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INVITED REVIEW

Anorexia nervosa in female adolescents: endocrine and bone mineral density disturbances

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Abstract

Anorexia nervosa (AN) is a chronic childhood psychiatric illness that involves a reduction in caloric intake, loss of weight and amenorrhea, either primary or secondary. The diagnostic criteria for AN have been established by the American Psychiatric Association. The prevalence of this disease amongst adolescents and young adults is between 0.5 and 1% and the incidence of new cases per year is approximately 5-10/100 000 between 15 and 19 years of age.

A number of endocrine and metabolic disturbances have been described in patients with AN including amenorrhea–oligomenorrhea, delayed puberty, hypothyroidism, hypercortisolism, IGF-I deficiency, electrolyte abnormalities, hypoglycemia and hypophosphatemia, among others. In addition to prolonged amenorrhea, osteopenia and osteoporosis are the most frequent complications leading to clinically relevant fractures and increased fracture risk throughout life. Patients exhibit an alteration in the hypothalamic–pituitary–gonadal axis, which is responsible for the menstrual disorders. The increase in gonadotropin secretion that can be observed after ponderal recuperation suggests that malnutrition could be the most important mechanism involved in the decrease in gonadotropin secretion.

The loss of fat tissue as a consequence of nutrient restriction has been associated with hypoleptinemia and abnormal secretion of peptides implicated in food control (neuropeptide Y, melanocortins and corticotropin-releasing factor, among others).

A review of the endocrine abnormalities, disturbances in neurotransmitters, as well as a detailed analysis of bone markers and bone mineral density in patients with AN is described.

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Introduction

Anorexia nervosa (AN) is a childhood psychiatric disorder that is characterized by patient-induced and -maintained weight loss that leads to progressive malnutrition and specific pathophysiological signs (disturbance of body image and fear of obesity). The diagnostic criteria for AN according to the American Psychiatric Association (1) are as follows: (a) refusal to maintain body weight at or above a minimally normal weight for age and height; (b) intense fear of gaining weight or becoming fat, even though underweight; (c) disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight; and (d) in postmenarcheal females, amenorrhea (absence of at least three consecutive menstrual cycles). Based on the presence or not of bulimic symptoms, AN appears in two specific subtypes, restricting and binge-eating/purging (1).

Although pure forms can rarely be seen, the prevalence of this disease in adolescents and young adults is between 0.5 and 1%. The incidence of new cases per year is approximately 5-10/100 000 per year between 15 and 19 years of age (2). A recent study of a Spanish female population with ages between 12 and 21 years showed a prevelance of 0.3% for AN, 0.8% for bulimia and 3.1% for non-specified eating disorders, resulting in a total sum of 4.1% of the population suffering from some type of eating disorder (3). Complications in many organ systems can be seen, including the cardiovascular and peripheral vascular systems, gastrointestinal, hematological, renal, skeletal, endocrine and metabolic systems, amongst others. These alterations are not only related to the state of malnutrition, but also to the conduct used by these patients to control their weight. The endocrine and metabolic disturbances include amenorrhea–oligomenorrhea, delayed puberty, hypothyroidism, hypercortisolism, insulin-like growth factor (IGF)-I deficiency, electrolyte abnormalities, hypoglycemia

and hypophosphatemia, amongst others, with numerous studies of AN patients indicating hypothalamic dysfunction as a possible basis. We have here reviewed the endocrine and metabolic aspects of this eating disorder with a special emphasis on bone mineral disturbances.

Appetite-regulating peptides

The hypothalamus is important in the control of energy metabolism. In effect, it is responsible for the sensation of hunger and satiety and, therefore, energy intake. In addition, via modulation of the sympathetic nervous system, it is also involved in thermogenesis and energy expenditure. A variety of neuropeptides control these functions (4). Numerous substances synthesized in different parts of the body send afferent signals back to these hypothalamic centers to modify, either positively or negatively, appetite or energy expenditure. Regulation of acute eating behavior incorporates a system of satiety signals that originate from the food ingested. Cholecystokinin, bombesin, gastrin-releasing peptide, amongst others, are involved in this signaling system and reach the brain via peripheral innervation or the circulation to activate their receptors in the brain (5).

Long-term energy balance is regulated via a system comprised of different hormones secreted in proportion to corporal adiposity, such as leptin and insulin, that act at the level of the central nervous system (CNS). These respond to changes in body fat by activating anabolic or catabolic pathways (6), the first through production of neuropeptide Y (NPY), which stimulates food intake, and the second via the hypothalamic melanocortin system, which reduces food intake and stimulates weight loss (7) (Fig. 1).

Leptin is synthesized by adipose tissue and is involved in the regulation of food intake and energy expenditure.

