

PERSPECTIVES

Skeletal Abnormalities in Anorexia Nervosa

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Abstract

Anorexia nervosa (AN) is a condition of self-imposed starvation associated with multiple endocrine abnormalities that contribute to low bone density in both males and females. Hormonal alterations in AN include a state of acquired growth hormone (GH) resistance with low IGF-1 levels, hypogonadism, relative hypercortisolemia, elevations in levels of ghrelin, peptide YY and adiponectin, and reductions in leptin. Weight gain associated with menstrual recovery leads to stabilization of bone density, whereas persistence of low weight and amenorrhea causes further decreases in bone density. Low bone density in AN is refractory to oral estrogen replacement, although a combination of oral estrogen with rhIGF-1 causes an increase in bone density in adult women with AN. In addition to low bone density, alterations in bone microarchitecture have been reported in AN, with reductions in apparent bone trabecular volume, trabecular thickness and number, and an increase in trabecular separation. AN may also impact stature adversely, particularly when it begins before epiphyseal fusion and is long-standing. *IBMS BoneKEy*. 2010 Feb;7(2):63-83.

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Introduction

Anorexia nervosa (AN) is a condition characterized by chronic, severe, self-induced malnutrition, resulting in hypogonadotropic hypogonadism, an acquired state of growth hormone resistance, relative hypercortisolemia, and dysregulation of various appetite-regulating hormones and adipocytokines, all of which have critical negative effects on skeletal maturation, growth and bone mass accrual. The lifetime prevalence of AN, as defined by the DSM-VI, is reported to be as high as 2.2% in women (1). It occurs in 0.2-1% of adolescent girls and 1-4% of college-age young women (2). Although AN affects females predominantly, up to 10% of cases are seen in males, and the incidence of

males with this condition appears to be increasing in recent years (3-5). While many of the clinical consequences of AN appear to be reversible with recovery, this may not hold true for its impact on the skeleton. In this *Perspective*, we will review the skeletal consequences resulting from hormonal changes of AN in both girls and boys.

Skeletal Events in the Adolescent Years

Hormonal alterations specific to the adolescent period are critical for stimulating both bone mass accrual (6) and the pubertal growth spurt (7-9). These events are essential in establishing optimal peak bone mass and in optimizing final adult stature. Both are a consequence of rising levels of gonadal steroids in early puberty, and

increases in growth hormone (GH) and insulin-like growth factor-I (IGF-I), which follow suit. Maximal increases in bone mass occur between 11 and 14 years in girls and 13 and 16 years in boys (10). Almost 25% of peak bone mass is formed in the 2 years surrounding peak height velocity, and more than 95% is achieved by the time an individual is 18 years old (11). Low bone mineral density (BMD) occurring at this time of life is of immense concern given the narrow window within which increases in bone mass occur, and because complete catch-up may not be possible within this window even with weight recovery, resulting in residual deficits in bone mass that persist

into adult life (12). Coincidentally, eating disorders have a bimodal peak of onset, first at 14 years, and then again at around 18 years (13); this timing can lead to a profound and lifelong impact on bone health and stature.

Hormonal Determinants of Adverse Skeletal Outcomes in Anorexia Nervosa

Growth Hormone Resistance and Low Insulin-like Growth Factor I

AN results in a nutritionally-acquired resistance to GH with impressively low levels of IGF-1 (14) (Fig. 1).

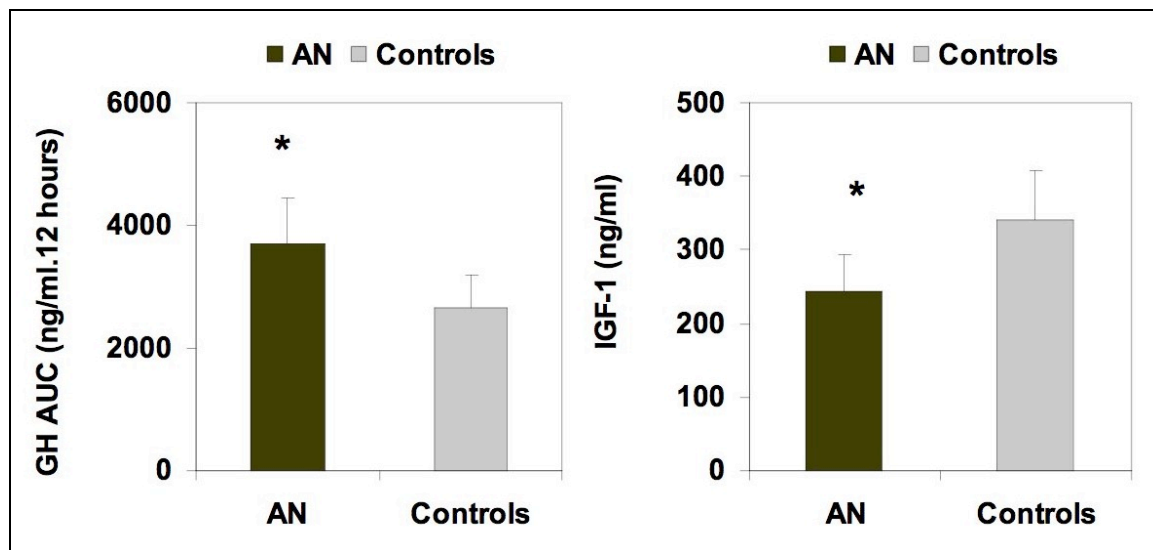


Fig. 1. Growth hormone (GH) and insulin-like growth factor-I (IGF-1) levels in adolescent girls with anorexia nervosa (AN) and controls. AUC for GH measured in serum over 12 hours was significantly higher in girls with AN than in controls, whereas measurements of IGF-1 were significantly lower in AN (* $p < 0.05$). (Adapted with permission from Misra *et al. J Clin Endocrinol Metab.* 2003 Dec;88(12):5615-23. Copyright Endocrine Society 2003).

