Clinical Study

Bone Mineral Density Accrual Determines Energy Expenditure with Refeeding in Anorexia Nervosa and Supersedes Return of Menses

Melissa Sum, Laurel Mayer, and Michelle P. Warren

Columbia University College of Physicians and Surgeons, 622 West 168th Street, PH 16-128, New York, NY 10032, USA

Correspondence should be addressed to Melissa Sum, ms2452@columbia.edu

Osteopenia and osteoporosis are major complications of anorexia nervosa (AN). Since bone is a tissue requiring large amounts of energy, we examined the disproportionate increase in resting energy expenditure (REE) that occurs with refeeding of AN patients to determine if it was related to bone accretion. Thirty-seven AN patients aged 23.4 ± 4.8 years underwent a behavioral weight-gain protocol lasting a median of 66 days; 27 remained amenorrheic, and 10 regained menses. Sixteen controls aged 25.1 ± 4.7 years were age- and % IBW matched with patients. REE was measured using a respiratory chamber-indirect calorimeter. Significant correlations were found between REE and changes in spine ($r = 0.48$, $P < 0.02$) and leg ($r = 0.43$, $P < 0.05$) BMDs in AN patients. Further subgroup analysis of the amenorrheics revealed significant correlation between REE and change in spine BMD ($r = 0.59$, $P < 0.02$) and higher IGF-1 after weight gain compared to controls. Amenorrheics also had lower BMDs. These findings were absent in the regained menses group. The increase in REE seen in women with AN during nutritional rehabilitation may be related to active bone formation, which is not as prominent when menses have returned.

1. Introduction

Anorexia nervosa (AN) is an eating disorder characterized by severe voluntary restriction of food with resultant major weight loss affecting up to 1% of women in Western societies [1]. Patients with AN typically have primary or secondary amenorrhea likely due to suppression of the reproductive axis by means of inadequate energy stores [2].

Another consequence of the decreased caloric intake in AN patients is a consistently reported decrease in resting energy expenditure (REE). REE, also known as resting metabolic rate, represents the amount of calories required in a 24-hour period by the body during a nonactive period. Metabolic rate is mainly a function of the activity of lean body mass also known as fat-free mass (FFM). Although a reduced REE theoretically facilitates weight gain during refeeding, REE has also been consistently found to increase with nutritional rehabilitation, thereby potentially rendering weight gain more difficult for patients. Several small studies have proposed explanations for this phenomenon, including

that the increase in REE is a reflection of lean body mass growth [3], a reversal of the initial adaptation to malnutrition [4], or a defense of low body weight [5]. Most puzzling is that others have found the increase in REE to be disproportionately greater than weight gain and deemed this clear evidence of a strong cellular "waste" phenomenon [6] or an energy drain of unknown source accounting for the use of extra calories. One possible hypothesis is that reversal of the metabolic consequences of caloric restriction necessitates activation of systems requiring energy.

Recently, bone has been recognized as a highly metabolically active tissue requiring energy [7] such that new bone formation is appropriately suppressed with inadequate nutrition. Indeed, reduction in bone mass to the degree of osteopenia and osteoporosis has been observed in over 90% of adolescents with AN who have been amenorrheic for more than 6 months [8]. Despite consideration of various therapies, weight restoration, which occurs prior to return of menses, along with menstrual recovery is regarded as the foundation of bone recovery [9]. Significant increases of

BMD have been noted prior to return of menses [10] with an increase in suppressed bone formation and a fall in bone resorption. Osteocalcin and *N*-telopeptide (NTX), established biochemical markers of bone formation and resorption, respectively, have been shown to appropriately increase and decrease, respectively, with weight recovery [10] accompanied with a 3-4% increase in BMD in as little as 4 months. In this longitudinal study in which we examine women with AN before and after weight normalization and compare them with healthy female control subjects, we propose that a substantial amount of the unexplained increase in REE during refeeding of AN patients is channeled towards BMD recovery such that significant bone rebuilding is required before energy stores become available for restoration of gonadal function.

