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Bone metabolism in anorexia nervosa and hypothalamic amenorrhea

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ABSTRACT

Anorexia nervosa (AN) and hypothalamic amenorrhea (HA) are states of chronic energy deprivation associated with severely compromised bone health. Poor bone accrual during adolescence followed by increased bone loss results in lifelong low bone density, degraded bone architecture, and higher risk of fractures, despite recovery from AN/HA. Amenorrhea is only one of several compensatory responses to the negative energy balance. Other hypothalamic-pituitary hormones are affected and contribute to bone deficits, including activation of hypothalamic-pituitary-adrenal axis and growth hormone resistance. Adipokines, particularly leptin, provide information on fat/energy stores, and gut hormones play a role in the regulation of appetite and food intake. Alterations in all these hormones influence bone metabolism. Restricted in scope, current pharmacologic approaches to improve bone health have had overall limited success.

1. Introduction

Anorexia nervosa (AN) is a relatively rare psychiatric disorder of polygenic etiology with many genetic variants, including psychiatric, educational, and medical phenotypes, all contributing a small effect size [1]. Lifetime prevalence is 0.9% among adult females in the United States and European countries [2]. The excessive food restriction results in a substantial energy deficit, hormonal aberrations, and deterioration of bone health. Prior to 2013, amenorrhea was one of four diagnostic criteria for AN in the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV, in addition to refusal to maintain body weight at or above a minimally normal weight, intense fear of gaining weight, and disturbance in self-perceived weight or shape [3]. The updated DSM-V has broadened the definition by eliminating the requirement of amenorrhea [4]. DSM-V has also defined atypical AN as meeting psychological diagnostic criteria with normal weight. Despite the more liberal DSM-V diagnostic criteria, BMD has been found to be equally low in women with AN by DSM-V criteria as by DSM-IV criteria [5]. Furthermore, women with atypical AN also had lower BMD than healthy women, despite normal weight [5]. 80% of women with AN by DSM-V criteria had BMD Z-score < −1.0 and 44% had Z-score < −2.0 at any site, and 69% and 25% of women with atypical AN had BMD Z-scores of <1.0 and <2.0, respectively [5]. Even with recovery of AN, bone density does not fully normalize, and there is a lifelong increased risk of fractures [6–9].

Up to 10% of patients with AN are male [10]. Bone density has been found to be similarly low in adolescent males as females with AN, and 32% of males with AN have lumbar spine BMD Z-score of <−2.0 [11]. However, while females with

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AN have an increased fracture risk at all ages, males with AN have a higher risk of fracture when >40 years old [12]. Women also had a higher risk of fractures at nearly all anatomic sites, particularly at the hip and pelvis, and men only had a higher risk of vertebral fractures [12].

Hypothalamic amenorrhea (HA) is a state of less severe chronic energy deficit from excess energy expenditure, insufficient nutritional intake, and/or stress, resulting in dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis with anovulation and cessation of menstrual cycles. It is a diagnosis of exclusion once other hormonal, ovarian, or anatomic causes are eliminated (e.g., hyperprolactinemia, thyroid dysfunction, polycystic ovarian syndrome, primary ovarian failure, etc.). HA is common, up to 60% among female athletes, particularly those involved in lean sports that emphasize endurance training and a lean physique (e.g., swimming, biking, running) [13]. Amenorrhea is one of several compensatory responses to chronic energy deprivation in HA with detrimental consequences in bone metabolism, although the hormonal and bone effects are less severe compared to AN. Prevalence of very low bone density (Z-score ≤ -2.0) and low bone density ($-2.0 <$ Z-score < -1.0) is up to 15.4% and 39.8%, respectively [13]. The female athlete triad was first used to describe the association of amenorrhea, disordered eating, and osteoporosis in 1993 [14]. To highlight the underlying pathophysiology, the International Olympic Committee introduced the term Relative Energy Deficit in Sports (RED-S) in 2014 [15].

Increasingly more hormonal changes are found to occur as an adaptive response to the chronic energy deficiency in HA and AN. Beyond the HPG axis, alterations in other hypothalamic-pituitary hormones, adipokines, and gut hormones contribute to the inhibition of bone accrual and stimulation of bone loss. Therapeutic interventions to improve bone health have targeted hormonal disruptions, including estrogen, androgens, insulin-like growth factor 1 (IGF-1), growth hormone, and leptin, with underwhelming results, as the fundamental energy deprivation needs to be addressed. Limited data on the use of bone active drugs approved for postmenopausal osteoporosis show potential for increased bone density but carry their own specific potential complications. This narrative review will focus on the status of bone health in AN/HA, the pathophysiology behind the bone deficits, and attempted treatment approaches.

2. Status of Bone Health

In clinical practice dual-energy X-ray absorptiometry (DXA) is used to measure areal bone mineral density (BMD). To identify compromised bone health early, the Endocrine Society recommends that patients with HA have a baseline BMD measured if there has been 6 or months of amenorrhea or if there is suspicion of severe nutritional deficiency, other energy deficit states, and/or skeletal fragility [16]. Other modalities of bone imaging, including quantitative computed tomography (CT), and computational modeling to determine bone strength, such as finite element analysis (FEA), are primarily research tools that have help describe altered bone density and quality in AN/HA.

