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Magnetic Resonance Imaging and Spectroscopy Evidence of Efficacy for Adrenal and Gonadal Hormone Replacement Therapy in Anorexia Nervosa

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Abstract

Purpose—Dehydroepiandrosterone (DHEA) + estrogen/progestin therapy for adolescent girls with anorexia nervosa (AN) has the potential to arrest bone loss. The primary aim of this study was to test the effects of DHEA+ estrogen/progestin therapy in adolescent girls with AN on bone marrow in the distal femur using magnetic resonance imaging (MRI) and spectroscopy.

Methods—Seventy adolescent girls with AN were enrolled in a double blind, randomized, placebo-controlled trial at two urban hospital-based programs.

Intervention—Seventy-six girls were randomly assigned to receive 12 months of either oral micronized DHEA or placebo. DHEA was administered with conjugated equine estrogens (0.3mg daily) for 3 months, then an oral contraceptive (20µg ethinyl estradiol/0.1mg levonorgestrel) for 9 months. The primary outcome measure was bone marrow fat by MRI and magnetic resonance spectroscopy (MRS).

Results—T2 of the water resonance dropped significantly less in the active vs. placebo group over 12 months at both the medial and lateral distal femur ($p=0.02$). Body mass index (BMI) was a significant effect modifier for T1 and for T2 of unsaturated ($T2_{\text{unsat}}$) and saturated fat ($T2_{\text{sat}}$) in the

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lateral distal femur. Positive effects of the treatment of DHEA + estrogen/progestin were seen primarily for girls above a BMI of about 18 kg/m².

Conclusions—These findings suggest treatment with oral DHEA+ estrogen/progestin arrests the age- and disease-related changes in marrow fat composition in the lateral distal femur reported previously in this population.

Keywords

bone marrow; hormone replacement therapy; dehydroepiandrosterone; eating disorders; adolescence

1. INTRODUCTION

Anorexia nervosa (AN) poses a threat to bone health in adolescent girls. The putative cause is malnutrition-associated hormonal alterations, which negatively impact bone marrow mesenchymal stem cell (MSC) differentiation towards adipocyte over osteoblast production, leading to increased marrow fat.^{1,2} The associated skeletal problems may be mechanistically linked to abnormalities in MSCs.^{3–6} Increasing evidence suggests that bone marrow activity alternates between osteoblast and adipocyte formation.^{3,7,8} Hormonal abnormalities likely mediate the preferential differentiation towards adipocytes, resulting in premature conversion from red marrow (RM) to yellow marrow (YM) and increased marrow fat.⁹ Some work has focused on marrow fat composition, defined by relative levels of saturated and unsaturated fat, in adult women with AN¹⁰ or osteoporosis^{11,12} and has identified an inverse relationship between saturated fat content in marrow and bone mineral density (BMD).

Because of the high prevalence of skeletal deficits in adolescents with AN,^{9,13} several therapies have been explored to counter the disease-associated bone loss. Prior studies testing oral estrogen monotherapy without the addition of dehydroepiandrosterone (DHEA) have not found BMD to improve in these patients.¹³ We previously showed an arrest of bone loss in older adolescents who received oral DHEA and estrogen/progestin replacement¹⁴, and significant favorable changes in hip structural geometry and strength variables after 18 months of treatment.¹⁵ Since underweight adolescents with AN are susceptible to estrogen-related side effects¹⁴, we administered low-dose conjugated estrogens for 3 months before advancing to a 20 µg ethinyl estradiol combined oral contraceptive while administering oral DHEA for the trial's duration.

We recently reported an inverse relationship between marrow fat content and both BMD and BMI in older girls with AN.¹⁶ However, the relationships in younger girls were more complex due to the dominant impact of age-related marrow fat maturation even in patients with this chronic disease.¹⁶ In that cohort of subjects with AN, the normal relationship between chronological age and physeal closure at the knee was preserved with respect to marrow composition. In the current study, we sought to determine the impact of DHEA + estrogen/progestin therapy on marrow fat composition as assessed by MRI/MR spectroscopy in a similar population of adolescents. We aimed to understand the mechanistic underpinnings of this hormonal therapy, including potential changes in bone marrow

composition that could reflect restoration of mesenchymal precursors of osteoblasts, and ultimately, increased bone formation.

MATERIAL AND METHODS

Subjects

Between January 2012 and January 2015, 76 adolescent girls (ages 11–18 years) were prospectively recruited from two urban hospital-based eating disorders programs (n=13 from Boston Children's Hospital, Boston, MA and n=57 from Hasbro Children's Hospital, Providence, RI) for participation in a clinical trial (ClinicalTrials.gov, NCT01343771) (Figure 1). Eligible patients were post-menarchal or had a bone age ≥ 13 years, and met Diagnostic and Statistical Manual, 5th edition¹⁷ diagnostic criteria for AN. Subjects were excluded if they had other medical conditions known to affect bone health or were receiving medications known to affect bone. Six subjects withdrew before baseline data collection. Eight subjects did not complete the trial for reasons not related to the protocol (e.g., move out of state). The local institutional review boards approved the protocol. Informed consent was obtained from participants or their parent/guardian. Minor subjects provided assent.

