Eating Disorders, Menstrual Dysfunction, Weight Change and DMPA Use Predict Bone Density Change in College-Aged Women

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Abstract:

Introduction: There are limited longitudinal studies that have evaluated bone mineral density (BMD) changes in college-aged women. Our objective was to simultaneously evaluate factors influencing 4-year BMD change. **Methods:** This was a longitudinal cohort study of healthy, physically active women in the US Military Academy (n=91; average age=18.4 years). Assessments over the four years included: height, weight, calcium intake, physical fitness, menstrual function (annual number cycles), oral contraceptives (OC) or depot-medroxyprogesterone acetate (DMPA) use, and eating disorder behavior (Eating Disorder Inventory; (EDI)). BMD was measured annually at the lumbar spine and total hip by dual x-ray absorptiometry and calcaneal BMD by PIXI. Slope of 4 year BMD change at each skeletal site (spine total hip and calcaneus) was calculated for each woman. **Results:** BMD gains occurred at the spine in 50% and the hip in 36% of women. In unadjusted analyses, spine bone gain was positively related to menstrual cycle frequency (p=0.04). Spine and hip BMD loss occurred in those using DMPA (p<0.01) and those with highest EDI quartile scores (p<0.05). BMD change was unrelated to OC use. Hip and calcaneus BMD decreased with weight loss (average 4.8+2.2 pound/year) as compared to those with stable weight/weight gain (p<0.05). In multivariable analysis, spine BMD increase was significantly related to African American (AA) race, normal EDI score and normal menses. Hip BMD increase was related to AA race, weight increase and normal menses. DMPA use was associated with spine, hip, and calcaneus bone loss.

Conclusion: On average, BMD may modestly increase in college-aged women, in the absence of risk factors. However, risk factors including subclinical eating disorders, weight loss, menstrual dysfunction and DMPA use can have significant detrimental effects on BMD in young healthy physically active women.

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Introduction:

A key prevention strategy to decrease the risk of osteoporosis is to increase early accrual of BMD. Higher BMD during youth is related to a decreased risk of childhood fractures and a higher peak bone mass may be associated with a decreased risk of osteoporosis related fractures later in life [1, 2]. Therefore, it is important to understand those modifiable factors that can improve the accrual of peak bone mass and optimal bone geometry [3, 4].

The accrual of bone during youth can be limited by menstrual dysfunction (oligo- and amenorrhea), whether due to weight loss, stress, or exercise [5-8]. Furthermore, the increased risk of stress fractures in women with a history of amenorrhea seen in some [9, 10] but not all studies [11] may also represent an adverse effect on the skeleton.

Oral contraceptive (OC) use may impact accrual of peak bone mass, although some of the data conflict and effects may be dose dependent with ultra-low dose pills causing a decrease in BMD accrual [12-15]. One study reported that either exercise or OC use was associated with a suppression of the normal increase in bone mass and mechanical strength in the femoral neck in women 18-31 years old, but the combination of exercise and OC use appeared to have a less suppressive effect [12]. The type and dose of contraception, lifestyle factors, and age at first use may alter the impact of OC use on bone health [16-18]. During adulthood, most studies have reported that OCs, even those containing a low dose of ethinyl-estradiol, had neutral or possibly beneficial effects on bone health. However, in adolescent girls, OC use may impede the development of peak bone mass, particularly when started within the teen years [19]. In addition, whether OC was started for menstrual dysfunction or for contraceptive indications may change

the effect on bone. The use of depot-medroxyprogesterone acetate (DMPA) has been shown to be detrimental to bone [20], particularly if used before the achievement of peak bone mass [21]. There are many other factors that can positively influence BMD acquisition, including adequate levels of physical activity and adequate calcium intake [22-24]. However, associated irregular menstrual periods in those who engage in excessive exercise or inadequate nutrition from eating disorders can have a negative impact on BMD [25, 26]. Cognitive eating restraint appears to predict lower BMD independent of age, BMI and amenorrhea [27]. The female athlete triad is now considered a spectrum of abnormalities in energy availability, menstrual function and BMD [28]. Prevalence estimates of all three triad conditions presenting simultaneously are generally low in female athletes, ranging from 0% to 16%. A greater percentage of these female athletes present with any two of the triad conditions, ranging from 3% to 27%, and an even greater proportion present with any one of the triad conditions ranging from 16% to 60%.[28].

Most of the information we have on the acquisition of peak bone mass is based on cross sectional data and a few short term longitudinal studies. The objective of this study was to evaluate prospective changes over 4 years in bone mineral density in a unique college-aged physically active population attending the United States Military Academy (USMA). We assessed comprehensively the importance of demographic factors such as race and changes in weight and height. We hypothesized that menstrual dysfunction, DMPA use, and eating disorders would have a negative impact, whereas physical fitness level, dietary calcium intake, and oral contraceptives would have a positive influence on bone mass accrual over 4 years.

