

Thin healthy women have a similar low bone mass to women with anorexia nervosa

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An association between anorexia nervosa (AN) and low bone mass has been demonstrated. Bone loss associated with AN involves hormonal and nutritional impairments, though their exact contribution is not clearly established. We compared bone mass in AN patients with women of similar weight with no criteria for AN, and a third group of healthy, normal-weight, age-matched women. The study included forty-eight patients with AN, twenty-two healthy eumenorrhoeic women with low weight (LW group; BMI < 18.5 kg/m²) and twenty healthy women with BMI > 18.5 kg/m² (control group), all of similar age. We measured lean body mass, percentage fat mass, total bone mineral content (BMC) and bone mineral density in lumbar spine (BMD LS) and in total (tBMD). We measured anthropometric parameters, leptin and growth hormone. The control group had greater tBMD and BMD LS than the other groups, with no differences between the AN and LW groups. No differences were found in tBMD, BMD LS and total BMC between the restrictive (*n* 25) and binge-purge type (*n* 23) in AN patients. In AN, minimum weight (*P*=0.002) and percentage fat mass (*P*=0.02) explained BMD LS variation (*r*² 0.48) and minimum weight (*r*² 0.42; *P*=0.002) for tBMD in stepwise regression analyses. In the LW group, BMI explained BMD LS (*r*² 0.72; *P*=0.01) and tBMD (*r*² 0.57; *P*=0.04). We concluded that patients with AN had similar BMD to healthy thin women. Anthropometric parameters could contribute more significantly than oestrogen deficiency in the achievement of peak bone mass in AN patients.

Anorexia nervosa: Bone mineral density

Anorexia nervosa (AN) is a common disorder in adolescent girls, with a prevalence of 0.2–1.0% in Western societies⁽¹⁾. The association of AN with low bone mineral density (BMD) has been conclusively and consistently demonstrated in adult women^(2–4) as well as in adolescent girls^(5–9). Age at diagnosis and duration of AN determine the severity of bone mineral loss, whereas factors involved in the location of bone loss are not clear in AN^(10,11). Adolescence represents a critical period for the achievement of peak bone mass, as most bone mineral is accumulated during the teenage years, and deficits arising during this time may be permanent. Low peak bone mass is an important risk factor for osteoporosis and osteoporosis-related fracture after menopause. Therefore, an understanding of the factors that impact on bone density during this period is of particular importance.

Multiple hormone, endocrine and nutritional factors, such as weight, BMI and degree of undernutrition, affect bone metabolism in patients with AN^(10,11). To date, the main hormonal mechanisms known to lead to low bone mass in AN involve abnormalities in the growth hormone–insulin-like growth factor I axis. These hormones increase during puberty and

stimulate the proliferation and differentiation of osteoblast precursors. Insulin-like growth factor I enhances bone formation and growth via effects on osteoblasts and collagen synthesis. Resistance to growth hormone with high growth hormone levels but low insulin-like growth factor I levels has been found in patients with AN^(6,12–14). High cortisol levels with a normal circadian cycle have been reported. Hypothalamic dysfunction or corticotropin-releasing hormone overproduction may contribute to hypercorticism^(5,13). Amenorrhoea is a diagnostic criterion for AN, and oestrogen deficiency has been described as a major source of bone loss in this condition. The mechanisms underlying this oestrogen deficiency involve multiple factors including hypothalamic dysfunction and weight loss^(15,16). Over recent years several other hormonal changes have been observed in AN patients, such as low levels of testosterone, leptin^(7,14), vitamin D⁽⁹⁾ and sex hormone-binding globulin⁽⁸⁾. The specific contribution of these factors to bone remodelling is not completely understood.

An association between AN and oestrogen deficiency has been reported, as has a correlation between bone loss and

Abbreviations: AN, anorexia nervosa; BMD, bone mineral density; BMD LS, bone mineral density in the lumbar spine; LW, low weight.

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the duration of amenorrhoea^(6,17). However, most studies failed to find total bone mass recovery with oestrogen–progesterin therapy^(18,19). Oestrogen deficiency does not fully explain the loss of bone mass associated with AN; malnutrition and nutrition-dependent factors therefore play a key role in the pathogenesis of bone loss associated with AN.

We hypothesised that at the beginning of the disease, when peak bone mass has not yet been reached, factors that involve bone mineral mass in AN patients are independent, at least in part, of oestrogen deficit and anthropometric variables and body composition will better predict bone mineral mass. To verify this hypothesis, we compared a group of patients with AN and oestrogen deficit with women of similar low weight with no criteria for AN (low-weight (LW) group; BMI < 18.5 kg/m²) and therefore with no oestrogen deficit, and a third group of healthy, normal-weight, age-matched women (control group; BMI 18.5–25 kg/m²).

