

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

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Abstract

In order to investigate the risk factors, pathogenesis and natural course of the osteoporosis frequently seen in anorexia nervosa, we measured the bone mineral density (BMD) of the lumbar spine using dual X-ray absorptiometry in 51 Japanese female patients with anorexia nervosa, and followed the change in BMD of 29 patients for 11 to 46 months. We also evaluated the serum osteocalcin and the urinary CrossLaps, degradation products of collagen I, in 103 samples obtained from 51 patients. There was a significant correlation between the spinal BMD and the duration of emaciation below a body mass index (BMI) of 15 kg/m² ($r = -0.652$, $P < 0.0001$) and 16 kg/m² ($r = -0.647$, $P < 0.0001$). The increase in BMD per year in the 29 patients significantly correlated with the BMI at the time of entry of each follow-up period ($r = 0.712$, $P < 0.0001$). The critical BMI for a positive increase in BMD was 16.4 ± 0.3 kg/m² (mean \pm S.E.M.). The serum osteocalcin declined, while the urinary CrossLaps increased in proportion to a decrease in BMI. Both markers were normalized in patients whose BMI was between 16.4 and 18.5 kg/m². The ratio of urinary CrossLaps to serum osteocalcin correlated with BMI ($r = -0.664$, $P < 0.0001$).

We conclude that the body weight history is the most important predictor of the presence of osteoporosis as well as of recovery. The BMD of patients does not increase to the normal range even several years after the recovery from this disorder, and they remain a high-risk group for osteoporosis in the future.

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Introduction

A decrease in bone mineral density (BMD) is frequently seen in patients with anorexia nervosa (1–12), and these patients have a higher incidence of fractures compared with healthy young women (13–15). Although both prospective and follow-up studies have been performed to investigate the risk factors for osteoporosis in anorexia nervosa, in which metacarpal index, single- and dual-photon absorptiometries or quantitative computed tomography have been used to measure BMD (1, 2, 5, 9, 11, 12, 16–20), the conclusions regarding the risk factors for osteoporosis remain controversial (5–12). Several longitudinal studies have revealed different recovery patterns of low BMD in the clinical course of anorexia nervosa patients complicated with osteoporosis (16–21). Because the duration of illness and amenorrhea in patients with anorexia nervosa is several years and extends over

adolescence, which is when their peak bone mass should be established, it is important to clarify the risk factors and natural history of osteoporosis in order to establish a strategy to prevent a further decrease in BMD.

Dual X-ray absorptiometry (DXA) is a recently developed method for measuring BMD. DXA is more accurate, with a coefficient of variation below 1%, and requires lower radiation exposure than other methods (22, 23). Consequently, we measured the BMD of the lumbar spine using DXA in Japanese patients with anorexia nervosa, and then followed them to clarify how BMD is affected by the degree of weight gain or resumption of menses. To evaluate the bone turnover in patients with anorexia nervosa, we measured serum osteocalcin, as a marker of bone formation, and urinary degradation products of collagen I (CrossLaps), as a new marker of bone resorption, by ELISA.

Table 1 An outline of diagnostic criteria for anorexia nervosa as determined by the Survey Committee for Eating Disorders of the Japanese Ministry of Health and Welfare (1990).

1. Weight loss of 20% below that expected, lasting longer than 3 months.
2. Abnormal eating behavior including restricting food, bulimic episodes, and eating by stealth.
3. Disturbance in the way in which one's body weight or shape is experienced and intense fear of gaining weight, even though underweight.
4. Onset under 30 years of age.
5. In females, amenorrhea.
6. Negation of illness including other psychiatric disorders that account for anorexia and weight loss.

Subjects and methods

Subjects

This study included 51 amenorrheic Japanese patients (age 19–42 years) who met the criteria for anorexia nervosa outlined in the Diagnostic and Statistical Manual IV (DSM IV) of the American Psychiatric Association, as well as the criteria determined by the Survey Committee for Eating Disorders of the Japanese Ministry of Health and Welfare (Table 1). Thirty-seven of the patients had Restricting type of anorexia nervosa and the remaining 14 had Binge-eating/Purging type. All were outpatients under the care of Dr Mari Hotta at Tokyo Women's Medical University. The age of onset of the disease in all 51 patients was higher than 15 years of age because the maximum bone mass is most likely to be present in 14- to 15-year-olds (24). There was no patient with primary amenorrhea. Five patients became amenorrheic before the start of weight loss, and 12 did so when they started to lose body weight. None of the patients had been treated with estrogen, vitamin D or calcium before or during this study. One patient had been consuming alcohol regularly, and five had a history of cigarette smoking. The clinical courses of 29 patients were followed for a period ranging from 11 to 46 months. The daily calcium intake, calculated from the patients' dietary records, was variable and was 400 mg/day at most. Two patients had been participating in aerobics for 30 min several times a week before entry into the study. However, their exercise habits changed during the follow-up period; they stopped playing sports.