Methods

Subjects

All cadets from one class at USMA in West Point NY were asked to participate in a longitudinal study to evaluate the determinants of peak bone mass in elite military cadets over a four year period. The USMA is a four year military college with strict entrance requirements that include academic and physical excellence. The Institutional Review Board (IRB) of Keller Army Community Hospital (KACH), West Point, NY, approved the study. There are stringent medical requirements for entrance into USMA that led to the exclusion of women with severe polycystic ovarian disease and other diseases that affect BMD, including persons with uncontrolled thyroid disease, epilepsy, gastrointestinal disease or long term steroid use [29], therefore the normal exclusion criteria were not needed. One-hundred-thirty-five women entering USMA consented to this study (70% of the class). At the time of graduation, 91 women (75 Caucasian, 9 Asian, and 7 African American) remained in the study, completed the assessments described below and are included in the analyses.

Assessments:

Menstrual function and use of OC and DMPA during the prior year were assessed annually by written questionnaire. Number of months of use was averaged over the four years. Menstrual function was assessed as mean number of cycles during the prior year and results were averaged over the 4 years. An average of greater than or equal to 10 menstrual cycles per year was considered normal and menstrual dysfunction was defined as 9 or fewer menstrual cycles each year [6, 30]. Years from menarche was dichotomized at ≤ 5 or > 5 years (on average, 5 years from menarche equates to an average menarchal age between 12 and 13 years. This value is

similar to national data from NHANES that indicates that 90% of all US girls are menstruating by 13.75 years of age, with a median menarchal age of 12.43 years [31].

Calcium intake was determined by annual food frequency questionnaire using the modified Block questionnaire [32] with 12 questions including intake of milk, cheese, tofu, ice cream, cheese dishes and green leafy vegetables for calcium, as well as calcium supplementation. We used the average calcium intake reported over the four years. We did not evaluate smoking as only 3% previously smoked and only 10% admitted to consumption of 2 alcoholic beverages per week prior to entry into the academy.

Eating disorders were assessed once by using 3 subscales of the Eating Disorder Inventory: EDI-DT (drive for thinness); EDI-B (bulimic tendencies) and EDI-D (body dissatisfaction) and this summary score was divided into quartiles, with higher quartile scores suggestive of symptoms consistent with an eating disorder. A score of 32 is average for Anorexia using these 3 subscales (DT=15; B=3; D=14) where the maximum possible score is 69 [26, 33]. Scores on the EDI have been shown to be stable in individuals of this age group, such that scores at the time the inventory was completed (during the last year) would be expected to correlate strongly with scores upon entry to USMA [34].

Physical measurements:

Height and weight were measured annually and body mass index was calculated (weight in kilograms/height in meters²). Weight change was assessed as gain or stable weight versus any loss. Height was assessed on a standard scale and categorized as either stable or increasing (>0.5

inch increase). Body fat was also measured using Bioelectric Impedance (BIA) annually. Every year each cadet took the Army Physical Fitness Test (APFT) that included individual scores for 2-mile run speed (cardiorespiratory endurance), number of push-ups in 2 minutes (upper body strength) and number of sit-ups in 2 minutes (abdominal and hip flexor strength), as well as a composite score of these three fitness variables. The final score serves as an overall indicator of fitness and has a range of 0 to 300 with faster running times, higher numbers of push-ups and sit-ups earning higher scores. The running event has been highly correlated with VO₂ max and is considered a good proxy measure for cardio-respiratory fitness [35].

During their time at USMA, in addition to a rigorous daily lifestyle routine, all cadets are required to march daily, attend 3 hours of physical education per week, and participate in NCAA, club or company level sports. All cadets would fall into the upper end of the exercise spectrum, since cadets participate in mandatory activity as well as a minimum of 4 and as many as 14 or more hours of sports practice a week. Due to constraints on the time allotted with each cadet by USMA, we did not capture specific sports activity while at USMA. However, we believe that the relatively small differences in optional activity compared to mandatory activity would have minimal additional impact. BMD (g/cm²) was measured annually in the calcaneus, spine, and total hip. BMD of the left calcaneus was measured by peripheral dual-energy x-ray absorptiometry (pDXA; PIXI, Lunar, Madison, WI). Total hip and spine BMD were measured using a mobile DXA scanner (DPX-IQ, Lunar, Madison WI). Hip structural analysis (HSA) software was used to determine femoral neck diameter [36]. Individuals (n=10) were scanned twice on each machine to calculate the short-term coefficient of variation for each measurement. The coefficient of variation for bone density in vivo was 1.0%, 1.5%, and 1.5% for the calcaneus, spine, and total hip respectively.

