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Sclerostin Levels and Bone Turnover Markers in Adolescents with Anorexia Nervosa and Healthy Adolescent Girls

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Abstract

Sclerostin, product of the *SOST* gene, is an important determinant of bone formation and resorption. Adolescents with anorexia nervosa (AN) have low bone density and decreased levels of bone turnover markers. However, sclerostin has not been examined in AN as a potential mediator of impaired bone metabolism. Our study objectives were to (i) assess associations of sclerostin with surrogate bone turnover markers in girls with AN and controls and (ii) examine effects of transdermal estradiol on sclerostin in AN. 69 girls (44 with AN and 25 normal-weight controls) 13–18 years old were studied at baseline. 22 AN girls were randomized to transdermal estradiol (plus cyclic medroxyprogesterone) or placebo in a double-blind study for 12 months. Sclerostin correlated positively with P1NP and CTX in controls (r = 0. 67 and 0. 53, p = 0. 0002 and 0. 005, respectively) but not in AN despite comparable levels at baseline. Changes in sclerostin over twelve months did not differ in girls randomized to estradiol or placebo. The relationship between sclerostin and bone turnover markers is disrupted in adolescent girls with AN. Despite an increase in BMD with estradiol administration in AN, estrogen does not impact sclerostin levels in this group.

Keywords

anorexia nervosa; sclerostin; osteoporosis

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The authors have nothing to declare.

1. 1 Introduction

Sclerostin is a member of the DAN (differential screening-selected gene aberrant in neuroblastoma) glycoprotein family and is secreted by osteocytes [1]. Sclerostin binds LRP5/6 to inhibit Wnt signaling in osteoblasts [2,3]. In addition, sclerostin increases osteoclast formation and activity *in vitro*, mediated by RANKL [4]. The function of sclerostin on bone biology is illustrated by the abnormal skeletal features of sclerosteosis and van Buchem disease, two diseases characterized by sclerostin deficiency. These conditions are characterized by osteoblast hyperactivity and bone overgrowth, most notably in the mandible and skull. Sclerosteosis is a recessive disorder resulting from inactivating mutations in the SOST gene, which encodes sclerostin [5]. Van Buchem disease is caused by deletion of SOST transcription regulatory elements [6].

The SOST gene promoter has multiple estrogen-response elements [7], and several studies have shown that serum levels of sclerostin are negatively regulated by estradiol in adults. Sclerostin levels are higher in postmenopausal women as compared to premenopausal women [8], and treatment with transdermal estradiol [9,10] or with raloxifene [11] significantly reduces sclerostin levels in this population. In elderly men, treatment with estradiol, but not testosterone, has been shown to reduce sclerostin levels [10]. Reductions in bone resorption markers were observed in parallel with the decrease in sclerostin [9–11].

Sclerostin levels have not previously been evaluated in anorexia nervosa (AN) or in children with disorders that alter bone metabolism. Individuals with AN have low levels of estrogen and the majority have decreased bone mineral density (BMD) [12,13]. Although adolescents with AN are hypogonadal, they typically have low levels of both bone formation and bone resorption markers [12,14,15]. Multiple metabolic and hormonal abnormalities are present in patients with AN, including relative IGF-1 deficiency, hypercortisolism, and alterations in leptin and ghrelin [16–19] which contribute to low levels of bone formation and resorption markers. It is not currently known whether changes in levels of bone turnover markers are also mediated by changes in sclerostin.

In this study we assessed associations of sclerostin with levels of surrogate markers of bone turnover in adolescent girls with AN and normal-weight controls. We have recently demonstrated in a randomized double-blind placebo-controlled study that physiologic estrogen replacement in adolescent girls with AN significantly improves BMD [20]. We utilized data from this study to examine the effects of 12 months of transdermal estradiol replacement or placebo on sclerostin levels in adolescent girls with AN. We hypothesized that at baseline, levels of sclerostin would be higher in estrogen deficient subjects with AN and would be inversely associated with a surrogate marker of bone formation (N-terminal propeptide of type 1 procollagen (P1NP)) and positively associated with a marker of bone resorption (CTX). We also hypothesized that sclerostin levels would decrease in subjects who received estradiol and predict changes in BMD.