Serum osteocalcin and urinary CrossLaps, used as bone metabolic parameters, were measured in 103 samples obtained from the 51 patients with anorexia nervosa. We also measured both markers in ten samples obtained from ten healthy women, who had a median age of 26.0 year (range, 24.8–30.2) and a median BMI of 21.2 kg/m² (range, 19.4–22.0).

Bone densitometry

The anteroposterior BMD of the lumbar spine (L₂–L₄) was measured by DXA using a Hologic QDR-2000 apparatus (Hologic Inc., Waltham, MA, USA). BMD measurements are expressed in grams per square centimeter (g/cm²). The BMD results from the patients

were compared with the mean plus 2 standard deviations (s.d.) established for healthy young women at Tokyo Women's Medical University.

Biochemical and endocrinological study

Laboratory evaluation included measurements of serum total protein, albumin, calcium and alkaline phosphatase. The plasma levels of intact parathyroid hormone (PTH), calcitonin, triiodothyronine (T₃), growth hormone (GH), insulin-like growth factor (IGF)-I, cortisol, estradiol and urinary excretion of free cortisol were measured by their specific RIAs or IRMAs. Bone turnover was assessed by measuring both the fragment (1–43) of osteocalcin as well as intact osteocalcin in serum (25) and CrossLaps in urine; CrossLaps is an eight amino acid fragment derived from the C-terminal telopeptide of collagen I (26). These markers were measured with ELISAs (Osteometer BioTech A/S, Rodovre, Denmark) (25, 26). The urinary concentration of CrossLaps was corrected for creatinine (Cr) as µg per mmol Cr. The serum and urine samples were collected between 0800 and 1000 h on the same day and stored at –80 °C until assayed.

Statistical analysis

For nonparametric data, Spearman's ranked correlations (*r*) were determined among the factors listed in Tables 2 and 3. The correlation between body mass index (BMI) and an increase in BMD during a follow-up period, and the correlation between BMI and the ratio of CrossLaps to serum osteocalcin were also tested by Spearman's ranked correlations test. To obtain the critical BMI for a positive increase in BMD, BMI at the time of entry of each follow-up period was log-transformed and both variables were tested for linear regression analysis. Differences between the patients with Binge-eating/Purging type and those with Restricting type, and differences in bone metabolic parameters among the groups were assessed using Student's *t*-test for parametric data, or the Mann–Whitney U test with a Bonferroni correction for nonparametric data.

Results

The clinical profile of each patient at the start of this study is shown in Table 2. Of the 51 patients, 25 had a

Table 2 Clinical data and correlation with spinal BMD ($n=51$).

	Range	Mean (\pm S.D.)	Correlation coefficient (r)	P
Age (months)	230–514	309 \pm 65	–0.258	0.0678
Age at onset (months)	189–411	249 \pm 42	0.018	0.8927
Age at onset of amenorrhea (months)	189–411	259 \pm 44	–0.008	0.9530
Height (cm)	144.5–171.0	157.6 \pm 5.6	0.093	0.5101
BMI before the onset of illness (kg/m^2)	15.6–26.7	20.0 \pm 2.4	0.526	0.0002
BMI on entry (kg/m^2)	11.0–17.0	15.1 \pm 2.5	0.396	0.0051
Minimal BMI (kg/m^2)	9.0–13.7	12.6 \pm 2.0	0.599	<0.0001
Duration of illness (months)	7–270	59 \pm 47	–0.439	0.0021
Duration of amenorrhea (months)	3–270	46 \pm 46	–0.404	0.0043
Duration of emaciation below BMI 17 kg/m^2 (months)	0–270	51 \pm 50	–0.621	<0.0001
Duration of emaciation below BMI 16 kg/m^2 (months)	0–262	35 \pm 49	–0.647	<0.0001
Duration of emaciation below BMI 15 kg/m^2 (months)	0–260	33 \pm 48	–0.652	<0.0001