Anthropometric Measures

At each study visit, height was obtained by stadiometer and weight on a calibrated, digital scale. Body mass index (BMI) was calculated.

Magnetic Resonance (MR) Imaging and Spectroscopy

All subjects underwent MR imaging of the left knee with 15 channel receive coils on one of three 3 Tesla scanners with identical software (Trio systems, Siemens Medical Inc., Erlangen, Germany) at baseline and 12 months. During the study, the three scanners were calibrated using a fat/water phantom to insure validity of results across all scanners. Coronal T1 weighted images (TR 600 msec, TE 9.7 msec, matrix 384×288, ETL 166, NEX 1, FOV 140 mm, 3 mm slice thickness, 0.3mm gap) were obtained through the knee with field of view of 16 cm to include distal femoral and proximal tibial metaphyses.

We also performed spin-lattice relaxation (T1) relaxometry consisting of nine fast spin echo (FSE) acquisitions of varying TR (TR=250 – 5000 msec, TE=17 msec, matrix 128×128, ETL = 3, flip angle 180 degrees, FOV 140 mm, 4 mm slice thickness, 1 mm gap).^{2,18}

MR spectroscopy was performed using single voxel PRESS acquisitions (TR=2500msec, bandwidth=2500Hz, 2048 time points, 32 signal averages, voxel size = 1×1×1 cm) with voxels placed in the distal medial and distal lateral femoral metaphysis (Figure 2A). These anatomical locations were chosen because they show the greatest difference in marrow fat fraction between adolescent girls with AN and age-matched controls, as previously reported² and because exclusion of the central metaphysis avoids contamination by the normal physeal undulation and posterior cortical depressions that occur centrally in the distal femoral metaphysis. The reproducibility of femoral MRS data is supported in the literature^{10,11}. Two spectra were acquired from each voxel at two separate echo times of 30 msec and 60 msec to allow for spectral T2 estimates of the major resonances.

Image Analysis

Visual assessment—Two blinded pediatric radiologists (KE and PTC) visually assessed all images for RM content, designated as areas of low signal intensity. The distal femoral and proximal tibial metaphyses were graded according to the following scale, validated in our previous study¹⁹: 0= no RM; 1= mild RM; 2= moderate RM; 3= extensive RM. Distal femoral and proximal tibial physes were assessed as open or closed.

T1 relaxometry—Voxel-wise T1 maps were generated from the variable TR scans using the following equation: $S_{TR} = S_{max} (1 - e^{-TR/T1})$, where S_{TR} is the signal intensity of the voxel for a particular TR and S_{max} is the signal intensity for a TR of infinity. S_{max} and T1 were calculated at each voxel and the corresponding maps generated using IDL software (Harris Geospatial Solutions, Melbourne, FL, USA). Regions of interest were placed corresponding to the spectroscopic voxel locations in the distal femur metaphyses, and the mean T1 values were recorded (ImageJ, NIH, Bethesda, MD). The anatomical locations of these regions were consistent for all subjects and were 1.2cm² in size (Figure 2A).

MR spectroscopy—Spectra were manually phased and baseline corrected using vendor supplied spectroscopic analysis software to achieve absorption mode spectra for spectral peak quantification. Spectral fits were then performed using the vendor-supplied software to obtain peak areas for the water resonance and five lipid resonances (lip 1–5) visually present in the bone marrow spectra (Figures 2B and 2C), as reported previously.¹⁶ Fat fractions of saturated fat (R_{sat}), unsaturated fat (R_{unsat}), and total lipid (R_{total}), the unsaturation index (UI), and the T2 relaxation time of water ($T2_w$), saturated fat ($T2_{sat}$) and unsaturated fat ($T2_{unsat}$) were calculated as outlined previously¹⁶.

Hormonal Measures

Hormonal measurements included serum estradiol and testosterone concentrations [CIA (Beckman Coulter, Fullerton, CA); %CV estradiol 8.0% and 7.0% for testosterone], leptin [RIA (Millipore, Billerica, MA), %CV 4.6–6.2]; human (total) ghrelin [RIA (Millipore, Billerica, MA), %CV 5.2–7.2%]; dehydroepiandrosterone sulfate (DHEAS) [double-antibody ELISA (Diagnostic Systems Laboratories, Webster, TX), %CV 4.8–5.3%]; and triiodothyronine [ECLIA (Labcorp, Burlington, NC, %CV 3.5–5.4%]. Safety studies included liver function tests every six months and a lipid panel at the beginning and end of the study.

Statistical Analysis

We assessed the effect of active drug on 12-month visual marrow assessment by analysis of covariance, adjusted for age and baseline visual score. We used repeated-measures analysis of variance (ANOVA) to compare the time course of each MRI/MRS variable between trial arms. Some MRS outcomes showed a skewed distribution and were log-transformed for analysis. We accounted for within-subject correlation using a compound-symmetric covariance structure. The primary null hypothesis was that the arms did not differ in change from baseline to 12 months, addressed by testing for treatment × time interaction. From parameters of the fitted ANOVA, we derived scalar estimates for the change in each arm from baseline to 12 months, as well as the difference between arms for those changes.