GH has direct stimulatory effects on the differentiation and proliferation of osteoblast precursors, and also acts indirectly through IGF-1 to stimulate the differentiation of these precursors (15;16). Since both are bone anabolic factors that rise in early puberty and peak in mid-puberty, low IGF-1 levels coupled with GH resistance appear to contribute to the low bone formation rates observed in AN, as indicated by low levels of bone formation markers. GH resistance occurs both at the level of the liver and bone, and the latter is suggested by a lack of association between GH concentrations

and levels of bone formation markers in girls with AN, whereas strong positive associations are observed in healthy controls (14). Some studies (17), but not all (18), have reported an adverse impact on stature in adolescents with AN, consistent with low IGF-1 levels and resistance to GH. However, an effect on stature is also dependent on the duration and severity of AN. Therefore, timing of AN onset within the adolescent period and in relation to pubertal onset, progression and the growth spurt has specific and differential consequences on both immediate and long-term skeletal

outcomes.

Hypogonadotropic Hypogonadism

AN is characterized by hypothalamic amenorrhea and consequent hypoestrogenic (19;20) and hypoandrogenic states (20;21). Most studies indicate an inverse association between bone density measures and duration of amenorrhea (22-25), and low estradiol levels in AN are predicted by the severity of nutritional status (19). In adolescence, estrogen increases bone mass accrual by two core mechanisms: first, progressive elevation of estrogen in early puberty stimulates increases in GH and IGF-1, which have longitudinal bone growth effects and increase periosteal bone apposition (16), and second, surges of estrogen levels in late adolescence have an osteoclast antiresorptive effect, especially at endosteal surfaces. The antiresorptive effects of estrogen on bone are mediated by the osteoprotegerin (OPG)-RANK-RANKL system and by inhibition of various proinflammatory cytokines that otherwise stimulate osteoclast differentiation and activation (26). Estrogen stimulates secretion of OPG, the soluble decoy receptor of RANK, which competes with RANKL for binding to RANK and inhibits osteoclastic activity. Interestingly, although estrogen levels are low in AN, OPG levels are high (27), likely an adaptive mechanism given the low bone density state. However, proinflammatory cytokines are elevated in adolescent girls with AN (28), and may contribute to the impaired state of bone metabolism.

In addition, androgen levels are depressed in girls (12;29-32) and boys with AN (33), and likely contribute to low BMD. Studies in adolescents (20) and adults (21) with AN have demonstrated that low testosterone levels predict low BMD and low lean mass, and the latter is a particularly important determinant of bone density measures in this population. Data regarding adrenal androgens, however, are contradictory, with some studies reporting reduced dehydroepiandrosterone sulfate (DHEAS) levels in young women with AN, which

correlate inversely with markers of bone turnover (34), whereas other studies have not been able to demonstrate a difference in DHEAS levels in adult women (30) and adolescents (20) with AN compared with controls. It should be acknowledged that hypogonadism likely acts synergistically with other deficient elements to impact BMD, as administration of oral estrogen alone, for example, does not improve BMD in adult women or in adolescents with AN (35;36). Of note, the issue of adherence to oral estrogen and compliance with the recommended regime has not been addressed in some of these studies.

Hypercortisolemia

A relative state of hypercortisolemia (Fig. 2), as evidenced by elevated urinary and serum cortisol levels compared with controls, has been described repeatedly in both adult women (37-43) and girls with AN (44), likely a consequence of both increased cortisol secretory burst frequency and decreased clearance (44;45). Cortisol suppresses bone formation by inhibiting the replication, differentiation and function of osteoblasts through various mechanisms, and by inducing apoptosis of mature osteoblasts and osteocytes (46-48). Cortisol potentiates osteoclast function, and also inhibits both renal tubular calcium reabsorption and calcium absorption from the GI tract (49). Furthermore, glucocorticoids may directly decrease the secretion of GH (50) and hence lower IGF-1, thus having further detrimental effects. Excess cortisol, both from exogenous and endogenous sources, has been consistently linked with low BMD (51-57), and an inverse association between BMD and cortisol levels has been reported amongst adults with AN (21). Furthermore, an inverse association has clearly been demonstrated between markers of bone formation, such as osteocalcin, and cortisol in adults (57) and adolescents (44) with AN.

