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Anorexia nervosa and osteoporosis

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Abstract Anorexia nervosa (AN), a condition of severe undernutrition, is associated with low bone mineral density (BMD) in adults and adolescents. Whereas adult women with AN have an uncoupling of bone turnover markers with increased bone resorption and decreased bone formation markers, adolescents with AN have decreased bone turnover overall. Possible contributors to low BMD in AN include hypoestrogenism and hypoandrogenism, undernutrition with decreased lean body mass, and hypercortisolemia. IGF-I, a known bone trophic factor, is reduced despite elevated growth hormone (GH) levels, leading to an acquired GH resistant state. Elevated ghrelin and peptide YY levels may also contribute to impaired bone metabolism. Weight recovery is associated with recovery of BMD but this is often partial, and long-term and sustained weight recovery may be necessary before significant improvements are observed. Anti-resorptive therapies have been studied in AN with conflicting results. Oral estrogen does not increase BMD or prevent bone loss in AN. The combination of bone anabolic and anti-resorptive therapy (rhIGF-I with oral estrogen), however, did result in a significant increase in BMD in a study of adult women with AN. A better understanding of the pathophysiology of low BMD in AN, and development of effective therapeutic strategies is critical. This is particularly so for adolescents, who are in the process of accruing peak bone mass, and in whom a failure to attain peak bone mass may occur in AN in addition to loss of established bone.

Keywords Anorexia nervosa · Bone density · Bone turnover · Hypogonadism · Hypercortisolemia · GH resistance

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1 Introduction

Anorexia nervosa (AN) is becoming increasingly common in affluent societies, and occurs in 0.2–1.0% of adolescent girls and 1.0–4.0% of college aged young women [1, 2]. The condition also occurs in males, and the prevalence of AN in adolescent boys and men appears to be increasing [3]. The association of AN with low bone mineral density (BMD) has been conclusively and consistently demonstrated in adult women [4–8] as well as in adolescent girls [9–15] and boys [16]. Of concern, the adolescent years, which are a critical time for bone mass accrual [17], are also a common time for the onset of this disorder. Maximal increases in bone mass accrual occur between 11–14 years of age in girls and 13–16 years in boys [17], with more than 90% of peak bone mass being achieved by the end of the second decade [18]. Evidence suggests that insults occurring during these critical adolescent years may well result in permanent deficits.

Bachrach et al. demonstrated that 12 out of 18 girls with AN had BMD more than two SDs below that in controls at the lumbar spine, and importantly, more than 50% of these 12 girls had been diagnosed with AN for less than a year [9]. Thus, not only was the extent of low BMD profound in this young population, but appeared to have occurred over a relatively short period of time. Subsequent studies have continued to report low BMD in adolescent girls with AN

[10–15]. We reported BMD *z*-scores of <−1 at one or more sites in 41% of 60 adolescent girls with AN, and BMD *z*-scores of <−2 in another 11% [11]. Similarly, Castro et al. reported that 35% of adolescent boys with AN had reduced BMD at the lumbar spine and femoral neck [16]. In addition, subsequent BMD is lower when AN begins in the adolescent years than when it occurs in adult life, even when the duration of illness is comparable [19]. Low BMD occurs at all skeletal sites, thus both trabecular and cortical bone are affected, although the spine is more likely to be affected than the hip [11, 20]. In adolescents, AN is associated with a decrease in levels of surrogate markers of bone turnover in comparison to the high levels of these markers in healthy adolescent girls, with particularly a marked suppression of bone formation [12, 13]. The net result is decreased bone mass accrual and low BMD.

In adult community dwelling women with AN, osteoporosis at one or more sites occurs in as many as 38% and osteopenia in 92% [20]. More than 50% of all women with AN have BMD below the fracture threshold [21], and there is a seven fold greater fracture risk compared with matched healthy controls [5]. Lucas et al. reported a 57% cumulative incidence of fractures at the spine, hip and radius 40 years after diagnosis of AN [1]. An uncoupling of surrogate markers of bone turnover occurs, with a decrease in bone formation and an increase in bone resorption markers, resulting in overall bone loss [21].

2 Pathogenesis of low bone density in anorexia nervosa and treatment options

Multiple factors contribute to low BMD in AN, and these include hypogonadism, undernutrition, low levels of nutritionally dependent insulin-like growth factor-I (IGF-I), an acquired resistance to growth hormone (GH), excessive exercise and hypercortisolemia. A critical role may also be played by alterations in hormones regulating food intake.

2.1 Hypogonadism

Undernutrition may be associated with acquired hypogonadotropic hypogonadism, and amenorrhea is currently a part of the DSM-IV criteria for the diagnosis of AN in females. The gonadal hormones, estrogen and testosterone, have a critical role to play in optimizing BMD. At the onset of puberty, release of inhibitory control of the hypothalamo–pituitary–gonadal axis is associated with increasing nighttime pulsatility of gonadotropins followed by daytime pulsatility [22]. Rising levels of gonadal hormones are followed closely by increases in GH and IGF-I, which peak about a year after attainment of maximal pubertal growth velocity [23]. Bone anabolic effects of GH and IGF-I, and anti-resorptive effects of estrogen are postulated to cause the marked increase in bone mass accrual that is typical of the adolescent years. The anti-resorptive effects of estrogen are mediated at least in part by (1) suppression of secretion of certain proinflammatory cytokines that stimulate osteoclast differentiation and action and prolong osteoclast survival, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α, and (2) an increase in secretion of transforming growth factor (TGF-)-β and osteoprotegerin (OPG), which suppress osteoclast activity and accelerate osteoclast apoptosis [24]. Estrogen may also inhibit osteoblast apoptosis [25, 26]. In both genders, aromatization of androgens to estrogen accounts for decreased bone resorption, and testosterone also has direct anti-resorptive and bone anabolic effects [27].

In AN, abnormalities in gonadotropin releasing hormone (GnRH) secretion cause gonadotropin pulsatility patterns to revert to pre- or early pubertal levels [22], and low estrogen levels are characteristic in both adults and adolescents [11–13, 28]. Adolescent girls and women with AN also have low testosterone levels [13, 29], and low levels of the adrenal androgen dehydroepiandrosterone (DHEA) have been reported in young women with AN [30]. Delayed menarche occurs in as many as 35% of adolescents with AN, and age at menarche is an important and independent predictor of low BMD in this population [11, 15]. Duration of amenorrhea predicts the extent of low BMD in adolescents [8, 9, 11, 12, 15, 31] as well as adults [4, 8, 19, 32] with AN. In addition, we have demonstrated a direct association between changes in testosterone levels and changes in bone formation markers in AN [13]. The contribution of hypogonadism to low BMD in AN independent of direct effects of undernutrition is indicated by lower bone density *T*-scores in women with AN who are amenorrheic and low weight compared with eumenorrheic women of similar weight [33]. However, the importance of nutritional factors is evident from the significantly lower BMD in amenorrheic women with AN compared to women with hypothalamic amenorrhea and normal weight with comparable estradiol levels [8].