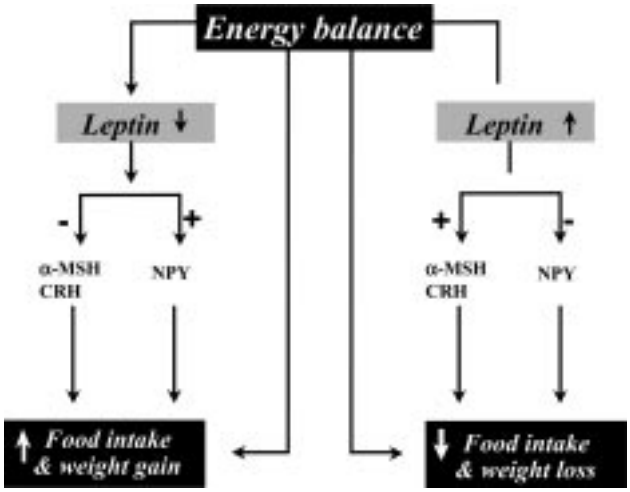

Figure 1 Model of energy balance regulation. α-MSH, α-melanocyte-stimulating hormone; CRH, corticotropin-releasing hormone.

The mechanisms of action of this hormone are largely unknown, but many studies demonstrate that its primary target is the hypothalamus (8). Plasma levels of leptin and its secretory pattern vary during the night and day and are influenced by food intake (9). Acting at the level of the hypothalamus, leptin effects a decrease in appetite, which in turn results in weight loss and activation of the gonadal axis by stimulating gonadotropin-releasing hormone (GnRH) secretion. Mutation analysis of the coding region and part of the promoter region of the leptin gene in patients with AN has yielded negative results, suggesting that involvement of this gene in the etiology of AN is unlikely (10).

NPY is produced throughout the CNS and acts as a central stimulator of feeding. Its actions, incrementing ingestion and decreasing thermogenesis (11) are, hence, opposite that of leptin. The neuropeptide YY5 and YY1 receptors in rats and humans are assumed to play a major role in NPY-induced food intake. The neuropeptide YY5 receptor gene (NPYY5R) is expressed in brain regions known to be involved in the central regulation of feeding behavior, including the lateral hypothalamus, the paraventricular nucleus and the arcuate nucleus (12). Systematic mutation screening within the coding region of the NPYY5R revealed a rare Glu-4-Ala variant in a single patient with AN. This allele was transmitted from the mother who had no history of any eating disorder. Association and transmission disequilibrium studies pertaining to variations and polymorphisms within the NPYY1R and NPYY5R and AN were negative (13).

Glucocorticoids are also implicated in energy regulation. Via their effects on NPY, they act as endogenous antagonists of leptin and insulin (14). Other neuropeptides that stimulate food intake and energy storage are melatonin-concentrating hormone and orexin A and B, which increase in response to fasting and stimulate appetite (15). The melanocortins (MC) are peptides derived from the precursor pro-opiomelanocortin (POMC) and act on specific receptors. The endogenous MC most implicated in food intake and body weight is α-melanocyte-stimulating hormone, which has a high affinity for the MC receptors, especially MC3 and MC4 (16). Mutation screening of the coding region of MC4 receptors in patients with AN and bulimia nervosa revealed two common polymorphisms in both groups. Allele and genotype frequencies did not differ between these groups and probands of different weight extremes (17).

Serotonin (5-hydroxytryptamine; 5-HT) is involved in a broad range of biological, physiological and behavioral functions. Its actions are regulated by biosynthetic enzymes (tryptophan hydroxylase), specific receptors, as well as by an inactivation system involving neuronal re-uptake (serotonin transporter) (18). The serotonergic system has been implicated in the development of eating disorders (19). Polymorphisms

within different 5-HT receptor genes and the tryptophan hydroxylase gene have been analysed and an association between the −1438 A allele of the −1438 G/A polymorphism within the promoter region of the 5-HT2A receptor gene and AN has been reported (20, 21). However, others studies could not confirm this association (22). Nacmias *et al.* (23) reported an association between the −1438 A allele and AN of the restricting subtype, whereas allele frequencies in patients with the binge/purging subtype did not differ from those of controls. Aubert *et al.* (24) found that the −1438 A allele was associated with lower energy intake and lower alcohol consumption in obese individuals, a finding which they assumed to be consistent with the higher frequency of the A allele in patients with AN. These studies suggest that patients with AN have enhanced 5-HT2A receptor binding and provide further evidence for a serotoninergic dysfunction in eating disorders, but these results need further confirmation (25).

Hypothalamic–pituitary–adrenal axis

Adrenocorticotropin (ACTH) and the endogenous opioids are derived from the same precursor, POMC. Increased ACTH secretion is preceeded by activation of the POMC system. The opioid system has both direct and indirect influences over food intake and the level of physical activity. In laboratory animals, opioid administration stimulates appetite via receptors in the paraventricular nucleus and opioid antagonists such as naloxone reduce appetite (26). Corticotropin-releasing hormone (CRH), synthesized in neurons of the paraventricular nucleus, is regulated, at least in part, by leptin and insulin and its administration i.c.v. induces a reduction in food intake and weight loss (27).