Neuroendocrine Gastrointestinal-Derived Peptides and Adipocytokines

Several peptide hormones have recently been linked to bone health in AN. These include neuroendocrine gastrointestinal-

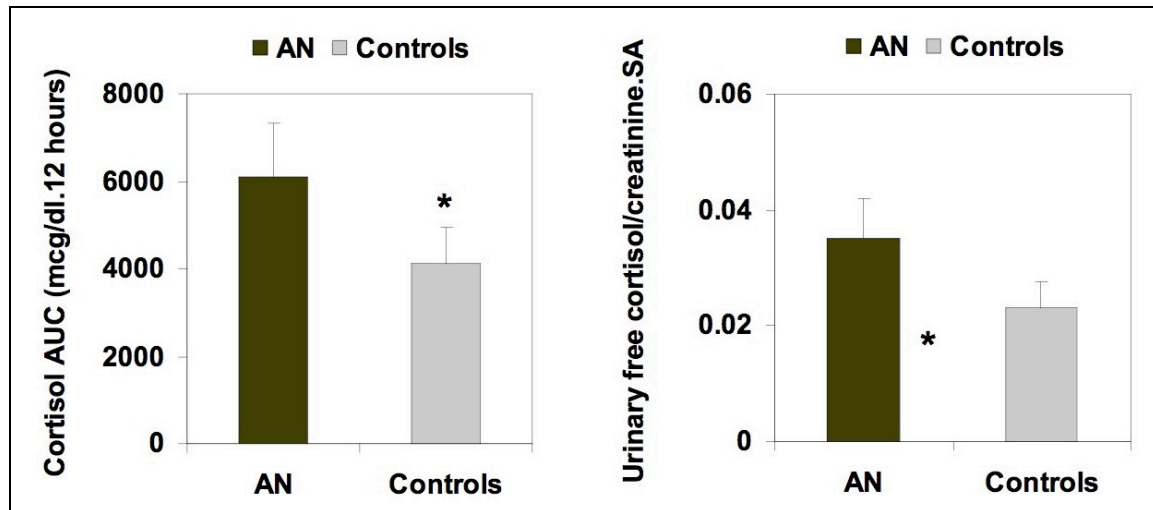


Fig. 2. Cortisol levels in adolescent girls with anorexia nervosa (AN) and controls. AUC for cortisol measured in serum over 12 hours was significantly higher in girls with AN than in controls, as were measurements of urinary free cortisol corrected for creatinine and surface area (* $p < 0.05$). (Adapted with permission from Misra *et al. J Clin Endocrinol Metab.* 2004 Oct;89(10):4972-80. Copyright Endocrine Society 2004).

derived peptides regulating food intake and certain adipocytokines.

Ghrelin

AN is associated with increased secretion of ghrelin, a gastric-derived orexigenic peptide, which is a GH secretagogue (58;59), stimulates osteoblast proliferation *in vitro* (60), and is expressed in cartilage (61) and osteoblasts. Elevated ghrelin levels in girls with AN predict high GH and cortisol burst frequency (58). Importantly, as described above, GH resistance and hypercortisolemia both negatively affect bone. Interestingly, in a follow-up study, ghrelin was demonstrated to strongly positively predict BMD in healthy girls, but not in girls with AN (62), suggesting that AN may confer a state of ghrelin resistance.

Leptin and Peptide YY (PYY)

The role of the leptin network and other hypothalamic hormones in regulating eating behaviors has received significant attention over the last decade. However, the potential additional function of this system in the central control of BMD is a relatively new area of focus. Leptin is an anorexigenic adipocytokine that is depressed in AN (63;64). Leptin-deficient and leptin-resistant mice are obese and hypogonadal and yet

have high trabecular (but low cortical) BMD (65;66). Furthermore, administration of leptin to leptin-deficient mice leads to a decrease in trabecular, but an increase in cortical, BMD, suggesting a negative impact of leptin on trabecular bone, and a positive impact on cortical bone. Takeda *et al.* demonstrated that leptin regulates bone formation via the sympathetic nervous system (67), whereby a β -adrenergic agonist decreases bone mass in leptin-deficient and wild-type mice, while a β -adrenergic antagonist increases bone mass in wild-type and ovariectomized mice without affecting body weight. In contrast to animal studies, a positive association between leptin and BMD has been reported in humans even at sites of trabecular bone (68). The relationship between leptin and bone metabolism in AN remains to be clarified.

Both leptin and peptide YY (PYY) are peripherally-released molecules that serve as long-term and short-term signals, respectively, of the energy-replete state (69;70). Their effects on the central nervous system are to decrease subsequent caloric intake. PYY is an intestinally-derived anorexigen that acts via the Y2 neuropeptide Y (NPY) receptor to decrease NPY secretion and inhibit caloric intake (71). The downstream mechanism connecting Y receptor activation to decreased BMD is yet

to be well-elucidated. However, it has been shown that Y2 receptor knockout mice have a two-fold increase in trabecular bone volume at the distal femoral metaphysis with increased trabecular number and thickness. Furthermore, osteoclast surface was not affected in these animals, but osteoclast number was reduced. Osteoblast surface and number, osteoid surface, and mineralizing surface were all unaffected but rates of mineral apposition and bone formation were increased. As there is no detectable Y receptor expression in bone, this was interpreted to represent an effect mediated within the central nervous system. These data suggest that signaling through the Y2 receptor suppresses bone formation, and more specifically trabecular bone development (71). In humans, PYY has been demonstrated to negatively correlate with markers of bone formation and resorption (72) and an association between elevated levels of PYY in AN and low BMD has also been reported (73), suggesting that PYY may act as a catabolic signal to bone. With the presumption that leptin and PYY both relay a catabolic signal to bone, the results of their skeletal effects have been examined in human models studying phenotypes at opposite extremes, obesity and AN. Obese individuals have elevated leptin and low PYY levels, whereas individuals with AN have low leptin and elevated PYY levels. Obesity is associated with increased BMD (74) and AN with decreased BMD (75), therefore it appears that if these peptides exert independent effects on bone, PYY may have a dominant effect compared to leptin. Conversely, Wortley *et al.* recently reported a PYY knock-out mouse model with an osteopenic phenotype, including a reduction in trabecular bone mass and a decrease in bone strength (76). These results suggest that elevated PYY levels in AN may reflect resistance at the level of bone to the positive effects of PYY.