1. 2 Subjects and Methods

The study was performed at the Clinical Research Center of Massachusetts General Hospital (MGH), Boston, MA, USA, and the Clinical Investigation Unit at the Hospital for Sick Children (SickKids), Toronto, ON, Canada. Written informed consent was obtained from all subjects prior to their participation. Parental consent was also obtained for subjects under the age of 18 years. The study was approved by the Institutional Review Board of Partners HealthCare, Boston, and the Research Ethics Board at SickKids, Toronto, Canada.

For this study, we assessed a subset of adolescent girls with AN and normal-weight controls 13–18 years of age from a prior study [20] based on their lumbar spine BMD Z-scores. The

subset included all subjects with AN who had a baseline lumbar BMD Z-score less than −0. 5 (n = 44) and normal-weight controls with lumbar BMD Z-scores between +1. 0 and −1. 0 (n = 25). These cutoffs were chosen to separate the groups based on bone density and thereby potentially maximize differences in sclerostin levels. The diagnosis of AN was confirmed by a study psychiatrist according to DSM-IV criteria. Exclusion criteria have been described previously [20] and included diseases and medications affecting bone metabolism, suicidality, psychosis or history of substance abuse. All subjects with AN were actively enrolled in treatment programs under the supervision of their primary providers during the course of the study.

Data from a subset of twenty-two AN subjects with spine BMD Z-scores of < −0. 5 and a bone age of ≥ 15 years were available for longitudinal analysis over 12 months. The treatment protocol has been described previously [20]. In brief, subjects with AN were randomized to transdermal estradiol (100mcg patch applied twice weekly; Novartis Pharmaceuticals, Inc.) or placebo in a double-blind study. Subjects randomized to transdermal estradiol also received medroxyprogesterone 2. 5mg daily for 10 days each month. Calcium and vitamin D were given to all subjects (1200mg calcium carbonate and 400 IU vitamin D daily). In the subset available for longitudinal analysis, thirteen girls with AN were randomized to transdermal estradiol and nine were randomized to placebo.

Blood samples were collected at baseline and at 12 months (samples from normal-weight controls were obtained during the early follicular phase of the menstrual cycle). Baseline and 12-month biochemical analysis included serum levels of estradiol, insulin-like growth factor 1 (IGF-1), leptin, C-terminal cross-linked peptides (CTX), N- terminal propeptide of type 1 procollagen (P1NP), parathyroid hormone (PTH), and sclerostin. Samples for serum 25(OH) vitamin D [25(OH)D] and 24-hour urinary free cortisol (24-hr UFC) were also collected at baseline. Serum sclerostin concentrations were measured by a sandwich ELISA assay (TECOmedical AG, Sissach, Switzerland; intra-assay coefficient of variation 3. 1%, inter-assay coefficient of variation 3. 5%; sensitivity 0. 170 ng/ml). Additional biochemical assays have been described previously [20].

BMD and body composition were determined by dual-energy x-ray absorptiometry (DXA) (Hologic 4500 A, Waltham MA). Bone age was assessed by a single pediatric endocrinologist according to the methods of Greulich and Pyle [21].

Statistical analysis was completed using JMP (version 9, SAS Institute). P values < 0. 05 were considered statistically significant. All values are shown as means±SEM. Baseline characteristics and longitudinal comparisons were performed by utilizing the Wilcoxon Rank Sum test. The effects of potential confounders were examined by multivariate analysis after log transformation. Univariate relationships were assessed by Spearman's correlation. Variables with p values ≤ 0. 05 on Spearman's correlation were subsequently log-transformed and entered into a stepwise mixed model multiple regression analysis to control for potential confounders and to determine independent determinants of bone turnover markers.

Because of the relatively small sample size available for longitudinal analysis, we performed a power calculation based on derivations from Modder et al [10]. With 9 subjects in each group and using a one-sided t-test, the study was powered at 84% to detect a net decrease in sclerostin levels with estrogen treatment of 1. 32*SD at an alpha level of 0. 05, assuming a SD of 1.

1. 3 Results

1. 3. 1 Baseline Characteristics

Comparisons between normal-weight controls and subjects with AN are shown in Table 1. The two groups did not differ for bone age (as per study design), height, Tanner staging, and exercise activity. As expected, girls with AN had lower body mass index (BMI), lean mass, and fat mass. Consistent with our prior report, subjects with AN had significantly higher calcium and vitamin D intake than controls [22]. BMD at the lumbar spine and hip were significantly lower in subjects with AN than controls.