spinal BMD greater than 2 s.d. below the age-matched standard in our hospital. The mean values of BMD of the 37 patients of Restricting type and the 14 patients with Binge-eating/Purging type were $0.823 \pm 0.123 \text{ g}/\text{cm}^2$ (mean \pm s.d.) and $0.852 \pm 0.143 \text{ g}/\text{cm}^2$ respectively, and there was no significant difference in the BMD between these two groups. Correlation between the spinal BMD and the clinical parameters is shown in Table 2. A significant correlation was found between the spinal BMD and the following variables: the duration of emaciation below a BMI of 15 kg/m^2 ($r = -0.652$, $P < 0.0001$), 16 kg/m^2 ($r = -0.647$, $P < 0.0001$) and 17 kg/m^2 ($r = -0.621$, $P < 0.0001$). The spinal BMD also correlated significantly with the minimal BMI ($r = 0.599$, $P < 0.0001$) and the BMI before the onset of illness ($r = 0.526$, $P = 0.0002$). The minimal BMI correlated significantly with the duration of emaciation below a BMI of 15 kg/m^2 ($r = -0.648$, $P < 0.0001$) and 16 kg/m^2 ($r = -0.613$, $P < 0.0001$). There was a lower degree of correlation between BMD and the duration of illness ($r = -0.439$, $P = 0.0021$), or the duration of amenorrhea ($r = -0.404$, $P = 0.0043$). None of the biochemical parameters including serum

total protein, albumin, calcium, alkaline phosphatase and hormones (Table 3) correlated significantly with the spinal BMD.

The BMD and BMI on each DXA of the 29 patients in the follow-up study are shown in Fig. 1. None of the patients had clinically apparent fractures before entry into this study. However, during the follow-up period, a metatarsal fracture was clinically noted in two patients (patients 3 and 19) (Fig. 1A and C). By the end of the study, of the 19 unrecovered patients, 5 had lost weight, of which 1 (patient 1) gained a positive increase in BMD (Fig. 1A). The change in BMI during each follow-up period was within 0.5 in three patients, of whom two (patients 6 and 7) showed a positive increase in BMD (Fig. 1B). Eleven patients had gained BMI of 0.7–8.0 kg/m^2 , of whom four (patients 9, 13, 15 and 16) had a positive increase in BMD (Fig. 1C). All of those patients whose BMD increased had a BMI greater than 15 kg/m^2 at the time of entry of each follow-up period. The ten recovered patients (patients 20–29) resumed regular menses and showed an increase in BMD of 0.5–7.5% per year after entry into the study (Fig. 1D). Only one (patient 25) of the six patients (patients 24–29)

Table 3 Correlation between spinal BMD and various biochemical and hormonal parameters.

	n	Range	Mean (\pm S.D.)	Correlation coefficient (r)	P
Intact PTH (ng/l)	40	4–83	29 \pm 14	–0.280	0.0800
Osteocalcin ($\mu\text{g}/\text{l}$)	51	3.4–115.9	16.5 \pm 15.8	–0.234	0.1203
Calcitonin (ng/l)	39	37–10	20 \pm 7	–0.295	0.0686
T_3 (nmol/l)	51	0.45–2.65	1.29 \pm 0.43	0.179	0.2044
IGF-I ($\mu\text{g}/\text{l}$)	51	4–529	182.4 \pm 99.8	0.201	0.1586
GH ($\mu\text{g}/\text{l}$)	43	0.1–89.2	11.6 \pm 7.9	–0.224	0.1472
Plasma cortisol ($\mu\text{mol}/\text{l}$)	41	0.25–1.85	0.62 \pm 0.29	–0.330	0.0867
Estradiol (pmol/l)	51	ND–349.8	66.8 \pm 59.1	0.340	0.0225
Urinary free cortisol (nmol/day)	16	84.7–1288.4	447.4 \pm 333.1	0.188	0.4660

ND = not detected.