Data regarding pro-inflammatory cytokines in AN are contradictory, with reports of increased [34, 35] or normal levels of IL-6 [36–40], normal [36, 38, 40] or decreased levels of IL-1β [39], and normal [34, 36, 40] or increased levels of TNF-α [35]. We observed high IL-6 levels in AN, however, IL-6 did not predict BMD or levels of bone markers in our subjects. Hypogonadism should cause a decrease in OPG levels associated with an increase in bone resorption markers and a decrease in BMD. However, levels of OPG are elevated in adolescents with AN and correlate inversely with nutritional markers and BMD [41]. This is likely a compensatory response rather than a contributor to low BMD.

2.1.1 Estrogen replacement

Although hypogonadism is an important cause of low BMD in AN, studies of estrogen replacement on bone metabolism have yielded disappointing results, and are consistent with the concept that other nutritionally dependent factors are important in bone loss and recovery in this population. In a cross-sectional analysis of 130 women with AN, we observed no differences in BMD at multiple sites in women with or without estrogen exposure [20]. In addition, in a randomized study, our group observed no increase in BMD at the spine in adult women with AN following 18 months of estrogen–progesterone replacement with calcium and vitamin D supplementation, compared with calcium and vitamin D supplementation alone (Fig. 1) [28]. A post hoc analysis did, however, indicate some beneficial effects in women with the lowest bone densities. Although a low dose of estrogen was used in this study, a subsequent randomized study for 9 months using an oral contraceptive showed no differences in BMD compared to no estrogen [42]. Similarly, in adolescents with AN, two studies have reported no increases in BMD with use of oral estrogen–progesterone combination pills for a year [43, 44]. Neither of the studies was randomized or placebo controlled. Definitive studies of effects of estrogen replacement on bone metabolism in adolescents with AN are thus lacking. Although estrogen use causes a suppression of bone resorption, the lack of observable effects on BMD may stem from (1) persistence of decreased bone formation, (2) IGF-I suppressive effects of oral estrogen, and (3) persistence of factors other than hypogonadism that contribute to low BMD in AN.

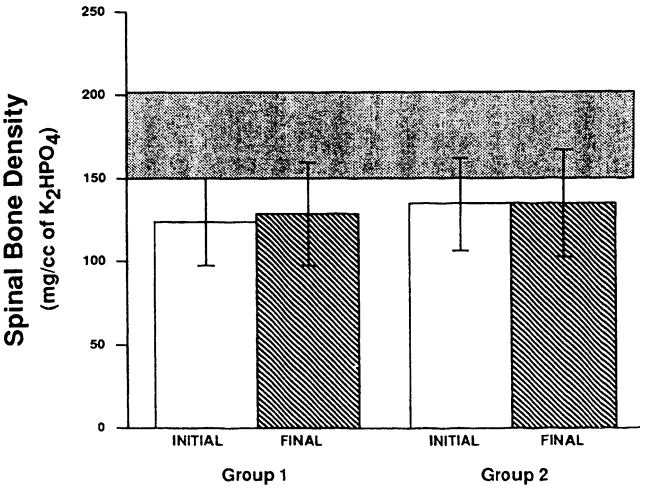

Fig. 1 Effects of administration of estrogen–progestin with calcium and vitamin D vs administration of calcium and vitamin D alone on bone density in women with anorexia nervosa. No differences in bone mineral density were observed before and after 18 months of estrogen–progestin administration in a group of women with anorexia nervosa in a randomized, prospective trial. (Reprinted with permission from the Journal of Clinical Endocrinology and Metabolism, 1995; 80: 900. Copyright © 1995 The Endocrine Society. All rights reserved).

2.1.2 Androgen replacement

Low testosterone levels are characteristic of AN [13], and we have reported in a pilot study that testosterone replacement to approximate levels in healthy women is associated with an increase in the C-terminal propeptide of type 1 procollagen (PICP), a bone formation marker (Fig. 2) [29]. Gordon et al. reported an increase in bone formation markers following 3 months of DHEA administration in young women with AN [30]. However, a subsequent yearlong study of DHEA administration failed to demonstrate an increase in BMD in AN after controlling for effects of weight gain (Fig. 3) [45]. Effects of androgen replacement on bone metabolism need to be further characterized, and of importance, the effect of androgen replacement in males with AN needs to be examined.

2.2 Undernutrition and low levels of IGF-I

Undernutrition and nutritionally dependent factors play a critical role in low BMD in AN. Effects of undernutrition on bone independent of hypogonadism are indicated by significantly lower BMD in AN, a condition of hypogonadism and undernutrition, than in conditions of hypogonadism not associated with undernutrition such as hyperprolactinemia [8]. Strong correlations are observed between BMD and nutritional indices such as BMI [8, 9, 31, 46], fat mass [11], lean body mass [8, 11, 12, 15, 47] and IGF-I [8, 12]. Lean body mass, in particular, is a strong and independent predictor of BMD in adults [8] and adolescents [11, 12]. Effects of lean body mass on bone are attributed to bone remodeling stimulated by biomechanical forces exerted by the pull of muscles on growing bone. Surrogate markers of bone formation are predicted by nutritional indices such as IGF-I [12, 13], BMI, body fat [21] and lean body mass [13], and short term fasting results in a 50% reduction in bone formation markers in healthy volunteers [48]. Similarly, weight recovery in AN is associated with a marked increase in bone formation markers, and an increase in bone formation markers predicts a subsequent increase in lumbar spine bone mineral content [13].