Statistical Analysis:

Analyses were performed using SAS software, Version 9.3 (Cary, NC). All analyses were based on those women completing the four years of evaluations. The pre-study power calculation was based on the impact of menstrual dysfunction on bone mass. Given 91 individuals, an alpha of 0.05 and a standard deviation of 0.02 for bone density we would have 80% power to detect a 5% difference in bone density between those women with menstrual dysfunction and normally menstruating women. Annual assessments of calcium intake, menstrual function, and OC and DMPA use were averaged over the four years. Individuals with an average use of OC or DMPA over the four years of more than 3 months per year (>12 months over 4 years) were considered OC or DMPA users. The 4 year change in weight or height was used to categorize weight loss or height gain. Slopes of change in spine, hip, and calcaneus BMD were calculated for each woman over the 4 years, and percent annualized or absolute annualized change was derived from the individual slopes. BMD slope of change within each of the above categories was evaluated using t-tests. Analysis of covariance (PROC GLM) was used to assess the impact of various risk factors on annualized change in BMD, controlling for potential covariates.

Results

Baseline data (Table 1)

The women were 82% Caucasian, 8% African American (AA) and 10% Asian. The average age, BMI and racial composition of the final population who completed a 4 year follow-up (n=91) was similar to the initial cohort of 135 women who did not complete the study. The average score on the APFT was 206.8 ± 50.0 , with a range of 66 to 280. Results on the APFT, including time to run 2 miles (14 minutes, 27 seconds) and number of pushups (n=32) revealed the high

level of physical fitness in entrants to the USMA as compared to female military recruits (17 min 24 sec for run and 22 for push-ups) [37]. The percent body fat was 21% by BIA and mean BMI was 23 kg/m². At baseline, mean BMD levels were above age matched controls from the Lunar normative DXA database for spine, hip, and calcaneus. The average daily calcium intake was 1078± 426 mg. Although 42% consumed less than a glass of milk per day they still had an average intake at 900 mg per day. The highest quartile of EDI (n=23 women) for the three subscales had a mean of 38.5 with a range of 25-57, a value within the range that can be seen in women with anorexia nervosa and may represent subclinical eating disorders [33, 38].

Change in variables over time (Table 2)

Approximately one half of the women gained height (average 0.9 inches). Two-thirds of the women who gained height were within 5 years of menarche. About one third of the women lost weight over the 4 years with an average weight loss of 4.8 ± 2.2 lbs. as compared to those with stable weight or weight gain (+10.5±7.5 lbs.; Table 2). The APFT improved (3 to 158 points) in all women over the 4 years (mean increase in total score=77.7).

In the entire group there were small changes in BMD (spine, hip, and calcaneus). However, 50% of the women had bone loss in at least one of these skeletal sites (spine, hip, calcaneus). There was also a small mean increase in femoral neck diameter that occurred in 85% of the women.

Unadjusted analysis of BMD changes (Table 3)

There was significantly more bone gained at the spine and hip in AA women as compared to all other races (p<0.05), but there were no racial differences in calcaneal BMD change.

The women who lost weight (n=28) had an annual loss in hip (0.8%/year) and calcaneal BMD (0.9%/year) over the four years that was significantly different from those women who had stable weight or weight gain. There were no significant differences between weight change and changes in spine BMD. Change in height was unrelated to BMD change at any skeletal site, but the change in femoral neck diameter was significantly greater in the women who were still gaining height as compared to those with stable height.

We compared those who had a four year average APFT score that was above versus below the median with change in BMD and femoral neck diameter. There was no significant relationship between the higher or lower average APFT score over the four years and BMD change at any skeletal sites (although spine $p=0.06$) or change in femoral neck diameter.

Women in the highest EDI quartile lost spine BMD while there was a gain in spine BMD in the other 3 quartiles. There was a similar trend with hip BMD ($p<0.10$), but no relationship with change in calcaneus BMD. The gain in BMD at any skeletal site was not significantly different whether calcium intake was above or below the recommended intake (1000 mg calcium/day). In addition, there was no association between calcium intake as a continuous variable and BMD in these women.

There was no relationship between years from menarche (dichotomized by ≤ 5 years or > 5 years) and BMD change at any site. Bone gain or stability at the spine and hip were seen in those with normal menstrual cycles whereas bone loss occurred at the spine and hip in those with menstrual dysfunction ($p<0.05$). There was no relationship between these categories of menstrual cycles and changes in calcaneus BMD.