We employed the following strategy to build appropriate ANOVA models for each measure. (1) The basic model comprised treatment (active drug or placebo), time (baseline and 12 months), and treatment $\times$ time interaction. (2) We tested potentially influential time-varying covariates and added to the basic model three that were consistently statistically significant: age, N<I, and physeal closure status. (3) We additionally evaluated serum hormone concentrations at baseline as potential time-varying covariates, ultimately including DHEAS and estradiol on the grounds of biological relevance (being components of the study medication) and leptin because it showed a statistically significant influence on the outcome variables. Total testosterone, ghrelin, and triiodothyronine (baseline) met neither criterion and were not included. All hormones showed skewed distributions and were log-transformed for analysis. (4) Finally, we tested for effect modification by baseline BMI, treated as a continuous variable (treatment $\times$ time $\times$ BMI interaction). In cases where the interaction was significant, we illustrated the effect modification by plotting adjusted 12-month change vs. BMI over the observed range (12.8–22.4 kg/m²). The differing linear relations between 12-month change and BMI in the two trial arms were calculated from the fitted model, as was the difference between arms.

We used SAS software for all computations (version 9.4, Cary, NC) and used $p < 0.05$ as the criterion for statistical significance.

RESULTS

Descriptive Statistics of Sample and Safety Parameters

Baseline characteristics of the 70 subjects who completed baseline assessments are provided (Table 1). Characteristics were compared between participants who withdrew ($n=8$) vs. completers ($n=62$), and no significant differences found (Supplemental Table 1). Only age was close, but did not reach significance ($p=0.07$), with those who completed the trial being younger by approximately one year. Liver function and lipid panels obtained at 6-month intervals remained within normal limits. No subject experienced hirsutism or exacerbation of acne. Five subjects reported irregular menstrual bleeding or spotting which did not differ by treatment group (3 placebo, 2 active). There were no significant changes in weight or hormonal covariates in either group, except for the expected DHEAS increase in the active group ($p=0.01$).

MR Imaging Analyses

Visual assessment—Of the 70 subjects assessed at baseline (35 active, 35 placebo), 62 subjects (32 active, 30 placebo) completed the 12-month trial and were evaluated by visual assessment of the femur at 12 months (Figure 1). Only 9 subjects showed a higher visual score, indicating increased RM, of which 4 were in the active group and 5, placebo. The majority of subjects ($n=53$, 85%) showed either a decrease or no change on visual assessment. There was no statistical difference between the active and placebo groups in visual score change over 12 months.

T1 relaxometry and MR spectroscopy—Results of analyses comparing active and placebo groups with respect to 12-month changes are provided (Table 2). All means were

adjusted for baseline age and BMI, physeal closure, and serum DHEAS, estradiol, and leptin concentrations. Baseline BMI was a significant effect modifier for T1, T2_{unsat} and T2_{sat} in the lateral location. Group differences in the influence of BMI on 12-month change are listed in Table 3 and detailed below.

MRT1 values: The lateral distal femur showed a decrease of 2 ± 14 msec (estimate $\pm$ SE) in mean T1 for the active group, as compared to a decrease of 25 ± 14 msec in those receiving placebo, indicating a higher conversion to YM during the trial period in the placebo group (Table 2). Baseline BMI was significant as an effect modifier (Table 3); for each 1 kg/m² increment change in baseline BMI, active subjects' 12-month change in T1 increased by 15 msec (Figure 3A), whereas the placebo subjects' change in T1 decreased by 16 msec (Figure 3B), a net difference of 31 msec per kg/m² ($p=0.01$; Figure 3C).

At the medial distal femur, T1 decrease was -4 ± 13 msec for the active group and -23 ± 13 msec for the placebo group. The group difference was not statistically significant at this site, and baseline BMI was not an effect modifier.

MRS T2 values: In the lateral distal femur, T2_w decreased slightly in the active group, and showed a greater decrease in those receiving placebo. In contrast, in the medial distal femur, T2_w increased in the active group, but decreased in the placebo group (Table 3). In both locations, these T2_w differences were statistically significant ($p=0.02$). T2_{unsat} increased significantly in the medial location in the active group compared to placebo ($p=0.04$), whereas group differences in the change in T2_{sat} were not significant. At the lateral site, the influence of BMI on the 12-month change was significantly different between the active and placebo arms for T2_{unsat} ($p=0.02$) and near significant for T2_{sat} ($p=0.07$), as detailed (Table 3) and illustrated (Figure 3).

MRS lipid ratios: In both lateral and medial locations, no group differences over time were noted for R_{total}, R_{sat} and R_{unsat}. At TE=30 msec, UI in the active group declined significantly compared to placebo ($p=0.02$) at the medial location, but not in the lateral distal femur ($p=0.63$; Table 3). There were no UI differences between groups at TE=60 msec.

DISCUSSION

Our results demonstrate several strong and independent indicators that combined adrenal and gonadal steroid replacement therapy can arrest the bone marrow changes associated with mild to moderately severe AN in adolescent girls as measured in the distal femoral metaphysis. The inclusion of young adolescents who were at varying degrees of physeal closure provides information of potential use to clinicians who care for patients with this disease across the adolescent age spectrum.

