review the suicidality item of each measure. If any subject scores a “2” or “3” on the BDI-II “Suicidal Thoughts or Wishes” item, or if the total score corresponds to “moderate” or “severe” depression (see below), the study psychologist/psychiatrist will be paged for further assessment with the participant. BDI-II scoring ranges are as follows:
    a. 0-13: minimal depression
    b. 14-19: mild depression
    c. 20-28: moderate depression
    d. 29-63: severe depression
(g) STAI: State Trait Anxiety Inventory questionnaire is a 40-item and four-point Likert scale self-report inventory assessing state and trait anxieties common in AN. (All subjects consent to standardized psychiatric tests, although they are treated as optional during the study. This component can also be completed at the screen visit, instead of the baseline visit.)

(h) Activity Assessment: Habitual exercise and activity will be assessed using a standardized exercise questionnaire, which provides a good estimate of past year physical activity in adolescents (26, 27). (All subjects consent to the activity assessment, although it is treated as optional during the study.)
(i) Bone Turnover Markers: Serum for PINP and C-telopeptide (CTX).
(j) Calcium Metabolism: Blood for calcium, phosphorus, 25(OH) D, $1,25(OH)_2D$, PTH.
(k) Hormonal Evaluation: Serum for IGF-1, IGFBP-3, SHBG, estradiol, leptin.
(l) If not obtained at screening visit: Blood laboratory work for glucose, TSH, LH, FSH, potassium and hematocrit, and IGF-1. Medications will be withheld until laboratory results are received and assessed.
(m) Bone Mass Density: lumbar spine (AP and lateral), hip, radius, whole body areal BMD, BMC and BA will be measured by dual energy x-ray absorptiometry (DXA: Hologic QDR 4500, Waltham, MA).
(n) Body Composition: Whole body DXA will be performed to assess fat and lean mass.
(o) Bone morphology, 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 (28).
(p) Safety Evaluation: Urine or Serum HCG (to rule out pregnancy) and fasting glucose.
(q) Thigh and abdomen MRI and an L4 MR spectroscopy will be performed to assess bone marrow fat. We will also add Pref-1 assessment to the already collected blood. This will only be repeated at 12 months for patients with anorexia nervosa.
    a. This study is optional for AN subjects and non-optional for HC subjects
(r) Study Drug Reminders: We will set up medication reminders on phone calendars for those who consent to the optional reminders. Subjects may decrease or turn off reminders at any time. All patient information will remain confidential, and no identifiers will be used. Additionally, all subjects will receive paper medication calendars to keep track of each dose taken and to return to study staff at each visit.
(s) 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). (All subjects consent to the cognitive assessments, with the intention that they will be completed to the extent that time, subject schedule, and circumstances allow during the study visit.)

All blood samples will be drawn fasting or at least two hours after food. Subjects who are unable to fast overnight will have the option to complete the metabolic test while fasting for two hours.

<u>Combined Screening and Baseline Visit</u>

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. 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:

(c) History and physical exam including Tanner stage, height and weight.

(d) Blood laboratory tests for glucose, TSH, LH, FSH, potassium and hematocrit, IGF-1, urine or serum pregnancy test and Bone age estimated using an x-ray of the left hand and wrist by standards of Greulich and Pyle (19).

(e) Anthropometric Measurements: Height will be measured in triplicate using a stadiometer, and weight on a single electronic scale in the Clinical Research Center (CRC).

(f) Nutritional Assessment: CRC dieticians at MGH will evaluate nutritional status using 4-day food records for macro- and micronutrient intake (Nutrition Data Systems, version 4, Minneapolis, MN) and indirect calorimetry (metabolic cart) to determine respiratory quotient (RQ) and resting energy expenditure**.** (All subjects consent to the metabolic cart, although it is treated as optional during the study. A food diary log may be provided at the screening visit or baseline visit. Although all subjects consent to the food diary log, its completion is treated as optional during the study.)