2.1. *Bone Density*

Areal BMD, as measured by DXA, is low in women with AN. Up to 92% of young adult women with AN have T-score of <−1.0 and 38% have T-score < −2.5 [17]. In a longitudinal study, women with AN continued to have a mean annual rate of decline of 2.6% at the spine and 2.4% at the hip [18]. Indeed, adult women with AN have decreased markers of bone formation and increased markers of bone resorption, indicating bone loss [19,20]. While unchecked bone resorption is the major concern in women with AN, adolescent girls with AN have impeded bone accrual during a time when it's normally at its maximum. This is evidenced by decreased markers of bone formation (e.g., osteocalcin and bone-specific alkaline phosphatase) and bone resorption (e.g., collagen type 1 crosslinked carboxyterminal telopeptide, urine deoxypyridinoline, urine N-telopeptide), compared to Tanner stage-matched, normal-weight controls [21,22]. Adolescent boys with AN have also been noted to have low bone turnover markers [23]. In healthy females, 39% of the total body bone mineral content is accrued in the circumpubertal years (around 10–14 years of age), and >95% peak bone mass is reached by 19 years of age [24]. AN-associated low bone accrual during adolescence is particularly worrisome as it results in low peak bone density and early onset of osteoporosis. Adult women with an onset of AN before age 18 have significantly lower BMD at the spine than those developing it later, independent of amenorrhea duration [25]. Women with HA also have low bone density but to a lesser degree than that compared to those with AN [26]. Predictors of low bone density include low weight, body mass index (BMI), lean body mass, and fat mass, later menarche, and greater duration of amenorrhea [17,27,28].

Measures of volumetric BMD, as assessed by quantitative CT, are independent of bone size (unlike areal BMD) and are also low in AN. In a study comparing women with AN, atypical AN, and healthy lean controls, vertebral volumetric BMD, including trabecular and cortical compartments, has been noted to be the lowest in women AN, intermediate in women with atypical AN, and highest in the control group [29]. In AN, vertebral volumetric BMD was positively associated with current and lowest achieved BMI and negatively associated with illness and amenorrhea duration [29]. Women with current amenorrhea with or without exogenous estrogens had lower vertebral volumetric BMD than those with spontaneous menses, even after controlling for BMI [29]. Trabecular and cortical volumetric BMD at the tibia has also been noted to be low in AN [30].

2.2. *Bone Quality*

Bone strength depends on not only bone density but also quality, which includes factors such as architectural integrity, bone geometry, bone turnover rate, accumulation of microdamage, degree of mineralization, and collagen status [31]. The trabecular bone score (TBS) reflects bone architecture by analyzing gray-level variation of pixels from lumbar spine DXA images. In a study of adolescent girls with AN, 11% of participants had degraded and 33% had partially degraded bone microarchitecture as assessed by TBS [32]. Although TBS has been shown to be better associated with fractures than

areal BMD in adult populations of type 2 diabetes mellitus and chronic glucocorticoid use, TBS was not found to be associated with fractures in this study of adolescent girls with AN of relatively mild severity (mean BMI of 18.9 kg/m^2) and short duration (mean of 4 months) [32–34]. TBS was also not found to correlate with disease duration in AN but did correlate with age, BMI, lean mass, areal BMD by DXA, and volumetric BMD by pQCT [32].

With a resolution of ~90 μm, high resolution peripheral quantitative CT (HR-pQCT) can be used to image bone microarchitecture. Females with AN have significantly lower trabecular bone volume fraction and trabecular thickness and greater trabecular separation at the ultradistal radius compared to healthy controls [35]. In a study of adolescent girls with AN, poor trabecular architecture was noted despite having areal BMD by DXA similar to controls [36]. Changes in cortical bone, including greater porosity and lower cortical area and thickness, have also been noted by HR-pQCT despite similar areal BMD by DXA at the radius [37]. Thus, detrimental changes to the bone microarchitecture appear to precede losses in areal BMD. The bone microarchitecture of young adult women with AN has been compared to that of healthy postmenopausal women aged 70–81 years. The women with AN were found to have better total, cortical, and trabecular BMD and cortical and trabecular thickness at the ultradistal radius [38]. However, trabecular number and spacing were comparable to that seen in osteoporosis and suggests early degradation of trabecular bone [38].

Along with decreased bone mass, women with AN have greater bone marrow fat compared to healthy controls, as assessed by proton magnetic resonance spectroscopy (1H-MRS), despite their overall low body fat [39]. Furthermore, marrow fat correlates inversely with BMD [39]. Bone marrow mesenchymal stem cells have the potential to differentiate into adipocytes or osteoblasts [40]. Several processes have been observed in AN that can lead to preferential differentiation to adipocytes over osteoblasts, including hypoleptinemia and hypercortisolism (see Section 3) [41,42]. Osteoporosis is also characterized by thinning trabecular and cortical bone and increased bone marrow fat [43]. However, the marrow fat composition in osteoporosis is noted to be higher in saturated lipids, which may be an additional biomarker of skeletal fragility, while the marrow fat composition in AN has been found to be normal [43–45].

2.3. *Fracture Risk*

Measurements estimating bone strength suggest an increased fracture risk in women with AN. Compared to lean, healthy controls, women with HA have a higher risk of hip fracture, as calculated by the ratio of force applied to the hip from a fall with respect to femoral strength and attenuation by overlaying trochanteric soft tissue [46]. Furthermore, this factor of risk at the hip has been associated with fragility fractures [46]. Other estimates of hip strength, including resistance to axial and bending loads and indices of whole bone strength, have been noted to be decreased in AN [47]. Women with HA have also been found to have high factor of risk at the spine with holding and bending, driven by low vertebral strength [46]. FEA is a computer-based simulation applied to QCT images to estimate bone fragility based on the distribution of bone mass and the biomechanical properties of the bone extracellular matrix. At the wrist, FEA estimates of failure load and stiffness have been found to be worse in girls with AN than controls, even after controlling for distal radius areal BMD [37]. The decrease in strength may be driven by trabecular deterioration as trabecular number has been found to be an independent predictor of failure load and stiffness, accounting for 57% of the variability [35].