2.2.1 Weight recovery

An increase in BMD was reported with weight recovery, even before menstrual recovery, by Bachrach et al. in adolescent girls with AN [49], demonstrating the critical role played by nutritional status on bone mass accrual. However, one third of girls recovering weight continued to have BMD *z*-scores more than two SDs below the mean in this study, indicating that improvement in BMD was not

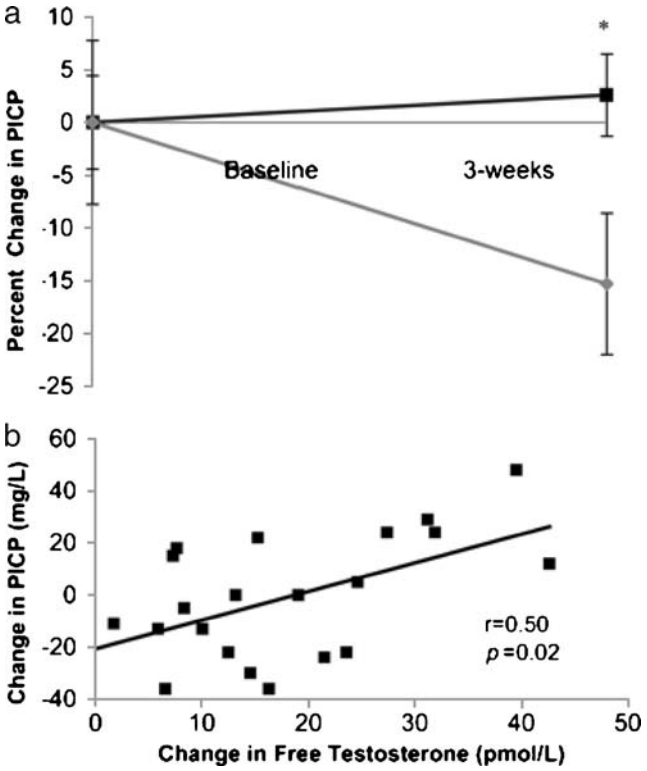

Fig. 2 Changes in PICP levels after testosterone or placebo administration in adult women with anorexia nervosa. (a) PICP levels were higher during testosterone administration (*black line*) than with placebo (*gray line*) (*$P = 0.03$). (b) Change in PICP correlated with change in serum-free testosterone ($r = 0.50$, $P = 0.02$). (Reprinted with permission from the Journal of Clinical Endocrinology and Metabolism, 2005; 90:1431. Copyright © 2005 The Endocrine Society. All rights reserved).

complete. In fact, many studies have been unable to demonstrate a significant increase in BMD despite short-term weight recovery. We reported a persistence of low BMD despite weight recovery in adolescents with AN followed for a year [13], and in half of all weight recovered adults with AN followed for an 18 month period [28]. In both studies, however, persistence of low weight led to a further decline in BMD. Thus, weight recovery protected against further bone loss. A longer period of weight recovery may be necessary before significant increases in BMD are observed. Indeed, in one study of adolescents with AN, BMD at the lumbar spine decreased at 7 months follow-up despite improved nutritional status, and an increase in BMD was observed only after an average of 21 months of sustained weight recovery [10].

Of concern, long-term follow-up studies indicate that despite some improvement in BMD with weight recovery, residual deficits may persist. For example, in one study of women with AN, BMD at the femoral neck was lower than in controls even after 21 years of weight recovery [50]. In another study, prevalence of osteopenia decreased from 35 to 13% and of osteoporosis from 54 to 21% with weight recovery, yet, low bone mass persisted in a large proportion of these women [51]. These data indicate that AN may result in permanent deficits in BMD despite recovery, and underscore the importance of timely diagnosis and treatment of AN.

2.2.2 Recombinant human IGF-I

IGF-I is secreted by the liver in response to GH, and is also nutritionally regulated. Undernutrition is associated with low levels of IGF-I despite normal [52] or elevated [53–57] GH levels, indicative of a nutritionally acquired resistance to effects of endogenous GH secretion. This is attributed to a downregulation of GH receptors in the liver with undernutrition. The increase in GH concentrations stem from increased basal GH secretion, as well as an increased

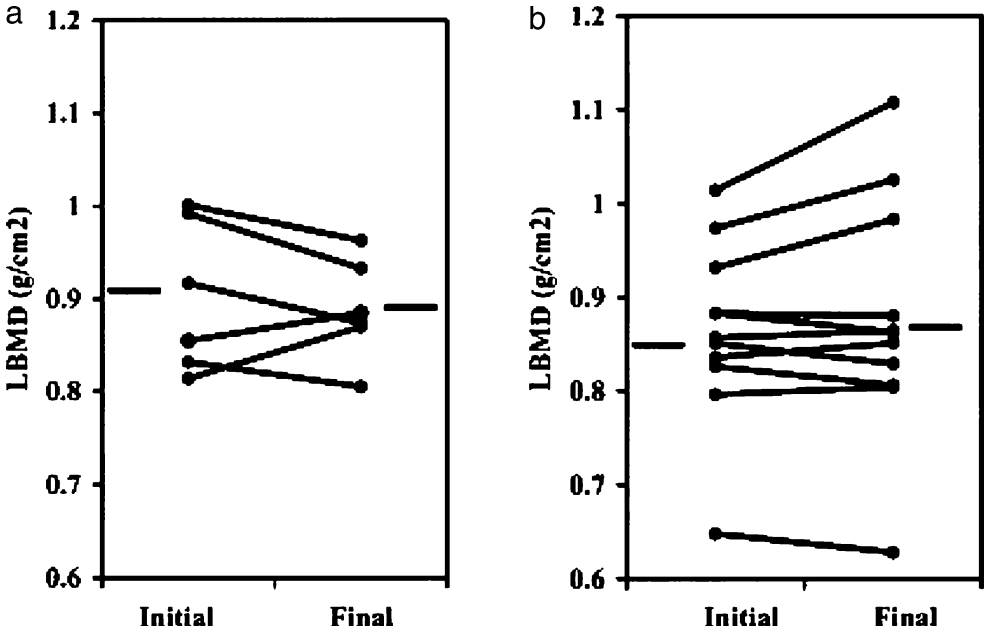

Fig. 3 Initial and final BMD in recovered and non-recovered adolescent girls with AN. No differences were observed between initial and final bone mineral density in adolescent girls with anorexia nervosa who did not recover weight (a) and who recovered weight (b) in a yearlong prospective study. (Reprinted with permission from the Journal of Clinical Endocrinology and Metabolism, 2002;87:4180. Copyright© 2002 The Endocrine Society. All rights reserved).

frequency of GH secretory bursts [54]. GH and IGF-I are bone anabolic and stimulate osteoblast differentiation and proliferation [58]. Consistent with these effects on bone formation, IGF-I is an important predictor of bone formation markers such as osteocalcin and bone specific alkaline phosphatase [12, 13]. We have demonstrated that GH levels strongly predict levels of bone turnover markers in healthy adolescents, but not in AN, suggestive of resistance to GH effects not only at the level of the liver, but also at the level of bone [54].