Levels of IGF-1, estradiol, leptin, CTX, and P1NP were significantly lower in AN than in controls. 24-hour urinary free cortisol and serum 25(OH) vitamin D levels were significantly higher in subjects with AN. However, despite higher 25(OH) vitamin D levels, PTH levels were higher in the AN group, although within the normal range.

1. 3. 2 Sclerostin Levels and Associations with Surrogate Markers of Bone Turnover

Baseline serum sclerostin levels are shown in Table 1. The groups did not differ for sclerostin even after controlling for levels of estradiol and PTH ($p = 0.69$) or after matching the groups for chronological age ($p = 0.33$). Baseline sclerostin values in the controls and girls with AN did not correlate with BMI nor exercise activity ($r = 0.12$, $p = 0.32$ and $r = 0.14$, $p = 0.29$, respectively).

Relationships between sclerostin and additional biochemical parameters were assessed using Spearman's correlation in subjects with AN and controls (Table 2). Sclerostin levels correlated strongly and positively with markers of bone formation and resorption in healthy controls. In contrast, this association was not seen in girls with AN (Figure 1). Bone age correlated inversely with sclerostin levels in controls, and we observed a trend towards an inverse association between chronological age and sclerostin. No correlations were evident with sclerostin in AN, although there were trends towards a positive association with 25(OH) vitamin D and an inverse association with estradiol.

We then utilized stepwise regression analysis to further assess associations between sclerostin and P1NP and CTX. Bone age was included as an independent variable for analysis of P1NP and CTX in the controls. Levels of IGF-1, leptin, 25(OH) vitamin D, and bone age were included in the analysis for P1NP, and bone age was incorporated for the analysis of CTX in the AN group. These parameters (and chronological age) are the only variables that significantly correlated with bone turnover markers on univariate analysis in AN or controls (data not shown). In controls, on regression modeling, the relationship between sclerostin and P1NP remained significant ($p = 0.005$), and the relationship between sclerostin and CTX was of borderline significance ($p = 0.05$) after controlling for bone age. However, there was no independent association of sclerostin with P1NP or CTX in girls with AN on regression modeling (data not shown). Conversely, P1NP was significantly associated with IGF-1 in girls with AN ($p = 0.02$).

1. 3. 3 Impact of Estradiol Replacement on Sclerostin Levels

Twenty-two adolescent girls with AN received transdermal estradiol or placebo for twelve months. At baseline, the two groups had similar characteristics with the exceptions that the placebo-treated group had significantly higher CTX levels. Sclerostin levels in the placebo and treatment groups did not differ at baseline (Table 3).

At twelve months, estradiol-treated subjects had significantly improved lumbar bone density compared to the placebo group. Body composition changes during the treatment period did

not differ between the groups. Despite the increase in bone density, changes in sclerostin levels over twelve months did not differ in girls with AN treated with transdermal estradiol versus placebo. These results did not change after adjusting for baseline age and weight change during the treatment period (Table 4). Similarly, replacing chronological age with bone age did not significantly change our results. In subjects who received estradiol, changes in sclerostin did not correlate with changes in lumbar bone density parameters (data not shown).

1. 4 Discussion

Sclerostin levels have not been previously examined in adolescents with anorexia nervosa (AN). In this study, we showed that levels of sclerostin are associated with surrogate markers of bone turnover in normal-weight girls. In contrast, this relationship is not seen in adolescent girls with AN although levels of sclerostin do not differ in girls with AN compared with normal-weight adolescents. Additionally, although studies in adults have shown that estrogen decreases sclerostin levels, transdermal estradiol replacement in adolescent girls with AN does not change sclerostin levels despite improving bone mass.

We observed strong positive associations between sclerostin and markers of both bone formation and resorption in normal-weight controls. These results are consistent with prior data demonstrating a significant positive relationship between markers of bone turnover and sclerostin levels in healthy girls [23]. However, sclerostin levels in subjects with AN did not correlate with markers of bone formation or resorption. These patterns were unchanged in a multiple regression model. In contrast to our initial hypothesis, sclerostin levels were not higher in subjects with AN compared to normal-weight controls. In addition, despite a significant improvement in lumbar BMD compared to placebo, treatment of AN subjects with transdermal estradiol did not significantly change levels of sclerostin. Together, our data suggests that the positive effect of estradiol replacement on BMD in adolescent girls with AN is mediated in a sclerostin-independent fashion. Further study would be valuable to determine whether sclerostin would represent a potential therapeutic target in this population.