There was no significant relationship between change in BMD at the spine, hip, or calcaneus and use of OC (median use was 6 months a year). However, annual increase in femoral neck

diameter was significantly greater in OC users (0.39 ± 0.05 mm) as compared to non-users (0.12 ± 0.07 mm; $p < 0.001$). DMPA users (median use was 6.3 months per year) had bone loss at the spine, hip and calcaneus, while those who never used DMPA maintained or gained bone at these sites ($p < 0.05$).

Multivariable analysis

Figure 1 A demonstrates that positive changes in spine BMD were seen in women who were African-American ($p = 0.035$), had an EDI score in the normal range ($p = 0.04$), and reported normal menses ($p = 0.001$) in models that included all of these variables (except for the variable that was graphed), in addition to weight change and baseline BMD. Loss in spine BMD was related to use of DMPA ($p < 0.001$), after controlling for race, weight change, EDI score, and baseline BMD (Figure 1A). Increases in hip BMD were related to African American race ($p = 0.023$), weight gain ($p = 0.008$), and normal menses ($p = 0.011$) after controlling for all of these variables as well as EDI score and baseline hip BMD. Use of DMPA was associated with a loss in hip BMD ($p < 0.001$) after controlling for race, weight change, EDI score and baseline BMD (Figure 1B).

There was a greater increase in neck diameter (mm/year) in those who were still gaining height as compared to those with stable height ($p < 0.01$). There was also a greater increase in femoral neck diameter for women on OCs as compared to non-users ($p < 0.01$), after controlling for race, height change, DMPA use, and baseline femoral neck diameter (Figure 1C).

The use of DMPA was also related to a loss in calcaneal BMD as compared to a gain in calcaneal BMD in those without DMPA use ($p = 0.05$) after controlling for race, weight change, and baseline calcaneal BMD (Figure 1D).

Because of potentially important racial differences, analyses were repeated in Caucasian women only (Table 4). In all Caucasians, there was a slight mean loss of bone at the spine and hip. When risk factors were present (weight loss, high EDI score, menstrual dysfunction or DMPA use), BMD loss was even greater. Because the majority of women had a small increase in femoral neck diameter, multivariate analyses were completed to control for the change in femoral neck diameter over the four years. There were no significant differences from the results that are presented in Figure 1B (data not shown).

Discussion:

In this cohort of young physically active women, we found that components of the female athlete triad including menstrual dysfunction and a high summary score based on 3 subscales of the EDI (indicative of subclinical eating disorders), as well as DMPA use had the strongest negative impact on accrual of BMD in young physically active women, even after controlling for important covariates. African American race and weight stability or weight gain were associated with BMD increase.

It is possible that the decrease in BMD, particularly notable in the presence of risk factors, could occur if bone size increases and bone mineralization lags behind. The multivariate analyses which show no impact of correcting for change in femoral neck diameter on our BMD results, suggests that BMC is increasing commensurate with an increase in bone size. However, in order to be sure, we would perhaps need to follow participants for a longer period of time.

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53 307 In this study, weight loss versus gain had an impact on accrual of bone mass, similar to a study in
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55 308 runners where weight gain was associated with increases in whole-body BMC, spine BMD, and
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58 309 hip BMD [18]. We did not see an association between dietary calcium intake and BMD accrual
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in this study, possibly because most women had adequate calcium intake (above the threshold for calcium intake) and perhaps because a majority of the calcium intake came from non-dairy sources. In contrast, in a prior study of older runners dietary calcium intake (primarily from low-fat dairy products) predicted bone gain regardless of menstrual function, even with a mean calcium intake at the recommended level, [42].

The association between overt disordered eating (Anorexia nervosa) and BMD has been shown in many studies [8]; however the relationship between subclinical eating disorder behavior and BMD is less well known. Our findings regarding the association between subclinical eating disorders (based on EDI scores) and BMD loss was robust and independent of multiple covariates. This is consistent with studies in young competitive female distance runners and varsity athletes [7, 43]. However, our findings suggest the possibility that there are potential skeletal impacts of subclinical eating disorders that may only come to light when assessed through surveys such as the EDI.

Physical activity has the capacity to increase BMD and modify bone structure and geometry by increasing mechanical loads to bones that stimulate osteogenic responses [44, 45]. During the four years at USMA, all women were exercising rather intensively due to USMA requirements for daily marching, physical education class (3 times a week) and participation in intramural or NCAA sports. In all women there was an increase in APFT scores perhaps related to more physical activity. The fact that even in these physically active women we still found bone loss related to high EDI scores, weight loss, menstrual irregularity or DMPA use indicates a need for further education and intervention in these areas in young women.