(g) EDI2:Eating Disorders Inventory-2 questionnaire, which has been validated in adolescent patients with eating disorders (25) and includes assessment of body image. This is a 91-item, 11 scale, six-point forced-choice self-report inventory assessing behavioral and psychological traits common in AN. (This can be completed at the screen visit)

(h) BDI-II: Beck Depression Inventory-2 questionnaire is a self-report inventory assessing depression and mood traits common in AN (All subjects consent to standardized psychiatric tests, although they are treated as optional during the study. This component can also be completed at the screen visit, instead of the baseline visit.). For safety concerns related to suicidality, after every administration of the BDI-II, a research assistant or study nurse will review the suicidality item of each measure. If any subject scores a “2” or “3” on the BDI-II “Suicidal Thoughts or Wishes” item, or if the total score corresponds to “moderate” or “severe” depression (see below), the study psychologist/psychiatrist will be paged for further assessment with the participant. BDI-II scoring ranges are as follows:

   a. 0-13: minimal depression
   b. 14-19: mild depression
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   d. 29-63: severe depression

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(j) Activity Assessment: Habitual exercise and activity will be assessed using a standardized exercise questionnaire, which provides a good estimate of past year physical activity in adolescents (26, 27). (All subjects consent to the activity assessment, although it is treated as optional during the study.)

(k) 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). (All subjects consent to the cognitive assessments, with the intention that they will be completed to the extent that time, subject schedule, and circumstances allow during the visit)

6-month and 12-month (AN Subjects Only): will include all the baseline procedures but the bone age x-ray.

Eligible consented subjects will be allowed to have CT scans performed between the screening and baseline visits if scheduling conflicts arise with the baseline visit. This will allow the subject to obtain a baseline assessment of bone microarchitecture prior to the initiation of rhIGF-1 supplementation. In the event that a CT cannot be performed on the scheduled baseline visit date due to unforeseen issues, willing subjects will be able to return within a 1 week window for this test. DXA scans can also be completed at screening visit if not able to be performed at baseline visit. MR/MRS will be repeated at 12 month visit for patients with anorexia nervosa. We will also repeat the Pref-1 levels for those who enrolled in this optional part of the study.

1-month, 4.5-month, 7.5-month and 10.5 month visits (AN Subjects Only): Evaluations on these visits will include:
(a) History and physical exam, including height and weight
(b) Pregnancy test
(c) Serum IGF-1 and glucose

3-month and 9-month visits (AN Subjects Only): Evaluations on these visits will include:
(a) History and physical exam, including height and weight
(b) Pregnancy test
(c) Serum IGF-1 and glucose
(d) Nutrition evaluation will be assessed by using 4-day food records for macro- and micronutrient intake (Nutrition Data Systems, version 4, Minneapolis, MN)
(e) Activity assessment: Habitual exercise and activity will be assessed using a standardized exercise questionnaire. (All subjects consent to the activity assessment, although it is treated as optional during the study.)
(f) Bone turnover markers and hormones

Subjects who do not live locally will be able to have their blood drawn offsite for levels of IGF-1 and bone markers. Subjects who choose this option will also have a history and physical performed by a local physician for each offsite visit. Tests that need to be run in real time will be done at local laboratories, with the results mailed to investigators. Otherwise, the samples will be mailed to investigators.

Subjects who are in residential treatment can have their visits at the treatment center with permission from the center, the subject's case manager, and the subject. The consent, history, and physical will be done by licensed study staff. This applies to all visits except for baseline, 6 month, and 12 month. Blood will not be drawn at the treatment center by study staff. If lab results are available for any labs that we need for the particular visit, we will use those labs. A release of information will be signed by the subject prior to obtaining any labs.

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 girls with anorexia nervosa. Weight gain is associated with some increase in bone density, but is difficult to attain (occurs in only 50%) and sustain. There are currently no FDA-approved therapies for anorexia nervosa-induced bone loss.

***Alternate strategies to address low bone density in AN*: (1) Recovery of weight and menses:** Although weight and menses recovery is associated with some increase in BMD, this increase is not sufficient to cause bone accrual to normalize, and BMD remains lower than in controls (54). Additionally, recovery can be hard to attain and sustain. **(2) Calcium and vitamin D supplementation:** Many studies have now shown that calcium and vitamin D supplementation is not sufficient to increase BMD in AN. BMD remains low in these girls despite higher calcium and vitamin D intake than in controls (9, 12, 54). **(3) Bisphosphonates**: Given their very long half life, bisphosphonates are typically avoided in young girls and women of reproductive age. In addition, data indicate that alendronate use is ineffective in increasing BMD at the spine in girls with AN (55). **(4) PTH**: Teriparatide increases BMD in post-menopausal women (56), however, PTH is not recommended in young people at this time given reports of osteosarcoma with PTH use in animal models (57, 58).