Because IGF-I functions as a bone trophic factor, administration of recombinant human (rh) IGF-I was hypothesized to cause an increase in bone formation markers and in BMD. Indeed, we have demonstrated a dose dependent increase in bone formation markers following short-term administration of rhIGF-I at a dose of 30 mcg/kg twice daily, without any change in bone resorption markers (Fig. 4) [21]. We have also shown that chronic rhIGF-I administration for 9 months at the same dose causes a significant increase in BMD, when given together with estrogen–progesterone combination pills [42]. Presumably, bone trophic effects of rhIGF-I couple with anti-resorptive effects of estrogen to cause this observed increase in BMD. Difficulties in obtaining rhIGF-I for this indication, lack of FDA approval, and twice daily injections currently limit the use of rhIGF-I to optimize BMD in AN.

2.2.3 Calcium and vitamin D intake

Abrams et al. demonstrated through calcium kinetic studies that calcium absorption is decreased and calcium excretion increased in AN [59], and Castro et al. recently reported that calcium intake of less than 600 mg is an important predictor of low BMD in AN [16, 31]. However, other studies have failed to demonstrate any relationship between calcium intake and BMD in this condition [11, 13]. In fact, we observed that more girls with AN than healthy adolescents met the RDI for calcium (47 *vs*. 33%) and vitamin D (47 *vs*. 22%) [12]. In addition, administration of calcium and vitamin D does not result in an increase in BMD in adults or adolescents with AN [13, 28]. Nevertheless, we do recommend optimizing calcium and vitamin D intake, and suggest a daily intake of 1,200–1,500 mg of elemental calcium and 400 IU of vitamin D.

2.3 Physical activity

The contribution of physical activity to low BMD in AN is unclear. Castro et al. did report that less than three hours/week of physical activity was a risk factor for low BMD in AN [16, 31]. Other studies, however, have not been able to demonstrate a relationship between physical activity and bone metabolism in this condition [4, 9, 12]. Muscle contractions generate strain signals that are picked up by the 'mechanostat' (presumed to be osteocytes) in bone, and this is postulated to activate remodeling resulting in an increase in BMD. A permissive role for estrogen appears necessary for this pathway to function intact [24]. Hypogonadism in AN may result in impaired functioning of the 'mechanostat', and lack of substrate availability may lead to a catabolic state, both of which would impair bone mass accrual. In addition, low BMD in AN predisposes this

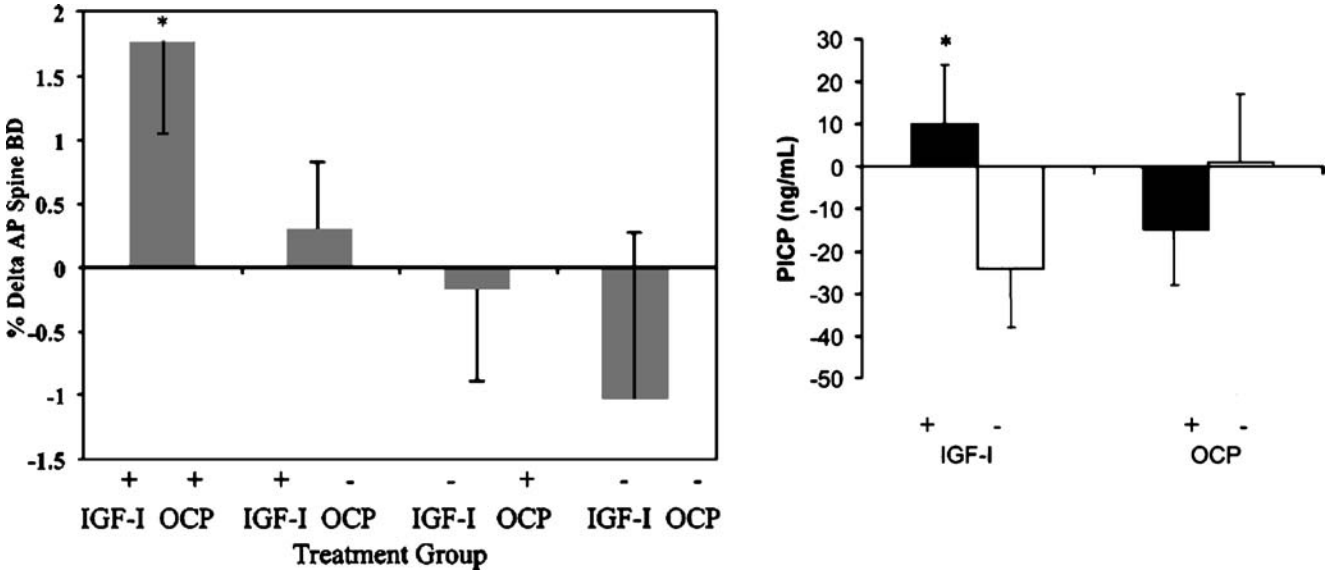

Fig. 4 Effect of rhIGF-I and oral contraceptive pills on bone density (*left*) and PICP levels (*right*). Administration of recombinant human-insulin-like growth factor-I (IGF-I) (30 μg/kg b.i.d. subcutaneously) increased bone density and PICP in adult women with anorexia nervosa. The effect on bone density was most marked when IGF-I was given with an oral contraceptive pill. (Reprinted with permission from the Journal of Clinical Endocrinology and Metabolism, 2002; 87:2888. Copyright © 2002 The Endocrine Society. All rights reserved).

population to fractures, and excessive activity may increase the risk of stress and other fractures.

2.4 Hypercortisolemia

High cortisol levels affect bone metabolism at many levels, the net effect being low BMD [60–64]. Hypercortisolemia stimulates osteoclastic bone resorption, inhibits osteoblast differentiation and action, and has an inhibitory effect on the GH–IGF–I axis, calcium absorption from the gut, and renal handling of calcium. AN is associated with increased cortisol secretory burst frequency resulting in increased cortisol concentrations [65]. Decreased clearance of cortisol may also contribute to hypercortisolemia in AN [65, 66]. Serum and urinary cortisol concentrations have been reported to be elevated in both girls [65, 66] and adults [6, 57, 66, 67] with AN. We have demonstrated strong inverse correlations between high cortisol levels and low levels of bone formation markers in AN, and an inverse association between cortisol and BMD [65]. Grinspoon et al. however, noted hypercortisolemia in only 22% of all AN women with bone loss as assessed by 24-h urinary free cortisol levels, suggesting that high cortisol levels are not a major cause of low BMD in AN [48].