Limitations in this study include the fact that the number of non-Caucasian women was small in this population based sample. Our interest was in determining factors that relate to maximal BMD accrual, however, the small increase in bone size could have confounded our results. All women were physically active so we could not evaluate physical activity on changes in bone density. In addition, due to time constraints, we did not collect data on specific activity intensity or duration for each woman and the food frequency questionnaire had to be abbreviated. There could have been inaccurate recall regarding menstrual function and OC use. Confounding by indication was also a concern, particularly when evaluating OCs that may have been prescribed for menstrual irregularity. Furthermore, there were too few women with amenorrhea to resolve the difference in impact between menstrual dysfunction and amenorrhea. The strengths of the study included the 4 year longitudinal follow-up period, the multiple risk factors evaluated and the multiple skeletal sites investigated in a multiracial cohort.

In conclusion, subclinical eating disorders, weight loss, DMPA and infrequent menstrual function all have significant independent detrimental effects on bone gain in young physically active women. In order to optimize BMD accrual during youth, the benefit of physical activity must not be counteracted by infrequent menstrual function or subclinical eating disorders.

REFERENCES:

- [1] Tveit M, Rosengren BE, Nilsson JA, Karlsson MK. Exercise in youth: High bone mass, large bone size, and low fracture risk in old age. *Scand J Med Sci Sports* 2014.
- [2] Barrack MT, Gibbs JC, De Souza MJ, Williams NI, Nichols JF, Rauh MJ, Nattiv A. Higher incidence of bone stress injuries with increasing female athlete triad-related risk factors: a prospective multisite study of exercising girls and women. *Am J Sports Med* 2014;42: 949-58.
- [3] Stagi S, Cavalli L, Iurato C, Seminara S, Brandi ML, de Martino M. Bone metabolism in children and adolescents: main characteristics of the determinants of peak bone mass. *Clin Cases Miner Bone Metab* 2013;10: 172-9.

- [4] Maimoun L, Georgopoulos NA, Sultan C. Endocrine disorders in adolescent and young female athletes: impact on growth, menstrual cycles, and bone mass acquisition. *J Clin Endocrinol Metab* 2014;99: 4037-50.
- [5] Juul A, Hagen CP, Aksglaede L, Sorensen K, Mouritsen A, Frederiksen H, Main KM, Mogensen SS, Pedersen AT. Endocrine evaluation of reproductive function in girls during infancy, childhood and adolescence. *Endocr Dev* 2012;22: 24-39.
- [6] De Souza MJ, Williams NI. Physiological aspects and clinical sequelae of energy deficiency and hypoeestrogenism in exercising women. *Hum Reprod Update* 2004;10: 433-48.
- [7] Cobb KL, Bachrach LK, Greendale G, Marcus R, Neer RM, Nieves J, Sowers MF, Brown BW, Jr., Gopalakrishnan G, Luetters C, Tanner HK, Ward B, Kelsey JL. Disordered eating, menstrual irregularity, and bone mineral density in female runners. *Med Sci Sports Exerc* 2003;35: 711-9.
- [8] Misra M, Klibanski A. Anorexia nervosa and bone. *J Endocrinol* 2014;221: R163-76.
- [9] Bennell KL, Malcolm SA, Thomas SA, Ebeling PR, McCrory PR, Wark JD, Brukner PD. Risk factors for stress fractures in female track-and-field athletes: a retrospective analysis. *Clin J Sport Med* 1995;5: 229-35.
- [10] Lappe J, Cullen D, Haynatzki G, Recker R, Ahlf R, Thompson K. Calcium and vitamin d supplementation decreases incidence of stress fractures in female navy recruits. *J Bone Miner Res* 2008;23: 741-9.
- [11] Cosman F, Ruffing J, Zion M, Uhorchak J, Ralston S, Tendy S, McGuigan FE, Lindsay R, Nieves J. Determinants of stress fracture risk in United States Military Academy cadets. *Bone* 2013;55: 359-66.
- [12] Burr DB, Yoshikawa T, Teegarden D, Lyle R, McCabe G, McCabe LD, Weaver CM. Exercise and oral contraceptive use suppress the normal age-related increase in bone mass and strength of the femoral neck in women 18-31 years of age. *Bone* 2000;27: 855-63.
- [13] Ruffing JA, Nieves JW, Zion M, Tendy S, Garrett P, Lindsay R, Cosman F. The influence of lifestyle, menstrual function and oral contraceptive use on bone mass and size in female military cadets. *Nutr Metab (Lond)* 2007;4: 17.
- [14] Nappi C, Bifulco G, Tommaselli GA, Gargano V, Di Carlo C. Hormonal contraception and bone metabolism: a systematic review. *Contraception* 2012;86: 606-21.
- [15] Ziglar S, Hunter TS. The effect of hormonal oral contraception on acquisition of peak bone mineral density of adolescents and young women. *J Pharm Pract* 2012;25: 331-40.
- [16] Weaver CM, Teegarden D, Lyle RM, McCabe GP, McCabe LD, Proulx W, Kern M, Sedlock D, Anderson DD, Hillberry BM, Peacock M, Johnston CC. Impact of exercise on bone health and contraindication of oral contraceptive use in young women. *Med Sci Sports Exerc* 2001;33: 873-80.
- [17] Teegarden D, Legowski P, Gunther CW, McCabe GP, Peacock M, Lyle RM. Dietary calcium intake protects women consuming oral contraceptives from spine and hip bone loss. *J Clin Endocrinol Metab* 2005;90: 5127-33.
- [18] Cobb KL, Bachrach LK, Sowers M, Nieves J, Greendale GA, Kent KK, Brown BW, Jr., Pettit K, Harper DM, Kelsey JL. The effect of oral contraceptives on bone mass and stress fractures in female runners. *Med Sci Sports Exerc* 2007;39: 1464-73.
- [19] Tremollieres F. Impact of oral contraceptive on bone metabolism. *Best Pract Res Clin Endocrinol Metab* 2013;27: 47-53.
- [20] Curtis KM, Martins SL. Progestogen-only contraception and bone mineral density: a systematic review. *Contraception* 2006;73: 470-87.