We and others have observed that plasma cortisol levels in female adolescents with AN are often elevated while its circadian rhythm is conserved (28). Dexamethasone can partially suppress this hypercortisolemia, which is similar to that observed in patients with depression and Cushing's disease. Recently, Foppiani *et al.* (29) demonstrated that DDAVP does not stimulate ACTH and cortisol in AN patients. This fact could be due to a down-regulation of hypophyseal 1-deamino-8D-arginine vasopressin (DDAVP) V3 receptors in AN (29). In acute situations of AN, the dexamethasone test has no medical significance; however, in patients that are recovering weight it may have prognostic value (30). Refeeding studies of anorexic patients have shown that, irrespective of the initial weight, weight gains as low as 10% are associated with the normalization of cortisol secretion (29).

The mean plasma half-life of cortisol is prolonged (31) and ACTH levels are within the normal range, but the ACTH response to CRH is inferior to that of control patients (32). Putignano *et al.* (33) demonstrated that, in untreated AN patients, plasma and salivary cortisol and urinary free cortisol concentrations are increased, and cortisol suppression after the dexamethasone suppression test is decreased in both plasma and saliva. These alterations were less pronounced in AN patients who were being treated (33). This hypercortisolemia could reflect a defect at the level of the hypothalamus, or even higher up, that results in the hypersecretion of CRH. Taken together, these observations suggest a situation of CRH hypersecretion or peripheral cortisol resistance (34).

Hypothalamic–pituitary–ovarian axis

Patients with AN exhibit isolated hypogonadotropic–hypogonadism of hypothalamic origin and uncertain etiology. Multiple factors could play a role, including hypothalamic dysfunction, weight reduction, sex steroid or neurotransmitter alterations and excess physical exercise, among others. Women with AN exhibit low basal levels of gonadotropins, as well as low estradiol levels, indicating abnormal functioning of the hypothalamic–pituitary–gonad axis (35). We have demonstrated that spontaneous secretion of luteinizing hormone (LH) during 24 h is decreased in both the frequency and the amplitude of the secretory bursts (36). Weight recuperation increases serum levels of both LH and follicle-stimulating hormone, suggesting that malnutrition could be implicated in the regulation of gonadotropin secretion. Disturbances in neurotransmitters implicated in GnRH regulation, including the dopaminergic system and endogenous opioids, have been described (37). Whether these alterations are primary or secondary to malnutrition remains to be elucidated.

Malnutrition could be responsible for the delayed puberty and reduction in growth in AN patients, as the shut-down of these two systems has been interpreted as an adaptation mechanism to a reduction in nutrients. Puberty is delayed if the symptoms of AN start during the prepubertal period. In contrast, if the illness begins once development has already started, puberty is detained and the growth spurt delayed and reduced (38). Finally, if symptoms appear when puberty has ended, secondary amenorrhea is present. One of the signals that begins the process of adaptation to malnutrition is hypoinsulinemia, which is present as a consequence of low glucose and amino acid levels (39). Furthermore, growth hormone (GH) abnormalities and low IGF-I levels contribute to poor growth in prepubertal patients, leading to a reduction in their final height (Fig. 2). Reliable data are not available to establish the percentage of growth lost in these patients, with the exception of one study where it was estimated that 3 cm is lost in some patients in relationship to their target height (40).