Adiponectin

Adiponectin is an adipokine that is known to affect bone metabolism (77-80), and consequently has been examined in the pathogenesis of low BMD in AN. Although

one would expect this fat-dependent hormone to be disturbed in this state of under-nutrition, the specific direction of derangement has been somewhat baffling such that adiponectin levels have been reported as normal in adolescents with AN (81), and both high (82) and low (83;84) in adults with AN. Of note, high adiponectin levels are associated with low BMD in healthy adults (85;86). Adiponectin receptors are expressed both on osteoblasts and osteoclasts (77;79;80;87) and a role for adiponectin in suppressing OPG and increasing expression of RANKL has been described, suggesting that high adiponectin levels may cause increased osteoclastic activity resulting in low BMD (78). Adiponectin, however, is also reported to increase osteoblastic activity, which theoretically should be associated with increased bone formation (79;80;87). Again, this puzzling inconsistency led to a study that aimed to integrate human phenotypes into the equation and determine associations of BMD with adiponectin (81). Interestingly in this study, adiponectin did not differ in AN subjects versus controls, although adiponectin levels 60 minutes after oral glucose did trend higher in the AN group. Despite the lack of difference in adiponectin levels between AN subjects and controls, an inverse and independent association between adiponectin and BMD in AN was established.

Bone Mineral Density in Anorexia Nervosa

AN is complicated by severe bone loss. Osteopenia is present in 92% and osteoporosis in 38% of young women with AN, with less than 15% of women having normal bone density, despite an average age only in the early twenties (75;88). Low bone density in adults with AN is associated with an uncoupling of bone turnover, with low levels of bone formation markers and high levels of bone resorption markers. In women with active AN, bone loss occurs rapidly, at an average annual rate of 2.5% (89), highlighting the importance of early intervention for women with AN. Data regarding skeletal effects of weight recovery in women with AN are inconsistent and not

particularly reassuring; some suggest that weight recovery results in increases in BMD, whereas others are not able to demonstrate significant improvement (25;90-93). Of concern, most studies do agree that some degree of residual bone loss is common even several years after recovery from AN (94;95). Additionally, body weight history appears to be a crucial predictor of both the presence of low bone density as well as of recovery; specifically, patients with a history of a critical body mass index less than $16.4 \pm 0.3 \text{ kg/m}^2$ appear to remain at high-risk for osteoporosis even several years after their recovery (95).

Bone Mineral Density in Adolescent Girls with Anorexia Nervosa

In teenagers with AN, low BMD is associated with a reduced bone turnover state, evidenced by a decrease in both bone formation and bone resorption markers (12). This is in sharp contrast to the vigorous bone turnover state characteristic of early puberty (96). Furthermore, compared to a 1-year follow-up period of continued spine bone mass accrual observed in healthy adolescents, girls with AN have a plateauing of bone density and bone mineral content (12). Teenage girls with AN have been reported to have Z-scores of < -1 at the spine and hip in as many as 50% and 30%, and Z-scores of < -2 at the spine and the hip in 9% and 10% (19). For whole body bone mineral content adjusted for height, 30% of girls with AN had Z-scores of < -1 and 4% had Z-scores of < -2 (19). In this particular study, investigators took note of the fact that areal bone density measurements by dual-energy X-ray absorptiometry (DXA) are affected by stature, leading to a potential underestimation of bone density in very short children. This is of concern given that some studies have reported short stature in AN, raising the possibility that reports of low BMD in AN are at least in part a consequence of shorter stature and impaired radial bone growth (97;98). However, these investigators observed that girls with AN also have lower bone mineral apparent density (BMAD), a surrogate measure for volumetric bone density that

adjusts for stature (98), and were not short, indicating that lower bone density observed in their cohort was not a consequence of short stature and consequently short bones (19).

With weight gain, there is a significant increase in markers of both bone formation and bone resorption in adolescent girls with AN, suggesting the potential to revert to a more physiological and pubescent state of increased bone turnover. Additionally, an increase in bone formation markers in the first 6 months after weight gain is predictive of increases in bone mineral content in the subsequent 6 months (12). In girls with AN who gained 10% of their BMI and resumed menses, modest increases in bone density and BMAD at the spine and the whole body were observed over a 1-year follow-up period (99), and these data suggest that sustained recovery of menses and weight should lead to a significant increase in bone mass and BMD. In contrast, girls not gaining weight are expected to have continued BMD and BMAD loss with progressive and significant decreases in their Z-scores over time (Fig. 3). Therefore, weight gain and resumption of menses are to be strongly encouraged since even modest improvements are clearly preferable to a continued detriment of bone health with persistence in underweight.