- [21] Cromer BA, Scholes D, Berenson A, Cundy T, Clark MK, Kaunitz AM, Society for Adolescent M. Depot medroxyprogesterone acetate and bone mineral density in adolescents--the Black Box Warning: a Position Paper of the Society for Adolescent Medicine. *J Adolesc Health* 2006;39: 296-301.
- [22] Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. *Bone* 2007;40: 14-27.
- [23] Pitukcheewanont P, Punyasavatsut N, Feuille M. Physical activity and bone health in children and adolescents. *Pediatr Endocrinol Rev* 2010;7: 275-82.
- [24] Kemmler W, Bebenek M, von Stengel S, Bauer J. Peak-bone-mass development in young adults: effects of study program related levels of occupational and leisure time physical activity and exercise. A prospective 5-year study. *Osteoporos Int* 2015;26: 653-62.
- [25] Soyka LA, Misra M, Frenchman A, Miller KK, Grinspoon S, Schoenfeld DA, Klibanski A. Abnormal bone mineral accrual in adolescent girls with anorexia nervosa. *J Clin Endocrinol Metab* 2002;87: 4177-85.
- [26] Sundgot-Borgen J, Torstveit MK. Prevalence of eating disorders in elite athletes is higher than in the general population. *Clin J Sport Med* 2004;14: 25-32.
- [27] Cano Sokoloff N, Eguiguren ML, Wargo K, Ackerman KE, Baskaran C, Singhal V, Clarke H, Slattery M, Lee H, Eddy KT, Misra M. Bone parameters in relation to attitudes and feelings associated with disordered eating in oligo-amenorrheic athletes, eumenorrheic athletes, and nonathletes. *Int J Eat Disord* 2015;48: 522-6.
- [28] Gibbs JC, Williams NI, De Souza MJ. Prevalence of individual and combined components of the female athlete triad. *Med Sci Sports Exerc* 2013;45: 985-96.
- [29] Army Regulation. Regulation 40-501, chapter 2. Standards of Medical Fitness. Rapid Action Revision issue date 2010.
- [30] Practice Committee of the American Society for Reproductive Medicine. Current evaluation of amenorrhea. *Fertility and sterility* 2004;82: 266-272.
- [31] Chumlea WC, Schubert CM, Roche AF, Kulin HE, Lee PA, Himes JH, Sun SS. Age at menarche and racial comparisons in US girls. *Pediatrics* 2003;111: 110-3.
- [32] Cummings SR, Block G, McHenry K, Baron RB. Evaluation of two food frequency methods of measuring dietary calcium intake. *Am J Epidemiol* 1987;126: 796-802.
- [33] Garner DMO, M.P., Polivi, J. Development and validation of a multidimensional eating disorder inventory for anorexia nervosa and bulimia *Int J Eat Disord* 1983;2: 15-34.
- [34] Garner DM. Eating disorder inventory-2: Professional manual: Psychological Assessment Resources (Odessa, Fla. P.O. Box 998, Odessa 33556); 1991.
- [35] Knapik J. The Army Physical Fitness Test (APFT): a review of the literature. *Mil Med* 1989;154: 326-9.
- [36] Yoshikawa T, Turner CH, Peacock M, Slemenda CW, Weaver CM, Teegarden D, Markwardt P, Burr DB. Geometric structure of the femoral neck measured using dual-energy x-ray absorptiometry. *J Bone Miner Res* 1994;9: 1053-64.
- [37] DiBenedetto M. Experience with a pre-basic fitness program at Fort Jackson, South Carolina. *Mil Med* 1989;154: 259-63.
- [38] Beals KA, Manore MM. Behavioral, psychological, and physical characteristics of female athletes with subclinical eating disorders. *Int J Sport Nutr Exerc Metab* 2000;10: 128-43.
- [39] Wegienka G, Baird DD. Potential bias due to excluding oral contraceptive users when estimating menstrual cycle characteristics. *Am J Epidemiol* 2003;158: 947-50.