Subjects and methods

We studied forty-eight Caucasian adolescent girls who were referred by general practitioners to our unit for AN (aged 19 (SD 5) years). All of them met the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) criteria for AN and secondary amenorrhoea (20 (SD 30) months) was present at the time of the study. None of them had been treated with Ca supplements, vitamin D preparations, hormone therapy, anti-resorptive therapy, thiazides, steroids, or other medications that might affect bone mass. The LW group was composed of twenty-two women, aged 18 (SD 4) years, recruited in primary care centres and who fulfilled the following criteria: BMI < 18.5 kg/m², eumenorrhoeic and no criteria for eating disorders. Women with co-morbid conditions known to be associated with secondary bone loss were excluded. After 5 years of follow-up, none presented any criteria for eating disorders. The control group included twenty healthy, Caucasian, female adolescents, aged 19.3 (SD 1.5) years, who were post-menarchal with regular menstrual periods and no history of eating disorders. Control subjects with a medical condition or receiving hormone or other medications known to affect bone metabolism were excluded. All the participants or their parents gave informed consent and the study was approved by the Research Committee of Virgen de la Victoria University Hospital (Malaga, Spain).

Bone mineral density and body composition assessment

BMD at the anterior-posterior lumbar spine L1–L4 (BMD LS) and total BMD and total bone mineral content were determined with dual-energy X-ray absorptiometry. The percentage of total body fat mass and lean body mass was quantified using the dual-energy X-ray absorptiometry method (Lunar DPX-L; Lunar Corp., Madison, WI, USA).

Biochemical measurements

Morning fasting samples of venous blood were taken. Serum was promptly separated and stored at –80°C until assay. Serum growth hormone (Immunotech IRMA kit Beckman Coulter; manufacturer's reference level <5 mU/l) was assayed. Serum leptin (RIA; Nichols Institute Diagnostics,

Heston, Middlesex, UK; manufacturer's reference range for a normal BMI (18.5–25 kg/m²): 3.7–11.1 µg/l; analytical sensitivity: 0.5 ng/ml) was also measured.

Statistical analysis

For the descriptive analysis all values are presented as mean values and standard deviations. The comparison between quantitative variables was done with the ANOVA test. Inter-group comparisons were performed using the Student *t* test and Mann–Whitney test in parametric and non-parametric comparisons, respectively. For the bivariate analysis we used simple linear (bivariate) correlations in all study groups to evaluate several interactions: anthropometric, nutritional and hormone parameters. Statistical significance was set at *P*<0.05. A multivariate analysis was carried out to evaluate the impact of anthropometric and hormone parameters on BMD LS and total BMD in the AN patients and the LW group as well as a multivariate linear regression (stepwise addition). Inclusion of hormone, clinical and anthropometric variables in the analysis was based on the strength of correlation with BMD. All statistical analyses were performed with SPSS 13.0 (SPSS, Inc., Chicago, IL, USA).

Results

Table 1 shows the clinical characteristics of the study population. The control group had a higher total BMD and BMD LS than the AN and LW groups after adjusting for age, height and weight. No significant differences were found between the AN and LW groups in age, weight, height or BMI. Leptin levels and the percentage of fat mass were significantly lower (both *P*<0.001) in the AN group compared with the LW group, regardless of age. No significant differences were found in bone mass parameters between the two groups for total bone mineral content, total BMD or BMD LS. Regarding the two types of AN patients, the anthropometric parameters were similar in all the patients, regardless of whether they had the restrictive or the binge–purge variant of AN. No significant differences were found in total bone mineral content, total BMD or BMD LS, or in the other parameters, including leptin and growth hormone (Table 2).

Bivariate analysis in the AN group showed positive correlations between bone parameters and anthropometric parameters such as minimum weight, weight and BMI (Table 3). No correlations were found between bone markers, age and duration of AN and amenorrhoea. In the LW group, anthropometric parameters such as weight and BMI were correlated with total bone mineral content and total BMD. No correlation was found between BMD LS and anthropometric parameters (Table 3).

In the multivariate analysis (Table 4), an important influence of the anthropometric parameters in BMD was shown in the AN group. Minimum weight during the illness and percentage fat mass accounted for 48% of the BMD LS variance, while 42% of the total BMD variance was determined by minimum weight during the illness. In the LW group, up to 72% of the variance in BMD LS was due to BMI, and 57% of the total BMD variance was explained by the BMI.