Indeed, fracture risk has been found to be significantly higher in women with AN. In one study, girls and young adults with AN had 60% more fractures than compared to normal-weight controls (31.0% v. 19.4%) [48]. Although bone density was lower in the women with AN, lower BMD within the AN cohort was not associated with higher risk of fractures, and fractures occurred even with normal BMD. In a longitudinal study over 18 months, 12.5% of women with AN were found to have incident vertebral fractures, all asymptomatic, and these fractures were not predicted by BMD, duration of illness, or severity of malnutrition [49]. The increased risk of fracture persists with time and has been estimated to be up to 2–3-fold higher, particularly at the hip, spine, and distal forearm [8,9]. Amenorrheic athletes also have higher lifetime fracture risk compared to eumenorrheic athletes and nonathletes (47% versus 25.6% and 12.5%, respectively), largely driven by stress fractures [50]. Predictors of fractures among the amenorrheic athletes include lower lumbar and whole body BMD and trabecular parameters as assessed by HR-pQCT [50].

Recovery of bone density is slow. Bone density tends to only stabilize during the first year after weight gain/restoration in adolescent females and eventually increases with long-term recovery [22,51]. However, BMD does not seem to reach normal range [6,7]. Recovery of menstrual function may be more important than weight for bone health, as one study found that women who regained menses had increased spine BMD but women who gained weight without the return of menses continued to lose bone [18]. Predictors of recovery from AN in general include higher baseline BMI, shorter duration of illness, lack of lifetime depression diagnosis, and greater self-esteem [52].

3. Pathophysiology

Certainly, the poor nutrition observed in AN/HA, particularly insufficient intake of calcium, dairy, and protein, contributes to inhibition of bone mass accrual during adolescence [53]. However, the levels of parathyroid hormone (PTH), which physiologically increase in response to inadequate calcium/vitamin D intake, have been found to be normal and similar to controls in AN [20,54]. Along with the poor nutrition, disturbances in a complex network of hormones emerge mainly as a compensatory response to the chronic energy deprivation at the expense of bone health. In addition to estrogen deficiency, other hormonal derangements also involve other hypothalamic-pituitary axes, adipokines, and gut hormones. All together, these hormones influence the bone microenvironment, affecting bone metabolism.

3.1. Bone Metabolism

The mechanism behind the decreased bone turnover in adolescents with AN and the uncoupling of bone formation and resorption in adult women has been an active area of investigation. However, most studies have been limited by the assessment of circulating factors that may not reflect the bone microenvironment. Early research has focused on the role of the receptor activator of nuclear factor-κβ ligand (RANKL)/RANK/osteoprotegerin (OPG) system in bone resorption. RANKL is expressed by osteoblasts and stimulates osteoclast differentiation and activation and inhibits osteoclast apoptosis [55]. OPG acts as a decoy receptor to sequester RANKL and prevent it from affecting osteoclasts [55]. Although circulating OPG levels have been found to be elevated in girls with AN compared to controls, possibly as a compensatory mechanism, levels of RANKL are also increased and so the OPG/RANKL ratio is actually reduced [21,56,57]. The net effect of the OPG/RANKL ratio, but not the individual levels, has been found to be an independent predictor of bone turnover markers [21,56]. OPG/RANKL ratio has also been found to be positively correlated with lumbar BMD, while OPG itself has been found to negatively correlated, possibly due to a compensatory but inadequate response [56,57]. Cytokines, including interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β1, may also play a role as they have been found to correlate with levels of OPG, RANKL, and OPG/RANKL ratio in girls with AN; however, studies have found inconsistent differences in inflammatory cytokines and one study found that IL-6 did not predict BMD [58–60].

More recent research has focused on the role of osteoblasts in AN. Sclerostin and dickkopf-1 (DKK-1) are secreted by osteocytes and inhibit Wnt/β-catenin signaling in osteoblasts to inhibit osteoblast differentiation, proliferation, and function and may regulate expression of OPG and RANKL [61]. In one study of girls and young adults with AN, sclerostin levels were elevated compared to controls and positively correlated with whole body BMD with a significant interaction with age [19]. Another study on girls with perhaps less severe AN (mean BMI 17.2 ± 0.21 versus 16.0 ± 1.6 in the former study) found no difference in sclerostin levels compared to controls [62]. In both studies, sclerostin levels correlated positively with bone turnover markers in the control groups but not in the AN females [19,62]. Furthermore, although estrogen has been shown to decrease sclerostin levels in postmenopausal women, there was no association between estradiol and sclerostin levels in adolescent girls with and without AN, and transdermal estradiol replacement in girls with AN did not affect sclerostin levels despite an increase in BMD [62,63]. These results suggest that alterations in other hormones, such as IGF-1, (see Section 3) may affect the role of sclerostin in bone metabolism in AN. DKK-1 levels have been found to be lower in females with AN than controls [19]. The increase in sclerostin levels in AN may contribute to the decreased osteoblast activity and inhibition of bone mass acquisition, while the decrease in DKK-1 levels has been hypothesized to be a compensatory response. Further studies are required to elucidate their roles in bone health in AN.

3.2. Hypothalamic-pituitary Axes

HA is characterized by suppression of gonadotropin-releasing hormone pulsatility, resulting in low normal luteinizing hormone and follicle-stimulating hormone levels, anovulation, and amenorrhea [64]. Estrogen suppresses bone remodeling by impeding both osteoblastogenesis and osteoclastogenesis [65]. Estrogen further inhibits bone resorption by directly affecting osteoclast activity and survival, suppressing RANKL production and increasing OPG production by osteoblasts, and suppressing production of pro-resorptive cytokines [65]. Estrogen deficiency results in primarily an increase in bone resorption with a relative deficit in bone formation, and newly postmenopausal women have accelerated bone loss of 1–2%, compared to stable bone density in premenopausal women [66,67]. However, estrogen deficiency alone does not wholly explain the bone deficits seen in AN/HA.