- [40] Leung K-C, Johannsson G, Leong GM, Ho KK. Estrogen regulation of growth hormone action. *Endocrine Reviews* 2004;25: 693-721.
- [41] Weissberger AJ, Ho K, Lazarus L. Contrasting Effects of Oral and Transdermal Routes of Estrogen Replacement Therapy on 24-Hour Growth Hormone (GH) Secretion, Insulin-Like Growth Factor I, and GH-Binding Protein in Postmenopausal Women*. *The Journal of Clinical Endocrinology & Metabolism* 1991;72: 374-381.
- [42] Nieves JW, Melsop K, Curtis M, Kelsey JL, Bachrach LK, Greendale G, Sowers MF, Sainani KL. Nutritional factors that influence change in bone density and stress fracture risk among young female cross-country runners. *PM R* 2010;2: 740-50; quiz 794.
- [43] Holm-Denoma JM, Scaringi V, Gordon KH, Van Orden KA, Joiner TE, Jr. Eating disorder symptoms among undergraduate varsity athletes, club athletes, independent exercisers, and nonexercisers. *Int J Eat Disord* 2009;42: 47-53.
- [44] Bielemann RM, Martinez-Mesa J, Gigante DP. Physical activity during life course and bone mass: a systematic review of methods and findings from cohort studies with young adults. *BMC Musculoskelet Disord* 2013;14: 77.
- [45] Lappe JM, Watson P, Gilsanz V, Hangartner T, Kalkwarf HJ, Oberfield S, Shepherd J, Winer KK, Zemel B. The longitudinal effects of physical activity and dietary calcium on bone mass accrual across stages of pubertal development. *J Bone Miner Res* 2015;30: 156-64.

Table 1: Demographic Data for 91 USMA Female Cadets

	Mean + SD
Age (years)	18.4 + 0.8
Years from Menarche	5.6 ± 1.4
Army Physical Fitness Test	206.8 ± 50.0
Height (cm)	170.0 ± 10.0
Weight (kg)	63.2 ± 7.6
BMI (kg/m ²)	22.8 ± 2.2
% Body fat by Bioelectric Impedence	20.9 ± 3.0
Spine BMD (g/cm ²)	1.253 ± 0.12
Spine Z-score	0.5 ± 1.2
Total hip BMD (g/cm ²)	1.155 ± 0.11
Total hip Z-score	1.5 ± 1.1
Calcaneal BMD (g/cm ²)	0.59 ± 0.1
Calcaneal z-score	1.14+ 1.27
Calcium intake (mg/day)	1073 + 420
Eating Disorders Inventory Score	Mean (range)
1st quartile - 3rd quartile	10.2 (0-24)
4th quartile	38.5 (25-57)

Table 2: Mean Changes in Weight, Height, APFT and BMD over 4 Years in 91 Women

	Mean (SD)	Range
Height Change (inches/4 years)	0.5 ± 0.5	0 to 2.0
Gain (n=46)	0.9 ± 0.3	0.5 to 2.0
No Change (n=45)	0	0
Weight Change (pounds/4 years)	5.0 ± 8.8	-12 to 35
Gain/No Change (n=58)	9.7 ± 6.5	0 to 35
Loss (n=28)	-4.8 ± 3.2	-12 to -0.5
APFT Score Change (4 years)	77.65	3 to 158
Annualized Percent Change		
Spine BMD	0.11 ± 1.02	-3.10 to 3.60
Gain/stable (n=50)	0.81 ± 0.10	0.00 to 3.60
Loss (n=41)	-0.75 ± 0.10	-3.10 to -0.10
Total Hip	-0.18 ± 1.15	-4.80 to 2.60
Gain/stable (n=42)	0.68 ± 0.10	0.00 to 2.60
Loss (n=49)	-0.91 ± 0.14	-4.80 to -0.10
Femoral Neck Diameter (mm/yr)	0.27 ± 0.39	-0.82 to 1.07
Increase (n=77)	0.39 ± 0.29	0.01 to 1.07
Decrease/no change (n=14)	-0.36 ± 0.27	-0.82 to -0.05
Calcaneus BMD	0.33 ± 3.3	-8.8 to 11.6
Gain/stable (n=39)	3.03 ± 0.46	0.00 to 11.60
Loss (n=45)	-1.82 ± 0.24	-8.80 to -0.20