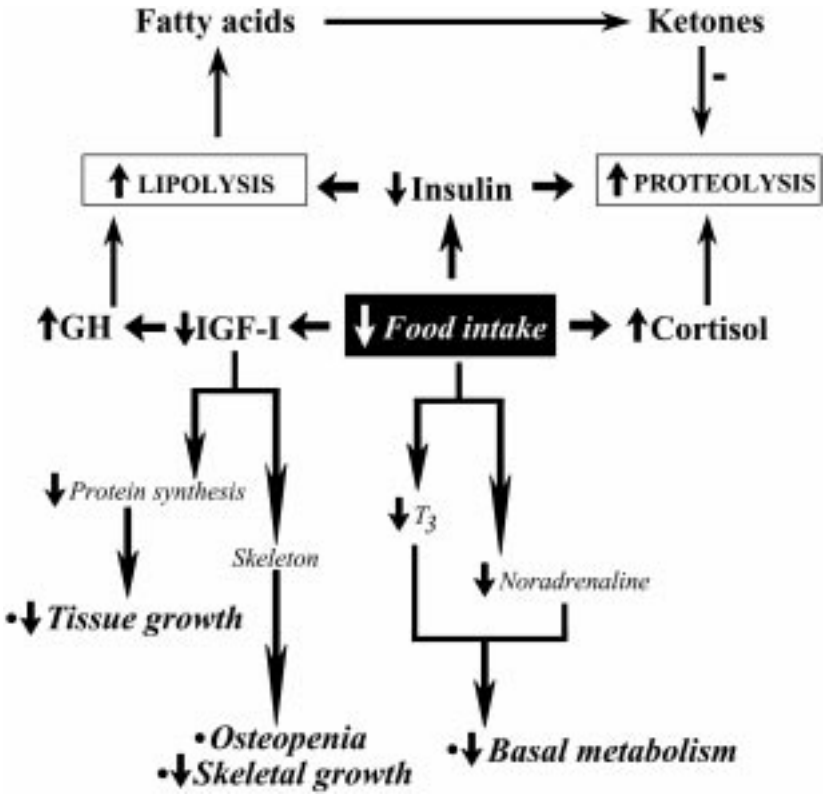

Figure 2 Hormonal response to malnutrition. T_3, tri-iodothyronine.

Leptin is also involved in gonadal development and reproductive function. Its receptors are expressed in the anterior pituitary and gonads, and the biological significance of this is a current object of investigation (41). It is suggested that leptin is involved in the regulation of GnRH secretion and plays a role in the initiation and maintainance of gonadal function in humans (42). Whether leptin is only a permissive factor or plays a central role in the onset of puberty remains unknown. Problems in the functioning of the reproductive system, including a reduction in serum sex-steroid levels, are frequent in subjects with reduced fat stores (43, 44), as occurs in patients with AN. This decrease in gonadal function could be related to the reduced serum leptin levels as a result of the loss of fat tissue. In contrast to this hypothesis, we have observed that in patients with AN during partial weight recuperation there is no significant increase in leptin levels or in the recovery of gonadal function, at least simultaneously (9) (Fig. 3). It is possible that a longer weight recuperation period is necessary for this to occur. Some studies suggest that body mass index (BMI) is the most important control factor for the secretion of leptin in situations of modified food intake (45) and that there is a loss in circadian rhythms in patients with AN (46). One aspect of great interest is whether leptin is necessary for the recovery of menstruation in these patients (47); however, more investigation is necessary in order to answer this question.

There is a clear association between melatonin levels and gonadal function in humans, with women with hypothalamic amenorrhea having elevated nocturnal melatonin levels (48). Patients with AN also have elevated nocturnal levels of 6-sulfatoximelatonin (the principal metabolite excreted in the urine) both at diagnosis and after weight recuperation if the amenorrhea persists (49, 50). This could be due to insufficient weight gain or the need for a longer period of weight recuperation.

The percentage of total body fat can now be evaluated by using dual-energy X ray absorptiometry and, in patients with AN and moderate malnutrition, the percentage of total body fat appears to be a better indicator of the nutritional state than BMI. There is a significant correlation between leptin levels and the percentage of total body fat that is not found between BMI and leptin (51, 52). It has been reported that if patients with AN recuperate weight to obtain at least 90% of the weight adequate for their height, their menstrual cycles will return within the following 6 months. It therefore follows that one of the decisive factors for the normalization of gonadal function is recovery of the nutritional state and body mass (53).

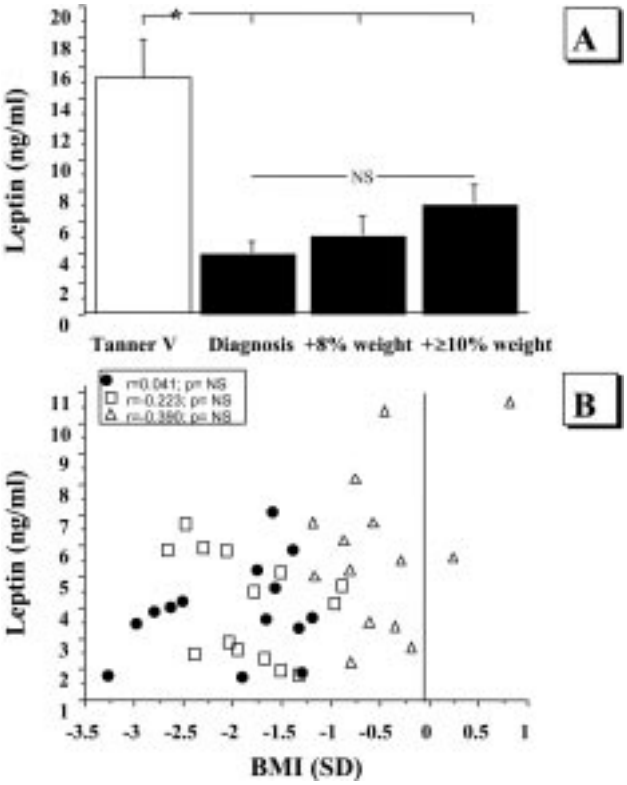

Figure 3 (A) Mean circulating leptin plasma levels (±S.E.M.) in women with AN, at the time of diagnosis, after recovery of 8% of their original body weight and after regaining at least 10% of their original body weight (approximately 1 year after starting treatment). These are compared with their age- and sex-matched controls (Tanner V females); one-way ANOVA, $*P < 0.0001$; NS, not significant. (B) Linear correlation between fasting serum leptin levels and body mass index, expressed as standard deviation (BMI (SD)), in women with AN, at the time of diagnosis and after at least partial recovery of their original body weight. Patients with AN at the time of diagnosis (●), patients with AN after 8% recuperation of their original weight (□) and patients with AN after recuperation of ≥10% of their original weight (△).