Long-term Bone Mineral Density Deficits in Women with a History of Anorexia Nervosa

Although bone mass accrual may improve with weight gain and menstrual recovery, significant residual deficits certainly persist into adulthood. In a study of adult women with AN, Biller *et al.* reported that women with an onset of amenorrhea before the age of 18 years had lower bone density than those who developed amenorrhea after 18 years, even after controlling for duration of amenorrhea (24). In another study of 19 women with teenage-onset AN, bone density at the femur, although not at the spine, was significantly lower than in controls even after full recovery from AN 14-23 years later (mean 21 years) (100). These investigations further emphasize that

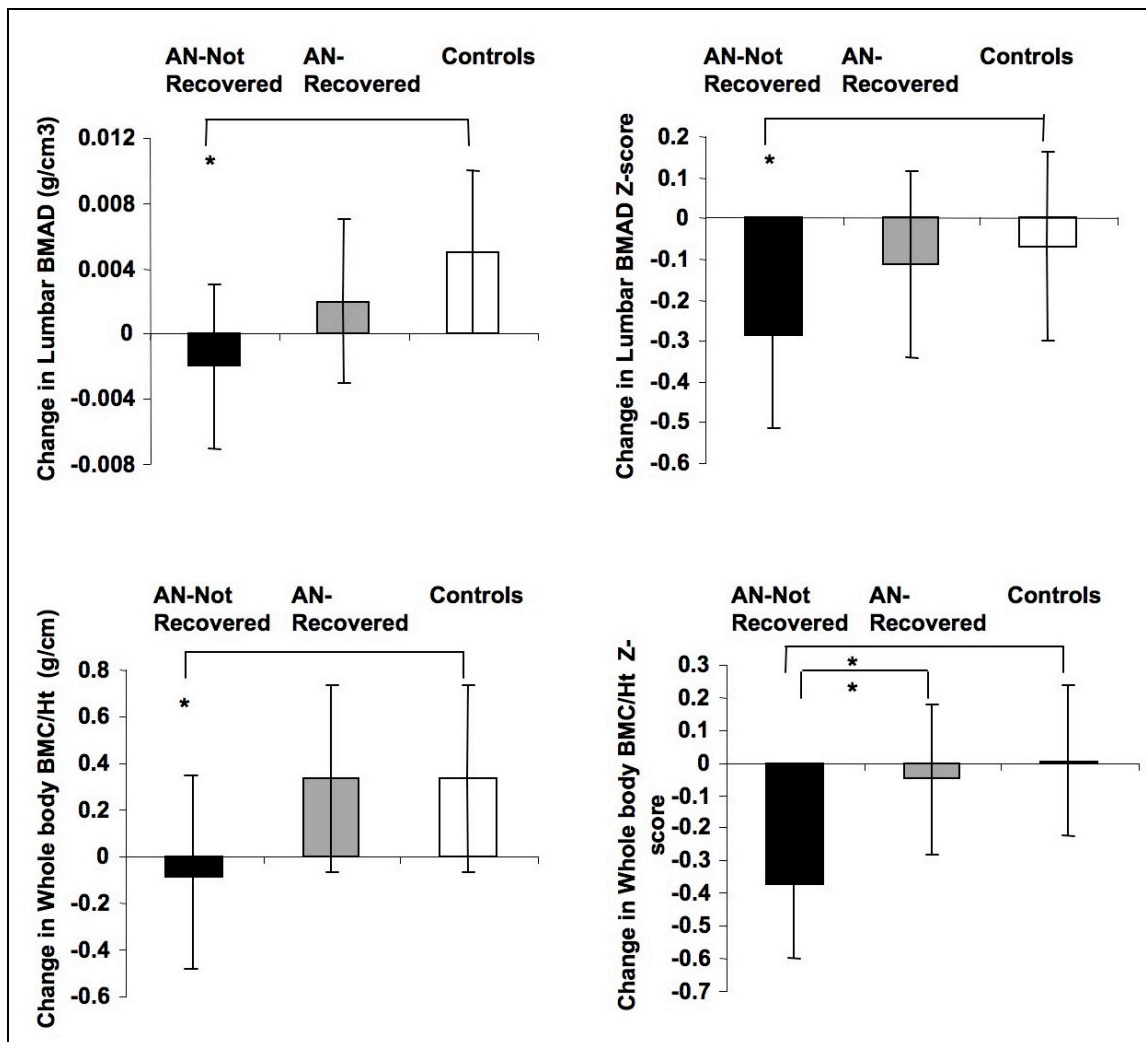


Fig. 3. Change in lumbar bone mineral apparent density (BMAD) and whole body (WB) BMC/Ht measures in anorexia nervosa (AN)-not recovered (black bar), AN-recovered (gray bar), and healthy adolescents (white bar) (Reproduced with permission from Misra *et al.* *J Clin Endocrinol Metab.* 2008 Apr;93(4):1231-7. Copyright Endocrine Society 2008).

adolescence serves as a critical period for establishing life-long bone health.

Most studies indicate an inverse association between BMD measures and duration of amenorrhea (23-25;101) as well as duration of illness (94) with few obvious or hopeful prognostic indicators of BMD recovery. For example, in a 25-month follow-up study of women aged 19-37 years (mean age 26 years) with a 1-17-year history of amenorrhea (mean 5.8 years), some of whom developed AN in the adolescent years, there was no significant difference in the mean change in bone density between women who attained greater than 80% of

ideal weight, resumed menses, took estrogen or calcium, or who exercised vigorously (100).

The primary import of BMD is to predict future fracture risk. However, there are only a few studies that have examined risk of fracture in the follow-up of women with AN. Interestingly, Wentz *et al.* (102) reported similar rates of fracture in women with AN versus controls after 11 years of onset of AN (4/36 AN and 5/43 controls). These authors, however, did not describe the nature of fractures observed, and their AN group was distinguishable from most reported in the literature in that the women in this study did

not differ in BMD from controls (102). In contrast, Rigotti *et al.* reported a 7.1-fold greater risk of non-spine fractures in women with AN during follow-up compared with normal women in the same age range (100). Analogously, Lucas *et al.* reported a 57% cumulative incidence of fractures at the hip, spine, and radius in women with AN 40 years after diagnosis of their eating disorder and a standardized incidence ratio of fractures of 2.9 compared to a population of healthy women (95% confidence interval, 2.0-3.9) (103). These later data suggest significant fracture risk resulting from AN.