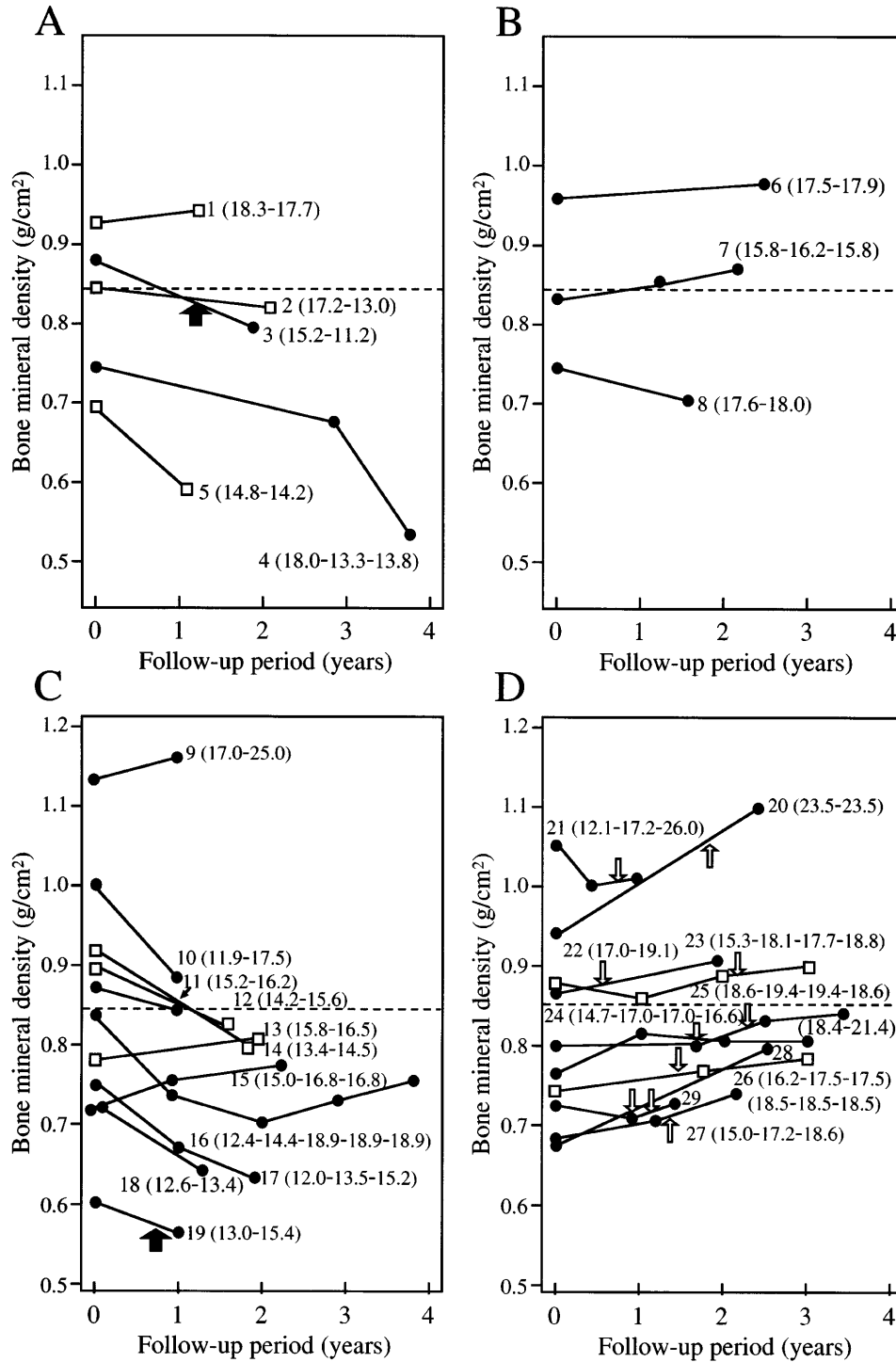


Figure 1 Changes in the spinal BMD of 5 anorexia nervosa patients who lost weight (A), 3 whose change in BMI was within 0.5 (B), 11 who gained weight (C), and 10 who recovered from anorexia nervosa (D) during follow-up. Patients are represented by numbers and their BMI values when BMD was measured. Closed circles and open squares indicate Restricting type and Binge-eating/Purging type of anorexia nervosa respectively. Black thick arrows indicate times when bone fractures happened and white arrows represent when menstruation recovered. The dotted line represents the mean BMD-2s.d. established at our hospital.

