STATISTICAL ANALYSIS PLAN

Title: Fat Mediated Modulation of Reproductive and Endocrine Function in Young Athletes

NCT00946192

Version Date: December 4, 2014

Statistical Analysis Plan

Biostatistical analysis: Hang Lee, Ph.D., a collaborator on this grant, is our CRC statistician and has co-authored many papers with us and other members of the endocrine division. Dr. Hang Lee and Dr. David Schoenfeld, Head of the Biostatistics Core, have been instrumental in study design and power analysis. Dr. Lee will also analyze the data at study completion. Analysis and interpretation of specific sub-aims are included in each section of the grant. Data generated will be largely longitudinal. Measures of hormone function, bone turnover, nutrition, activity and body composition will be collected every 6 months. In addition, there will be baseline variables. Subject groups include: AA randomized to transdermal estrogen, AA randomized to oral estrogen, AA randomized to observation, EA and controls. Randomization will be performed centrally by our research pharmacy.

The first step in data analysis is to determine models for longitudinal data collected. These models are usually based on our knowledge of how these data behave and details of the study. These models can also be checked to see whether they fit the data by viewing data for each patient without regard to patient group. For instance, BMD can be assumed to increase linearly over the study duration. We hypothesize that this linear increase will be slowest in untreated AA patients and intermediate in those on oral estrogen, and the smallest effect size would be the difference between groups randomized to transdermal versus oral estrogen. We would check this linearity by looking at data from individual patients and checking that actual measurements are normally distributed around the patient's linear trajectory by looking at residuals (difference between observed value and trend line). It is possible that in some cases linearity and normality might hold after a log or other transformation. If linearity holds for actual or transformed values, variables will be analyzed by mixed model ANOVA, a common method for analyzing longitudinal data. One advantage of this method is that it allows us to use data from subjects that drop out early. The linear model is appropriate for BMD, but will not be appropriate for variables that may be relatively constant during treatment. These might include measurements of bone turnover and hormones. These would be modeled assuming that each patient has a constant level. After examining how well the assumed model fits the data, repeated measures ANOVA will be used for analysis. In this model there is a fixed treatment term of primary interest. Measurements at each time are assumed to be a multivariate observation with an unspecified variance covariance matrix.

Power calculation for primary endpoint: Power analysis is based on the primary endpoint, i.e. average change in BMD over one year in a randomized trial comparing mean change in spine BMD in young women 14-25 years old with AA randomized to transdermal estrogen versus oral estrogen or no therapy for one year. Girls with AA will be stratified based on spine BMD Z-scores (≤ -1 or > -1).

Our preliminary data indicate a relative increase in spine BMD of 4.7% over one year with transdermal estrogen vs. no therapy. In addition, one study in AA women has reported a 1.5% relative increase in BMD in AA women following oral estrogen vs. placebo. Our preliminary data indicate that the SD for change in lumbar BMD over a year is 2.7%. Based on these data, using the Dunnett's test, a total of 99 AA (33 each in the two treatment arms no-treatment arm) will be needed to detect a difference of 3.2% (4.7%-1.5%) in mean %increase in spine BMD in groups receiving transdermal vs. oral estrogen, with a power of 80%, using a two sided $p=0.05$ level test.

The estimated sample size of 99 subjects with AA does not take into account study drop-outs. Based on previous studies, we expect an 8- 10% drop-out rate over one year. We thus estimate that we will need to recruit 109 subjects with AA (37 in the transdermal estrogen arm, and 36 each in the oral estrogen and no-treatment arms) to have 99 evaluable subjects at the end of the study. An independent Data and Safety Monitoring Board (consisting of an adolescent medicine physician, a statistician, a bone

expert, a pediatric endocrinologist and a psychiatrist) will review data regarding adverse events, study deviations and violations at study initiation and every six months. BMD information will be presented to the DSMB at each meeting, and study termination considered for subjects demonstrating >10% decrease in spine BMD from baseline, if randomized to estrogen.