Prior studies have implicated multiple factors in the regulation of sclerostin. In healthy girls, levels of sclerostin gradually decline after the onset of puberty [23]. In our study we observed an inverse relationship between sclerostin and bone age and chronological age in normal-weight adolescents. These data suggest that increasing maturity is associated with decreasing levels of sclerostin, as previously reported. Additionally, after matching for chronological age, sclerostin values did not differ between the groups. We also considered that serum sclerostin levels may be proportional to osteocyte number. After controlling for total body bone mineral content as a surrogate for osteocyte number, sclerostin values did not differ in the controls and subjects with AN.

PTH may also affect sclerostin levels. An acute infusion of PTH lowers sclerostin in adult healthy men [24] and intermittent PTH treatment reduces sclerostin levels in postmenopausal women [25]. Adult patients with primary hyperparathyroidism have lower levels of sclerostin than healthy controls or hypoparathyroid patients, and sclerostin normalizes after successful parathyroidectomy [26–29]. *In vitro* and *in vivo* experiments in mice also support an inverse relationship between PTH and sclerostin [30]. In our study mean PTH levels were significantly higher in the AN group than in the controls (although within the normal range). It is possible that the higher PTH in girls with AN prevents the increase in sclerostin expected in a state of estrogen deficiency. However, even after adjustment for PTH, sclerostin levels did not differ in subjects with AN compared with controls. We did not observe a significant correlation between PTH and sclerostin in

subjects with AN or in controls. Our data are consistent with a prior study of sclerostin in healthy adolescent girls, which also did not show an association between sclerostin and PTH [23]. In contrast, normative data in a large study of healthy adult premenopausal women showed a weak inverse correlation between sclerostin and PTH [31].

The lack of an association between levels of estradiol and sclerostin in girls with AN or controls consistent with normative data from another adolescent study [23]. In contrast, data from healthy premenopausal and postmenopausal adult women show an inverse association between estradiol and sclerostin [31]. Those associations were derived from a large range of estradiol levels, which contrasts to our study population's relatively narrow range of estradiol values. Contrary to observations in postmenopausal women, in whom estrogen replacement decreases sclerostin levels, transdermal estradiol replacement in subjects with AN did not change levels of sclerostin [9,10]. However, therapy with estradiol did result in a significant improvement in BMD in our study, suggesting an uncoupling of sclerostin from estradiol effects in adolescents with AN. *In vivo* mouse experiments suggested that glucocorticoids may stimulate sclerostin expression [32]. However, hypercortisolism does not appear to be the mechanism for the apparent dissociation between estradiol and sclerostin in adolescents with AN, as sclerostin and 24-hr urine free cortisol did not correlate in subjects with AN or controls.

In our study sclerostin strongly correlated with CTX and P1NP in the control group, but this relationship was not present in subjects with AN. Although sclerostin is typically believed to impact bone formation by inhibiting osteoblast activity, data indicate that sclerostin also increases osteoclast formation and activity by increasing RANKL expression [4]. The positive association between sclerostin and CTX, a marker of bone resorption, is consistent with the latter effect. The positive association between sclerostin and P1NP is unexpected, but may be a consequence of the coupling of bone resorption with bone formation. Our findings of positive associations of sclerostin with both CTX and P1NP in normal-weight controls are also consistent with prior observations in healthy adolescent girls [23]. Studies examining the relationship between sclerostin and bone formation/resorption markers in adults have yielded disparate results depending on the study population and disease state. For example, sclerostin appears to have an inverse relationship with P1NP and CTX in patients with primary hyperparathyroidism but correlates positively with these markers in patients with hypoparathyroidism [28]. It is intriguing that the observed association of sclerostin with markers of bone turnover in controls was lost in girls with AN. It is possible that alterations in other hormones such as IGF-1 in girls with AN supercede effects of sclerostin on markers or bone turnover. Consistent with this, in girls with AN, IGF-1 was an important determinant of bone turnover markers.