2. Materials and Methods

2.1. Subjects. We studied 37 patients aged 23.4 ± 4.8 (range 18–36 years) with AN and 16 healthy control women aged 25.1 ± 4.7 (range 18–35 years). Patients were receiving inpatient treatment on the Eating Disorders Research Unit at the New York State Psychiatric Institute, Columbia University Medical Center (NYSPI, CUMC). All met criteria for AN from the 4th edition of the *Diagnostic and Statistical Manual of Mental Disorders*. Subjects were recruited, screened, and subjected to exclusion criteria as previously described [10].

The 16 healthy control women were recruited from the New York City area and the Columbia University campus by public advertisements. All were healthy, eumenorrheic, and matched with patients by age and percentage of ideal body weight (IBW) as previously described [10]. None of the control women had a history of an eating disorder or psychiatric or medical illness. Potential control subjects receiving hormonal or other medications known to affect reproductive function or bone metabolism were excluded. All of the control women exercised less than 3 h/wk.

All subjects provided written informed consent. The study protocol was approved by the IRB of the NYSPI, CUMC, and St. Luke's-Roosevelt Hospital Center. Procedures were followed according to approved ethical guidelines.

2.2. Bone Density, Body Fat, Fat-Free Mass Index (FFMI), and Fat-Free Mass (FFM). Spine, pelvis, leg, and total BMDs were determined using dual energy X-ray absorptiometry (DEXA) from a DPX scanner (Lunar Corp., Madison, WI) using version 3.6 software. The reports from the DPX-L scanner (GE Systems, Madison, WI) were analyzed with the use of version 3.6 software and were used to determine regional BMD of the hip and spine, total body bone mineral content, and total percentage of body fat. The CVs for BMD measurements ranged from 0.5% to 1.0% [11]. When measured by DXA, percentage of body fat is independent of BMD because this value is measured directly by recognized standard means in fat depots at sites where bone is not present [12]. FFM is calculated by the following: fat mass = weight $\times$ fat% and FFM = weight $-$ fat mass. FFM is also known as lean body mass [13]. FFMI is calculated as FFM/height^2 [14].

2.3. REE. Each subject's REE was measured in a respiratory chamber-indirect calorimeter. The chamber was equipped with a high-precision gas (oxygen and carbon dioxide) exchange measurement system. A linear state space model converts gas exchange measurements to energy expenditure estimates. REE was measured early in the morning, with subjects in a fasted state for 12–15 h before the experiment. Subjects remained in the chamber for 1 h, and REE calculations were based on the average of 3 10-minute readings after 30 minutes had elapsed [15].

2.4. Biochemical Analyses. Serum osteocalcin was measured using a human immunoradiometric assay (Immunotopics International, San Clemente, CA) with a sensitivity of 0.5 ng/mL and an interassay CV of 5.5–6.7%. Urine NTX was measured with the use of an enzyme-linked immunoassay (Ostex International Inc, Seattle, WA) with a detection limit of 20 nmol bone collagen equivalent and an interassay CV of 4.1%. Assays for estradiol, FSH, LH, PRL, testosterone, DHEAS, T_3 and T_4 (both total and free), TSH, and cortisol were performed as previously described [10]. IGF-1 was assessed by RIA after alcohol extraction with an intra-assay coefficient of variation of 2.4–3.0% (Nichols Institute Diagnostics, San Juan Capistrano, CA). Leptin and total ghrelin levels were measured using commercial ELISA kits (Diagnostic Systems, Webster, TX). Assay sensitivity was 0.1 ng/mL for each. Both hormones were measured in nonfasting subjects. For leptin, the intra-assay coefficient of variation was 3.6%, and the interassay coefficient of variation was 4.9%. For ghrelin, the intra-assay coefficient of variation was 4.9%, and the interassay coefficient of variation was 5.5%, based on five assays. Blood and urine samples from patients were obtained at the initiation of hospitalization and at maintenance of weight gain to 90% IBW for ≥ 2 weeks except for one patient who reached 79% IBW. Venous samples from controls and menstruating subjects were obtained during the follicular phase of the menstrual cycle (days 3–10) as determined by a take-home ovulation test kit to confirm ovulatory cycles.