GH–IGF-I axis

Most studies indicate that a large percentage of patients with AN have elevated basal and GH-releasing hormone (GHRH)-stimulated GH levels (54). A number of abnormalities in GH response to different stimuli have been described (decreased response after hypoglycemia, clonidine, hexarelin and dexamethasone), as well as paradoxical hormone responses (elevated GH after thyrotropin-releasing hormone (TRH), thyrotropin (TSH) or i.v. glucose), although these responses are heterogeneous (55–57). Few studies have analyzed spontaneous GH secretion (SGHS) in AN patients. Scacchi *et al.* (58) reported that increased GH secretion could be the result of an increased frequency of secretory bursts superimposed on enhanced tonic GH secretion. We studied SGHS in a group of anorexic patients at the time of diagnosis and two different times during weight recuperation and found that at the time of diagnosis SGHS is heterogeneous (59). In 40% of these subjects, mean 24-h GH secretion was greater than 3 ng/ml (the lower limit of normality) and the remaining 60% had levels below the normal range. The difference between these groups and the controls was due to modification in the amplitude of the GH peaks and not to the frequency. In both groups, recuperation of at least 10% of their initial weight resulted in the normalization of the parameters of SGHS. More recently, Støving *et al.* (60) analyzed SGHS in eight patients with AN by using the deconvolution method and found that the half-life of GH in these patients is normal. The integrated concentration of GH was ten times greater in AN patients compared with controls. The frequency of the GH peaks, their duration and the amount of GH secreted in each peak, as well as basal GH levels, were elevated. These parameters all correlated negatively with BMI.

These observations suggest that the alterations in GH secretion in these patients are due to modifications in its neuroendocrine control, with an increase in GHRH release and decreased somatostatin tone. The GH pattern in conjunction with the negative correlation between basal and pulsatile GH secretion and BMI suggests that the alterations in GH secretion observed in AN are directly related to malnutrition (59). One possible mechanism could involve the reduced IGF-I levels produced by malnutrition. Gianotti *et al.* (61) showed that a low IGF-I dose inhibits, though does not normalize, spontaneous and stimulated GH secretion in patients with AN. These findings indicate the existence of a defective hypothalamic control of GH release (61). This would effect a reduction in the negative feedback action that IGF-I exerts on GH secretion at the level of both the hypothalamus and the pituitary (60). Another variable that could be involved in the alterations in GH secretion is the hypoestrogenism that accompanies amenorrhea. It has been suggested that malnutrition underlies the increase in the amount of GH secreted in each pulse and that hypoestrogenism is responsible for the increased pulse frequency. However, these studies are still insufficient to draw definite conclusions.

Recently it has been demonstrated that a gastrointestinal peptide hormone, ghrelin, stimulates GH secretion in rats and humans. Ghrelin also plays an important role in the regulation of energy balance. Plasma ghrelin levels are regulated by acute and chronic changes in energy balance, e.g. fasting increases while feeding decreases circulating ghrelin concentrations (62). Otto *et al.* (63) showed that plasma ghrelin levels in patients with AN are significantly increased and rapidly normalize after partial weight recovery. While decreased leptin levels in AN patients might simply reflect reduced body fat mass, increased gastric ghrelin secretion in AN might, however, reflect a physiological effort to compensate for the lack of nutritional intake and stored energy (64).

Serum GH-binding protein (GHBP) levels in patients with AN are dramatically reduced (58, 65, 66) and tend to normalize with weight recuperation (58, 67). This reduction in GH receptors is most likely one of the principal causes of GH resistance. It has been suggested that in malnutrition the low GHBP levels could be related to hypoinsulinemia, alterations in thyroid function or hypoestrogenism (68, 69). On the other hand, many studies have demonstrated a correlation between serum GHBP levels and BMI or the percentage of body fat, and more specifically, with visceral fat (70). Given that it has not been demonstrated that circulating GHBP is uniquely or even preferentially derived from liver GH receptors, it is possible that other tissues, such as adipose tissue, could contribute to plasma GHBP levels. If this is the case, the extreme reduction in adipose tissue in patients with AN could result in the observed decrease in serum GHBP levels (71, 72).

Patients with AN have extremely reduced serum IGF-I levels that tend to normalize with weight recuperation (58, 73); however, the time necessary for this to occur, as observed in other forms of malnutrition, may be prolonged (74). Circulating IGF-I is largely dependent on GH, but it is also very sensitive to nutritional changes. In AN patients, serum IGF-I levels do not correlate with GH secretion (58), which suggests that the decrease in IGF-I is independent of GH and probably directly due to the state of malnutrition. Thus, the coexistence of reduced IGF-I levels and normal or elevated GH secretion suggests that these patients exhibit resistance to GH action.