Although the relationship between sclerostin and bone turnover markers was disrupted in girls with AN, dysregulation of Wnt signaling may contribute to decreased bone formation in AN. Additional extracellular factors, such as Dickkopf-1 (Dkk1), secreted frizzled-related proteins, Wnt inhibitory factor-1, and sclerostin domain containing 1 modulate Wnt signaling and have not yet been studied in subjects with AN. Treatment of postmenopausal women with transdermal estradiol does not appear to affect levels of Dkk1 [9], although a recent study in a similar population reported that treatment with raloxifene lowered Dkk1 [33]. Dkk1 is expressed by human preadipocytes [34], and like other adipokines, its secretion may be altered in AN.

A potential limitation of the longitudinal component of our study is the absence of samples from additional data timepoints other than at 12 months after treatment initiation. Although the effect of estrogen on levels of sclerostin could theoretically be transient, prior studies suggest that the influence of estrogen is more durable. In a study by Modder et al. , serum

sclerostin levels were lower in postmenopausal women after 4 months of treatment with transdermal estradiol compared to placebo [9]. In a retrospective study, postmenopausal women treated with raloxifene for a mean of 19 months had significantly lower serum levels of sclerostin compared to controls [11].

The lack of standardization of the measurement of sclerostin is an additional factor which complicates data interpretation. Interassay correlation between two popular commercial tests (including the assay employed in this study) was poor in a recent study by McNulty et al [35]. Studies suggest that sclerostin exists in blood in both free and protein-bound forms [35,36]. The physiologic significance of these forms is unknown, and the ability of any assay to detect these forms may potentially differ [35].

A particular strength of our study is that the cross-sectional analysis of normal-weight controls and girls with AN is complemented and supported by longitudinal data showing a consistent pattern of a disrupted relationship between sclerostin and markers of bone formation and resorption and an uncoupling of sclerostin from the effects of estradiol administration in AN. These findings imply the presence of additional unspecified factors which regulate sclerostin in adolescents with AN. Further study of sclerostin in adolescents with AN would be beneficial prior to consideration as a potential therapeutic target.

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Highlights

- Serum sclerostin is positively associated with bone turnover markers in healthy adolescent girls.
- The relationship between sclerostin and bone turnover markers is disrupted in adolescent girls with anorexia nervosa.
- Transdermal estradiol replacement in adolescent girls with anorexia nervosa does not change sclerostin levels despite improving bone mass.