2.5. Treatment. Treatment for patients with AN followed a predominantly behavioral approach at the NYSPI aimed at normalizing weight and eating. Target weight was a minimum of 90% IBW based on 1959 Metropolitan Life Actuarial Tables. All but one patient reached a minimum of 90% IBW; that patient remained amenorrheic at 79% IBW. Median number of days of admission was 66. On admission, patients were fed a standard hospital diet of 1800 kcal ($\approx 55\%$ of energy from carbohydrates, 15% of energy from protein, and 30% of energy from fat), given as 3 meals/d and a snack. Patients were observed to eat 100% of the food prescribed and for 1 h afterwards. If patients did not gain weight, calories were increased in 400-kcal increments in food or liquid nutritional supplement (Ensure Plus; Abbott Laboratories, Abbott Park, IL). After a 1-2 wk medical stabilization period, patients began the active weight-gain treatment phase that

TABLE 1: Age and anthropometric measures (mean ± SD).

Characteristic	Patients with AN at admission (*n*)	Patients with AN at 90% IBW (*n*)	Amenorrheics at 90% IBW (*n*)	Regained menses at 90% IBW (*n*)	Controls (*n*)
Age[a] (years)	23.4 ± 4.8 (36)	23.6 ± 4.7 (36)	23.1 ± 4.2 (26)	24.8 ± 5.8 (10)	24.8 ± 4.7 (16)
Weight[a,b] (kg)	41.5 ± 5.4 (37)	53.8 ± 4.7 (37)	53.6 ± 5.0 (27)	54.5 ± 3.9 (10)	56.7 ± 4.4 (16)
BMI[a,b,c] (kg/m^2)	15.8 ± 1.6 (37)	20.4 ± 1.0 (37)	20.3 ± 1.1 (27)	20.8 ± 0.6 (10)	21.2 ± 0.9 (16)
REE[a] (kcal/d)	1087 ± 128 (23)	1378 ± 191 (23)	1395 ± 211 (17)	1327 ± 113 (6)	1451 ± 135 (12)
Fat free mass index[a] (kg/m^2)	14.4 ± 1.3 (36)	15.5 ± 1.1 (36)	15.6 ± 1.2 (27)	15.4 ± 0.8 (9)	15.6 ± 1.0 (16)

Significance set at $P < 0.05$ for all comparisons and is noted in the table.
[a]Significant difference between patients with AN at admission and patients with AN at 90% IBW.
[b]Significant difference between patients with AN at 90% IBW and controls.
[c]Significant difference between amenorrheics at 90% IBW and controls.

continued until patients reached 90% IBW with weight gain rates as previously described [10]. Formal exercise was not allowed during hospitalization although no effort was made to control for previous exercise load. Next, a 4–6 wk period of weight maintenance ensued during which patients gained independence and transitioned to outpatient care. Mean caloric intake on discharge was 2600 kcal. No calcium or vitamin D supplements were given.

2.6. Statistical Analysis. Data was analyzed using multiple *t*-tests, and the probability level was adjusted using the Bonferroni correction to compare patients at baseline and after weight gain, those with amenorrhea, those who regained menses, and controls. All data are expressed as mean ± SD. We used independent and dependent *t*-tests to measure differences between groups. Multiple comparisons were made with use of the Bonferroni method, available in SPSS software (version 12; SPSS Inc, Chicago, IL). Significance was set at $P < 0.05$ for all comparisons. Initially, patients were analyzed as one group with the use of independent *t*-tests; later, menstrual status was taken into account. For comparisons with controls, 2-factor repeated-measures analysis of variance with Bonferroni correction was conducted. Repeated-measures analyses were performed for LH, FSH, estradiol, testosterone, DHEAS, serum osteocalcin, urine NTX, and leptin after a log transformation that resulted in a SD < 20% value for each parameter. Correlations between REE and change in BMD and between REE and FFMI were tested using the Pearson linear correlation.

3. Results

Thirty-seven patients and 16 healthy control subjects entered and completed the study. Of the 37 study patients, 10 regained normal menstruation at 90% IBW. Age and anthropometric measures are shown in Table 1. Bone marker, BMD, and hormone data are shown in Table 2.