Women with AN may also have low levels of testosterone and dehydroepiandrosterone sulfate (DHEAS), which is produced by the adrenal glands and converted into androgens and estrogens, while normal-weight woman with HA seem to maintain normal androgen levels [68–70]. During puberty, androgens stimulate longitudinal and radial bone growth [71]. Along with estrogens, but to a lesser degree, androgens maintain trabecular bone in adulthood by decreasing osteoclastogenesis, stimulating osteoclast apoptosis, and preventing osteoblast apoptosis [71]. However, the major effect of androgens is likely from aromatization to estrogen. In addition, androgens upregulate transforming growth factor (TGF)-β and IGFs to stimulate bone formation and downregulate IL-6 to suppress osteoclastogenesis [71]. In AN DHEAS levels positively correlate with markers of bone formation and inversely with markers of bone resorption [69,70]. Lower total and free testosterone and DHEA levels predict lower bone density [68].

Beyond the HPG axis, HA is also associated with other neuroendocrine dysfunction, including growth hormone (GH) resistance with increased basal and pulsatile GH secretion but decreased IGF-1 activity; increased levels of corticotropin-releasing hormone, adrenocorticotropic hormone, and cortisol; and low normal levels of thyrotropin, decreased levels of thyroid hormone, and increased levels of the inactive reverse triiodothyronine similar to a sick euthyroid state [54,64,72–77]. These neuroendocrine disturbances further contribute to the inhibition of bone accrual and loss of bone.

The resistance to growth hormone has been proposed to be a protective response to chronic starvation in order to slow growth-related processes (Golden 1994). IGF-1 stimulates osteoblast activity by upregulating processes that increase collagen synthesis and decrease collagen degradation [78]. Indirectly through stabilizing β-catenin in the Wnt canonical signaling pathway, IGF-1 may also encourage osteoblastogenesis [78]. Along with the effects of estrogen, GH and IGF-1 have key roles in skeletal maturation, pubertal growth, and bone mass accrual during the early adolescent years [79]. In postmenopausal women IGF-1 levels correlated positively with BMD [80]. The availability of IGF-1 is regulated by binding proteins. In addition to low serum IGF-1 levels, women with AN also have lower IGFBP-3 and higher IGFBP-2 levels [81]. While IGFBP-3 may enhance IGF activity by upregulating IGF-1 delivery to cell surface receptors, IGFBP-2 prevents IGF-1 from

acting on osteoblasts [78]. In AN, IGF-1 levels are positively associated with markers of bone formation, inversely associated with markers of bone resorption, and positively associated with BMD and parameters of trabecular microarchitecture; similar associations with IGFBP-3 levels have been observed as well [69,82–84].

It is unclear if hypercortisolism in AN is in response to chronic nutritional deprivation and psychiatric stress, contributing to it, or both. It is furthering poor bone metabolism. In girls with AN, strong inverse correlations were observed between markers of bone formation and cortisol levels, compared to no relation in healthy controls; no correlation was found with a marker of bone resorption [74]. Thus, hypercortisolism may inhibit bone accrual in girls with AN. Continued hypercortisolemia in woman may also result in accelerated bone loss as cortisol has been noted to be the best predictor of spine BMD variability [73]. The effects of exogenous glucocorticoids on the uncoupling of bone formation and resorption has been well studied. Excess glucocorticoids inhibits osteogenic over adipocyte differentiation of bone marrow stromal cells and induces osteoblast apoptosis to deter bone formation [42]. In addition, excess glucocorticoids stimulate bone resorption by increasing RANKL expression, reducing OPG expression, and promoting osteoclast survival [42]. Patients with subclinical hypercortisolism (i.e., without symptoms) have been found to have low levels of bone formation markers, increased bone loss, low bone density particularly at the spine, and increased vertebral fracture risk, regardless of age, gender, gonadal status, and BMD [85]. Cortisol may also inhibit gastrointestinal absorption and increase renal excretion of calcium.

The hypothalamic dysfunction also involves hormones released from the posterior pituitary. Oxytocin has major roles in childbirth and lactation and may also be involved in appetite and energy regulation. Oxytocin decreases food intake, possibly through effects on reward and cognitive processes [86]. Overnight levels of oxytocin have been found to be low in women with AN and HA compared to healthy controls, even after controlling for estradiol levels [87,88]. In contrast, postprandial oxytocin secretion is higher in AN [89]. This increase in postprandial oxytocin, measured peripherally, has been proposed to paradoxically reflect attenuated central signaling of oxytocinergic satiety in the starved state [89]. Postprandial oxytocin levels were positively associated with increased severity of disordered eating and associated with differential activation of food motivation neural pathways as assessed by functional magnetic resonance imaging (MRI) [89]. Oxytocin has also been shown to stimulate osteoblast differentiation in vitro and increase BMD when administered peripherally, but not centrally, to mice [90]. In women with AN, overnight oxytocin levels were positively associated with BMD at the spine but not at the hip [88]. Overnight oxytocin levels have also been found to be positively associated with greater integrity of trabecular and cortical microarchitecture as well as strength at the distal radius in women with HA [87]. There were weaker associations at the tibia, a site that likely benefits from weight-bearing exercise [87]. No association was seen in ovulatory athletic women [87]. Thus, low oxytocin levels may contribute to poor bone quality only in the setting of estrogen deficiency.

Antidiuretic hormone (ADH), another hormone secreted from the posterior pituitary, regulates water homeostasis and osmolality by promoting the retention of water at the collecting ducts of the kidneys. Abnormal osmoregulation of vasopressin has been observed in AN with an erratic release of vasopressin being common [91]. Overproduction of ADH can lead to hyponatremia. Anorectic women with hyponatremia have been found to have lower BMD at the spine and hip, even after adjusting for other variables [92,93]. As females with AN have similar levels of ADH both in the cerebrospinal fluid and serum, other factors likely contribute to hyponatremia in AN, including excessive water ingestion, hypovolemia from long-term sodium restriction or the misuse of diuretics, impaired cell membrane integrity, and use of psychiatric medications like selective serotonin reuptake inhibitors [94,95].