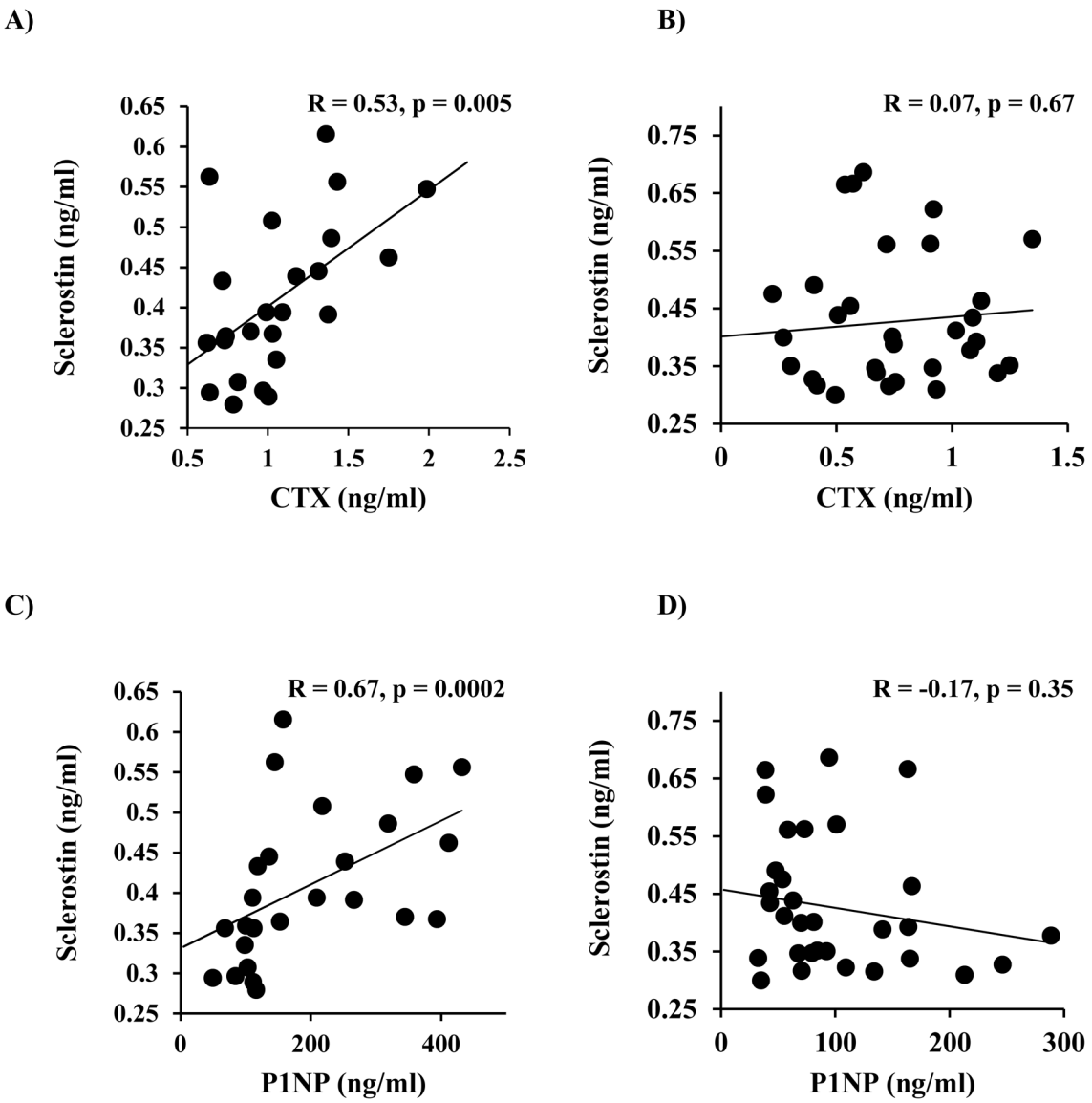

Figure 1. Correlations between sclerostin and bone markers in adolescent girls. R and p values were derived from Spearman's correlation

Panel A: Normal-weight controls, CTX

Panel B: AN, CTX

Panel C: Normal-weight controls, P1NP

Panel D: AN, P1NP

Table 1

Comparison of Normal-Weight Controls and Adolescent Girls with Anorexia Nervosa

	Controls (n=25)	Anorexia Nervosa (n=44)	p (Controls vs. AN)
Age (y)	15. 7±0. 23	16. 7±0. 22	0. 006
Bone age (y)	15. 7±0. 25	16. 2±0. 17	NS
Weight (kg)	57. 2±1. 4	46. 7±0. 8	<0. 0001
BMI (kg/m2)	21. 11±0. 55	17. 2±0. 21	<0. 0001
Tanner stage (breasts)	4. 66±0. 09	4. 59±0. 10	NS
Amenorrhea duration (y)	-	0. 935±0. 123	-
Exercise Activity (h)	16. 2±2. 2	17. 0±1. 9	NS
Calcium intake (mg)	1096±102	2055±139	<0. 0001
Vitamin D intake (IU)	174±31	570±42	<0. 0001
DXA Measures			
Fat mass (kg)	15. 1±0. 8	8. 8±0. 4	<0. 0001
Lean mass (kg)	41. 78±0. 81	37. 31±0. 69	0. 0003
Lumbar BMD (g/cm2)	0. 944±0. 014	0. 833±0. 010	<0. 0001
Lumbar BMD Z-score	−0. 14±0. 11	−1. 37±0. 09	<0. 0001
Biochemical Parameters			
25(OH) vitamin D (ng/ml)	22. 2±1. 1	30. 2±1. 4	<0. 0001
PTH (pg/ml)	9. 6±2. 1	21. 3±2. 0	<0. 0001
Estradiol (pg/ml)	43. 1±2. 0	29. 3±2. 4	<0. 0001
24-hr UFC (mcg)	38. 7±2. 3	65. 1±6. 6	0. 003
IGF-1 (ng/ml)	366±20	227±13	<0. 0001
Leptin (ng/ml)	11. 2±1. 1	5. 0±0. 6	<0. 0001
P1NP (ng/ml)	194. 8±23. 6	97. 2±11. 2	<0. 0001
CTX (ng/ml)	1. 04±0. 07	0. 73±0. 06	0. 002
Sclerostin (ng/ml)	0. 408±0. 018	0. 422±0. 016	0. 70