***Alternate strategies to assess bone microarchitecture and strength*:** MRI of the peripheral skeleton has lower resolution, longer scan time and increased susceptibility to image post-processing. QCT could also be used to assess trabecular bone structure and density of the spine. However, its relatively high radiation dose at the spine precludes its use in adolescents. HR-pQCT low radiation and technique precision makes it the optimal choice. We have compared bone structure as assessed by HR-pQCT in girls with AN and healthy adolescent girls. Finally, we have demonstrated our ability to perform FEA from assessments of bone structure in a population of women with AN, and have shown that bone strength is associated strongly with bone trabecular volume (59). FEA is easily derived using HR-pQCT data (28, 60-63).

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.

Risks from medications:

RhIGF-I administration in the dose being used in this study is associated with few side effects. This medication is approved by the FDA for use in children with primary IGF-I deficiency at a dose of 40-80 mcg/kg sc twice daily (up to 120 mcg/kg sc twice daily). As per the package insert for rhIGF-I for this indication: mild or moderate hypoglycemia (very rarely severe), thought to be related to the drug's insulin-like activities, may occur in up to 42% of patients on this medication during the course of therapy. More severe hypoglycemia is rare. The peak hypoglycemic effect of IGF-I occurs 30 minutes to one hour after injection. Risk of hypoglycemia is generally avoided when a meal or snack is consumed 30 minutes prior to rhIGF-I administration. We have not observed hypoglycemia in subjects in our pilot studies with rhIGF-I shots. Our subjects were instructed to take rhIGF-I within half an hour of a meal and these recommendations will hold in the proposed study, in which the doses we will use will be much lower than those used in primary IGF-I deficiency. We will also assess fasting glucose levels at all study visits. The safety and efficacy of rhIGF-I has been demonstrated by our group in a study in adult women with AN (64, 65), in which 30 adult women with AN received 30

mcg/kg of rhIGF-I sc twice daily. RhIGF-I was well tolerated in this study (65). Other occasionally reported side effects of rhIGF-1 include bruising and redness at the injection site, lymphoid hypertrophy and snoring, lipohypertrophy, chronic middle ear effusions, raised intracranial pressure (headaches, vision changes, vomiting), slipped capital femoral epiphysis and progression of scoliosis in subjects who experience rapid growth, and arthralgias. Rotating injection sites will be advised and a close watch on other clinical features implemented. Also, as the drug is a pharmaceutical protein, anti-IGF-1 antibody formation can occur. 14 of 23 subjects treated for 2 years with the drug in one study experienced some degree of anti-IGF-1 antibody formation, though no clinical consequences (allergic reaction or loss of efficacy) were observed (from package insert). However, as with any exogenously administered protein, local or systemic allergic reactions may occur. Parents and subjects will be informed that such reactions are possible and that if an allergic reaction occurs, treatment should be interrrupted and prompt medical attention sought. Because we will be giving a smaller dose of rhIGF-1 (30 mcg/kg sc twice daily), we expect to see fewer side effects than indicated in the package insert. These risks of side effects are justified in light of the anticipated benefit of improved bone density and lowered immediate and lifetime fracture risk with treatment during this critical period of bone mass accrual. The rhIGF-1 dose will be increased or decreased by up to 25% to maintain IGF-1 levels in the upper half of the normal range. We will titrate the dose using a maximum of two dose increments. Thus the maximum dose will be 46.88 mcg/kg twice daily.

Estrogen administration in the doses being administered is associated with minimal possible side effects. Occasional side effects may occur, the most common of which are mild headaches, and very rarely some irritation or redness at the application site for transdermal estrogen. 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, gallbladder 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. These are more commonly seen in older women and in women with a family history of clotting disorders. Continuous estrogen administration without cyclic progesterone can predispose the uterus to cancer. Because we will be administering progesterone for ten days every month, this should not be a concern. We will monitor the subjects' blood pressure at each visit and will also determine through careful questioning and examination the appearance of any side-effect at each visit. Estrogen administration in pregnancy may be teratogenic. We will thus perform a pregnancy test prior to administering estrogen.