We chose to assess the distal femur because of its robust metabolic activity (contributing 40% of the linear growth of the entire lower extremity²⁰) and because residual hematopoietic marrow is predictably present in the distal femoral metaphyses of normal adolescents.²¹ Given the more rapid red to yellow marrow conversion in the extremities compared to the axial skeleton, and the predictable progression of this conversion in a distal to proximal

direction, we hoped to increase our ability to detect relatively small changes in marrow fat content by interrogating the distal femur rather than the spine or hip. Noteworthy is the fact that other groups have assessed the vertebral spine in adults with AN¹ and have also shown increased marrow adiposity at this skeletal site, similar to our observations in the juvenile appendicular skeleton.^{2,16}

Previously, it has been shown that girls with AN exhibit lower T1 when compared to age-matched healthy controls,² consistent with disease-associated increased marrow fat. Additionally, these patients show an age-related decrease in T1,²² indicative of increased fatty YM. In healthy children, there is a predictable physiologic conversion of hematopoietic to lipid-rich marrow throughout growth, but especially during puberty. This conversion appears to be accelerated in teenage girls with AN.^{1,2} In the current study, T1 in the active group was virtually unchanged over the 12-month trial, indicating that residual RM was preserved with treatment. Conversely, the placebo group showed a significant decrease in T1, indicating further accumulation of fatty marrow.

Interestingly, when examining our cohort as a whole, the T1 change in the lateral distal femoral metaphyses in the placebo group is strikingly similar to our previous data²² showing a comparable change per year in the same location as determined by regression analysis of the baseline cohort. This finding is a strong indicator of the robustness of the data and analyses, and suggests that this treatment arrests both age- and disease-related transformation to fatty marrow.

T2_w also remained virtually unchanged over the trial in the girls treated with combined DHEA + estrogen/progestin, whereas the placebo group showed a significant decrease, consistent with increased YM in the placebo group over the same period. Our baseline data showed a strong correlation between decreased T2_w and AN, thought to be related to the restricted motion of water with increasing marrow fat.²² This hypothesis is bolstered by recent work²³ in rats that identified distal 'constitutive' marrow adipose tissue (cMAT), characterized by larger adipocytes and low haematopoiesis, which remained differentially preserved upon systemic challenges when compared to the smaller regulated MAT (rMAT) adipocytes. It is possible that a disease-associated loss of rMAT in our human population leaves behind the larger cMAT adipocytes, leading to the restricted water motion and the lower T2_w observed in our study. In addition, it has been shown that decreased T2_w correlates with increased ferritin levels.²⁴ Serum ferritin levels have recently been shown to be elevated in AN.²⁵ It may be that the decrease in T2_w seen in the placebo group could be associated with increased ferritin compared to the active group that showed no such change. Unfortunately, ferritin levels were not measured in the current investigation, but should be assessed in future studies.

In the lateral femoral location, BMI proved to be a significant effect modifier for T1, T2_{unsat}, and T2_{sat} when comparing the change between trial arms. In other words, BMI had a significantly different effect on the active and placebo groups during the trial. Analysis of our baseline data showed a consistent decrease in T1 and T2_{unsat} and increase in T2_{sat} with increasing age and disease duration. All of these trends were found to be reversed or arrested in the active group, but were apparent in the placebo group, particularly in adolescents with

a higher BMI. The girls with a BMI around 18 kg/m² receiving treatment exhibited an arrest in change in T1, T2_{unsat} and T2_{sat} at the trial's end, whereas girls with a higher BMI saw reversal of these age- and disease-related trends. These findings are in keeping with previous work by DiVasta et al.¹⁴ showing bone mass to be preserved in girls with AN receiving the same adrenal/gonadal steroid replacement. The treatment effect was much more pronounced in girls with a BMI of 18 kg/m² or higher, suggesting that DHEA + estrogen/progestin is effective in preserving bone mass and marrow composition in adolescent girls with AN who are of a higher weight and BMI. The fact that the BMI effect was seen consistent among the disparate variables assessed using different MR modalities points to the robustness of the data and analysis. Our cohort was skewed towards girls with higher BMI. Therefore, studying the effects of this therapy in a cohort with more severe disease is warranted.

Several recent studies support the importance of saturated/unsaturated marrow fat estimates. Recent work^{7,10,11,26–28} has focused on the composition of marrow adipose tissue and its role in bone turnover in AN, osteoporosis, and other diseases, as well as in normal adolescents and adults. Yeung et al. studied 35 postmenopausal women with osteoporosis or osteopenia vs. 27 healthy control women in a first report to examine the relationship between marrow fat saturation with osteoporosis.¹² They showed higher fat content in patients with osteoporosis and a decreasing trend in the unsaturation index of lumbar vertebrae from normal to osteoporotic subjects. These results suggest that as fat content increases with reducing BMD, saturated fats increase preferentially relative to unsaturated fats. A more recent study reported increased total lipid and saturated fat in the femoral neck of post-menopausal women with osteoporosis versus healthy controls, but failed to detect group differences between osteoporotic and healthy women in UI.¹¹ Similarly, examining the femur in adult women with AN, Bredella et al.¹⁰ failed to detect group differences in UI between anorexic and healthy subjects, but found increased levels of total lipids, and saturated and unsaturated fats. Our data consistently show increases in these levels in the placebo group at both TE=30ms and TE=60ms and at both locations studied, although with considerable variation. The variation between medial and lateral distal femur is indicative of the spatial variation in bone turnover in adolescents with AN, as evidenced by the fact that BMI proved to be a significant effect-modifier at the lateral site, but not at the medial site.. Further studies are required to continue to elucidate the clinical implications of these findings for adolescents with AN, and recent animal studies involving adipocyte size and hormonal effects on MAT may shed light on future work in this regard.^{23,29,30}