3.3. *Adipokines*

Leptin may be the key mediator of the observed abnormalities in the hypothalamic-pituitary axes. Produced primarily in adipose tissue, leptin levels are directly related to amount of body fat [96–98]. Leptin levels, along with fat mass, are lower in females with AN as well as women with HA compared to weight-matched and activity-matched eumenorrheic controls, and leptin levels increase after recovery [99–102]. Leptin levels also acutely fall to 10% of baseline within 72 h of starvation, out of proportion to any loss of fat mass [103]. Physiologic replacement of leptin prevented starvation-induced changes in the gonadal, growth hormone, and thyroid axes [103]. Thus, hypoleptinemia signals a state of energy deficiency and seems to trigger adaptive mechanisms to conserve energy: suppression of HPG axis to prevent pregnancy, growth hormone resistance to limit growth, and sick euthyroid syndrome-like status to slow metabolism [104]. Leptin may affect bone metabolism through these altered hypothalamic-pituitary axes. In women with AN, leptin levels are positively correlated with bone density at the hip and spine as well as parameters of trabecular microarchitecture at the distal radius, independent of BMI [84]. The administration of leptin to women with HA has been show to help normalize neuroendocrine dysfunction and increase BMD (see Section 4) [105–107]. Leptin does not seem to directly regulate other adipokines, including vaspin and visfatin, or gut hormones, including amylin, ghrelin, and peptide YY, suggesting redundant regulation of energy homeostasis [108–111].

Although not confirmed in humans, leptin may also have central effects on bone via noradrenergic and serotonergic signaling as well as direct effects on bone [112]. Mouse studies suggest that leptin induces cortical bone formation via β1 sympathetic activation and/or the GH-IGF-1 axis, but causes trabecular bone loss via β2 sympathetic activation and inhibition of brainstem-derived serotonin synthesis [113,114]. Leptin may also act directly on bone marrow stromal cells to increase expression of osteogenic genes, resulting in differentiation along the osteogenic over the adipocytic pathway [41]. Leptin also stimulates stromal cells to increase OPG and decrease RANKL expression, suppressing osteoclastogenesis [115]. In vitro studies have also shown that leptin increases de novo collagen synthesis and mineralization [116]. Peripheral administration of leptin increases bone in leptin-deficient, but not normal, mice [117]. Thus, leptin may be the link among fat

stores, energy availability, reproductive function, and bone health.

The effects of other adipokines on bone metabolism in AN/HA have been less studied and their roles remain unclear. Adiponectin is also secreted by adipose tissue and is involved in insulin sensitization, promotion of beta cell function and survival, and anti-inflammatory activity [118]. Although adiponectin levels are inversely associated with central obesity in the general population, levels in females with AN have varied widely with observations of lower, similar, and elevated adiponectin levels compared to healthy controls [119–122]. Adiponectin levels have been found to be negatively associated with BMD in adolescent females with AN [119,120]. In exercise-induced HA, levels of adiponectin did not differ among athletes with or without amenorrhea and controls and were not found to predict markers of bone turnover or BMD [123]. Adiponectin has been found to have variable effects on bone. In in vitro studies adiponectin has been shown to stimulate the differentiation and proliferation of osteoblasts but may also act on osteoblasts to suppress OPG and increase RANKL expression to stimulate osteoclastic activity [124,125]. In a more recent mice model, adiponectin decreased proliferation and increased apoptosis of osteoblasts in young mice to hinder bone mass accrual but increased bone mass via inhibitory effects on the sympathetic nervous system in later life [126]. Overall, in adolescents and young women with AN, adiponectin appears to have a net negative effect.

Preadipocyte factor 1 (pref-1) is an epidermal growth factor secreted by preadipocytes and is an important regulator of mesenchymal cells. In vitro studies show that pref-1 inhibits both adipocyte and osteoblast differentiation, and in mice overexpression of pref-1 results in decreased body weight, fat mass, and bone mass [127]. Women with AN and HA have increased level of pref-1 compared with normal-weight controls, and pref-1 has been found to correlate positively with bone marrow fat and negatively with bone density [128,129]. In healthy controls, pref-1 is negatively correlated with bone marrow fat [130]. Thus, the effects of pref-1 may depend on the nutritional status. It has also been proposed that pref-1 may be cleaved and released in the circulation when preadipocytes differentiate into adipocytes in women with HA, explaining the elevated circulating level of pref-1 in relation to high bone marrow fat [130]. Although mouse studies have suggested that GH negatively regulates pref-1 [127], levels of and longitudinal changes in pref-1 and IGF-I have not been found to correlate in females with AN or healthy controls [131]. Pref-1 has been found to be negatively regulated by estradiol in girls with AN but not influenced by leptin [129,131]. Women who have recovered from AN have lower pref-1 levels and lower bone marrow fat than those with active AN, comparable to healthy controls [130].