The available data in respect to serum levels of free IGF-I are limited and contradictory. Some authors find them to be normal (58), while others report them to be decreased (75); however, in both cases weight recuperation tended to increase free IGF-I levels. A similar phenomenon is seen with IGF-II levels. We (58) and others (59) found serum IGF-II levels to be normal at the time of diagnosis and to increase with weight recuperation. In contrast, Counts *et al.* (66) reported IGF-II levels to be decreased, although not significantly, and to normalize with weight recuperation. Serum levels of free IGF-II are reported to be decreased in patients with AN and to increase with weight recuperation (59).

Patients with AN have elevated serum IGF-binding protein (IGFBP)-1 and IGFBP-2 levels that tend to normalize with weight recuperation (58, 66). Both factors are reported to be GH independent and very sensitive to nutritional regulation. The increase in IGFBP-1 in these patients is most likely related to hypoinsulinism, although other metabolic or hormonal factors, such as increased glucagon and glucocorticoids levels, as well as decreased intracellular glucose or other specific substrates, may be involved (76). In AN, as in other forms of malnutrition, the increase in IGFBP-2 most likely depends on the combined influence of caloric-protein restriction, hypoinsulinism and GH resistance (76).

Serum IGFBP-3 levels are decreased in AN patients as a consequence of GH resistance and tend to normalize after weight recuperation (58, 66). Indeed, all of the components of the trimolecular complex formed by the union between IGFBP-3, IGF and the acid-labile subunit (ALS) are decreased (68). Given that these proteins are all GH dependent and regulated by the nutritional state, this is not unexpected. IGFBP-3 decreases significantly with caloric restriction, but in adults only with protein restriction (77). In contrast to that observed in other catabolic situations, increased proteolysis of IGFBP-3 has not been observed in AN (78).

Although present in the serum in low concentrations, both IGFBP-4 and IGFBP-5 are very important in the process of bone formation, where at the cellular level they regulate the actions of the IGFs. In AN, serum levels of both of these IGFBPs are dramatically reduced and do not normalize with partial weight recuperation (79).

The biological significance of the changes in IGFBPs that occur in AN or malnutrition is difficult to explain, in part because the physiological roles of the binding proteins are not totally understood. The decrease in serum IGFBP-3, and therefore the trimolecular complex IGFBP-3–IGF–ALS, impedes the retention of IGF in the vascular space, favoring the decrease in plasma levels (59). On the other hand, the increase in IGFBP-1 and IGFBP-2, two proteins with low molecular weights that can cross the vascular barrier, would favor even more the movement of the IGFs to the tissues where they can act. Therefore, modification of the serum and tissue levels of the IGFBPs could be one of the mechanisms by which malnutrition regulates the concentration and actions of the IGFs and the somatotrope axis (68).

Hypothalamic–pituitary–thyroid axis

The majority of patients with AN present a condition called 'low T_3 syndrome' characterized by low triiodothyronine (T_3), normal or low thyroxine (T_4) and normal TSH (80). We have observed that T_3 levels are reduced at the time of diagnosis and normalize with weight gain, while T_4 and TSH levels are within the normal range at both time-points (81). The extremely reduced T_3 levels in these patients is due to the existence of altered peripheral deiodination that preferentially transforms T_4 into the inactive metabolite, reverse T_3. These thyroid alterations normalize with weight recuperation (82).

Stoving *et al.* (83) determined thyroid volume by ultrasonographic methods in patients with AN and demonstrated that this gland is markedly reduced in comparison with age- and sex-matched controls. This thyroid atrophy is not due to low TSH levels, as TSH levels are usually normal in AN. However, thyroid size is influenced by IGF-I, and the low IGF-I levels in these patients could contribute to thyroid atrophy

(83). A blunted and delayed TSH response to exogenously administered TRH has been reported in about 25–50% of AN patients. Hence, there is evidence that AN is generally associated with hypothalamic–pituitary–thyroid dysfunction (82).

Bone mineral density and bone markers

Osteopenia is a frequent and often persistent complication of AN, leading to clinical fractures and increased fracture risk throughout life (84, 85). According to the existing international literature, more than 50% of AN patients present with osteopenia at the time of diagnosis. Anorexic patients with an average illness duration of 5.8 years were found to have an annual fracture rate seven times greater than that of healthy women of the same age (86). Estrogen deficiency is an important risk factor for bone loss and osteoporosis, whereas malnutrition and low body weight may also increase the risk of osteoporosis by estrogen-dependent and non-estrogen-dependent mechanisms (87).