We will not enroll subjects with the following absolute contraindications to estrogen therapy:

- 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.

Relative contraindications such as migraines and smoking history will be decided on a case by case basis using history and phyical findings

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.

Use of replacement or higher doses of estrogen before epiphyseal closure could accelerate growth plate closure resulting in an inability to reach the full potential for adult height. To avoid

this possible side effect, we plan to administer replacement estrogen doses transdermally to girls with a bone age of 14 or more years, who have achieved 98% of their adult height.

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.

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.

Physician Availability: 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.

Subjects will be instructed to call us if they develop any side-effects from the study medications, or if symptoms of hypoglycemia are experienced. They will be advised to take 4 oz orange juice or two glucose pills if they are experiencing symptoms of hypoglycemia.
Serious side effects related to study drug will necessitate protocol discontinuation. If a subject develops a minor side effect, 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 one week will necessitate discontinuation of the study.

To minimize the risk from radiation, DXA scans will not be performed on subjects until a negative pregnancy test result is obtained. If lab abnormalities are discovered, the subject and their physician, if authorized by the subject, will be notified.
If subjects are uncomfortable with any portion of any study visit, this portion will be discontinued or not performed.
Discontinuation criteria for subjects:

1. Pregnancy (for any subject)
2. Identification of characteristics meeting criteria for exclusion (for any subject)

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.

Complications of surgical and non-surgical procedures: There is a risk of superficial bruising and discomfort at the venepuncture site. Rarely, fainting (from a vasovagal episode) or a treatable infection may occur. An intravenous catheter will be in place for frequent sampling overnight at baseline and at the end of the study. This may cause discomfort for the duration of

the study. EMLA or ELA-MAX, a numbing cream, can be used to minimize the pain and discomfort associated with blood sampling.

Fasting for prolonged periods of time can cause blood sugar levels to fall resulting in light-headedness, dizziness, sweating, hunger or headaches. Because fasting will only occur between midnight and approximately 8 a.m. the following morning, a short period of time, we do not expect any of these side effects.

The risks from radiation exposure are minimal due to the low doses of radiation involved in bone age x-rays, CT scans, and DXA (~0.16 mSv for DXAs, bone age film and fpVCT for each subject, which is equivalent to about 5% of the effective dose (3mSv) everyone gets from the background radiation per year).

Risks from medications:

RhIGF-I administration in the dose being used in this study is associated with few side effects. This medication is approved by the FDA for use in children with primary IGF-I deficiency at a dose of 40-80 mcg/kg sc twice daily (up to 120 mcg/kg sc twice daily). As per the package insert for rhIGF-I for this indication: mild or moderate hypoglycemia (very rarely severe), thought to be related to the drug's insulin-like activities, may occur in up to 42% of patients on this medication during the course of therapy. More severe hypoglycemia is rare. The peak hypoglycemic effect of IGF-I occurs 30 minutes to one hour after injection. Risk of hypoglycemia is generally avoided when a meal or snack is consumed 30 minutes prior to rhIGF-I administration. We have not observed hypoglycemia in subjects in our pilot studies with rhIGF-I shots. Our subjects were instructed to take rhIGF-I within half an hour of a meal and these recommendations will hold in the proposed study, in which the doses we will use will be much lower than those used in primary IGF-I deficiency. We will also assess fasting glucose levels at all study visits. The safety and efficacy of rhIGF-I has been demonstrated by our group in a study in adult women with AN (64, 65), in which 30 adult women with AN received 30 mcg/kg of rhIGF-I sc twice daily. RhIGF-I was well tolerated in this study (65). Other occasionally reported side effects of rhIGF-1 include bruising and redness at the injection site, lymphoid hypertrophy and snoring, lipohypertrophy, chronic middle ear effusions, raised intracranial pressure (headaches, vision changes, vomiting), slipped capital femoral epiphysis and progression of scoliosis in subjects who experience rapid growth, and arthralgias. Rotating injection sites will be advised and a close watch on other clinical features implemented. Also, as the drug is a pharmaceutical protein, anti-IGF-1 antibody formation can occur. 14 of 23 subjects treated for 2 years with the drug in one study experienced some degree of anti-IGF-1 antibody formation, though no clinical consequences (allergic reaction or loss of efficacy) were observed (from package insert). However, as with any exogenously administered protein, local or systemic allergic reactions may occur. Parents and subjects will be informed that such reactions are possible and that if an allergic reaction occurs, treatment should be interrrupted and prompt medical attention sought. Because we will be giving a smaller dose of rhIGF-1 (30 mcg/kg sc twice daily), we expect to see fewer side effects than indicated in the package insert. These risks of side effects are justified in light of the anticipated benefit of improved bone density and lowered immediate and lifetime fracture risk with treatment during this critical period of bone mass accrual. The rhIGF-1 dose will be increased or decreased by up to 25% to maintain IGF-1 levels in the upper half of the normal range. We will titrate the dose using a maximum of two dose increments. Thus the maximum dose will be 46.88 mcg/kg twice daily. Because changes in IGF-1 levels and bone density can occur due to multiple factors, IGF-1 results and DXA results after the baseline visit will not be shared with participants in order to avoid misleading assumptions, until after the 12 month treatment period.