Omentin-1 is mainly expressed by visceral adipose tissue and may modulate obesity-related metabolic dysfunction and atherosclerosis via an anti-inflammatory mechanism [132]. Levels have found to be lower in obesity, type 2 diabetes, and polycystic ovarian syndrome [132]. Ometin-1 may also have a role in bone metabolism, as levels are inversely associated with bone turnover and density in healthy premenopausal women, but not in postmenopausal women [133]. Adolescent girls with AN have been found to have higher levels of omentin-1 than healthy controls, and associations with bone parameters follow the premenopausal pattern despite amenorrhea with inverse relationships with OPG/RANKL ratio, bone turnover markers, and BMD [120,134]. In in vitro studies, omentin-1 has been found to inhibit osteoblast differentiation while stimulating OPG and inhibiting RANKL expression and secretion to suppress osteoclast formation [135]. Thus, omentin-1 may decrease bone turnover in adolescent girls with AN but mouse studies suggest a more complicated picture. Although overexpression of omentin-1 in mice with and without ovariectomy decreased serum levels of bone turnover markers, it partially restored BMD and femur bone strength in the ovariectomized mice and did not affect control mice [135].

Vaspin is another visceral adipose tissue-derived hormone that has roles in insulin sensitivity and cardiovascular disease, though levels are higher with obesity and may serve as a compensatory mechanism [132]. In one study adolescent girls with AN had higher levels of vaspin compared to controls, and levels negatively correlated with OPG/RANKL ratio but was not associated with bone turnover markers [136]. BMD was not reported. In in vitro studies, vaspin has been found to protect osteoblasts from apoptosis and inhibit RANKL-induced osteoclastogenesis [137,138]. The role of vaspin in bone metabolism in AN may be further complicated with regulation by thyroid hormones, growth hormone, adiponectin, and leptin [136].

3.4. *Gut Hormones*

Gut hormones may also contribute to the coupling between fat and bone tissue. Insulin and amylin from pancreatic β cells and other gastrointestinal hormones, particularly ghrelin and peptide YY (PYY), involved in appetite regulation may have direct effects on bone cells. Observed differences in these gut hormones in AN/HA, mostly in response to starvation, may contribute to a negative bone balance.

Fasting insulin levels and measures of insulin resistance are lower in girls with AN compared to healthy controls [119,139]. Although these insulin measures were positively associated with both markers of bone formation and resorption, they were not associated with bone density in multivariate analysis [119]. In vitro studies have shown that insulin promotes osteoblast proliferation and differentiation and may act synergistically with IGF-1 and PTH, and rodent studies have shown that local administration of insulin to the hemicalvaria increases histomorphometric indices of bone formation 2- to 3-fold [140–142].

Amylin is co-secreted with insulin from pancreatic β cells and has been shown to directly stimulate osteoblast proliferation and inhibit osteoclast differentiation and activity [143]. One study showed that fasting amylin levels are lower in women with AN than healthy controls, and levels were positively associated with hip BMD, independent of weight and percent fat [144]. Another study in women with HA did not find any difference in amylin levels compared to BMI-matched healthy controls [108]. While administration of amylin improved bone density and strength in normal rodents and those with streptozotocin-induced type 1 diabetes, amylin treatment for 1 year was not found to affect bone turnover markers or BMD in patients with type 1 diabetes mellitus [145–147].

Fibroblast growth factor-21 (FGF-21) is predominantly expressed by the liver in response to starvation via activation of peroxisome proliferator-activator receptor α (PPARα). It is involved in regulating glucose and lipid metabolism, possibly through stimulation of adiponectin [148]. One study in anorectic women found that FGF-21 levels were lower than healthy controls, while another study in anorectic women with higher BMI (mean 17.8 versus 15.9 kg/m^2 in the former study) found no difference [149]. A proposed explanation for lower FGF-21 levels in AN is the lack of fat stores and thus free fatty acids to activate PPARα [150]. Since levels of FGF-21 decrease with realimentation therapy as expected, FGF-21 levels may be maximally induced in AN [149,150]. Lower FGF-21 levels in AN may be advantageous as it is associated with better trabecular microarchitecture and bone strength at the radius [150]. In mice, overexpression and administration of FGF-21 results in decreased bone mass by inhibiting osteoblastogenesis and stimulating adipogenesis from bone marrow mesenchymal stem cells via PPARγ [151].

Gut hormones such as ghrelin and PYY serve as peripheral signals to the hypothalamus to regulate appetite and food intake. Ghrelin, which is primarily secreted from the gastrointestinal tract, is an orexigenic hormone released during fasting and acts centrally to stimulate appetite and food intake and induce secretion of GH [143]. Adolescent girls with AN have higher levels of ghrelin compared to normal controls, and female athletes with amenorrhea have higher levels of ghrelin compared to eumenorrheic athletes and healthy controls [119,123,152,153]. The high ghrelin level has been attributed as an adaptive response to the caloric deficit in AN [154]. Independent of IGF-1 levels, ghrelin levels were found to be positively associated with BMD measures in AN but not in HA; ghrelin levels were inversely associated with bone turnover markers in HA [119,123]. Ghrelin may directly promote bone formation by increasing osteoblast proliferation and differentiation, as the administration of ghrelin to rats increases BMD even in the setting of growth hormone deficiency [155].

Peptide YY (PYY) is secreted from the endocrine L cells of the intestine in response to food intake and has been shown to have anorectic effects in normal-weight and obese persons [156,157]. Levels of PYY are low in obesity and high in females with AN and HA compared to normal controls [119,123,152,158]. It has been hypothesized that the elevated levels of PYY may be due to delayed gastric emptying in AN patients [159]. PYY may be contributing to appetite suppression in AN/HA, and levels correlate with drive for thinness in women with HA [152]. PYY levels are inversely associated with BMD, independent of BMI [119,123,158,160]. In mice studies, PYY overexpression reduced bone mass by both decreasing osteoblast activity and increasing bone resorption [161].