The accrual of optimal bone mineral deposits in the skeleton during infancy, adolescence and adulthood depends on the interaction of nutritional factors, hormones and lifestyle (88). The bone mass of an individual increases with age, weight and height. It is estimated that approximately 35% of the bone mineral content is acquired during the first 3 years of life. Between 4 years of age and the start of puberty another 20% is obtained and during adolescence the remaining 45% of bone mineral content is acquired (89). Bone density and bone metabolism change dramatically during adolescence, and the onset of AN during this critical time may interfere with the achievement of peak bone mass. In addition, significant changes in body weight and composition, pubertal development and pubertal hormones, such as estradiol and IGF-I, occur in AN and may affect bone metabolism. However, the effect of AN on bone turnover in adolescent girls remains poorly understood (90).

It is possible that the degree of osteopenia depends on the age at which the amenorrhea began, as well as its duration. Indeed, it has been demonstrated that patients with primary amenorrhea have more severe osteopenia than those that present with secondary amenorrhea. Other factors, such as bone mass at the onset of the illness, genetic factors and the level of physical activity of the patient, also play important roles in this process (91). Zipfel *et al.* (92) investigated the course of bone mineral density (BMD) in patients with AN over a 3.6 year follow-up period. Non-recovered AN patients with the binge-eating/purging subtype had a significantly reduced BMD compared with patients with the restricting subtype. These results suggest that patients of the binge-eating/purging subtype are at a higher risk for osteoporosis and may need additional therapy to prevent bone loss. The BMI at follow-up and subtype of AN were reported to be the best predictors of lumbar spine BMD (92). Another study showed the value of the frequency of vomiting in predicting lumbar spine BMD (93).

Karllson *et al.* (94) reported that a substantial proportion of the bone mass deficit in anorexic patients was due to smaller bone size. Illness recovery was associated with near normal bone size and volumetric BMD. However, incomplete recovery of lean and fat mass may account for part of the remaining deficit in bone size, but not volumetric BMD (94). Another study, examining 19 recovered anorexic women at 21 years post-recovery, while showing reduced BMD at the femoral neck, found that BMD of the lumbar spine was not significantly different from that of controls (95).

Administration of estrogens and gestagens to adolescents with reduced bone mass and amenorrhea for at least 1 year indicated that the osteopenia cannot be reversed (96). However, in those patients capable of spontaneously recuperating menstruation, a 20% increase in bone mass compared with the time of diagnosis was seen (87). Karlsson *et al.* (94) recently reported that AN occurring during adolescence impaired both mineral accrual, as measured by volumetric BMD, and bone size. Whereas reduced volumetric BMD could be related to estrogen deficiency, reduced bone size no longer existed after adjusting for fat and lean mass (94). Weight, but not estrogen use, is a significant predictor of BMD in anorexic women at all skeletal sites. The reason why estrogens are incapable of increasing bone mass in adolescents with AN and amenorrhea is currently not known. It may be due to failure to administer estrogen therapy at diagnosis or to poor compliance, or perhaps to the short duration of recovery. Furthermore, in addition to the deficit in estrogens, the decrease in other nutritional and hormonal factors is also involved in the pathogenesis of bone mass loss (97, 98). Therefore, estrogen replacement alone may not be sufficient for recovery of BMD.

The *in vivo* effects of leptin on bone metabolism are controversial. Ogueh *et al.* (99) found an inverse association between fetal blood levels of leptin and the cross-linked carboxyterminal telopeptide of type 1 collagen, a marker of bone resorption. They concluded that leptin might decrease bone resorption with the overall effect of increasing bone mass in the human fetus (99). Pasco *et al.* (100) reported that in non-obese women there is a positive correlation between bone mass and serum leptin levels independent of body weight and fat mass. There is also an inverse relationship between serum leptin and a marker of bone formation and BMD in healthy adult men (101). Therefore, leptin may be a regulator of BMD in humans.

Ducy *et al.* (102) reported an increase in bone mass in rats with a non-functional mutation in the leptin gene or of the leptin receptor gene, demontrating that this

increase was due to increased osteoblast activity, since osteoclast function was normal (102). Intraventricular infusion of leptin caused a loss of bone mass in these animals. Therefore, leptin could regulate osteoblast metabolism via a hypothalamic mechanism (103).

The effect of leptin administration compared with estrogen therapy in ovariectomy-induced bone loss in rats has also been reported (104). Leptin was effective in reducing trabecular bone loss, trabecular architectural changes and periosteal bone formation. These findings suggest that leptin could regulate bone remodeling and this effect may be, at least in part, mediated by the osteoprotegerin (OPG)/receptor for activation of the nuclear factor κB (RANK) ligand pathway. RANK and RANK ligand (RANKL) are members of the tumor necrosis factor (TNF) and TNF receptor superfamilies, which are essential for osteoclast differentiation. In the bone microenvironment, the stimulatory effects of RANKL are neutralized by the secreted decoy receptor, OPG (105, 106). It follows that the balance between OPG and RANKL secretion by stroma cells is critical for the regulation of osteoclast formation (107). The dramatic decline in leptin levels observed in AN may be one of the major hormonal factors involved in the pathogenesis of the associated bone fragility through diminishing cortical bone formation rates and skeletal growth. Leptin may play an important protective role in bone metabolism by inhibitory bone resorption (108, 109).