Estrogen administration in the doses being administered is associated with minimal possible side effects. Occasional side effects may occur, the most common of which are mild

headaches, and very rarely some irritation or redness at the application site for transdermal estrogen. 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, gallbladder 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. These are more commonly seen in older women and in women with a family history of clotting disorders. Continuous estrogen administration without cyclic progesterone can predispose the uterus to cancer. Because we will be administering progesterone for ten days every month, this should not be a concern. We will monitor the subjects' blood pressure at each visit and will also determine through careful questioning and examination the appearance of any side-effect at each visit. Estrogen administration in pregnancy may be teratogenic. We will thus perform a pregnancy test prior to administering estrogen.

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.

Use of replacement or higher doses of estrogen before epiphyseal closure could accelerate growth plate closure resulting in an inability to reach the full potential for adult height. To avoid this possible side effect, we plan to administer replacement estrogen doses transdermally to girls with a bone age of 14or more years, who have achieved 98% of their adult height.

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.

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.

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.

There are no direct known benefits to study volunteers from the study. However, there is the prospect of direct benefit to study volunteers as they may have improved bone density and bone microarchitecture after study treatment. This research may lead to an improved understanding of the disordered bone metabolism in teenage girls with AN and whether administration of IGF-1, a key bone anabolic factor, with low-dose estrogen therapy, is effective in increasing peak bone mass in this population compared with estrogen alone. The potential benefits are thought to warrant the risks described above.

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.

All subjects will be female as the vast majority of patients with AN are female. We will be recruiting adolescents and young women 14-22 years old as there have been no treatment thus far has been successful in increasing bone density in adolescent girls with anorexia nervosa.

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. PHRC policy on Obtaining and Documenting Informed Consent of Subjects who do not speak English will be followed. For this study, potential subjects will be given a written translation in a language understandable to them of the "short form" or of the entire English version of the consent form approved by the Partners Human Research Committee (PHRC).

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.

1) Subjects will be recruited from a large population of patients with AN from the following locations:
Dr. Anne Klibanski and Kamryn Eddy Ph.D have organized a group of over 30 AN health care providers in New England (The New England Eating Disorders Research Collaborative). This group includes the directors of 15 major eating disorders with in- and out-patient services throughout New England and representatives from many major colleges and University health services. The group meets quarterly for two hour sessions to discuss recruitment strategies for MGH AN studies, review MGH research findings and discuss published data from other groups. This group represents a concerted and collaborative effort to recruit subjects with AN to the MGH studies and funnels patients into these studies from all over the New England area. It has been developed into an effective recruitment tool. The group includes:

- Representatives from the leadership of major eating disorder treatment centers and groups in the larger area, including: 1) Lori Ciotti, Clinical Director, The Renfrew Center of