The roles of other gut hormones in AN/HA have been explored. Levels of glucose-dependent insulinotropic polypeptide (GIP), which is secreted in response to feeding and induces insulin and glucagon secretion, are low in AN [143,144]. Although GIP may have anabolic effects on bone, levels did not correlate with BMD in women with AN [143,144]. Glucagon-like peptide-1 (GLP-1) and GLP-2 levels increase after food intake and stimulate insulin secretion and nutrient absorption, respectively [143]. While GLP-1 does not seem to directly affect osteoblasts or osteoclasts, it may have indirect effects via stimulation of calcitonin release, and GLP-2 may directly reduce bone resorption [143]. Women with AN/HA have been found to have similar levels of GLP-2 and GLP-1 levels as healthy controls, and GLP-2 has not been found to predict BMD [144,152].

The pervasive alterations in the above described hormones in aggregate affect bone metabolism with a negative net effect. In a multivariate analysis that included body composition, pituitary-related hormones, adipokines, and gut hormones, independent predictors of higher bone density were higher BMI/lean mass and levels of estradiol, growth hormone, and IGF-1 and lower levels of cortisol, leptin, adiponectin, and PYY [119].

4. Pharmacologic Treatment Approaches

Since the pervasive endocrine dysfunction described above is primarily in response to a state of chronic energy deficit, management is focused on addressing this underlying cause. The treatment of AN focuses on weight rehabilitation and involves a multidisciplinary approach, including psychological, medical, and nutritional aspects. The primary goal for women with HA is to normalize energy status, and thus weight, in a similar manner via modifications in diet and/or exercise training. Once energy balance is restored, menses may return on the order of months, followed by an increase BMD on the order of years. Aside from the long time course of recovery, success itself is variable. About half of patients with AN have a full recovery, a third improve, and a fifth suffer a chronic course [162]. Thus, pharmacologic approaches targeting bone health have been studied.

Although estrogen in the form of oral contraceptives (OCPs) is widely prescribed for females with AN and HA for bone health, it has not been found to increase bone density at the spine or hip after controlling for changes in weight [163–166]. For HA, OCPs have been shown to stabilize or slightly increase spine bone density (1% per year), compared to placebo [167–169]. Treatment with OCPs, however, does not affect stress fracture incidence in athletes [167]. Estrogen therapy may lack efficacy in addressing bone health in AN/HA because it has a primarily anti-resorptive effect and bone turnover is abnormally low in adolescents with AN. In addition, estrogen treatment only addresses one hormonal abnormality and may worsen others. Estrogen may decrease free testosterone levels by increasing sex hormone binding globulin levels, and oral estrogen can decrease IGF-1 levels [170]. In girls excessive exogenous estrogen may lead to premature fusion of epiphysis leading to short stature. More recently, physiologic estrogen replacement has been studied. In a randomized controlled trial using physiologic estrogen replacement for 18 months, girls with AN on estrogen had increased BMD at the spine and stabilized BMD at the total hip, compared to no change in BMD at the spine and slight bone loss at the hip for those on placebo [171]. Although bone accrual increased in the anorectic girls treated with physiologic estrogen replacement, rates were similar to the untreated healthy controls and did not result in normalization of BMD [171]. The main concern with both OCPs and physiologic replacement of estrogen is the masking of return of menses, a key indicator of recovery.

As androgens have anabolic effects on bone, the efficacy of testosterone and DHEA treatments have been studied alone and in combination with estrogen. Testosterone treatment in women with AN and relative testosterone deficiency over 3 weeks increased 1 of 3 markers of bone formation analyzed and did not affect a marker of bone resorption [172]. Although treatment with DHEA over 1 year was shown to transiently increase markers of bone formation compared to OCP and decrease markers of bone resorption similar to OCP, DHEA did not significantly increase BMD once corrected for weight gain [166]. The combination of androgen and estrogens may fair a little better. One study found that the combination of DHEA and OCP over 18 months maintained BMD, while there was a decrease in BMD with placebo [173].

IGF-1 therapy has also been tried with and without estrogen with some promising results. First of all, a supraphysiologic dose of recombinant human GH was not able to overcome GH resistance in women with AN, as it did not affect IGF-1 levels or bone turnover markers [174]. Short-term (~1 week) administration of recombinant IGF-1 in girls with AN did increase a marker of bone formation and decrease a marker of bone resorption [175]. In a trial that randomized women with AN to 1) recombinant IGF-1 and OCP, 2) IGF-1 alone, 3) OCP alone, or 4) no treatment for 9 months, only the combined treatment group increased spine BMD by 1.8%, which was not enough to normalize BMD [176].

Leptin monotherapy may address multiple hormone abnormalities to more effectively improve bone health. In clinical trials, administration of subcutaneous leptin in physiologic doses has been shown to restore the HPG axis in HA women, increasing LH levels, LH pulse frequency, and estradiol and progesterone levels, and reestablish menses [105,106]. Furthermore, leptin decreased cortisol levels and increased thyroid hormone and IGF-1 levels [72,105,106]. In the short-term, leptin increased markers of bone formation by week 4 and prevented an increase in a marker of bone resorption [105,106]. After 2 years of treatment, the only change from baseline was a decrease in a marker of bone resorption [107]. Two years of leptin treatment in an open-label extension trial resulted in an increase in lumbar BMD of 4% but no effect at the hip, radius, and whole body [107]. Leptin was also found to decrease RANKL/OPG ratio but did not affect serum levels of sclerostin or DKK-1 [177]. Finally, leptin also decreased PTH levels, possibly through a direct effect or through restoration of estradiol, which increases calcium absorption from the intestine and decreases calcium renal excretion [177]. Overall, it seems that the improvement in bone mass is not due to a direct effect of leptin as leptin levels did not correlate with BMD [177]. As would be expected, women treated with leptin lost weight and fat mass but maintained lean body mass [105,178]. Other less concerning safety issues include injection site reactions and the development of nonneutralizing antibodies. The clinical trials to date show that leptin is a safe, effective therapeutic option in women with HA, improving both neuroendocrine and bone outcomes; however, further studies are needed before clinical adaptation. Leptin would not be an option for women with AN but, with careful monitoring and dose adjustments, may have potential for women with mild HA in conjunction with lifestyle changes.