2.5 Leptin, ghrelin and peptide YY

The appetite regulating peptides, leptin, ghrelin and peptide YY (PYY), have all been implicated in the regulation of bone metabolism. Leptin is an anorexigenic adipocytokine [68, 69], levels of which are low in AN [13, 70–76]. In rodent models, leptin deficient and leptin resistant animals are obese and hypogonadal, yet have high BMD. Leptin administration is known to decrease bone formation in these animals through its actions on the central nervous system [77]. Conversely, leptin deficiency in AN is associated with low BMD [8, 13], and leptin is not an independent predictor of BMD after controlling for other nutritionally dependent factors such as body mass index.

Ghrelin is an orexigenic peptide [78, 79] that is also a GH [80–84] and ACTH [80, 82, 85] secretagogue. In addition, ghrelin suppresses LH pulsatility [85] and thus may cause a decrease in secretion of sex steroids. Ghrelin administration has been reported in one study to increase osteoblast proliferation *in vitro* [86]. Levels of ghrelin are elevated in AN [73, 87–89], and we have demonstrated that ghrelin is an important predictor of BMD at the spine and the hip. This relationship between ghrelin and BMD may be mediated via effects of ghrelin on GH and cortisol secretion, which in turn have known effects on bone turnover [90].

PYY is an anorexigenic peptide secreted by L-cells of the colonic mucosa [91, 92], and PYY levels are elevated in AN [93]. The first indication of a role for PYY in bone metabolism came from a study demonstrating that selective deletion of the receptor for PYY (the Y2 receptor) results in increased bone formation [94]. Since then, we have reported that high PYY levels predict low levels of bone formation and bone resorption markers in adolescent girls with AN and healthy adolescents, and PYY is an independent predictor of markers of bone turnover on regression analysis [93]. Further studies are necessary to better characterize the role of PYY in bone metabolism.

2.6 Bisphosphonates and other options for increasing bone density

Bisphosphonates increase BMD by suppressing osteoclastic bone resorption, and the role of bisphosphonates in increasing BMD has been examined in adults and adolescents with AN. Golden et al. reported no effects of alendronate on BMD in adolescents with AN after controlling for effects of weight gain [95]. In adult women, our group has reported a 4% increase in BMD at the spine after 6 months and a 5% increase after 9 months of treatment with risedronate (Fig. 5) [96]. However, this study was not randomized and used historical controls. Longer-term studies are now underway. The absence of IGF-I suppressive effects of bisphosphonates (unlike oral estrogen) confers an advantage to this medication in treatment of low BMD in conditions associated with increased bone resorption. The rationale for the use of bisphosphonates in adolescents with AN is less clear given that bisphosphonates suppress bone turnover, whereas healthy adolescents are in a state of increased bone turnover. As a consequence, these medications may further aggravate pathology in AN.

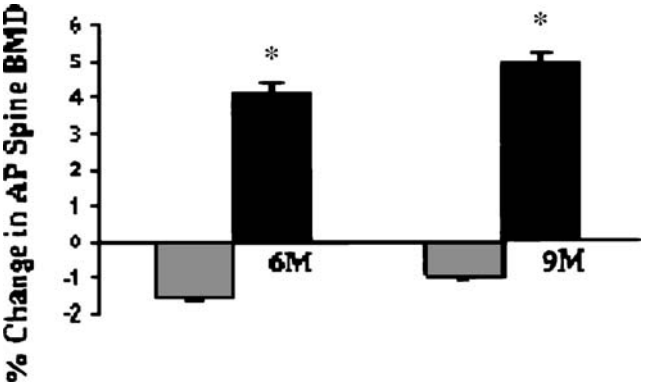

Fig. 5 Percent change in AP spine bone density in women with anorexia nervosa receiving risedronate (*black bars*) vs controls (*gray bars*) at 6 and 9 months of treatment. Bone density at the AP spine increased with risedronate use at 6 and 9 months. *$P < 0.05$ compared with baseline and controls. (Reprinted with permission from the Journal of Clinical Endocrinology and Metabolism, 2004; 89:3905. Copyright © 2004 The Endocrine Society. All rights reserved).

3 Conclusions

Low BMD is an important co-morbid condition associated with AN, and is of particular concern in adolescents, who are in the process of accruing peak bone mass. The pathogenesis underlying low BMD includes an uncoupling of bone turnover in adults with AN, with a decrease in bone formation and an increase in bone resorption markers, whereas in adolescents with AN, there is an overall decrease in bone turnover, in contrast to the state of increased bone turnover in healthy adolescents. Contributors to low BMD in AN include hypogonadism, undernutrition, an associated state of GH resistance, hypercortisolemia, and possibly high ghrelin and PYY levels. Oral estrogen is not effective in improving BMD, however, rhIGF-I with estrogen has been shown to cause an increase in BMD in adults with AN. A potential for therapeutic use of low dose testosterone and bisphosphonates also exists in adults with AN. Currently, there are no therapeutic options for improving BMD in adolescents with AN with the exception of sustained weight recovery. Weight recovery and adequate calcium and vitamin D intake should be encouraged in all patients with AN, and the possible detrimental effects of excessive exercise emphasized.

4 Key unanswered questions

These include the following:

- Role of androgens in optimizing BMD in adults with AN
- Role of gonadal hormone replacement in adolescents with AN
- Development of strategies with bone anabolic effects, unlike the anti-resorptive effects of estrogen and bisphosphonates
- Role of ghrelin and PYY in bone mass accrual in healthy individuals, and in low BMD in AN

