**Effects of Anorexia Nervosa on Peak Bone Mass**
**NCT01301183**
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**Analytical Plan**

## ANALYTIC PLAN

David A. Schoenfeld, Ph.D., a Professor of Biostatistics at Harvard Medical School and co-investigator, has co-authored papers with us for more than 20 years and has been instrumental in our study design and will analyze these data. Data generated will be largely longitudinal. Measures of hormone function, bone turnover markers, nutrition, activity, body composition, bone density, bone microarchitecture and strength will be collected every three to six months for 12 months. There are three study groups: AN randomized to rhIGF-1 and transdermal estrogen, estrogen alone with placebo, and normal adolescents. Randomization will be performed by the MGH Research Pharmacy.

**Analysis and interpretation are listed below for each aim and sub-aim:**

**Sample Size Determination:** The study is powered to our primary endpoints, (i) change in lumbar spine BMD (specific Aim 1A), and (ii) change in trabecular bone density (specific Aim 3A) in girls with AN who receive estrogen and rhIGF-1 versus estrogen alone. **Specific Aim 1A:** The primary analysis for this Specific Aim and the study is to compare the change in BMD over time in subjects with AN randomized to receive rhIGF-1 and estrogen (n=50) with the corresponding change in estrogen treated subjects (n=50). Using data from our previous study, we found that the variance covariance matrix of the random effects model was as follows: variance-intercept 0.01056, variance-slope 0.001523, covariance -0.001, and the residual variance around each subject's trajectory 0.000533. Based on these parameters, with 100 subjects with AN (50 in each group), we will have an 80% chance of detecting a significant (p=0.05 two sided) difference in change in lumbar spine BMD between girls who receive estradiol and rhIGF-1 versus estradiol alone if the true difference in slope between the groups is 0.03 $g/cm^2$/year. This is a medically significant difference in that lumbar spine BMD in adolescents with AN is about 0.08 $g/cm^2$ lower than that in healthy adolescents at baseline. Estradiol alone has a net effect of 0.02 $g/cm^2$.year in increasing bone density, so an additional effect of 0.03 $g/cm^2$ (with rhIGF-1) added to 0.02 $g/cm^2$ (from estradiol alone) would be a net effect of 0.05 $g/cm^2$/year (from estradiol and rhIGF-1). This would allow girls with AN to catch up to healthy adolescents. The sample size calculation, which assumes a 20% dropout rate, is based on the methods described by Yi and Panzarella (38) using the program http://hedwig.mgh.harvard.edu/biostatistics/node/35. The same model can be used with covariates and possible mediators of treatment effects such as baseline nutritional status, body composition, hormonal status, exercise and pubertal maturation. We will also explore the possibility that effects of rhIGF-1 with estradiol persist after discontinuation of treatment by examining bone density 12 months after stopping therapy.

**Specific Aim 1B.** We will analyze the impact of rhIGF-1 with estrogen (n=50) versus estrogen alone (n=50) on levels of the bone turnover markers, PINP and CTX, in girls with AN at 3, 6, 9 and 12 months using a repeated measures ANOVA. The primary planned analysis will be the difference in mean levels by treatment group during the 3, 6, 9 and 12 month observations. As for Specific Aim 1A, we will examine whether effects of combination therapy persist following discontinuation of therapy by examining these markers after 12 months of stopping therapy.

**Specific Aim 1C**. We will use the longitudinal model described above to compare the rate of bone mass accrual in girls with AN who receive rhIGF-1 with estrogen (n=50) with that seen in healthy controls (n=36). We expect the change in BMD in AN girls to exceed that in controls because of a "catch up" effect when rhIGF-1 is used with estradiol. We will control for multiple comparisons in our analysis when comparing against controls. Both unadjusted and multiplicity adjusted analyses will be presented.