Bone active medications are approved for the treatment of postmenopausal osteoporosis and include anti-resorptive (e.g., bisphosphonates) and anabolic (e.g., teriparatide) approaches. In a randomized controlled trial in adolescents with AN, alendronate treatment for 1 year was found to increase BMD at the lumbar spine by 3.5 ± 4.6% and femoral neck by 4.4 ± 6.4%, but similar increases were seen in the placebo group [179]. Both groups had gained weight, and body weight was the most important determinant of BMD [179]. In another randomized controlled trial, risedronate for 1 year also increased BMD at the spine by 3% and at the hip by 2%, which was significant when compared to placebo; the addition of low dose testosterone did not affect the results [180]. The major concern with the use of bisphosphonates in a premenopausal population is its long half-life coupled with its teratogenic potential. The anabolic agent teriparatide, an analog of PTH, had more impressive results, at least at the spine. In a small randomized controlled trial over 6 months in women with AN, teriparatide increased spine BMD by 6 ±1.4%, compared to 0.2 ± 0.7% with placebo, but did not affect hip BMD, a pattern similar to that seen in postmenopausal women [181,182]. Due to risk of osteosarcoma observed in rats, but not yet seen in humans, teriparatide is contraindicated in adolescents and young adults who have open epiphyses [183,184]. Given that RANKL levels are increased in AN, denosumab, a human monoclonal antibody to RANKL that acts as an inhibitor and has anti-resorptive effects, may be beneficial for bone health in adult women with AN who have increased bone resorption; no clinical trials have been performed to date. Finally, romosozumab, a sclerostin inhibitor, may also be useful as it increases bone formation and decreases bone resorption, at least in the first 9 months of treatment; this medication is currently pending U.S. Food and Drug Administration approval [185].

Clinically available treatments for low bone density include estrogen, testosterone, DHEA, and bone active agents. Practice guidelines for HA have been recently updated. Due to the high risk for bone stress injuries and fractures in athletes, the 2014 Female Athlete Triad Coalition has specified guidelines for the management of bone health. The Female Athlete Triad Coalition recommended consideration of transdermal estradiol if Z-scores are ≤−2.0, there are ongoing risk factors, and there has been a lack of response to 1 year of nonpharmacologic therapy (BMD loss or new fracture) and reserving bone active agents for those who have failed or have contraindications to estrogen replacement (see Fig. 1) [186]. The 2017 Endocrine Society guidelines suggested against using OCPs in patients with hypothalamic amenorrhea for the sole purpose of regaining menses or improving BMD [16]. Furthermore, the Endocrine Society specifically suggests that HA patients on OCPs for contraception should be educated that OCPs may mask the return of spontaneous menses and that bone loss may continue [16]. Instead of OCPs, short-term use of transdermal estradiol with cyclic oral progestin is suggested for adolescents and women who have not had return of menses after 6 to 12 months of nutritional, psychological, and/or modified exercise intervention [16]. The Endocrine Society suggested against using bisphosphonates, denosumab, testosterone, and leptin to improve BMD and the consideration of short-term use of teriparatide in adults with delayed fracture healing and very low BMD [16].

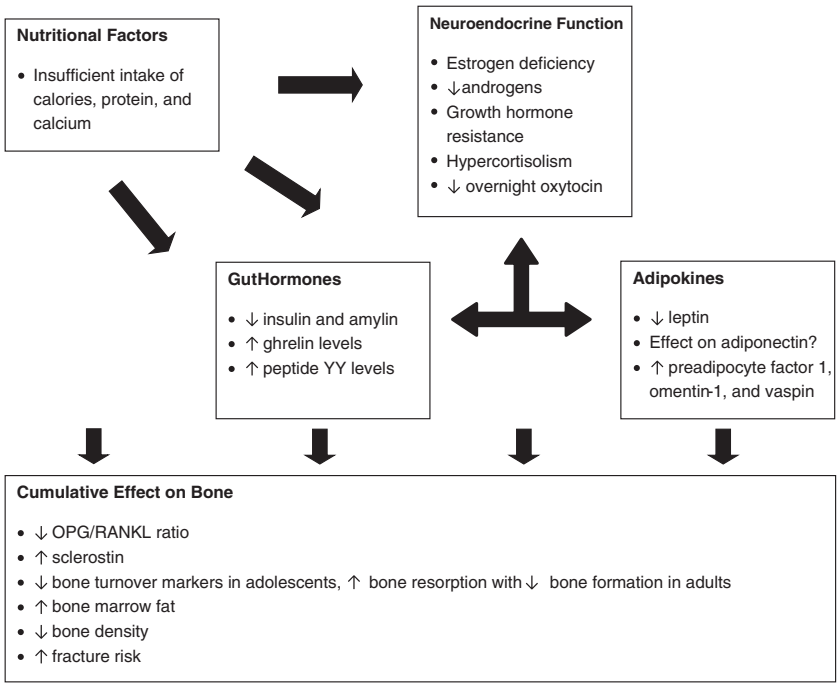

Fig. 1 – The multifactorial effect of AN on bone health.

5. Conclusion

As women with AN have similarly low BMD regardless of menstrual status and estrogen therapy does not conclusively improve BMD in women with AN and HA, the lack of estrogen itself is not the main driver of poor bone health. Amenorrhea is only one of several compensatory responses to the chronic energy deficiency in AN/HA. Other hormones of the hypothalamus/pituitary, adipose tissue, and gastrointestinal system are affected and in total harm bone metabolism. Thus, addressing one hormonal aspect is not sufficient to improve bone density, and first-line therapy remains achieving a positive energy balance through lifestyle changes.

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Conflicts of Interest

None.

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