Boston, 2) Seda Ebrahimi, PhD, Director, Cambridge Eating Disorders Center, 3) Anne Robinson, Director, Massachusetts Eating Disorder Association, 4) Perry Belfer, Director, Newton-Wellesley Behavioral Care, 5) Thomas Weigel, MD, Klarman Center for Eating Disorders, 6) Diane Mickley, Director, Wilkins Center for Eating Disorders, Greenwich, CT; 7) Mark Goldstein, M.D., Chief, Adolescent Medicine, MGH, 8) Suzanne Gleysteen, Beth Israel Deaconess Medical Center, 9) Patrice Lockhart, MD, Mercy Hospital Eating Disorder Treatment Program, Portland, ME, 10) Celina Pereira, MD, Director, University of Rhode Island Health Services, 11) Bethany Block, M.D., Brigham and Women's Hospital, 12) Laura Koenigs, M.D., Baystate Medical Center, 13) Heather Bell, RD, Brown University Health Services, 14) Sarah Kelley, Harvard University, 15) Laura Clauss, ARNP-C, Center for Eating Disorders Management, Inc., New Hampshire.

- Health care personnel for many of the suburban high schools and independent schools such as Dr. Suzanne Boulter in Concord, MA, Dr. Rebecca Niloff and Dr. John Robinson for Milton Pediatrics in Milton, MA, as well as various pediatric primary care practices in the Boston community are contacted regularly and invited to attend as well.

(ii) The Eating Disorders Unit at Massachusetts General Hospital, which is the largest operating eating disorders clinic in New England. The Eating Disorders Unit in the Massachusetts General Hospital Child Psychiatry Service and the Adult Eating and Weight Center together form the largest outpatient Eating Disorders Clinic in the New England area. Since its inception in 1981, the Clinic has evaluated more than 4000 patients with AN and/or bulimia nervosa. Over 200 new patients are evaluated in the Clinic each year and over 150 patients are in treatment at any one time. Patients in the Clinic, many of whom were recruited for an ongoing, prospective, longitudinal study of eating disorders, are interested and willing to participate in research projects. In addition, Clinic patients have participated in studies of low bone density, obsessive compulsive symptomatology, childhood sexual trauma, and pharmacological treatment.

(iii) The Harris Center, previously the Harvard Eating Disorders Center, in which Kamryn Eddy, Ph.D is involved, brings together many of the eating disorders programs at Harvard Hospitals, including Deaconess Waltham Hospital. It also hosts annual dinners and sends out biannual newsletters that inform clinicians and families of eating disordered patients about new research opportunities. The Harris Center is a national non-profit organization whose goal is to promote the healthy development of women and others at risk through research, training, outreach and prevention. The Harris Center web-site is a frequently hit ED site, and details about the study will be available on that site. The Harris Center networks regularly with major treatment centers for eating disorders in Connecticut, Vermont, Rhode Island, New Hampshire, and Maine and with the 250 college health centers in the Greater Boston area;

(iv) Walden Behavioral Care. The Neuroendocrine Unit is invited to give monthly educational talks to patients and provide information about ongoing studies at the inpatient, partial hospitalization and residential programs. The service admits about 280 in- and outpatients with eating disorders each year

(v) The Massachusetts Eating Disorders Association, which runs support groups for women and teenagers with eating disorders throughout eastern Massachusetts;

(vi) Health services at local colleges and universities such as Harvard University, Massachusetts Institute of Technology, Boston University, Boston College, Brandeis University, Emerson College, and Wellesley College

(vii) Facebook Advertisements. We also plan to use Facebook advertising to reach potentially interested subjects living in the Boston area. Women ages 18-45 living in New England will see advertisements for this research study in the "News Feed" section of their Facebook and be directed to an IRB approved RedCap prescreen survey. These advertisements will in no way be

designed to target individuals who indicate they may be struggling with an eating disorder on Facebook. The ads are shown to individuals based on the demographic requirements but have no link to any specific personal, profile or browsing history information. Examples of these advertisements are attached to this study protocol in Insight. A general Facebook page has also been created for this study (a Facebook requirement) and includes only basic contact and study information. A copy of this page is also attached to this study protocol in Insight.