Bone Mineral Density in Adolescent Boys with Anorexia Nervosa

Although AN is primarily a disease of females, it is increasingly being recognized in males as well. Regardless, there are only a few controlled studies investigating BMD, bone turnover markers, or their predictors in

adolescent boys with AN. In one uncontrolled study (104), which did not examine bone turnover markers, boys with the lowest BMD had the longest duration of illness, and the lowest physical activity and calcium intake. A recent study examined absolute and height-adjusted measures of BMD and levels of bone turnover markers in adolescent boys with AN; this cohort was found to have lower BMD and corresponding Z-scores at the spine, hip, femoral neck, trochanter, intertrochanteric region, and whole body, compared with controls (Fig. 4) (33). Height-adjusted measures (lumbar BMAD and whole body bone mineral content/height) were lower and bone formation and resorption markers were reduced as well, indicating decreased bone turnover. IGF-I was shown to be an important predictor of bone turnover markers, and, as expected, testosterone levels and lean mass predicted BMD (33).

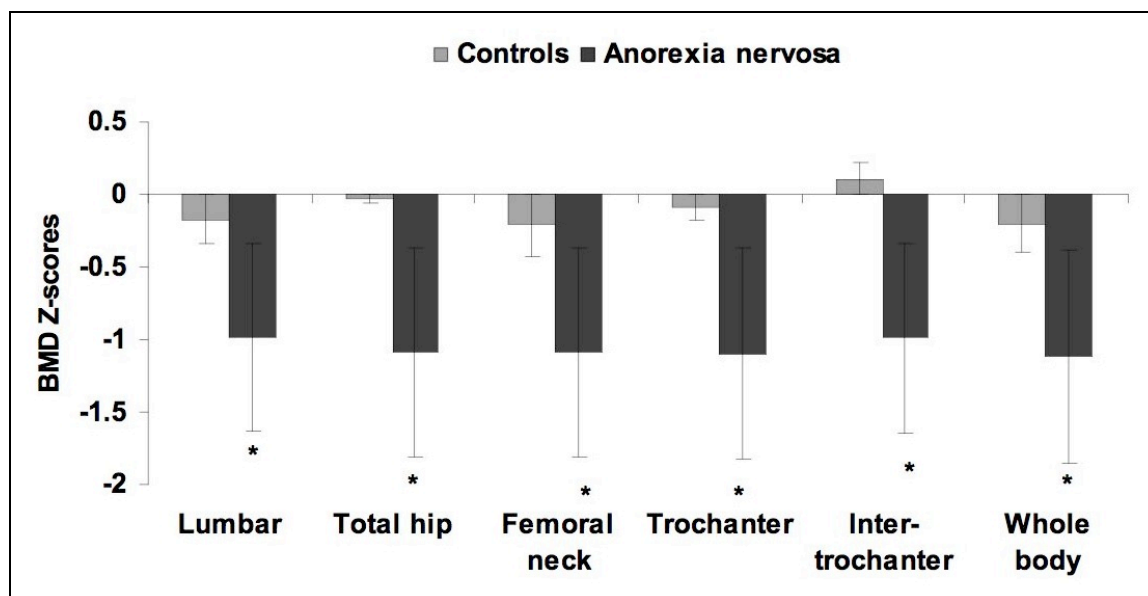


Fig. 4. Bone density Z-scores in adolescent boys with anorexia nervosa and controls. Bone density Z-scores at the lumbar spine, total hip, and its subregions (femoral neck, trochanter, intertrochanteric region) and whole body was significantly lower in boys with AN than in controls. (* $P < 0.05$) (Reproduced with permission from Misra *et al.* *J Clin Endocrinol Metab.* 2008 Aug;93(8):3029-36. Copyright Endocrine Society 2008).

Long-term Bone Mineral Density Deficits in Men with a History of Anorexia Nervosa

Analogous to AN, age-onset osteoporosis

has traditionally been considered a disease of women, however, men also incur substantial bone loss with aging and experience vertebral fractures at approximately one-third the rate of women

(105). The pattern of bone loss in men is distinctly different from that seen in females. In females, BMD begins to decline slowly after the age of 40 until the age of 55, when the loss of bone density accelerates. The pattern among males is a slower decline after the age of 40 such that only after age 60 does their BMD decrease to levels almost equivalent to females of equal age (106). The lower prevalence of osteoporosis in men is a consequence of greater accumulation of skeletal bone mass during the adolescent and young adult years, greater bone size, and the absence of a distinct equivalent of menopause. However, recognition of the significant vulnerability older men still have to low BMD is an important impetus to investigate long-term effects of AN in adult men.

There are few data reflecting long-term changes in bone density in men with teenage-onset AN. One study reported lower bone density in three men with AN compared with controls after 11 years of AN recovery; this difference was significant for the whole body, but not for the spine or hip (102). However, the small sample size makes globalization of these findings challenging. In reaction to scant investigations addressing long term osteoporosis risk in men with AN, Mehler *et al.* (107) studied severity of bone loss in male patients with eating disorders, compared results to females with AN, and identified factors that contribute to low BMD in these men. They demonstrated a strikingly high prevalence of low BMD in male patients with a history of AN. Moreover, the severity of deficiency was greater than in females with the same eating disorder, especially for those with a very low BMI and longer duration of illness. Their report strongly implies that osteoporosis in adult men with AN is likely under-reported and/or under-investigated, and serves to heighten awareness of the risk for osteoporosis in male patients with eating disorders.