who showed a starting BMD greater than 2 s.d. below the age-matched standard in our hospital rose to the lower normal limit over the follow-up period (Fig. 1D). The increase in BMD per year in these 29 followed patients did not correlate significantly with their ages ($r=0.393$, $P=0.0085$) or increases in BMI ($r=-0.133$, $P=0.3735$). The increase in BMD per year correlated significantly with the BMI at the time of entry ($r=0.712$, $P<0.0001$) (Fig. 2) and the final BMI ($r=0.589$, $P=0.0013$) during each of 46 follow-up periods. The critical BMI for a positive increase in BMD was $16.4 \pm 0.3 \text{ kg/m}^2$ (mean \pm s.e.m.) (Fig. 2).

The bone turnover in anorexia nervosa patients was assessed by analysis of serum osteocalcin and urinary CrossLaps. The patients were divided into five groups according to their BMI because the changes in BMD correlated with BMI. The mean serum osteocalcin, urinary CrossLaps, and the ratio of urinary CrossLaps to serum osteocalcin (C/O) are shown in Fig. 3. The concentrations of serum osteocalcin, urinary CrossLaps, and C/O in healthy young women were $13.8 \pm 1.1 \mu\text{g/l}$ (mean \pm s.e.m.), $176.5 \pm 25.5 \mu\text{g per mmol Cr}$, and

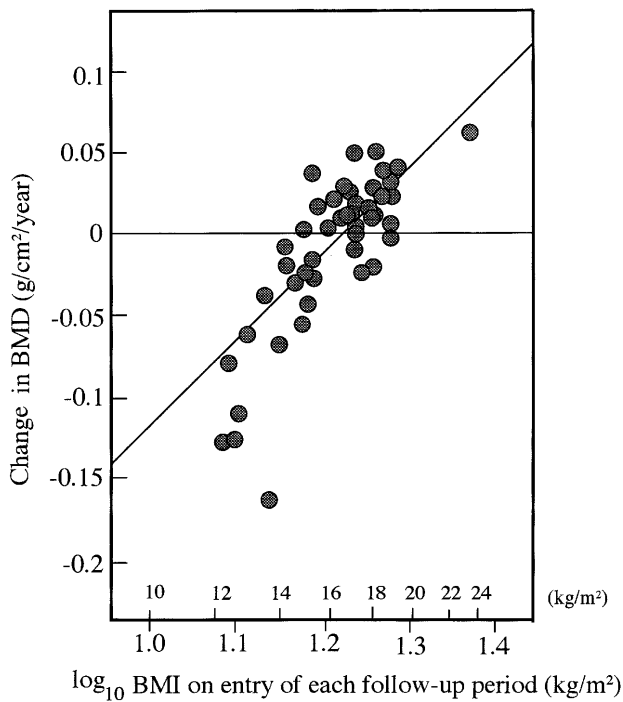


Figure 2 The relationship between the BMI at the time of entry and the incremental change in BMD per year during each of 46 follow-up periods in 29 patients with anorexia nervosa. The change in the BMD per year of these patients correlated significantly with the BMI at the time of entry of each follow-up period using Spearman's ranked correlations test ($r = 0.712$, $P < 0.0001$). The critical BMI for a positive increase in the BMD was $16.4 \pm 0.3 \text{ kg/m}^2$ (mean \pm s.e.m.), which was obtained from linear regression analysis of log-transformed BMI and a change in BMD per year. The regression equation was a change in BMD per year = $-0.732 + 0.602 \times \log [\text{BMI on entry of each follow-up period}]$ ($r^2 = 0.624$).

13.0 ± 3.1 respectively. The mean serum osteocalcin level of patients whose BMI was below 12.5 kg/m^2 was $8.3 \pm 1.0 \mu\text{g/l}$ and rose in accordance with an increase in BMI. The mean urinary CrossLaps concentration of patients whose BMI was below 12.5 kg/m^2 increased to $534.4 \pm 97.9 \mu\text{g per mmol Cr}$, and decreased in accordance with an increase in BMI. Both markers and C/O were normalized in patients whose BMI was between 16.4 and 18.5 kg/m^2 . Although both markers increased in patients whose BMI was over 18.5 kg/m^2 , the value of C/O showed no significant difference from that of control. The values of C/O significantly correlated with BMI ($r = -0.664$, $P < 0.0001$).