The available data regarding useful markers of bone remodeling in patients with AN are few and contradictory. However, the identification during the past few years of new peptides involved in this process has significantly improved the sensitivity and specificity of bone formation studies in different anomalies. Regarding markers of bone formation, the bone isoenzyme of alkaline phosphatase (bAP) and the amino-terminal pro-peptide of procollagen I (PNIP) have the greatest diagnostic sensitivity in detecting anomalies in bone remodeling, at least in osteoporetic women (110). Amongst the markers of bone resorption, the telopeptide carboxyterminal of the α1 chain of type 1 collagen (CTX) shows good specificity and sensitivity in the investigation of bone metabolism (111).

Studies from our group have demonstrated that serum levels of bAP are directly correlated with the degree of osteopenia, with no correlation between PNIP and osteopenia (112). It is interesting to note that we did not find differences in serum bAP levels between patients with AN and controls. However, when the data were analyzed individually, we found that the anorexic patients could be divided into three groups, those with elevated, normal or decreased serum bAP levels. The patients with the lowest levels of bAP had the lowest degree of osteopenia, and the smallest decrease in BMI and of α- and β-CTX. On the other hand, those patients with the greatest loss of bone mass had the highest levels of bAP, as well as of α- and β-CTX (113). Hence, the level of osteopenia correlates with their levels of bAP. The urinary fragments of CTX in patients with AN are derived primarily from old bone (β-CTX), while in young adolescents they are primarily from new bone (α-CTX). Therefore, α-CTX is more adequate for measuring bone resorption in controls, while β-CTX is more adequate in anorexic patients (113, 114).

Grinspoon *et al.* (115) demonstrated that the rate of bone resorption was significantly increased and bone formation significantly decreased in a group of AN patients before treatment. Markers of bone metabolism were reported to be normal in AN patients whose BMI reached at least 16.5 kg/m^2 (116). Anorexic patients at baseline and a subgroup of non-recovered patients at follow-up have also been shown to have elevated bone resorption, but normal bone formation. Patients with binge-eating/purging AN showed the highest level of bone resorption. Recovered patients who had an unchanged lumbar spine BMD at follow-up had low bone turnover, with both bone resorption and bone formation activity being decreased (90).

Nutritional factors play an important role in the process of bone mineralization, supplying essential factors for its realization. Deficiency in growth factors (116), especially IGF-I, most likely due to the state of malnutrition, as well as the slow recuperation of their plasma levels with weight gain, is known to occur in these patients. However, we do not know whether these patients would benefit from the administration of biosynthetic GH or recombinant IGF-I (117). Several trials have analyzed the effect of recombinant human (rh) IGF-I on bone formation in AN patients (118, 119). Administration of rhIGF-I at a dose of 100 μg/kg, s.c., twice a day for 6 days increased metabolic markers of bone formation, as well as of bone resorption. However, injection of rhIGF-I at dose of 30 μg/kg, s.c., per day stimulated only the production of bone formation markers (115). Hotta *et al.* (116) demonstrated that an improvement in nutritional status in AN patients via i.v. hyperalimentation therapy rapidly increases serum IGF-I levels, which is followed by a progressive increase in osteocalcin, suggesting an immediate start of bone formation. However, increased bone resorption appears to continue for at least 5 weeks (116).

In patients with AN, the mechanism of bone loss does not appear to be due to an increase in absorption over formation with respect to that seen in controls. It is possible that the increase in bone remodeling that is observed is a mechanism developed in an attempt to restore bone mass (120, 121). However, the large deficit of calcium in these patients (the loss of exogenous sources due to the deficit in alimentation produces liberation of bone calcium to maintain the homeostasis of extracellular fluid) and the deficit in amino acids as a result of fasting make it very difficult to restore bone mass (122).

Conclusions

The existing evidence suggests that there is a hypothalamic dysfunction in patients with AN and in general this normalizes with weight recuperation. Disturbances in various neurotransmitter, neuropeptide and neuroendocrine systems have been reported in both acutely ill and follow-up patients. In the face of extremely reduced macro- and micro-nutrient intake, non-vital processes, such as growth, pubertal development and reproduction which increase energy output and are not necessary for survival, are shut down until the nutritional situation improves. Early detection and implementation of appropriate psychological and nutritional therapy is the best treatment for preventing osteopenia/osteoporosis in these patients. The subtypes of AN and BMI at follow-up appear to be the best predictors of BMD and leptin may play an important protective role in bone metabolism.

Analysis of the genetic mechanisms underlying weight regulation is now progressing very rapidly. The genetic analysis of AN will possibly help to define new drug targets and lead to new treatment strategies.

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