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References

1. Lucas AR, Beard CM, O'Fallon WM, Kurland LT. 50-year trends in the incidence of anorexia nervosa in Rochester, MN: a population-based study. Am J Psychiatry 1991;148:917–22
2. Von Ranson K, Iacono W, McGue M. Disordered eating and substance abuse in an epidemiological sample. 1. Associations within individuals. Int J Eat Disord 2002;31:389–403
3. Andersen A, Woodward P, LaFrance N. Bone mineral density of eating disorder subgroups. Int J Eat Disord 1995;18:335–42
4. Hay P, Delahunt J, Hall A, Mitchell A, Harper G, Salmond C. Predictors of osteopenia in premenopausal women with anorexia nervosa. Calcif Tissue Int 1992;50:498–501
5. Rigotti N, Neer R, Skates S, Herzog D, Nussbaum S. The clinical course of osteoporosis in anorexia nervosa. A longitudinal study of cortical bone mass. JAMA 1991;265:1133–8
6. Bolton J, Patel S, Lacey J, White S. A prospective study of changes in bone turnover and bone density associated with regaining weight in women with anorexia nervosa. Osteoporos Int 2005;16:1955–62
7. Lennkh C, de Zwaan M, Bailer U, Strnad A, Nagy C, El-Giamal N, et al. Osteopenia in anorexia nervosa: specific mechanisms of bone loss. J Psychiatr Res 1999;33:349–56
8. Grinspoon S, Miller K, Coyle C, Krempin J, Armstrong C, Pitts S, et al. Severity of osteopenia in estrogen-deficient women with anorexia nervosa and hypothalamic amenorrhea. J Clin Endocrinol Metab 1999;84:2049–55
9. Bachrach L, Guido D, Katzman D, Litt I, Marcus R. Decreased bone density in adolescent girls with anorexia nervosa. Pediatrics 1990;86:440–7
10. Jagielska G, Wolanczyk T, Komender J, Tomaszewicz-Libudzic C, Przedlacki J, Ostrowski K. Bone mineral density in adolescent girls with anorexia nervosa—a cross-sectional study. Eur Child Adolesc Psychiatry 2002;11:57–62
11. Misra M, Aggarwal A, Miller KK, Almazan C, Worley M, Soyka LA, et al. Effects of anorexia nervosa on clinical, hematologic, biochemical, and bone density parameters in community-dwelling adolescent girls. Pediatrics 2004;114:1574–83
12. Soyka L, Grinspoon S, Levitsky L, Herzog D, Klibanski A. The effects of anorexia nervosa on bone metabolism in female adolescents. J Clin Endocrinol Metab 1999;84:4489–96
13. Soyka L, Misra M, Frenchman A, Miller K, Grinspoon S, Schoenfeld D, et al. Abnormal bone mineral accrual in adolescent girls with anroexia nervosa. J Clin Endocrinol Metab 2002;87:4177–85
14. Schneider M, Fisher M, Weinerman S, Lesser M. Correlates of low bone density in females with anorexia nervosa. Int J Adolesc Med Health 2002;14:297–306
15. Turner J, Bulsara M, McDermott B, Byrne G, Prince R, Forbes D. Predictors of low bone density in young adolescent females with anorexia nervosa and other dieting disorders. Int J Eat Disord 2001;30:245–51
16. Castro J, Toro J, Lazaro L, Pons F, Halperin I. Bone mineral density in male adolescents with anorexia nervosa. J Am Acad Child Adolesc Psychiatry 2002;41:613–8
17. Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko P, et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 1992;75:1060–5
18. Bachrach L. Acquisition of optimal bone mass in childhood and adolescence. Trends Endocrinol Metab 2001;12:22–8
19. Biller B, Saxe V, Herzog D, Rosenthal D, Holzman S, Klibanski A. Mechanisms of osteoporosis in adult and adolescent women with anorexia nervosa. J Clin Endocrinol Metab 1989;68:548–54
20. Grinspoon S, Thomas E, Pitts S, Gross E, Mickley D, Miller K, et al. Prevalence and predictive factors for regional osteopenia in women with anorexia nervosa. Ann Intern Med 2000;133:790–4
21. Grinspoon S, Baum H, Lee K, Anderson E, Herzog D, Klibanski A. Effects of short-term recombinant human insulin-like growth factor I administration on bone turnover in osteopenic women with anorexia nervosa. J Clin Endocrinol Metab 1996;81:3864–70
22. Boyar R, Katz J, Finkelstein J, Kapen S, Weiner H, Weitzman E, et al. Anorexia nervosa. Immaturity of the 24-hour luteinizing hormone secretory pattern. N Engl J Med 1974;291:861–5