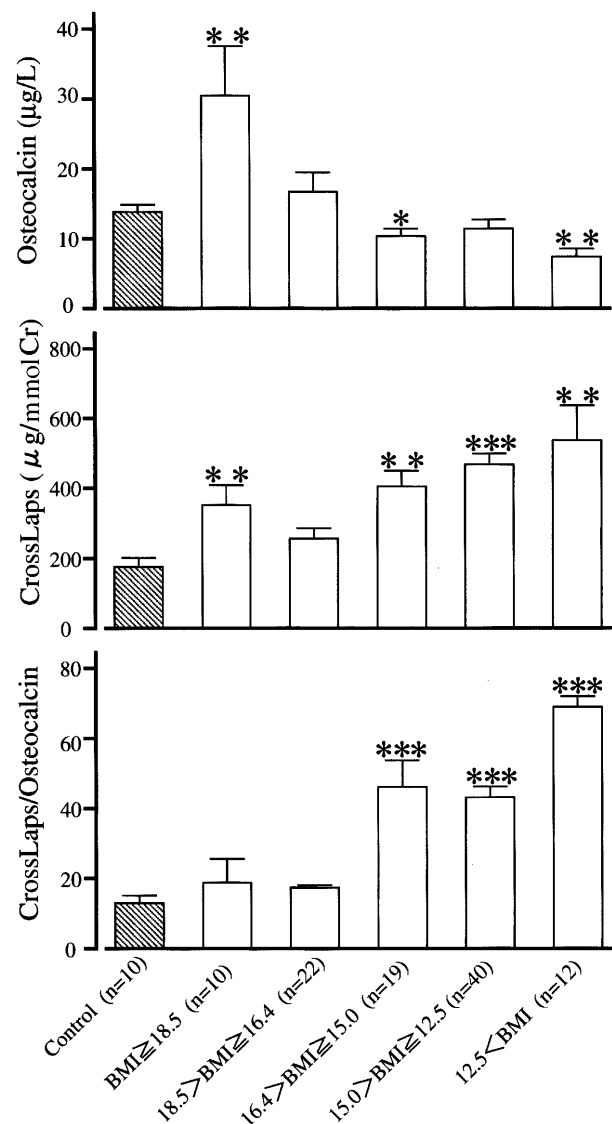


Figure 3 Bone turnover in patients with anorexia nervosa and age-matched healthy women. Upper, middle and lower panels represent serum osteocalcin, urinary CrossLaps, and the ratio of CrossLaps to osteocalcin respectively. Data are expressed as means \pm s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control.

Discussion

This is the first report of a cross-sectional and longitudinal study of the BMD measured by DXA and also the first assessment of bone turnover by a recently developed marker, urinary CrossLaps, in Japanese female patients with anorexia nervosa. Since it usually takes several years for anorexia nervosa patients to restore body weight to 85% or more of their ideal body weight and resume regular menses, it is important to clarify risk factors for osteoporosis to prevent further loss of BMD in this disorder. The present study revealed that the spinal BMD in anorexia nervosa patients correlates strongly and negatively with the duration of emaciation, especially below a BMI of 15 kg/m². Furthermore, the results showed that the period when the body weight is over a BMI of 16.4 ± 0.3 kg/m² (mean ± S.E.M.) during the course of illness contributes to an increase in BMD, even when patients are still amenorrheic. Thus, the period of emaciation, especially below a BMI of 15 kg/m², is the period when patients are at the highest risk of developing osteoporosis. There has been only one report in which the spinal BMD, measured by quantitative computed tomography, correlated negatively with the duration of BMI remaining below 16 kg/m². However, the correlation coefficient was below 0.4 (11). The measurement of BMD by DXA seems to be precise enough to reveal a higher correlation coefficient between the BMD and the duration of a BMI below 15 or 16 kg/m² than that in the other report (11).