(viii) Hasbro Children's Hospital at Rhode Island Hospital. We work with Dr. Abigail Donaldson and her team of providers to recruit subjects from Hasbro. This recruitment site will also function as an offsite clinic for follow up visits to provide Hasbro patients the opportunity to participate in the study with greater convenience. Subjects who are interested in the study can complete the eligibility portion of the screening visit at Hasbro and come to MGH for the consent process and baseline, 6 month, and 12 month visits. They will have the option to complete the entire 1, 3, 4.5, 7.5, 9, and 10.5 month visits at Hasbro.

To maximize enrollment of adolescent subjects with AN, a number of referral mechanisms have been put into place over the past year that utilize the collegial relationships between Dr. Klibanski, Kamryn Eddy, Ph.D and various directors of area eating disorder programs, as well as the Head of Child Psychiatry and major pediatric primary care practices. The investigators attend a monthly eating disorders support group at Walden Behavioral Care, which typically has an attendance of 70-80 patients, families, health professionals, and educators. The investigators also give talks on the endocrine and metabolic complications of AN to patients on the inpatient eating disorders units at Walden Behavioral Care, Center for Eating Disorders at Cambridge, Laurel Hill and HRI on a monthly or bi-monthly basis. Additionally, the Neuroendocrine Unit at Massachusetts General Hospital, in collaboration with the Harris Center, sponsors primary care symposia annually to educate the primary care clinician about eating disorders. These sessions also inform clinicians about research opportunities for eating disorder patients.

Control subjects will be recruited from pediatric practices at MGH, MGH-affiliated health care centers and community practices. Recruitment will occur through collaboration with physicians and caregivers at the above centers, and advertisement (i.e. posters, newsletters, and papers) in the community.

All subjects 18 years and older, and guardians of subjects younger than 18 years will sign an approved consent form, and all subjects 14-17 years old will sign their assent in this PHRC-approved Consent Form detailing study procedures. The consent and assent forms will be read to subjects and their guardians and signed in the presence of the study investigator. Minor subjects who turn 18 years of age during the course of the study will be asked to sign the approved consent form as an adult. In cases where the parents live out of state and find it difficult or inconvenient to come to Boston, we will ask the IRB for an exception to the protocol such that the study can be explained to parents and subjects over the phone and consent forms mailed/faxed to the parents/subjects to sign and send back to us. We will conduct the consent interview by telephone when the parent can read the consent as it is discussed. PHRC policy on Obtaining and Documenting Informed Consent of Subjects who do not Speak English will be followed. Potential subjects will be given a written translation in a language understandable to them of the "short form" of the consent form or of the entire English version of the consent form approved by the PHRC.

Psychologist co-investigators who obtain consent from subjects, if they are also treating psychologists, will not recruit their own patients for the study.

2) Subjects will also be recruited by using a REDCap survey, where all email correspondence will be done using encryption, as per institutional policy. Subjects can express

interest in the study by sharing their contact information and filling out eligibility criteria by using this survey.

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

AN subjects will receive $90 for completing each outpatient visit, except for the screening visit. AN subjects will also receive $50 each for completing the optional Baseline and 12 Month MRI, as well as reimbursement up to $25 per visit for parking and/or transportation costs to MGH. Healthy subjects will receive $100 for completing the study visit, as well as reimbursement up to $25 for parking and/or transportation costs to MGH. AN subjects can receive up to $910 for completing this study and its MRI sub-study, and healthy subjects can receive up to $100.

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.

Parent/guardian consent and child assent will be obtained after describing the purpose of the study and its requirements to the subjects and their parents/guardians, and prior to performing any procedures. Per institutional guidelines, those study volunteers who are 14 -17 years will document their written assent on the same PHRC approved consent form as their parents/guardians. The permission of one parent/guardian will be sufficient to document on the consent form. The consent form will be signed in the presence of a licensed physician or psychologist investigator or study nurse practitioner. Psychologists obtaining consent will hold a PhD or PsyD degree, be licensed to practice psychology in Massachusetts, and be listed as co-investigators on study staff. When consent is being obtained by a nurse practitioner or clinical psychologist, an optional consultation with a licensed physician investigator must be offered to all potential subjects before they sign the consent form. This offer, and its acceptance or refusal by the potential subject, must be documented in the research record. Any issues or problems with

the consent process must be reported to the PHRC as they occur, as "Other Event," not logged and held for continuing review. Subjects will have up to 6 weeks to consider participation. Minor subjects who turn 18 years of age during the course of the study will be asked to sign the approved consent form as an adult.