**Specific Aim 2A.** In a cross-sectional design, we will compare the described HR-pQCT endpoints and bone strength (as assessed by FEA) in girls with AN (n=100) to those in healthy adolescents (n=36). We will use the Student t test when variables are normally distributed or when transformations can be performed to approximate a normal distribution; otherwise, we will use non-parametric tests such as the Wilcoxon rank sum test. Our preliminary data indicate lower trabecular bone density in 8 girls with AN compared with 6 controls (162±27 vs. 209±36 g/cm$^3$, p=0.02). Based on these data, with 100 girls with AN and 36 controls in the study, we have >99% power for detecting a significant difference in trabecular bone density between the groups at an alpha level of 0.05. Another component of this specific aim is to determine the extent to which DXA parameters and possibly clinical parameters predict bone microarchitecture in the 100 adolescents with AN. This will help elucidate the role of DXA in the future clinical evaluation of adolescents with AN. Linear regression followed by regression modeling will be performed to determine hormonal, body composition, nutritional and exercise predictors of bone microarchitecture and bone strength in girls with AN and healthy adolescent girls.

**Specific Aim 2B.** The goal of this specific aim is to determine prospectively changes in bone microarchitecture and bone strength in girls with AN who receive rhIGF-1 and estrogen therapy (n=50) compared with changes in AN receiving estrogen alone (n=50). Bone microarchitecture will be assessed using HR-pQCT and bone strength using FEA at baseline, six, 12 and 24 months of the study. Our second primary study endpoint is the change in trabecular bone density in the two treatment groups over the course of the study. With 100 subjects (50 in each group) we will have an 80% chance of detecting a significant difference (p=0.05 two sided) in change in trabecular bone density in girls with AN who receive rhIGF-1 and estrogen compared with estrogen alone if the true relative change is 3.5% over the 12 month period. This assumes a standard deviation of relative change of 6%, as was found in the group of AN women who gained weight in a study by Milos *et al* (39). To understand the clinical meaning of such a change we note that AN women had a 20% decrease in trabecular bone density compared to normal women. The analysis will be accomplished using a longitudinal mixed model ANOVA with a random patient and time (slope) effect. The measure of interest will be the group time interaction. We estimate a 20% dropout rate based on our previous study. A secondary analysis will be a joint model with weight gain as described for Specific Aim 1. We will also compare changes in total cross-sectional area, cortical thickness, TbN, TbTh and TbSp between AN receiving rhIGF-1 and estrogen with AN receiving estrogen alone. Although this is not a specific aim, it will be interesting to compare the changes in BMD with the change in bone strength. An issue is that BMD and bone strength are measured in different units. In order to make the comparisons understandable, we will transform both to percent predicted using the data from healthy adolescent girls. Then we will fit a joint model for both measurements to assess the relationship between the two and which measure is affected most by treatment. In addition, we will examine these endpoints 12 months after stopping therapy to determine whether effects persist after discontinuing combination therapy. When comparing with healthy adolescent girls, we will adjust for multiple comparisons. Both unadjusted and multiplicity adjusted analyses will be presented.

**Specific Aim 2C.** The random effects model can also be used where the “treatment” variable is replaced by a continuous variable such as IGF-1 level. There are two possible analyses for this Specific Aim. One analysis would use all randomized subjects, and this would be very similar to the analysis for the treatment effect except that it may be more powerful if there is a large variation in IGF-1 levels in response to treatment with rhIGF-1. The other is to restrict analysis

to the group receiving IGF-1 to test whether the effect of treatment on bone microarchitecture parameters and bone strength is associated with the IGF-1 level. We will control for effects of nutrition, pubertal status, hormonal status (including estradiol and leptin), calcium metabolism and exercise in this analysis. As in most clinical trials, there will be measurements made during the trial of parameters which may or may not mediate the treatment effect. For instance we do not expect short term changes in nutritional status, weight, and hormonal status to affect the difference between treatment effects of estrogen versus rhIGF-1 plus estrogen on BMD or bone strength but we may find the bone turnover markers such as P1NP do mediate the effect of treatment. Mediation analysis in longitudinal trials is difficult and requires strong assumptions. We will explore mediation using the framework described by Ditlevsen *et al* (40).