Alterations in Bone Microarchitecture in Anorexia Nervosa

Alterations in bone microarchitecture in AN may explain fracture risk independent of BMD. Advances in CT imaging allow for noninvasive evaluation of trabecular microstructure at peripheral sites *in vivo*. Lawson *et al.* recently performed a cross-sectional study of 23 women (12 with AN and 11 healthy controls) to determine hormonal predictors of trabecular bone microarchitecture (108). Bone microarchitectural measures, including apparent (app.) bone volume fraction, app. trabecular thickness, and app. trabecular number, were reduced and app. trabecular spacing was increased in AN versus controls, consistent with other studies (109;110). Moreover, decreased structural integrity at the ultradistal radius was associated with decreased BMD at all sites except the total hip. IGF-I, leptin, testosterone, and free testosterone levels predicted greater structural integrity based on all microarchitectural parameters measured. The investigators concluded that bone microarchitecture is abnormal in women with AN and that endogenous IGF-I, leptin, and androgen levels predict disrupted bone microarchitecture independent of BMI (108). While techniques used in this study require further validation, they may prove crucial in revealing additional information about bone fragility and fracture risk in AN. A similar study has been performed in adolescents with AN. As in adults, adolescents with AN had lower bone trabecular volume and trabecular thickness, and greater trabecular separation than controls (111). Of concern, these changes were observed despite the fact that DXA measures of bone density did not differ between girls with AN and normal weight controls. These data suggest that changes in bone microarchitecture may occur even before changes in bone density become evident.

Therapeutic Strategies to Increase Bone Density in Anorexia Nervosa

Weight gain and resumption of menses

Several studies show that BMD is not significantly improved by weight gain in AN (20;99). However, Soyka *et al.* showed that low levels of bone turnover markers, present in girls with AN at baseline, did significantly increase with improvement in nutritional status (20). Furthermore, increases in surrogate markers of bone turnover correlated with an improvement in lumbar and total BMC and BMD in their AN group. Mika *et al.* reported corroborating data and demonstrated that weight rehabilitation in a 2-year period following an inpatient feeding program led to restoration of bone formation activity in adolescents with AN, despite a lack of increase in BMD (112). Interestingly, Iketani *et al.* demonstrated specific benefit to spine BMD with weight gain, although not to the level of that in controls (113). Not surprisingly, BMD further increased with resumption of menses. A recent study (99) demonstrated that even short-term weight gain with menstrual recovery is associated with stabilization of BMD measures in adolescent girls with AN. Moreover, even in the absence of menstrual recovery, weight gain seemed to have some positive effect on whole body parameters, albeit to a lesser extent than that noted associated with menstrual recovery (Fig. 3). Importantly, in this study there was no improvement in bone parameters at the spine solely with weight gain, restating the significance of gonadal steroids in optimizing trabecular BMD (99).

Estrogen Replacement

Hypothalamic hypogonadism is a synergistic rather than sole element in the etiology of low BMD in AN, and multiple studies have shown that estrogen replacement alone does not significantly improve BMD in this condition, despite its anti-resorptive effects (35;36;114;115). In fact, a study by Miller *et al.* demonstrated that despite findings indicating that resumption of menstrual function was important for improvement of spine BMD and weight gain critical for

improvement in hip BMD, no increase in BMD was observed in women with AN receiving oral contraceptive pills (OCPs) even with a mean weight gain of 11.7% (89). This result is speculatively attributed to the IGF-1- and androgen-suppressive effects of high doses of estrogen in OCPs (116-118). Conversely, transdermal estrogen preparations appear to be less IGF-1-suppressive (117), and their role in treating low BMD in AN remains to be determined. These results call into question the standard clinical practice of prescribing OCPs to adolescents and women with AN.

Recombinant Human Growth Hormone (rhGH) and Insulin-like Growth Factor-1 (rhIGF-1)

Although, hypoestrogenemia is an important contributing factor to low BMD in AN (119), the lack of improvement in BMD with estrogen-containing OCPs (36;120) suggests that correction of other nutritionally dependent factors, independent of, or in addition to, estrogen are necessary to improve BMD in AN. As discussed earlier, AN is associated with an acquired resistance to GH and consequent low levels of IGF-1 (14), a nutritionally dependent bone trophic hormone that stimulates osteoblast function and collagen formation (16). In other conditions of GH resistance, such as liver disease or renal failure, supraphysiologic doses of (rh)GH have been utilized therapeutically to overcome this resistance (121;122); it would thus follow that similar interventions may be effective in increasing BMD in AN. In a recent study, Hashizume *et al.* reported that administration of high doses of (rh)GH to AN subjects caused an increase in IGF-1 levels (123). A confounder of this study was that an increase in BMI was also observed, which could independently result in increased IGF-1 levels. In contrast, IGF-1 acts downstream of GH, and investigators have also examined the impact of (rh)IGF-1 administration on bone formation markers in adult women with AN. In this study, (rh)IGF-1 administration led to a normalization of the low levels of IGF-1 and caused a significant increase in markers of bone turnover (124).