23. Cara J, Rosenfield R, Furlanetto R. A longitudinal study of the relationship of plasma somatomedin-C concentration to the pubertal growth spurt. Am J Dis Child 1987 May;141:562–4
24. Riggs BL, Khosla S, Melton LJ, III. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 2002;23:279–302
25. Gu G, Hentunen T, Nars M, Harkonen P, Vaananen H. Estrogen protects primary osteocytes against glucocorticoid-induced apoptosis. Apoptosis 2005;10:583–95
26. Iu M, Kaji H, Naito J, Sowa H, Sugimoto T, Chihara K. Low-dose parathyroid hormone and estrogen reverse alkaline phosphatase activity suppressed by dexamethasone in mouse osteoblastic cells. J Bone Miner Metab 2005;23:450–5
27. Michael HHP, Vaananen HK, Hentunen TA. Estrogen and testosterone use different cellular pathways to inhibit osteoclastogenesis and bone resorption. J Bone Miner Res 2005;20:2224–32
28. Klibanski A, Biller B, Schoenfeld D, Herzog D, Saxe V. The effects of estrogen administration on trabecular bone loss in young women with anorexia nervosa. J Clin Endocrinol Metab 1995;80:898–904
29. Miller KK, Grieco KA, Klibanski A. Testosterone administration in women with anorexia nervosa. J Clin Endocrinol Metab 2005;90:1428–33
30. Gordon C, Grace E, Emans S, Goodman E, Crawford M, Leboff M. Changes in bone turnover markers and menstrual function after short-term oral DHEA in young women with anorexia nervosa. J Bone Miner Res 1999;14:136–45
31. Castro J, Lazaro L, Pons F, Halperin I, Toro J. Predictors of bone mineral density reduction in adolescents with anorexia nervosa. J Am Acad Child Adolesc Psychiatry 2000;39:365–70
32. Iketani T, Kiriike N, Nakanishi S, Nakasuji T. Effects of weight gain and resumption of menses on reduced bone density in patients with anorexia nervosa. Biol Psychiatry 1995;37:521–7
33. Miller KK, Grinspoon S, Gleysteen S, Grieco KA, Ciampa J, Breu J, et al. Preservation of neuroendocrine control of reproductive function despite severe undernutrition. J Clin Endocrinol Metab 2004;89:4434–8
34. Pomeroy C, Eckert E, Hu S, Eiken B, Mentink M, Crosby R, et al. Role of interleukin-6 and transforming growth factor-beta in anorexia nervosa. Biol Psychiatr 1994;36:836–9
35. Kahl KG, Kruse N, Rieckmann P, Schmidt MH. Cytokine mRNA expression patterns in the disease course of female adolescents with anorexia nervosa. Psychoneuroendocrinology 2004;29:13–20
36. Monteleone P, Maes M, Fabrazzo M, Tortorella A, Lin A, Bosmans E, et al. Immunoendocrine findings in patients with eating disorders. Neuropsychobiol 1999;40:115–20
37. Raymond N, Dysken M, Bettin K, Eckert E, Crow S, Markus K, et al. Cytokine production in patients with anorexia nervosa, bulimia nervosa, and obesity. Int J Eating Disord 2000;28:293–302
38. Vaisman N, Hahn T, Karov Y, Sigler E, Barak Y, Barak V. Changes in cytokine production and impaired hematopoiesis in patients with anorexia nervosa: the effect of refeeding. Cytokine 2004;26:255–61
39. Corcos M, Guilbaud O, Chaouat G, Cayol V, Speranza M, Chambry J, et al. Cytokines and anorexia nervosa. Psychosom Med 2001;63:502–4
40. Brambilla F, Monti D, Franceschi C. Plasma concentrations of interleukin-1-beta, interleukin-6 and tumor necrosis factor-alpha, and of their soluble receptors and receptor antagonist in anorexia nervosa. Psychiatry Res 2001;103:107–14
41. Misra M, Soyka LA, Miller KK, Herzog DB, Grinspoon S, de Chen D, et al. Serum osteoprotegerin in adolescent girls with anorexia nervosa. J Clin Endocrinol Metab 2003;88:3816–22
42. Grinspoon S, Thomas L, Miller K, Herzog D, Klibanski A. Effects of recombinant human IGF-I and oral contraceptive administration on bone density in anorexia nervosa. J Clin Endocrinol Metab 2002;87:2883–91
43. Golden NH, Lanzkowsky L, Schebendach J, Palestro CJ, Jacobson MS, Shenker IR. The effect of estrogen–progestin treatment on bone mineral density in anorexia nervosa. J Pediatr Adolesc Gynecol 2002;15:135–43
44. Munoz M, Morande G, Garcia-Centenera J, Hervas F, Pozo J, Argente J. The effects of estrogen administration on bone mineral density in adolescents with anorexia nervosa. Eur J Endocrinol 2002;146:45–50
45. Gordon CM, Goodman E, Emans SJ, Grace E, Becker KA, Rosen CJ, et al. Physiologic regulators of bone turnover in young women with anorexia nervosa. J Pediatr 2002;141:64–70
46. Hotta M, Shibasaki T, Sato K, Demura H. The importance of body weight history in the occurrence and recovery of osteoporosis in patients with anorexia nervosa: evaluation by dual X-ray absorptiometry and bone metabolic markers. Eur J Endocrinol 1998;139:276–83
47. Kooh S, Noriega E, Leslie K, Muller C, Harrison J. Bone mass and soft tissue composition in adolescents with anorexia nervosa. Bone 1996;19:181–8
48. Grinspoon S, Baum H, Peterson S, Klibanski A. Effects of rhIGF-I administration on bone turnover during short-term fasting. J Clin Invest 1995;96:900–6
49. Bachrach L, Katzman D, Litt I, Guido D, Marcus R. Recovery from osteopenia in adolescent girls with anorexia nervosa. J Clin Endocrinol Metab 1991;72:602–6
50. Hartman D, Crisp A, Rooney B, Rackow C, Atkinson R, Patel S. Bone density of women who have recovered from anorexia nervosa. Int J Eat Disord 2000;28:107–12
51. Zipfel S, Seibel MJ, Lowe B, Beumont PJ, Kasperk C, Herzog W. Osteoporosis in eating disorders: a follow-up study of patients with anorexia and bulimia nervosa. J Clin Endocrinol Metab 2001;86:5227–33
52. Golden N, Kreitzer P, Jacobson M, Chasalow F, Schebendach J, Freedman S, et al. Disturbances in growth hormone secretion and action in adolescents with anorexia nervosa. J Pediatr 1994;125:655–60
53. Counts D, Gwirtsman H, Carlsson L, Lesem M, Cutler G. The effect of anorexia nervosa and refeeding on growth hormone-binding protein, the insulin-like growth factors (IGFs), and the IGF-binding proteins. J Clin Endocrinol Metab 1992;75:762–7
54. Misra M, Miller K, Bjornson J, Hackman A, Aggarwal A, Chung J, et al. Alterations in growth hormone secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. J Clin Endocrinol Metab 2003;88:5615–23
55. Argente J, Caballo N, Barrios V, Munoz M, Pozo J, Chowen J, et al. Multiple endocrine abnormalities of the growth hormone and insulin like growth factor-I axis in patients with anorexia nervosa: effect of long- and short-term weight recuperation. J Clin Endocrinol Metab 1997;82:2084–92
56. Scacchi M, Pincelli AI, Caumo A, Tomasi P, Delitala G, Baldi G, et al. Spontaneous nocturnal growth hormone secretion in anorexia nervosa. J Clin Endocrinol Metab 1997;82:3225–9
57. Stoving RK, Veldhuis JD, Flyvbjerg A, Vinten J, Hangaard J, Koldkjar OG, et al. Jointly amplified basal and pulsatile growth hormone (GH) secretion and increased process irregularity in women with anorexia nervosa: indirect evidence for disruption of feedback regulation within the GH-insulin-like growth factor I axis. J Clin Endocrinol Metab 1999;84:2056–63
58. Ohlsson C, Bengtsson B, Isaksson O, Andreassen T, Slootweg M. Growth hormone and bone. Endocr Rev 1998;19:55–79
59. Abrams S, Silber T, Esteban N, Vieira N, Stuff J, Meyers R, et al. Mineral balance and bone turnover in adolescents with anorexia nervosa. J Pediatr 1993;123:326–31
60. Abad V, Chrousos G, Reynolds J, Nieman L, Hill S, Weinstein R, et al. Glucocorticoid excess during adolescence leads to a major