Early onset of amenorrhea and longer duration of the hypogonadal state are risk factors for a decrease in BMD (2), and spinal BMD has been shown to correlate negatively with the duration of amenorrhea in anorexia nervosa (5, 11). On the other hand, no significant correlation between BMD and either duration of amenorrhea (7, 8) or the plasma level of estradiol (1, 7) has been reported. The present study also revealed a lower correlation of BMD with duration of amenorrhea than duration of emaciation below a BMI of 15 or 16 kg/m², and no significant correlation of BMD with either age at onset of amenorrhea or the plasma level of estradiol. One study has shown that estrogen replacement cannot prevent progressive osteoporosis in young women with anorexia nervosa except in those with severe emaciation to less than 70% of the ideal body weight (21). The authors of that study suggest that there may be a distinct difference between the resumption of menses and estrogen therapy (21). We believe that osteoporosis in patients with anorexia nervosa may be caused by emaciation-induced multiple factors including estrogen deficiency. In most of the patients in the present study whose BMI was below 15 kg/m², plasma estradiol was not detected. The plasma levels of estradiol began to increase in patients whose BMI was between 15 and 16 kg/m², and an exaggerated response of gonadotropins to intravenously administered gonadotropin-releasing

hormone was observed in patients whose BMI was between 16.5 and 18 kg/m² (data not shown). Therefore, the duration of emaciation below a BMI of 15 or 16 kg/m² appears to be associated with severe hypogonadism.

The present study revealed no biochemical or endocrinological parameters that significantly correlated with BMD. The markers of bone turnover, including osteocalcin, collagen I carboxy-terminal propeptides, pyridinoline, and deoxypyridinoline, decline significantly with short-term fasting, even in normal females (27). It suggests that those parameters change rapidly and prior to a detectable change in BMD.

There have been several longitudinal studies (9, 17–20) and a cross-sectional study (16) that have examined the clinical course of osteoporosis in patients with anorexia nervosa. One of those studies demonstrated that patients who recover from anorexia nervosa show a spinal BMD as high as that of healthy women (16). On the other hand, a 4-year follow-up study found that the cortical BMD of patients whose body weight reached at least 80% of their ideal body weight with a resumption of menses does not change significantly, indicating that a reduction in the cortical BMD appears not to be rapidly reversed after recovery from the disorder (9). Weight rehabilitation and the resumption of menses are important in increasing BMD in anorexia nervosa; however, deficits in bone mineral that would ordinarily be acquired during adolescence appear not to be completely reversible (18–20). The results of the present study are in accordance with all of the above findings. We also found that recovery of BMD depended on the BMI at the time of entry of the follow-up period. This result is reasonable because it takes several months to detect a change in BMD even measured by DXA. We emphasize that the BMD of anorexia nervosa patients with a severe degree of osteoporosis did not reach even the lower level of the normal range during the follow-up period.

Values in CrossLaps by ELISA correlate well with values obtained by HPLC for the established bone resorption markers, pyridinoline (28, 29), deoxypyridinoline (26), and hydroxyproline (26). The increase in CrossLaps in menopausal women and its decrease in patients receiving estrogen replacement therapy are significantly greater than such changes in pyridinoline (29, 30). Therefore, CrossLaps is a useful parameter for assessment of bone resorption. Urinary CrossLaps and serum osteocalcin increase from control values in peri- and post-menopausal women (30), patients with Paget's disease (28), hyperparathyroidism (28) and hyperthyroidism (28, 31). We clarified first the BMI-dependent change of bone turnover in patients with anorexia nervosa. Our study showed that the serum osteocalcin declined, while the urinary CrossLaps increased in proportion to a decrease in BMI in patients whose BMI was below 16.4 kg/m². Thus, the mechanism of osteoporosis in anorexia nervosa is unbalanced

bone turnover, which is a combination of a decrease in bone formation and an increase in bone resorption. Our results partly confirm the finding that the mean level of serum osteocalcin was reduced and mean concentrations of bone resorption markers such as deoxypyridinoline and N-telopeptide were increased in 22 patients with anorexia nervosa whose BMIs ranged between 13 and 19 kg/m² (32). Both bone turnover markers were normalized in patients whose BMI was between 16.4 and 18.5 kg/m², which was compatible with the finding that the critical BMI for a positive increase in the BMD was 16.4 kg/m², as indicated in our study using DXA. Although both markers increased in patients whose BMI was greater than 18.5 kg/m², the value of C/O had no significant difference from that of age-matched healthy women, indicating that the bone turnover is enhanced, but balanced, in patients who restored body weight.

In conclusion, the most important factor for increasing the spinal BMD in anorexia nervosa is weight gain over a BMI of 16.4 kg/m², in which bone metabolism is normalized.

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