The impact of (rh)IGF-1 administration on bone formation in children with AN had not been studied until most recently. Bone accretion in children is a physiologically different state from the maintenance of bone mass in adults, with the former being a high formation and resorption state (96) leading to a net increase in bone mass, while in the latter, similar rates of bone formation and resorption result in no net changes in bone mass. IGF-1 is an important determinant of the pubertal increase in bone mass (125), therefore, effects of (rh)IGF-1 may differ in adolescent AN girls from those observed in adults with this disorder. Additionally, the safety of rhIGF-1 administration in adolescents with this disorder was unknown. To investigate the hypothesis that subcutaneous (rh)IGF-1 administration in adolescents with AN would stimulate bone formation, investigators examined responses of surrogate markers of bone formation, N-terminal propeptide of type 1 procollagen (PINP), and of bone resorption, C-telopeptide (CTX), to short-term (rh)IGF-1 administration (7-9-day period) in 10 consecutive adolescent girls with AN. They compared results to those in 10 age-matched girls with AN who did not receive (rh)IGF-1 and found that this intervention, when given in a dose of 30-40 mcg/kg twice daily, successfully increased IGF-1 levels to a high normal range and was associated with significant selective increases in levels of PINP without a concomitant increase in CTX (126). This study revealed that the effects of (rh)IGF-1 were immediate and well-tolerated, and the associated increase in bone formation markers suggests that there may be a role for this medication as adjunctive therapy for low BMD in adolescents with AN.

It is notable that when (rh)IGF-1 is given in conjunction with OCPs, the result is a small but significant increase in BMD in adult women with AN (114). This is likely because of the combination of the anabolic effects of (rh)IGF-1 and the anti-resorptive effects of estrogen. This combination has not been studied in adolescent girls with AN.

Bisphosphonates

Thus far, only two studies have been published examining the role of bisphosphonates in treating low BMD in AN. Bisphosphonate administration results in decreased osteoclast bone resorption and is an effective and commonly implemented treatment for post-menopausal osteoporosis. In a double-blind, randomized, placebo-controlled study in adolescents with AN, Golden *et al.* demonstrated that alendronate treatment resulted in a modest within-group increase in both spine and femoral neck BMD. However, BMD improvement in this group was primarily determined by weight restoration, and increases were not statistically significant when compared with their placebo group (127). In contrast, in an uncontrolled study of adults with AN receiving risedronate, Miller *et al.* showed a decrease in bone resorption with an increase in BMD, even without significant weight gain (128). It is crucial to note that at this time there is a lack of safety data regarding use of bisphosphonates in woman of reproductive age, and bisphosphonates are not FDA-approved in the U.S. for premenopausal women (other than for those receiving glucocorticoids). Therefore, these medications should remain confined to the research arena until more safety and efficacy data are available, particularly in adolescents.

Effects of Anorexia Nervosa on Adult Stature in Boys and Girls

The onset and duration of AN in relation to the adolescent pubertal growth spurt, achievement of peak height velocity and epiphyseal closure, determine adult height and also may, at least partially, explain the discriminate outcome in girls versus boys. Diminished stature in comparison with target height, both in boys and girls, has been reported in earlier studies (129-132), however, more recent studies do not fully corroborate these data (18). In fact, one study even indicated greater than expected height in girls with AN (133).

The hypogonadal state, associated with AN, causes a delay in bone age (BA), allowing

for a longer duration available for growth, and may offset deficits incurred by low IGF-I levels, as long as duration of illness is not prolonged (18). Therefore, a delay in BA may contribute to preservation of height potential in some adolescents with AN. Recent data also indicate that in girls with AN whose BA is <15 years, an inverse association exists between change in height standard deviation scores (SDS) over a 1-year period and the delay in BA in relation to chronological age. These data suggest that girls with AN who have a delay in BA are more likely to catch up for height SDS than those without delay. It should also be noted that other important determinants of height measures in girls with AN in this study were duration of illness and severity of growth deficits before weight rehabilitation (18). Despite the robust power of this study, Lantzouni *et al.* reported contradictory data, and showed that following nutritional rehabilitation in their cohort of girls with AN, an acceleration in growth velocity was not sufficient to prevent statural deficits (134). This discrepancy in outcome may reflect greater severity of illness in subjects in the latter study.

Because the pubertal growth spurt occurs later in boys than in girls (8;9), boys with onset of AN in the teenage years may be at greater risk for short stature than girls with this disorder. The pubertal growth spurt begins approximately 2 years later in boys than in girls, and peak height velocity occurs at Tanner stage 4 in boys versus Tanner stage 3 in girls (7-9). Furthermore, growth is almost complete at a BA of 15 years in girls versus 17 years in boys (135). Girls may thus be close to their target height at the time of onset of AN, whereas significant growth potential may exist in boys who develop AN at the same time. Correspondingly, Modan-Moses *et al.* recently reported significant short stature in boys with onset of AN in the adolescent years, and although weight restoration was associated with some catch-up growth in this study, complete catch-up did not occur (17).

Conclusion

AN, a condition of severe self-induced under-nutrition, is associated with low BMD, abnormal bone microarchitecture, and possibly statural deficits in men and in women, particularly when the disorder begins during the crucial adolescent years. Improvement in skeletal parameters may occur with weight and menstrual recovery, however, residual bone deficits typically persist. As the pathophysiology of hormonal alteration and bone loss in AN continues to be elucidated, therapeutic strategies to optimize bone mass accrual in adolescents and bone density in adults should become available.

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