When the AN patients were refed to 90% IBW, significant increases were observed in BMI, REE, FFMI, osteocalcin, spine and pelvis BMDs, LH, FSH, estradiol, leptin, total T_3, and IGF-1 while cortisol and ghrelin decreased. As the metabolic rate is a function of metabolically active tissues, all of them contained in the FFM, we controlled for differences in FFM between patients with anorexia and controls by using REE/FFM and REE/FFMI [16]. At 90% IBW, significant correlations were observed between REE and changes in spine ($r = 0.48$, $P < 0.02$) and leg ($r = 0.43$, $P < 0.05$) BMDs as shown in Figures 1 and 2, respectively. At 90% IBW, both BMI and weight correlated with change in leg BMD, and total T_3 correlated with change in spine BMD ($P < 0.05$). The REE correlations with spine and leg BMDs were even more significant after controlling for weight gain, an important predictor of BMD ($r = 0.65$, $P < 0.005$) and ($r = 0.56$, $P < 0.02$), respectively. The correlations remained significant after controlling for T_3. No significant correlations were found between REE and FFMI at baseline or at 90% IBW or between REE and the hormonal parameters including leptin, ghrelin, and IGF-1.

Comparison of the patients with AN at 90% IBW to controls showed significantly lower BMI, spine, pelvis, leg, and total BMDs, LH, FSH, estradiol, and free T_4 and higher urine NTX and IGF-1. Osteocalcin was higher in patients with AN but not significantly so. In contrast to the patients with AN, controls exhibited high correlation between REE and FFMI ($r = 0.71$, $P < 0.01$).

As a further analysis, patients were divided into two groups according to menstrual status at the time of treatment after 90% IBW testing: those who remained amenorrheic and those who regained menses. Of note, there was no difference in baseline weight (41.1 kg versus 42.3 kg, amenorrheic versus regained menses). After weight rehabilitation, no differences were observed between the groups in weight, BMI, or REE, but the amenorrheic group had higher urine NTX ($P < 0.02$) and lower estradiol ($P < 0.006$) than those

Table 2: Bone marker, BMD, and hormone data (mean ± SD).

Characteristic	Patients with AN at admission (*n*)	Patients with AN at 90% IBW (*n*)	Amenorrheics at 90% IBW (*n*)	Regained menses at 90% IBW (*n*)	Controls (*n*)
Osteocalcin[a] (ng/mL)	8.1 ± 3.0 (33)	11.1 ± 5.9 (34)	11.4 ± 6.6 (25)	10.1 ± 3.6 (9)	8.7 ± 3.6 (12)
Urine NTX[b,d] (nmol/mmol Cr)	72.4 ± 32.7 (34)	70.9 ± 46.8 (30)	80.0 ± 50.3 (22)	45.9 ± 22.7 (8)	48.3 ± 14.4 (11)
Total BMD[b,c] (g/cm^2)	1.05 ± 0.09 (37)	1.05 ± 0.09 (37)	1.04 ± 0.09 (27)	1.07 ± 0.06 (10)	1.13 ± 0.05 (16)
Spine BMD[a,b,c] (g/cm^2)	0.89 ± 0.15 (37)	0.94 ± 0.14 (37)	0.92 ± 0.14 (27)	0.98 ± 0.13 (10)	1.07 ± 0.09 (16)
Pelvis BMD[a,b,c] (g/cm^2)	0.91 ± 0.13 (37)	0.94 ± 0.12 (37)	0.93 ± 0.12 (27)	0.98 ± 0.09 (10)	1.07 ± 0.06 (16)
Legs BMD[b,c] (g/cm^2)	1.10 ± 0.15 (37)	1.09 ± 0.13 (37)	1.07 ± 0.14 (27)	1.12 ± 0.09 (10)	1.19 ± 0.07 (16)
IGF-1[a,c] (ng/ml)	244.4 ± 103.1 (34)	343.6 ± 120.1 (35)	345.7 ± 121.4 (26)	337.6 ± 123.2 (9)	247.9 ± 76.5 (12)
Leptin[a] (ng/ml)	2.9 ± 2.7 (28)	14.7 ± 17.8 (31)	13.1 ± 15.6 (23)	19.3 ± 23.8 (8)	11.5 ± 5.9 (10)
Ghrelin[a] (pg/ml)	2025 ± 748 (24)	1567 ± 669 (25)	1588 ± 654 (21)	1835 ± 1213 (4)	1738 ± 542 (11)
LH[a,b] (mIU/ml)	1.4 ± 2.9 (30)	2.8 ± 2.8 (35)	2.5 ± 2.7 (25)	3.6 ± 3.1 (10)	4.0 ± 2.3 (13)
FSH[a,b] (mIU/ml)	1.8 ± 2.1 (29)	2.7 ± 1.5 (34)	2.6 ± 1.3 (24)	3.0 ± 1.8 (10)	3.9 ± 1.3 (13)
Estradiol[a,b,c,d] (pg/ml)	23.7 ± 6.4 (32)	32.4 ± 14.0 (35)	28.2 ± 10.1 (25)	42.7 ± 17.3 (10)	52.4 ± 31.7 (14)
Testosterone (ng/dl)	76.2 ± 36.7 (26)	81.8 ± 36.7 (31)	79.3 ± 38.6 (23)	89.1 ± 31.8 (8)	63.1 ± 21.0 (16)
DHEAS (μg/dl)	153.1 ± 59.6 (25)	142.0 ± 81.1 (29)	139.8 ± 86.9 (23)	150.2 ± 59.3 (6)	175.8 ± 78.3 (16)
Cortisol[a] (μg/dl)	20.5 ± 5.5 (24)	16.7 ± 6.3 (27)	16.6 ± 5.9 (21)	16.2 ± 7.6 (6)	12.0 ± 4.3 (10)
TSH (mIU/L)	2.0 ± 1.2 (23)	1.7 ± 1.0 (26)	1.9 ± 1.0 (21)	1.2 ± 0.4 (5)	1.9 ± 1.0 (12)
Total T_3[a] (ng/dl)	76.8 ± 18.3 (27)	106.0 ± 28.3 (32)	103.8 ± 27.8 (25)	113.9 ± 30.9 (7)	120.1 ± 22.9 (12)
Free T_4[b] (ng/dl)	1.0 ± 0.2 (27)	1.0 ± 0.2 (32)	1.0 ± 0.2 (25)	1.0 ± 0.1 (7)	1.1 ± 0.1 (12)