Table 3: Annualized percent change in spine, hip and calcaneal BMD and femoral neck diameter as a Function of Selected Variables (unadjusted analysis: mean (standard error))

Variable	% Change in spine BMD	% Change in hip BMD	% Change in Calcaneus BMD	Change in femoral neck diameter (mm)
Race				
African-American (n=7)	0.84 (0.57) *	0.74 (0.53) *	0.35 (0.37)	0.05 (0.13)
Caucasian/Asian (n=84)	0.05 (0.10)	-0.25 (0.12)	1.50 (1.27)	0.29 (0.04)
Weight Change				
Gain or stable (n=58)	0.19 (0.13)	0.11 (0.12) **	1.00 (0.47) *	0.29 (0.05)
Loss (n=28)	-0.13 (0.20)	-0.78 (0.26)	-0.89 (0.53)	0.29 (0.08)
Height Change				
Gain (n=43)	0.20 (0.15)	-0.13 (0.18)	0.64 (0.54)	0.38 (0.36)*
Stable (n=43)	0.02 (0.15)	-0.22 (0.16)	0.22 (0.49)	0.16 (0.41)
APFT				
Above median (n=46)	0.31 (0.17) #	-0.10 (0.19)	0.82 (0.55)	0.29 (0.05)
Below median (n=45)	-0.09 (0.13)	-0.25 (0.15)	0.01 (0.47)	0.25 (0.07)
EDI in highest quartile				
Yes (n=23)	-0.31 (0.17) *	-0.55 (0.19)#	0.57 (0.46)	0.22 (0.37)
No (n=68)	0.25 (0.13)	-0.05 (0.14)	0.00 (0.42)	0.23 (0.40)
Calcium Intake				
>1000 mg/day (n=41)	0.08 (0.91)	-0.18 (0.86)	0.55 (3.32)	0.26 (0.46)
<1000 mg/day (n=33)	0.19 (1.12)	-0.18 (1.46)	0.25 (2.38)	0.26 (0.35)
Years from menarche				
>5 (n=60)	0.05 (0.14)	-0.19 (0.16)	0.79 (0.47)	0.24 (0.40)
≤5 (n=30)	0.28 (0.17)	-0.13 (0.19)	-0.28 (0.54)	0.34 (0.39)
Menstrual Function				
10-12 cycles/year (n=66)	0.31 (0.11) **	0.02 (0.11) *	0.50 (0.45)	0.29 (0.36)
0-9 cycles/year (n=25)	-0.43 (0.23)	-0.68 (0.32)	0.25 (0.56)	0.21 (0.48)
Use of DMPA				
Yes (n=19)	-0.65 (0.24) *	-1.00 (0.37)*	-1.0 (0.33) **	0.20 (0.47)
No (n=72)	0.31 (0.11)	0.04 (0.10)	0.76 (0.43)	0.29 (0.37)

# p<0.10

* p<0.05 by t-test

** p<0.01 by t-test

Table 4: Least squares means of BMD annualized change in spine and hip BMD as a Function of Selected Variables (controlling for all others in the table)

		All Races			Caucasian Only		
		Yes	No	p-value	Yes	No	p-value
Spine	EDI High Score	-0.12	0.36	0.04	-0.47	-0.01	0.04
	DPMA	-0.37	0.58	<0.001	-0.82	+0.10	0.001
	Menstrual Dysfunction	-0.25	0.49	0.001	-0.60	+0.19	0.001
Total Hip	EDI High Score	-0.23	0.05	0.26	-0.69	-0.33	0.003
	DPMA	-0.62	0.37	<0.001	-1.3	-0.17	<0.001
	Menstrual Dysfunction	-0.42	+0.24	0.011	-0.98	-0.21	0.003
	Weight Loss	-0.39	+0.35	0.003	-0.95	-0.24	0.004

Figure Legend:

Figure 1: Least squares means of BMD Annualized Change and Femoral Neck Diameter

Annual Change after Controlling for Other Factors (see below).

Spine models (1A) were adjusted for race, weight change, normal menses, EDI score and baseline BMD except for the variable that was graphed (In addition, DMPA model excluded normal menses).

Hip models (1B) were adjusted for race, weight change, normal menses, EDI score and baseline hip BMD except for the variable that was graphed (In addition, DMPA model excluded normal menses).

Femoral neck diameter models (1C) included race, height change, DMPA use, OC use and baseline neck diameter.

Calcaneal models (1D) for DMPA included race, weight change and baseline calcaneal BMD.

557

1B

558

1C

1D