In the current study, R_{sat}, R_{unsat} and R_{total} all showed a trend towards lesser increase in the cohort receiving DHEA + estrogen/progestin versus placebo at TE=60msec. These differences, while not statistically significant, are nevertheless indicators that active treatment arrests the increase in these ratios in AN.²² The fact that the treatment effect is more apparent and statistically significant in T1 and T2 calculations as opposed to the lipid ratios also suggests the changes in composition of saturated and unsaturated lipids occurring in AN are more noteworthy than previously thought. These data suggest the need for further studies that include T2 analyses of marrow fat to characterize disease-related changes in bone composition, as outlined in our baseline analysis.²²

The current treatment was found to be safe, with no changes noted in liver function tests, lipid outcomes, or physical examination (e.g., hirsutism). This treatment may augment the positive impact of nutritional repletion and psychological recovery towards the improvement of bone health in adolescents with restrictive eating disorders. Of note, our group previously reported beneficial psychological changes, including significantly decreased anxiety and mood enhancement, after short-term oral DHEA monotherapy in adolescents with AN.³¹

Study limitations merit discussion. This study enrolled subjects with AN based upon newer and broader DSM-5 diagnostic criteria. As a result, we included some patients with relatively mild disease. Our recruitment strategy aimed to increase the generalizability of the findings by examining bone health across a broader disease spectrum than has been captured in earlier reports. Our findings are also limited to one anatomical site within the peripheral skeleton, the distal femur. Thus, conclusions about marrow fat compartments within other skeletal sites must be drawn with caution. Lastly, we acknowledge that some measures that would have been informative in terms of mechanistic insights were not evaluated, such as ferritin. Future studies should also examine in greater depth how psychological measures and physical activity may influence bone marrow composition.

In conclusion, our findings suggest that combined treatment with DHEA + estrogen/progestin preserves bone mass and bone marrow composition in adolescents with AN, particularly those with a higher BMI at the time of presentation. Whether these findings ultimately translate to higher peak bone mass, lower fracture risk, or other beneficial bone health outcomes warrants further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bredella MA, Fazeli PK, Miller KK, et al. Increased Bone Marrow Fat in Anorexia Nervosa. *J Clin Endocrinol Metab.* 2009; 94(6):2129–2136. [PubMed: 19318450]
2. Ecklund K, Vajapeyam S, Feldman HA, et al. Bone Marrow Changes in Adolescent Girls With Anorexia Nervosa. *J Bone Miner Res.* 2010; 25(2):298–304. [PubMed: 19653811]
3. Di Iorgi N, Rosol M, Mittelman SD, Gilsanz V. Reciprocal relation between marrow adiposity and the amount of bone in the axial and appendicular skeleton of young adults. *J Clin Endocrinol Metab.* 2008; 93(6):2281–2286. [PubMed: 18381577]
4. Hess R, Pino AM, Rios S, Fernandez M, Rodriguez JP. High affinity leptin receptors are present in human mesenchymal stem cells (MSCs) derived from control and osteoporotic donors. *J Cell Biochem.* 2005; 94(1):50–57. [PubMed: 15517602]
5. Gimble JM, Nuttall ME. Bone and fat: old questions, new insights. *Endocrine.* 2004; 23(2–3):183–188. [PubMed: 15146099]