Significance set at $P < 0.05$ for all comparisons and is noted in the table.
[a] Significant difference between patients with AN at admission and patients with AN at 90% IBW.
[b] Significant difference between patients with AN at 90% IBW and controls.
[c] Significant difference between amenorrheics at 90% IBW and controls.
[d] Significant difference between amenorrheics at 90% IBW and regained menses at 90% IBW.

who regained menses. Osteocalcin was also highest in this group, but not significantly so. Compared to controls, the amenorrheic group at 90% IBW had lower BMI ($P < 0.03$), estradiol ($P < 0.001$), spine ($P < 0.001$), pelvis ($P < 0.0005$), leg ($P < 0.008$), and total ($P < 0.001$) BMDs and higher IGF-1 ($P < 0.05$). Significant correlation between REE and

change in spine BMD ($r = 0.59$, $P < 0.02$) was exhibited in the group who remained amenorrheic without correlation between REE and change in leg BMD ($P < 0.09$). Controlling for weight gain was limited by the number of patients in the group. No difference in BMI, estradiol, IGF-1, or BMDs was exhibited in the group who regained menses compared with
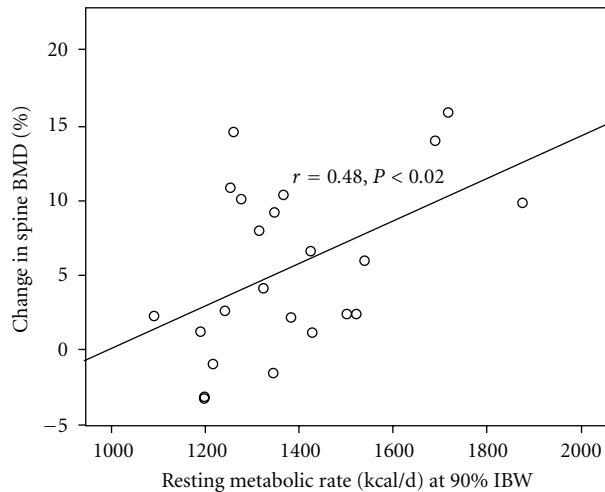
FIGURE 1: Correlation between resting metabolic rate of patients at 90% IBW and % change in spine BMD. Each circle represents the data point of a patient.
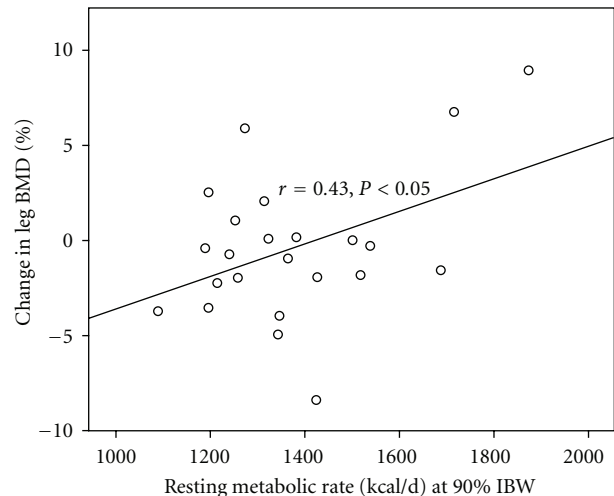