NS: not significant

Table 2

Correlations Between Serum Sclerostin and Additional Parameters in Normal-Weight Controls and Adolescent Girls with Anorexia Nervosa

	Sclerostin (Controls, n=25)	Sclerostin (AN, n=44)
P1NP	0. 67*	−0. 17
CTX	0. 53*	0. 07
PTH	−0. 001	−0. 20
Estradiol	0. 09	−0. 28**
IGF-1	0. 004	0. 01
25(OH)D	0. 18	0. 27**
24-hr UFC	−0. 08	−0. 11
Leptin	−0. 04	−0. 08
Bone age	−0. 50*	−0. 10
Chronological age	−0. 37**	−0. 11

*denotes $p < 0.05$

**denotes $p \leq 0.1$

Table 3

Baseline Comparison of Untreated AN (E−) versus Treated AN (E+)

	E−(n=9)	E+(n=13)	p (E− vs. E+)
Age (y)	16. 8±0. 4	17. 2±0. 3	NS
Bone age (y)	16. 0±0. 3	16. 7±0. 2	NS
Weight (kg)	45. 2±2. 3	47. 5±1. 3	NS
BMI (kg/m2)	16. 7±0. 4	17. 4±0. 4	NS
Tanner stage (breasts)	4. 66±0. 23	4. 69±0. 13	NS
Amenorrhea duration (y)	0. 84±0. 13	0. 90±0. 19	NS
Exercise Activity (h)	16. 5±3. 3	15. 4±3. 1	NS
Calcium intake (mg)	2033±312	1756±231	NS
Vitamin D intake (IU)	622±93	460±78	NS
DXA Measures			
Fat mass (kg)	6. 8±0. 8	9. 2±0. 8	NS
Lean mass (kg)	37. 3±2. 1	37. 7±1. 0	NS
Lumbar BMD (g/cm2)	0. 821±0. 028	0. 847±0. 020	NS
Lumbar BMD Z-score	−1. 53±0. 27	−1. 33±0. 20	NS
Biochemical Parameters			
25(OH) vitamin D (ng/ml)	35. 6±4. 1	27. 7±2. 2	NS
PTH (pg/ml)	17. 8±3. 5	30. 4±4. 4	NS
Estradiol (pg/ml)	24. 3±3. 6	34. 3±5. 1	NS
24-hr UFC (mcg)	50. 4±15. 2	54. 5±10. 3	NS
IGF-1 (ng/ml)	259±37	219±21	NS
Leptin (ng/ml)	3. 0±1. 1	4. 9±0. 7	NS
P1NP (ng/ml)	107. 7±34. 6	90. 8±13. 5	NS
CTX (ng/ml)	0. 99±0. 09	0. 62±0. 08	0. 02
Sclerostin (ng/ml)	0. 483±0. 040	0. 427±0. 030	0. 27

NS: not significant

Table 4

Changes in BMD Measures, Body Composition, Bone Turnover Makers, and Sclerostin in Untreated (AN E−) and Treated (AN E+), Baseline vs. 12 months

	AN E− (n=9)	AN E+(n=13)	p (AN E− vs. AN E +)	p*
Δ CTX (ng/ml)	−0. 075±0. 181	−0. 115±0. 081	NS	NS
Δ P1NP (ng/ml)	−26. 9±61. 8	−8. 4±19. 5	NS	NS
Δ Sclerostin (ng/ml)	−0. 128±0. 042	−0. 101±0. 031	NS	NS
Δ LBMD	0. 005±0. 012	0. 035±0. 007	0. 02	0. 01
Δ % LBMD	−0. 46±1. 5	4. 38±1. 02	0. 02	0. 02
Δ LBMD Z-score	−0. 19±0. 10	0. 21±0. 08	0. 01	0. 01
Δ Weight (kg)	4. 4±1. 3	3. 5±1. 7	NS	
Δ BMI (kg/m2)	1. 52±0. 52	1. 10±0. 67	NS	
Δ Fat mass (kg)	3. 3±1. 2	1. 6±1. 3	NS	
Δ Lean mass (kg)	1. 9±0. 6	0. 8±0. 8	NS	

NS: not significant

p*: controlled for baseline age and weight change