6. Gimble JM, Zvonic S, Floyd ZE, Kassem M, Nuttall ME. Playing with bone and fat. *J Cell Biochem.* 2006; 98(2):251–266. [PubMed: 16479589]
7. Fazeli PK, Horowitz MC, MacDougald OA, et al. Marrow fat and bone-new perspectives. *J Clin Endocrinol Metab.* 2013; 98(3):935–945. [PubMed: 23393168]
8. Veldhuis-Vlug AG, Rosen CJ. Mechanisms of marrow adiposity and its implications for skeletal health. *Metabolism.* 2017; 67:106–114. [PubMed: 28081773]
9. Donaldson AA, Gordon CM. Skeletal complications of eating disorders. *Metabolism.* 2015; 64(9): 943–951. [PubMed: 26166318]
10. Bredella MA, Fazeli PK, Daley SM, et al. Marrow fat composition in anorexia nervosa. *Bone.* 2014; 66:199–204. [PubMed: 24953711]
11. Di Pietro G, Capuani S, Manenti G, et al. Bone Marrow Lipid Profiles from Peripheral Skeleton as Potential Biomarkers for Osteoporosis: A ¹H-MR Spectroscopy Study. *Acad Radiol.* 2016; 23(3): 273–283. [PubMed: 26774740]
12. Yeung DK, Griffith JF, Antonio GE, Lee FK, Woo J, Leung PC. Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: a proton MR spectroscopy study. *J Magn Reson Imaging.* 2005; 22(2):279–285. [PubMed: 16028245]
13. Misra M, Golden NH, Katzman DK. State of the art systematic review of bone disease in anorexia nervosa. *Int J Eat Disord.* 2016; 49(3):276–292. [PubMed: 26311400]
14. DiVasta AD, Feldman HA, Giancaterino C, Rosen CJ, LeBoff MS, Gordon CM. The effect of gonadal and adrenal steroid therapy on skeletal health in adolescents and young women with anorexia nervosa. *Metabolism: Clin and Exp.* 2012; 61(7):1010–1020.
15. DiVasta AD, Feldman HA, Beck TJ, LeBoff MS, Gordon CM. Does hormone replacement normalize bone geometry in adolescents with anorexia nervosa? *J Bone Miner Res.* 2014; 29(1): 151–157. [PubMed: 23744513]
16. Ecklund K, Vajapeyam S, Mulkern RV, et al. Bone marrow fat content in 70 adolescent girls with anorexia nervosa: Magnetic resonance imaging and magnetic resonance spectroscopy assessment. *Pediatr Radiol.* 2017; 47(8):952–962. [PubMed: 28432403]
17. American Psychiatric Publishing. DSM-5 Task Force. Diagnostic and statistical manual of mental disorders: DSM-5. 5. Washington, D.C: 2013.
18. Fennessy FM, Fedorov A, Gupta SN, Schmidt EJ, Tempny CM, Mulkern RV. Practical considerations in T1 mapping of prostate for dynamic contrast enhancement pharmacokinetic analyses. *Magn Reson Imaging.* 2012; 30(9):1224–1233. [PubMed: 22898681]
19. Ecklund K, Kleinman PK, Vajapeyam S, et al. MRI assessment of metaphyseal bone marrow fat content in adolescents girls with anorexia nervosa. *Pediatr Radiol.* 2009; 39(Suppl 2):S264.
20. Ogden, JA. Skeletal injury in the child. 3. New York: 2000.
21. Moore SG, Dawson KL. Red and yellow marrow in the femur: age-related changes in appearance at MR imaging. *Radiology.* 1990; 175(1):219–223. [PubMed: 2315484]
22. Ecklund K, Vajapeyam S, Mulkern RV, et al. Bone marrow fat content in 70 adolescent girls with anorexia nervosa: Magnetic resonance imaging and magnetic resonance spectroscopy assessment. *Pediatr Radiol.* 2017
23. Scheller EL, Doucette CR, Learman BS, et al. Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues. *Nature Communications.* 2015; 6:7808–7820.
24. Ishijima H, Ishizaka H, Aoki J, Inoue T, Endo K. T2 relaxation time of bone marrow water and lipid: correlation with serum ferritin in normal individuals. *Journal of computer assisted tomography.* 1997; 21(3):506–508. [PubMed: 9135666]
25. Wanby P, Berglund J, Brudin L, Hedberg D, Carlsson M. Increased ferritin levels in patients with anorexia nervosa: impact of weight gain. *Eating and weight disorders: EWD.* 2016; 21(3):411–417. [PubMed: 26830429]
26. Liney GP, Bernard CP, Manton DJ, Turnbull LW, Langton CM. Age, gender, and skeletal variation in bone marrow composition: a preliminary study at 3.0 Tesla. *J Magn Reson Imaging.* 2007; 26(3):787–793. [PubMed: 17729356]

27. Patsch JM, Li X, Baum T, et al. Bone marrow fat composition as a novel imaging biomarker in postmenopausal women with prevalent fragility fractures. *J Bone Miner Res.* 2013; 28(8):1721–1728. [PubMed: 23558967]
28. Scheller EL, Rosen CJ. What's the matter with MAT? Marrow adipose tissue, metabolism, and skeletal health. *Annals of the New York Academy of Sciences.* 2014; 1311(1):14–30. [PubMed: 24650218]
29. Cawthorn WP, Scheller EL, Parlee SD, et al. Expansion of Bone Marrow Adipose Tissue During Caloric Restriction Is Associated With Increased Circulating Glucocorticoids and Not With Hypoleptinemia. *Endocrinology.* 2016; 157(2):508–521. [PubMed: 26696121]
30. Devlin MJ, Brooks DJ, Conlon C, et al. Daily leptin blunts marrow fat but does not impact bone mass in calorie-restricted mice. *Journal of Endocrinology.* 2016; 229(3):295–306. [PubMed: 27340200]
31. Gordon CM, Grace E, Emans SJ, et al. Effects of oral dehydroepiandrosterone on bone density in young women with anorexia nervosa: a randomized trial. *J Clin Endocrinol Metab.* 2002; 87(11):4935–4941. [PubMed: 12414853]

HIGHLIGHTS

- Previous studies have shown increased marrow fat in adolescent girls with anorexia nervosa.
- Changes in marrow composition may be mechanistically linked to changes in skeletal turnover and bone accretion in young adolescents.
- Treatment with oral dehydroepiandrosterone + estrogen/progestin arrested age- and disease-related changes in bone marrow composition in adolescents with anorexia nervosa.