FIGURE 2: Correlation between resting metabolic rate of patients at 90% IBW and % change in leg BMD. Each circle represents the data point of a patient.

controls, indicating near recovery of bone mass and gonadal function. Interestingly, no significant correlation between REE and changes in BMD was found in this group.

4. Discussion

The etiology of the increase in REE with refeeding in AN patients has been the subject of much debate. This is the first report showing significant correlations between REE and changes in BMD with nutritional rehabilitation. This correlation is apparent in only women who have not yet regained menses, suggesting a physiologic event with bone as a possible metabolic sink or energy drain.

Previous studies have not attempted to correlate REE and FFMI in AN patients although REE was disproportionately elevated compared to increases in lean body mass [5]. This is the first study to show no correlation between REE and height-normalized FFM indices in AN patients despite high correlation in the normal population [17]. The absence of correlation found in our study along with the disproportionate REE elevations previously reported suggest that the increase in REE with refeeding is not accounted for by lean body mass but by another physiologic system undergoing marked energy utilization.

During refeeding, the significant correlations between REE and changes in BMD along with the elevated osteocalcin and the increase in IGF-1 to induce bone collagen formation suggest bone recovery from the osteopenic or osteoporotic state as a source of the increased REE. The observed correlation of REE with change in BMD in amenorrheic subjects with low BMDs and the lack of correlation in the group with resumed menses and near-normal BMDs suggest that the latter group no longer needs to channel the energy towards bone. Indeed, whereas the group who remained amenorrheic continued to exhibit elevated urine NTX, the group who regained menses demonstrated normalization of urine NTX, indicating a return to the normal state of

bone resorption. The recent discovery of bone remodeling as a physiologic phenomenon requiring a large amount of energy but serving an evolutionary advantage [7] further supports the notion of bone as a metabolic sink, for the utilization of REE for bone recovery would serve a survival purpose. Indeed, the endocrine manifestations of AN typically result from mechanisms designed to preserve energy [18].

Many studies relate osteopenia and osteoporosis in AN to nutrition, but observations linking resumption of menses to bone recovery [19–21] may be explained by the notion that only after substantial bone recovery occurs are sufficient energy stores available for gonadal recovery. The etiology of bone loss in AN is multifactorial, and nutritional factors, particularly IGF-1, have been recognized to likely have an important role [22]. The dramatic increases in REE seen with nutritional rehabilitation and weight gain appear to be related to bone recovery. Indeed, a comprehensive study in AN following bone indices showed the powerful anabolic effect of nutritional rehabilitation on bone [10]. However, in this study, the authors state that menstrual resumption was required to suppress bone resorption and allow maximal appropriate bone recovery. Yet a quantitative effect of regained menses on bone mass remains unclear. In our study, the group who regained menses had near-normal BMDs and no correlation between REE and changes in BMD, suggesting that the majority of bone recovery had already occurred such that resumption of menses would not lead to further significant change. Instead of favorably predicting BMD recovery, menstrual return may indicate adequate replacement of energy stores.