Table 1. Clinical characteristics of the study population
(Mean values and standard deviations)

Characteristic	Control group		AN group		LW group	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	19.30	1.55	19.00	5.1	19.65	5.34
Height (cm)	160.72	5.83	159.90	6.69	159.92	5.29
Weight (kg)	54.4	5.39	44.28*	5.0	43.44*	5.29
BMI (kg/m ²)	22.30	1.59	16.13*	1.53	16.65*	1.02
Age at onset of illness (years)	–	–	16.51	3.31	–	–
Duration of amenorrhoea (months)	–	–	20.45	31.4	–	–
Total fat mass (%)	–	–	15.37	5.63	20.63†	3.40
Lean body mass (g)	–	–	35 404.26	3706.23	34 358.10	3915.21
Total bone mineral content (g)	–	–	2010.86	383.73	1939.60	327.10
Bone mineral density in the lumbar spine (g/cm ²)	1.049	0.06	0.958*	0.13	0.956*	0.13
Total bone mineral density (g/cm ²)	1.115	0.08	1.075*	0.11	1.067*	0.09
Leptin (ng/ml)	8.96	1.91	1.90*	1.76	4.36*†	2.01
Growth hormone (ng/ml)	–	–	3.67	2.93	3.07	5

AN, anorexia nervosa; LW, low weight.

* Mean value was significantly different from that of the control group ($P < 0.001$).† Mean value was significantly different from that of the AN group ($P < 0.001$).

Discussion

Previous studies have shown that osteopenia occurs in 50% of young women with AN at both trabecular and cortical bone sites. Fractures are common in these young osteoporotic patients^(2–8). These abnormalities result from the uncoupling of bone remodelling, including decreased bone formation and increased bone resorption⁽²⁰⁾. Factors responsible for low bone mass in AN patients involve interrelated multiple hormone and nutritional impairments that damage osteoblast and/or osteoclast activities. This model differs from the postmenopausal osteoporosis model, where oestrogen deficiency only determines an increase in bone resorption. Several hormone mechanisms are currently known to lead to low bone mass. The specific contribution of these factors on bone remodelling, though, is not completely understood^(5–9,13–16).

In the present study, we compared three different groups of teenagers and young adult women, all of similar age: a group of healthy, normal-weight women, a group of eumenorrhoeic

women with BMI $< 18.5 \text{ kg/m}^2$ with no criteria for AN (LW group) and a group of AN patients having a mean of 18 months' disease evolution and of similar weight to the LW group. The study of these three different groups enabled us to evaluate the independent contribution of oestrogen deficit and weight to bone mineral mass at the onset of the disease. The association between the hypo-oestrogenic state and early onset bone loss in AN patients⁽²¹⁾ and the need to reach normal oestrogen levels during this period of bone mass acquisition⁽²²⁾ have been reported previously. Klibanski *et al.* showed in a prospective study that oestrogen administration was effective in preventing progressive osteopenia in lean AN patients only when associated with weight gain⁽¹⁶⁾. The reason why oestrogen replacement alone is not sufficient to promote bone accretion in young patients with AN is currently unknown, but it may well mean that proper nutrition and recovery of normal circulating bone trophic factors such as insulin-like growth factor I are required for oestrogen action on bone.

Table 2. Differences between restrictive and purgative types within the anorexia nervosa group
(Mean values and standard deviations)

	Purgative (n 23)		Restrictive (n 25)		Significance
	Mean	SD	Mean	SD	
Minimum weight (kg)	39.18	3.72	38.5	5.59	NS
Age at onset of illness (years)	16.85	2.47	16.32	3.8	NS
Duration of amenorrhoea (months)	29.4	44.91	16.00	19.16	NS
Weight (kg)	41.52	4.98	41.13	5.28	NS
Height (m)	1.60	0.06	1.59	0.069	NS
BMI (kg/m ²)	16.15	1.59	16.12	1.53	NS
Leptin (ng/ml)	2.8	2.32	1.33	1.01	NS
Growth hormone (ng/ml)	4.91	3.67	3.3	2.64	NS
Bone mineral density in the lumbar spine (g/cm ²)	0.948	0.111	0.964	0.121	NS
Total bone mineral density (g/cm ²)	1.073	0.099	1.076	0.105	NS
Total bone mineral content (g)	2012.27	258.79	2009.45	353.17	NS
Lean body mass (g)	34 877.44	2778.73	35 787.41	4280.33	NS
Total fat mass (%)	16.60	6.15	14.47	5.18	NS

Table 3. Correlation analysis of the biological variables studied (Pearson's *r*) in the anorexia nervosa (AN) and low-weight (LW) groups

	BMD LS		Total BMD		Total BMC	
	AN	LW	AN	LW	AN	LW
Age	0.01	0.27	0.06	0.28	0.13	0.41
Illness duration	-0.08	-	-0.14	-	-0.12	-
Minimum weight	0.60**	-	0.69**	-	0.83**	-
Weight	0.46*	0.47	0.59**	0.32*	0.76**	0.84**
BMI	0.51**	0.53	0.49**	0.54*	0.45**	0.73**
Months of amenorrhoea	-0.06	-	-0.11	-	-0.21	-
Percentage total fat mass	0.24	0.35	0.14	0.22	0.16	0.27
Lean body mass	0.36	0.32	0.54**	0.22	0.76**	0.84**
Leptin	0.02	-0.01	0.01	0.02	0.06	-0.23
Growth hormone	0.13	0.26*	0.19	0.29*	0.16	0.69**

BMD LS, bone mineral density in the lumbar spine; BMD, bone mineral density; BMC, bone mineral content.