Figure 1. Subject Recruitment (depicted through consort diagram for clinical trial)

The study was a two-site, double blind, randomized, placebo-controlled trial. The hospital pharmacy dispensed drug or placebo in identical gelatin capsules. Assignments (computerized blocked randomization) were not revealed aside from the pharmacist and statistician until the trial's conclusion. The treatment arm received 12 months of oral micronized DHEA (50 mg daily; Belmar Pharmacy; IND 52192) with conjugated equine estrogens (0.3mg daily; Premarin®, Wyeth) for the first 3 months, followed by an oral contraceptive (20µg ethinyl estradiol/0.1mg levonorgestrel; Alesse®, Wyeth) for 9 months. The other group received placebo.

Figure 2.

Figure 2A shows the baseline T1-weighted image of the right knee of a 16-year-old girl with anorexia nervosa. The MR spectroscopic voxels are shown as black (medial) and white (lateral) rectangles. Figures 2B and 2C show the spectra from the lateral voxel with TE=30 msec and TE=60 msec respectively. The 5 relevant lipid peaks along with the water peak are seen, along with the fits and peak areas measured by the vendor-supplied software.

Figure 3. 12-Month Change in T1, T2_{unsat} and T2_{sat}:

Figure 3 shows the adjusted 12-month change in T1, T2_{unsat}, and T2_{sat} in the lateral distal femur, plotted as a function of BMI. BMI was a significant effect modifier for all 3 variables. The top row shows the changes in T1 in the active cohort, the middle row shows the placebo cohort, and the bottom row plots the difference between them. The gray shaded area in the bottom row shows 95% confidence limits for the difference. T2_{unsat} and T2_{sat} are plotted on a log₁₀ scale, and therefore the dashed lines at 1 on the y-axis (Figures 3F and 3I) correspond to a raw value of 0, which denotes no difference between active and placebo groups. Positive effects of the treatment of DHEA + E/P are seen primarily for girls above a BMI of about 18 kg/m². As seen from the graphs, our sample is skewed toward higher BMI girls, leading to a larger standard error at low BMI.

Table 1**Baseline Characteristics of Sample**

	Active drug(n=35)	Placebo (n=35)	<i>P</i> *
<i>Mean ± SD</i>			
Age, yr.	15.5 ± 1.9	15.5 ± 2.0	0.97
Height, cm	159.5 ± 7.4	161.5 ± 8.5	0.30
Weight, kg	48.0 ± 6.2	48.8 ± 6.9	0.60
BMI, kg/m ²	18.8 ± 1.6	18.7 ± 1.8	0.69
BMI Z-score	−0.55 ± 0.71	−0.66 ± 0.86	0.58
Percentage of IBW, %	92 ± 8	90 ± 10	0.49
<i>Median (Min–Max)</i>			
Time since AN diagnosis, mo.	4 (1 – 36)	6 (1 – 60)	0.17
Time since last menses, mo. †	4 (1 – 17)	4 (1 – 18)	0.67
Dehydroepiandrosterone sulfate, mcg/dL	185 (46 – 445)	125 (39 – 431)	0.01
Estradiol, pg/mL	29 (1 – 208)	26 (2 – 274)	0.52
Total testosterone, ng/dL	21 (2 – 274)	12 (1 – 130)	0.01
Leptin, ng/mL	4.3 (0.1 – 13.8)	2.7 (0.6 – 15.2)	0.33
Ghrelin, pg/mL	425 (50 – 1679)	389 (50 – 1082)	0.57
Triiodothyronine, ng/dL	88 (55 – 255)	90 (49 – 160)	0.80
Insulin-like growth factor I, ng/mL	303(126 – 734)	317 (154 – 550)	0.77
<i>N (%)</i>			
Race:			
Caucasian	30 (86)	31 (89)	1
Other	5 (14)	4 (11)	
Ethnicity:			
Hispanic	3 (9)	1 (3)	0.61
Non-Hispanic	32 (91)	34 (97)	
History of fracture:			
Yes	10 (29)	12 (34)	0.80
No	25 (71)	23 (66)	
Family history of osteoporosis:			
Yes	9 (26)	8 (23)	1
No or unknown	26 (74)	27 (77)	
Physal closure: Both open	5 (14)	12 (34)	0.17
Femur open, tibia closed	9 (26)	6 (17)	
Both closed	21 (60)	17 (49)	

Notes:

* Student t, Wilcoxon rank-sum, or Fisher Exact test comparing distribution in active drug and placebo arms.

† Eleven pre-menarchal participants excluded (4 active drug, 7 placebo).

Abbreviations: BMD, bone mineral density; DXA, dual-emission x-ray absorptiometry; pQCT, peripheral quantitative computed tomography; IBW, ideal body weight; BMI, body mass index

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MRI and MRS measures of femoral marrow content, as affected over 12 months by randomly assigned treatment (32 active drug or 30 placebo).