One potential regulator linking disordered eating, amenorrhea, and bone is leptin, a fat cell hormone disproportionately lowered by fasting. Leptin thresholds have been associated with increased gonadotropins [2] and leptin administration with resumption of ovulation in hypothalamic amenorrheic women [23]. Recently, leptin receptors were also

discovered in bone [24], thereby providing a possible mechanism for interaction between nutritional and metabolic bone axes and resumption of menses.

Our data are consistent with other studies examining the bone and hormonal changes that occur with refeeding in AN [2, 9]. In this present study, however, we searched for and found significant correlations between REE and changes in BMD during nutritional rehabilitation. The main limitation was the size of the study population, for a larger sample would allow for control of various variables while examining correlations and would facilitate detection of differences between those who remained amenorrheic versus those who regained menses. Of note, it would have been interesting to follow the bone markers of the amenorrheics at 90% IBW to see if continuation of adequate nutritional intake would reverse bone loss and amenorrhea, two of the many important complications of AN [25].

In conclusion, we explore here an alternate hypothesis of the increase in REE during refeeding of AN patients to relate the increased energy expenditure to bone formation such that only after substantial BMD recovery are sufficient energy stores available for gonadal recovery. Bone recovery from osteopenic and osteoporotic states may take priority because of its survival advantage, a notion consistent with the reproductive then bone loss that occurs in AN. Further research is necessary to identify why one group of AN patients recovers bone mass and menses while undergoing the same nutritional rehabilitation as another group yet to recover either. Nonetheless, our hypothesis that bone recovery utilizes a vast amount of energy offers the exciting possibility that prolonged nutritional rehabilitation may lead to recovery from osteopenia and osteoporosis and resumption of menses in the women who remain amenorrheic with low BMD.

Disclosure

Acknowledgments

References

[1] H. W. Hoek and D. van Hoeken, "Review of the prevalence and incidence of eating disorders," *International Journal of Eating Disorders*, vol. 34, no. 4, pp. 383–396, 2003.

[2] K. Holtkamp, C. Mika, I. Grzella et al., "Reproductive function during weight gain in anorexia nervosa. Leptin represents a metabolic gate to gonadotropin secretion," *Journal of Neural Transmission*, vol. 110, no. 4, pp. 427–435, 2003.

[3] J. C. Melchior, D. Rigaud, R. Rozen, D. Malon, and M. Apfelbaum, "Energy expenditure economy induced by decrease in lean body mass in anorexia nervosa," *European Journal of Clinical Nutrition*, vol. 43, no. 11, pp. 793–799, 1989.

[4] N. Vaisman, M. F. Rossi, M. Corey, R. Clarke, E. Goldberg, and P. B. Pencharz, "Effect of refeeding on the energy metabolism of adolescent girls who have anorexia nervosa," *European Journal of Clinical Nutrition*, vol. 45, no. 11, pp. 527–537, 1991.

[5] E. Obarzanek, M. D. Lesem, and D. C. Jimerson, "Resting metabolic rate of anorexia nervosa patients during weight gain," *American Journal of Clinical Nutrition*, vol. 60, no. 5, pp. 666–675, 1994.

[6] M. Moukaddem, A. Boulier, M. Apfelbaum, and D. Rigaud, "Increase in diet-induced thermogenesis at the start of refeeding in severely malnourished anorexia nervosa patients," *American Journal of Clinical Nutrition*, vol. 66, no. 1, pp. 133–140, 1997.

[7] C. B. Confavreux, R. L. Levine, and G. Karsenty, "A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms," *Molecular and Cellular Endocrinology*, vol. 310, no. 1-2, pp. 21–29, 2009.

[8] N. H. Golden, L. Lanzkowsky, J. Schebendach, C. J. Palestro, M. S. Jacobson, and I. R. Shenker, "The effect of estrogen-progestin treatment on bone mineral density in anorexia nervosa," *Journal of Pediatric and Adolescent Gynecology*, vol. 15, no. 3, pp. 135–143, 2002.

[9] Y. Jayasinghe, S. R. Grover, and M. Zacharin, "Current concepts in bone and reproductive health in adolescents with anorexia nervosa," *British Journal of Gynaecology*, vol. 115, no. 3, pp. 304–315, 2008.