In cases where the parents live out of state and find it difficult or inconvenient to come to Boston, we will ask the IRB for an exception to the protocol such that the study can be explained to parents and subjects over the phone and consent forms mailed/faxed to the parents/subjects to sign and send back to us. The consent interview will be conducted by telephone when the parent can read the consent as it is discussed.

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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.

Unanticipated problems or adverse events involving risks to subjects that are related to the study or study drugs will be reported within 5 working days or 7 calendar days of the date the study investigator becomes aware of the problem per PHRC guidelines. Mild and moderate adverse events will be reported to the Partners IRB per guidelines.

Data Safety Monitoring Board: One central Data Safety Monitoring Board (DSMB) will be convened at MGH every six months to review all adverse events at both sites. Moreover, additional meetings may be called to consider urgent issues. The following personnel have served on the DSMB for the current grant for the past 5 years and have agreed to continue to serve on the DSMB:

- Ellen O'Donnell, PhD. Instructor of Psychology, Harvard Medical School. Staff Psychologist, Massachusetts General Hospital for Children
- Dr. Harold Jueppner, MD, Pediatric Endocrine Unit, Massachusetts General Hospital
- Eray Savgan-Gurol, M.D., Pediatric Endocrine Unit, Massachusetts General Hospital

- Meredith M. Regan, Sc.D., Biostatistician, Biometrics Center, Beth Israel Deaconess Medical Center, Instructor in Medicine, Harvard Medical School
- Alissa S. White, RD., LDN, CNSC, Nutrition and Food Services, Massachusetts General Hospital

Serious Adverse Events that are related to the study or study drugs will be telephoned into MGH within one working day, and forms faxed or mailed within 72 hours. These events will be promptly related to the IRB and FDA by the Study Coordinator. If the Data and Safety Monitoring Board wishes to be notified, we will initiate this notification as well.

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

Serious Related Adverse Events will be reported within 10 days per PHRC guidelines. These events will be promptly related to the IRB and FDA by the Study Coordinator. Mild and moderate adverse events will be reported to the Partners IRB per guidelines. All adverse events will also be reviewed every six months during the DSMB meetings.

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: Serious Related Adverse Events will be reported within 10 days per PHRC guidelines. These events will be promptly related to the IRB and FDA by the Study Coordinator. Mild and moderate adverse events will be reported to the Partners IRB per guidelines. All adverse events will also be reviewed every six months during the DSMB meetings.

Data Quality Control: All data will be recorded on specified Case Report Forms. Completed forms will be sent to Massachusetts General Hospital and retained at the remote site. As forms are received at Massachusetts General Hospital, they will be logged into a forms calendar, which contains all subjects' code numbers and dates of each follow-up visit. In our experience, this has facilitated an easy, accurate, and global check of each subject's status, as well as providing recruitment and drop-out information. After each form is logged, it will be carefully checked for legibility and completeness and for ambiguity. Data from the forms, including laboratory results, will be entered into an electronic database. Forms that have missing or inconsistent data will also be entered into the database, except that a missing value indicator will take the place of the missing or inconsistent information. A missing information form will be filled out to indicate the items that are missing.

Forms that are complete will be filed in a dedicated locked filing cabinet organized by subject code number. Forms that have missing data will be kept separately for ease of notifying coordinators.

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 100 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_events.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.

Serum will be sent to Dr. Kristina Rother and Dr. Jenny Blau at the NIH for analysis of INSL-5 levels in girls with anorexia nervosa for a collaborative study. All samples and clinical data will be coded so that only the study staff at MGH can link the identities. The NIH researches will only receive coding to link the samples with the clinical data and will not receive any subject-specific identifying information. The clinical data associated with the samples will include anthropometric and menstrual information.

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.

No data and specimens collected at MGH will be stored at other sites.

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.

Subjects who do not live locally will be able to have their blood drawn in a local laboratory for levels of IGF-1 and bone markers, these results and/or specimens will be sent to MGH.