Table 2

Measure [†]		TE(msec)	12 mo. – baseline, mean ± SE		p
			Active	Placebo	
<i>Lateral</i>	T ₁ , msec	—	-2 ± 14	-25 ± 14	[‡]
	T _{2w} , msec	—	-1.3 ± 1.4	-5.7 ± 1.3	0.02
	Log ₁₀ T _{2unsat}	—	-0.07 ± 0.05	-0.12 ± 0.04	[‡]
	Log ₁₀ T _{2sat}	—	-0.03 ± 0.03	-0.03 ± 0.02	[‡]
	UI	30	0.002 ± 0.003	0.005 ± 0.003	0.63
		60	-0.007 ± 0.003	-0.008 ± 0.003	0.86
	Log ₁₀ R _{sat}	30	0.12 ± 0.03	0.04 ± 0.03	0.07
		60	0.12 ± 0.07	0.26 ± 0.06	0.13
	Log ₁₀ R _{unsat}	30	0.11 ± 0.03	0.07 ± 0.03	0.37
		60	0.07 ± 0.06	0.20 ± 0.06	0.12
<i>Medial</i>	Log ₁₀ R _{tot}	30	0.11 ± 0.03	0.04 ± 0.03	0.08
		60	0.13 ± 0.06	0.24 ± 0.06	0.22
	T ₁ , msec	—	-4 ± 13	-23 ± 13	0.34
	T _{2w} , msec	—	4.1 ± 2.4	-3.8 ± 2.2	0.02
	Log ₁₀ T _{2unsat}	—	0.12 ± 0.04	0.00 ± 0.04	0.04
	Log ₁₀ T _{2sat}	—	-0.03 ± 0.02	-0.00 ± 0.02	0.41
	UI	30	-0.014 ± 0.004	-0.001 ± 0.004	0.02
		60	-0.001 ± 0.003	-0.005 ± 0.003	0.35
	Log ₁₀ R _{sat}	30	0.20 ± 0.05	0.15 ± 0.04	0.44
		60	0.11 ± 0.07	0.22 ± 0.06	0.24
	Log ₁₀ R _{unsat}	30	0.13 ± 0.04	0.11 ± 0.04	0.74
		60	0.08 ± 0.06	0.19 ± 0.05	0.18
	Log ₁₀ R _{total}	30	0.21 ± 0.05	0.12 ± 0.05	0.19
		60	0.10 ± 0.06	0.21 ± 0.06	0.19

Notes:

R_{total}: ratio of total lipid to water. Means and standard errors are adjusted for baseline age, body-mass index (BMI), physical closure status, and serum concentration of DHEAS, estradiol, and leptin. p tests for equal 12-mo. change in the two trial arms.

[‡] Measures preceded by 'Log10' showed strongly skewed distributions and were log-transformed for analysis. Changes and standard errors for these measures are in log10 units. Each change of 0.10 unit corresponds to 1.26-fold (26%) increase or decrease; each 0.01 unit to a 1.02-fold (2%) increase or decrease.

[‡] These measures displayed significant or near-significant effect modification by baseline BMI (BMI $\times$ arm $\times$ visit interaction). Adjusted mean changes are estimated at the median of the effect modifier (18.9 kg/m²). Active-Placebo difference depended on baseline BMI as detailed in Table 3. Effect modification by baseline BMI was non-significant for other measures.

Abbreviations: MRI: magnetic resonance imaging. MRS: magnetic resonance spectroscopy. UI: unsaturation index (ratio of olefin to total lipid). R_{Sat}: ratio of saturated fat to water. R_{unsat}: ratio of unsaturated fat to water.

MRI and MRS measures of lateral distal femoral marrow content, as affected over 12 months by randomly assigned treatment (n=32 active drug or n=30 placebo): effect modification by baseline body-mass index. *

Table 3

Measure [‡]	Influence of BMI on 12-mo. change, per kg/m ² , estimate ± SE					
	Active	p	Placebo	p	Difference	P _{int}
T ₁ , msec	15 ± 9	0.10	-16 ± 8	0.04	31 ± 12	0.01
Log ₁₀ T _{2unsat}	0.034 ± 0.027	0.22	-0.055 ± 0.023	0.02	0.089 ± 0.036	0.02
Log ₁₀ T _{2sat}	-0.037 ± 0.020	0.06	0.007 ± 0.014	0.63	-0.044 ± 0.024	0.07

Notes: Estimates are adjusted for baseline age, body-mass index (BMI), physical closure status, and serum concentration of DHEAS, estradiol, and leptin. p tests for influence of BMI on 12-mo. change; Pint tests for equal influence in the two trial arms.

[‡] Measures preceded by 'Log10' showed strongly skewed distributions and were log-transformed for analysis. Estimates for these measures are in log10 units. Each increment of 0.10 unit corresponds to 1.26-fold (26%) increase or decrease; each 0.01 unit to a 1.02-fold (2%) increase or decrease.

Abbreviations: *MRI: magnetic resonance imaging. MRS: magnetic resonance spectroscopy