persistent deficit in bone mass and an increase in central body fat. J Bone Miner Res 2001;16:1879–85
61. Cino M, Greenberg G. Bone mineral density in Crohn's disease: a longitudinal study of budesonide, prednisone, and nonsteroid therapy. Am J Gastroenterol 2002;97:915–21
62. Di Somma C, Pivonello R, Loche S, Faggiano A, Marzullo P, Di Sarno A, et al. Severe impairment of bone mass and turnover in Cushing's disease: comparison between childhood-onset and adulthood-onset disease. Clin Endocrinol (Oxf) 2002;56:153–8
63. Lettgen B, Jeken C, Reiners C. Influence of steroid medication on bone mineral density in children with nephrotic syndrome. Pediatr Nephrol 1994;8:667–70
64. Hermus A, Smals A, Swinkels L, Huysmans D, Pieters G, Sweep C, et al. Bone mineral density and bone turnover before and after surgical cure of Cushing's syndrome. J Clin Endocrinol Metab 1995;80:2859–65
65. Misra M, Miller K, Almazan C, Ramaswamy K, Lapcharoensap W, Worley M, Neubauer G, Herzog D, Klibanski A. Alterations in cortisol secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. J Clin Endocrinol Metab 2004:Submitted.
66. Boyar R, Hellman L, Roffwarg H, Katz J, Zumoff B, O'Connor J, et al. Cortisol secretion and metabolism in anorexia nervosa. N Engl J Med 1977;296:190–3
67. Walsh B, Katz J, Levin J, Kream J, Fukushima D, Hellman L, et al. Adrenal activity in anorexia nervosa. Psychosom Med 1978;40:499–506
68. Campfield L, Smith F, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 1995;269: 546–9
69. Pelleymounter M, Cullen M, Baker M, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 1995;269:540–3
70. Grinspoon S, Gulick T, Askari H, Landt M, Lee K, Anderson E, et al. Serum leptin levels in women with anorexia nervosa. J Clin Endocrinol Metab 1996;81:3861–3
71. Misra M, Miller KK, Almazan C, Ramaswamy K, Aggarwal A, Herzog DB, et al. Hormonal and body composition predictors of soluble leptin receptor, leptin, and free leptin index in adolescent girls with anorexia nervosa and controls and relation to insulin sensitivity. J Clin Endocrinol Metab 2004;89:3486–95
72. Misra M, Miller KK, Kuo K, Griffin K, Stewart V, Hunter E, et al. Secretory dynamics of leptin in adolescent girls with anorexia nervosa and healthy adolescents. Am J Physiol Endocrinol Metab:00041.2005
73. Tolle V, Kadem M, Bluet-Pajot M-T, Frere D, Foulon C, Bossu C, et al. Balance in ghrelin and leptin plasma levels in anorexia nervosa patients and constitutionally thin women. J Clin Endocrinol Metab 2003;88:109–16
74. Heer M, Mika C, Grzella I, Heussen N, Herpertz-Dahlmann B. Bone turnover during inpatient nutritional therapy and outpatient follow-up in patients with anorexia nervosa compared with that in healthy control subjects. Am J Clin Nutr 2004;80:774–81
75. Herpertz S, Albers N, Wagner R, Pelz B, Kopp W, Mann K, et al. Longitudinal changes of circadian leptin, insulin and cortisol plasma levels and their correlation during refeeding in patients with anorexia nervosa. Eur J Endocrinol 2000;142:373–9
76. Audi L, Mantzoros C, Vidal-Puig A, Vargas D, Gussinye M, Carrascosa A. Leptin in relation to resumption of menses in women with anorexia nervosa. Mol Psychiatr 1998;3:544–7
77. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 2000;100:197–207
78. Tschop M, Smiley D, Heiman M. Ghrelin induces adiposity in rodents. Nature 2000;407:908–13
79. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. Nature 2001;409:194–8
80. Tassone F, Broglio F, Destefanis S, Rovere S, Benso A, Gottero C, et al. Neuroendocrine and metabolic effects of acute ghrelin administration in human obesity. J Clin Endocrinol Metab 2003;88:5478–83
81. Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, et al. Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab 2000;85:4908–11
82. Arvat E, Maccario M, Di Vito L, Broglio F, Benso A, Gottero C, et al. Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. J Clin Endocrinol Metab 2001;86:1169–74
83. Tannenbaum GS, Epelbaum J, Bowers CY. Interrelationship between the novel peptide ghrelin and somatostatin/growth hormone-releasing hormone in regulation of pulsatile growth hormone secretion. Endocrinology 2003;144:967–74
84. Kojima S, Nakahara T, Nagai N, Muranaga T, Tanaka M, Yasuhara D, et al. Altered ghrelin and peptide YY responses to meals in bulimia nervosa. Clin Endocrinol (Oxf) 2005;62:74–8
85. Vulliemoz NR, Xiao E, Xia-Zhang L, Germond M, Rivier J, Ferin M. Decrease in luteinizing hormone pulse frequency during a five-hour peripheral ghrelin infusion in the ovariectomized rhesus monkey. J Clin Endocrinol Metab 2004;89:5718–23
86. Maccarinelli G, Sibilia V, Torsello A, Raimondo F, Pitto M, Giustina A, et al. Ghrelin regulates proliferation and differentiation of osteoblastic cells. J Endocrinol 2005;184:249–56
87. Hotta M, Ohwada R, Katakami H, Shibasaki T, Hizuka N, Takano K. Plasma levels of intact and degraded ghrelin and their responses to glucose infusion in anorexia nervosa. J Clin Endocrinol Metab 2004;89:5707–12
88. Misra M, Miller KK, Herzog DB, Ramaswamy K, Aggarwal A, Almazan C, et al. Growth hormone and ghrelin responses to an oral glucose load in adolescent girls with anorexia nervosa and controls. J Clin Endocrinol Metab 2004;89:1605–12
89. Misra M, Miller KK, Kuo K, Griffin K, Stewart V, Hunter E, Herzog DB, Klibanski A. Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescents. Am J Physiol Endocrinol Metab:00615.2004
90. Misra M, Miller K, Stewart V, Hunter E, Kuo K, Herzog D, Klibanski A. Ghrelin and bone metabolism in adolescent girls with anorexia nervosa and healthy adolescents. J Clin Endocrinol Metab:Jul 5 2005; 90:5082–7
91. Batterham R, Cowley M, Small C, Herzog H, Cohen M, Dakin C, et al. Gut hormone PYY(3–36) physiologically inhibits food intake. Nature 2002;418:650–4
92. Stock S, Leichner P, Wong ACK, Ghatei MA, Kieffer TJ, Bloom SR, et al. Ghrelin, Peptide YY, glucose-dependent insulinotropic polypeptide, and hunger responses to a mixed meal in anorexic, obese, and control female adolescents. J Clin Endocrinol Metab 2005;90:2161–8
93. Misra M, Miller K, Tsai P, Gallagher K, Lin A, Lee N, et al. Elevated Peptide YY levels in adolescent girls with anorexia nervosa. J Clin Endocrinol Metab 2006;91:1027–33
94. Baldock PA, Sainsbury A, Couzens M, Enriquez RF, Thomas GP, Gardiner EM, et al. Hypothalamic Y2 receptors regulate bone formation. J Clin Invest 2002;109:915–21
95. Golden NH, Iglesias EA, Jacobson MS, Carey D, Meyer W, Schebendach J, et al. Alendronate for the treatment of osteopenia in anorexia nervosa: a randomized, double-blind, placebo-controlled trial 10.1210/jc.2004-1659. J Clin Endocrinol Metab 2005;90:3179–85
96. Miller KK, Grieco KA, Mulder J, Grinspoon S, Mickley D, Yehezkel R, et al. Effects of risedronate on bone density in anorexia nervosa. J Clin Endocrinol Metab 2004;89:3903–6