* $P < 0.05$, ** $P < 0.001$.

The first outstanding result in the present study was that the LW women had the same bone mineral mass as the AN patients. Both groups had lower total BMD and BMD LS than healthy women of a similar age. In the present study, BMD LS and total BMD in AN and LW patients were significantly related to anthropometric variables, though the duration of amenorrhoea did not explain the changes in BMD in the AN patients. Consequently, these results suggest that anthropometric parameters could contribute more significantly than oestrogen deficit in the AN patients of short evolution. A similar decrease in BMD in the LW group and the AN patients strengthens this theory. The relationship between BMI and BMD in AN patients and healthy women has been reported previously. Several studies have demonstrated that body weight in healthy premenopausal women is an independent and determinant factor for adequate peak bone mass in this population^(23–25). Bone loss is inversely correlated with weight in AN patients. Greater weight loss^(26,27) and a longer duration of the low weight state⁽²⁸⁾ produce a higher bone loss. Similarly, there was a correlation with lean mass, which is supported by previous studies where the loss of

lean mass was considered an important prognostic factor to predict the bone mass loss, with a significant correlation between lean mass and BMD LS and total BMD^(29,30).

The present study revealed no differences between the two forms of AN, the restrictive and the binge-purge types, either in bone or anthropometric parameters. Previous studies comparing the bone density between the two different forms of AN have yielded inconsistent findings^(31–34). The present results are consistent with data suggesting that the degree of underweight is an important predictor of low BMD, whereas other behaviour such as bingeing and purging seems to be of relatively minor importance. Discrimination between the two AN types is based on behavioural symptoms (for example, purging) that frequently change during the course of the illness^(35,36). This heterogeneity is associated with a masking of the differences in effects that restrictive and binge-purge eating behaviours might have on bone parameters.

Leptin exerts a critical role in the regulation of body energy balance and as a protective factor inhibiting bone resorption^(37–39). However, the exact mechanism underlying the effect of variation in this adipokine on bone anomalies in patients with AN is still not clear. The decrease in leptin levels observed in AN patients could contribute to BMD loss due to the reduction in bone formation⁽¹⁰⁾. Additionally, the correlation between leptin and bone resorption markers in AN patients during the refeeding process suggests a possible cause-effect relationship between serum levels of leptin and bone metabolism⁽⁴⁰⁾. Conversely, leptin deficiency in AN was not an independent predictor of BMD after controlling for other nutrition-dependent factors such as BMI. Despite the marked decrease in serum leptin levels in the AN group compared with the LW group no changes in BMD were revealed.

The present study has several limitations. First, hypo-oestrogenism was defined by a surrogate value, duration of amenorrhoea, because oestradiol or gonadotrophin serum levels were unavailable. Second, in our study, comparison groups were small. However, there are not many papers reporting differences between patients with AN, healthy women with LW and women with normal weight. Third, BMD could not be adjusted for 25-hydroxyvitamin D levels, an important cause in the pathophysiology of bone alterations in AN.

Table 4. Multiple regression analysis in the anorexia nervosa (AN) and low-weight (LW) groups*

Variables	AN			LW		
	R^2	SE (β)	P	R^2	SE (β)	P
BMD LS						
Independent variables						
Minimum weight	0.48	0.46	0.002			
% Fat mass		0.44	0.02			
BMI				0.72	0.85	0.01
Total BMD						
Independent variables						
Minimum weight	0.42	0.65	0.002			
BMI				0.57	0.72	0.04

BMD LS, bone mineral density in the lumbar spine; BMD, bone mineral density.

*Dependent variables: BMD LS and total BMD. Independent variables: age, minimum weight, lean body mass, percentage fat mass, time of disease evolution, months of amenorrhoea, BMI, leptin and growth hormone (minimum weight, time of disease evolution, months of amenorrhoea not included in the LW model). Statistical significance set at $P < 0.05$.

In conclusion, the present results support the hypothesis that anthropometric parameters could contribute more significantly to low bone mass than oestrogen deficiency in AN patients of short evolution. Therefore, teenagers and adult women with low weight have an increased risk for low bone mass similar to that of AN patients.

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There are no conflicts of interest to declare.

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