[10] J. Dominguez, L. Goodman, S. S. Gupta et al., "Treatment of anorexia nervosa is associated with increases in bone mineral density, and recovery is a biphasic process involving both nutrition and return of menses," *American Journal of Clinical Nutrition*, vol. 86, no. 1, pp. 92–99, 2007.

[11] M. Russell-Aulet, J. Wang, J. Thornton, and R. N. Pierson, "Comparison of dual-photon absorptiometry systems for total-body bone and soft tissue measurements: dual-energy X-rays versus gadolinium 153," *Journal of Bone and Mineral Research*, vol. 6, no. 4, pp. 411–415, 1991.

[12] R. B. Mazess, H. S. Barden, J. P. Bisek, and J. Hanson, "Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition," *American Journal of Clinical Nutrition*, vol. 51, no. 6, pp. 1106–1112, 1990.

[13] B. A. Kaufman, M. P. Warren, J. E. Dominguez, J. Wang, S. B. Heymsfield, and R. N. Pierson, "Bone density and amenorrhea in ballet dancers are related to a decreased resting metabolic rate and lower leptin levels," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 6, pp. 2777–2783, 2002.

[14] T. B. VanItallie, M. U. Yang, S. B. Heymsfield, R. C. Funk, and R. A. Boileau, "Height-normalized indices of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status(1,2)," *American Journal of Clinical Nutrition*, vol. 52, no. 6, pp. 953–959, 1990.

[15] S. B. Heymsfield, D. B. Allison, F. X. Pi-Sunyer, and Y. Sun, "Columbia respiratory-chamber indirect calorimeter: a new approach to air-flow modelling," *Medical and Biological Engineering and Computing*, vol. 32, no. 4, pp. 406–410, 1994.

[16] P. Webb, "Energy expenditure and fat-free mass in men and women," *American Journal of Clinical Nutrition*, vol. 34, no. 9, pp. 1816–1826, 1981.

[17] D. Gallagher, D. Belmonte, P. Deurenberg et al., "Organ-tissue mass measurement allows modeling of ree and metabolically active tissue mass," *American Journal of Physiology*, vol. 275, no. 2, pp. E249–E258, 1998.

[18] M. P. Warren, "Endocrine manifestations of eating disorders ," *The Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 2, pp. 333–343, 2011.

[19] C. M. Gordon, E. Goodman, S. J. Emans et al., "Physiologic regulators of bone turnover in young women with anorexia nervosa," *Journal of Pediatrics*, vol. 141, no. 1, pp. 64–70, 2002.

[20] A. Caillot-Augusseau, M. H. Lafage-Proust, P. Margaillan et al., "Weight gain reverses bone turnover and restores circadian variation of bone resorption in anorexic patients," *Clinical Endocrinology*, vol. 52, no. 1, pp. 113–121, 2000.

[21] M. P. Warren, J. Brooks-Gunn, R. P. Fox et al., "Persistent osteopenia in ballet dancers with amenorrhea and delayed menarche despite hormone therapy: a longitudinal study," *Fertility and Sterility*, vol. 80, no. 2, pp. 398–404, 2003.

[22] I. Legroux-Gerot, J. Vignau, F. Collier, and B. Cortet, "Factors influencing changes in bone mineral density in patients with anorexia nervosa-related osteoporosis: the effect of hormone replacement therapy," *Calcified Tissue International*, vol. 83, no. 5, pp. 315–323, 2008.

[23] C. K. Welt, J. L. Chan, J. Bullen et al., "Recombinant human leptin in women with hypothalamic amenorrhea," *New England Journal of Medicine*, vol. 351, no. 10, pp. 987–997, 2004.

[24] T. Thomas, F. Gori, S. Khosla, M. D. Jensen, B. Burguera, and B. L. Riggs, "Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes," *Endocrinology*, vol. 140, no. 4, pp. 1630–1638, 1999.

[25] J. E. Mitchell and S. Crow, "Medical complications of anorexia nervosa and bulimia nervosa," *Current Opinion in Psychiatry*, vol. 19, no. 4, pp. 438–443, 2006.