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13. ABSTRACT ***(Maximum 200 Words)***

This is a two-year randomized trial of the effects of oral contraceptives on bone mass and stress fracture incidence among 150 female competitive distance runners in the age range 18-25 years. The Coordinating Center is at Stanford University and bone mass is being measured at five sites: Massachusetts General Hospital, University of California Los Angeles, University of Michigan, Stanford University/Palo Alto VA Medical Center, and Helen Hayes Hospital in West Haverstraw NY. Athletes are currently being recruited from the areas around these five clinical sites. To date, over all five clinical sites, 133 have been randomized and an additional 4 successfully screened and are being scheduled for their bone densitometry. We expect to complete baseline examinations and randomization in early 2003. Final results will thus not be available until early 2005.

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(5) INTRODUCTION

Highly trained female athletes may experience loss of menses because of their participation in intense physical activity. Previous cross-sectional research has shown that women with exercise-induced menstrual irregularities have a significantly higher frequency of stress fractures and low bone mass than normally menstruating controls. Longitudinal studies suggest that these women are losing bone mass over time. Low serum estrogen levels are believed to be a principal cause of the bone loss. If so, re-establishing normal estrogen levels in these women should prevent or retard bone loss and decrease the incidence of stress fracture. This study is a two-year randomized trial of the effect of oral contraceptives on bone mass and stress fracture incidence among 150 female cross country runners in the age range 18-25 years. The Coordinating Center is at Stanford University and bone mass is being measured at five sites: the Massachusetts General Hospital, the University of California Los Angeles, the University of Michigan, Stanford University/Palo Alto VA Medical Center, and the Helen Hayes Hospital in West Haverstraw, NY. Athletes are being recruited from the areas around these five clinical sites.

(6) BODY

Below we summarize (a) our progress through year 4, (b) the status of recruitment as of the end of year 4, and (c) our plans for completing the study.

(a) Progress through year 4 (excluding recruitment, which will be described under [b] below):

As of the time of this writing (October 2002), 133 runners have been randomized, 79 have completed their first year follow-up visits, and 46 have had both follow-up visits and have thus completed the study protocol. Another 4 have been screened and their clinic visits are pending.

Eight have withdrawn from the study. During year 4 specifically, 32 new runners were recruited and randomized, 25 attended their first follow-up visit, and 30 completed the study.

During year 3 we had submitted a manuscript, Disordered Eating, Menstrual Irregularity, and Bone Mineral Density in Female Runners, to *Medicine and Science in Sports and Exercise*. During year 4 it was revised in response to reviewers' comments. The results are summarized below under Key Research Accomplishments. During year 4, another paper, Dietary Protein Intake and Bone Mineral Density in Young Female Distance Runners, was prepared from baseline data on the (inverse) relationship between protein intake and bone mineral density. A draft of the abstract is attached.

As in our previous annual progress reports, our main problems have been slow recruitment and long delays in obtaining IRB approval when we have tried to expand our recruitment strategies.

During the first year of the study, recruitment was our biggest problem. First we added the Helen Hayes Hospital in West Haverstraw, NY, to our clinical sites. Then we revised our recruitment methods and expanded the scope of the study eligibility to include non-collegiate highly competitive runners in the age range 18-25 years. Although difficulty in recruitment has to some extent continued to slow us down, our main problem since the first year has been the length of time it has taken to work out wording of the informed consent form that is mutually agreeable to the Army IRB and the IRBs at our clinical sites.

During year 2 of the study, athletes recruited during the first year were followed, additional runners were recruited, and, after lengthy negotiations, the Army and Helen Hayes Hospital IRBs

agreed upon a consent form. Negotiations with the Army and UCLA IRBs regarding the UCLA informed consent form for non-collegiate runners were begun.

Not until August of 2001 near the end of the third year of the study did the Army and UCLA IRBs reach agreement on the wording of all components of the informed consent form for non-collegiate runners in the Los Angeles area. Since then we have established procedures for having the participants seen by a physician at UCLA and have been actively recruiting non-collegiate runners. However, new glitches developed during year 4, and additional IRB/recruitment issues have again delayed recruitment. First, in our recruitment of "non-collegiate" runners in Los Angeles from road races, websites, and other such sources, we realized that several of the potential participants were in fact collegiate runners who had been recruited through means other than their colleges and whose colleges were not part of our study. Although the Stanford consent form allowed this, the UCLA consent form did not. Upon notification of this discrepancy, the UCLA IRB gave approval for changes in the title and wording of their consent form to permit enrollment of these runners, provided these runners were cleared for eligibility by the study physician at UCLA. However, the Army IRB's permission was also needed. Unfortunately, at about this same time (May, 2002), we learned that the Principal Investigator at the Massachusetts General Hospital (MGH) had neglected to obtain annual approval of the protocol and consent form. We immediately suspended all operations at the MGH and notified the Army IRB. The Army IRB withheld approval of the changes in the UCLA recruitment procedures until the situation at MGH was corrected. As of the time of this writing (October 2002), the IRB at MGH has not given approval to resume operations there, despite our having addressed their questions and having tried to hasten their decision. The Army IRB is currently trying to make direct contact with the MGH IRB, and if the situation cannot be

resolved, the MGH operations will be permanently suspended and the modified methods of recruitment at UCLA allowed. In the meantime, we have lost several potential participants in the Los Angeles area, although we have continued to recruit slowly from our previous sources. (If the MGH activities are stopped, we would lose the second follow-up visit of 7 participants and 2 runners who have been screened but not yet been randomized.)

(b) Recruitment through year 4: The focus of our recruitment efforts in the second, third, and fourth years of the study shifted from colleges to other sources such as road races, websites, and advertisements. At the time of this writing, 52 collegiate runners and 81 runners identified from other sources have been randomized, for a total of 133 randomized participants. Four more runners have been successfully screened and are in the process of scheduling their initial clinic appointments. This brings us to a total of 137 runners who have so far been randomized or who are scheduled to be examined and randomized.

Having already saturated the area around Stanford, our recent efforts to recruit 150 participants have focused mainly on the Los Angeles area. In the fourth year, we hired one person each in the Los Angeles and San Diego area whose sole duty is to recruit new participants. Our recruiter in Los Angeles has contacted coaches of local track clubs, including the Pacific West Track Club and Elite Track Club, and asked them to pass on information about the study to their eligible women runners via team websites, newsletters, and brochures. She has also attended several local marathons and road races, such as the Manhattan Beach 10K and the Brentwood Run, to hand out brochures containing information about the study. Flyers have been posted in retail sporting goods stores that tend to serve competitive runners. In addition, we have put information about the study on several running-related websites as well as in local newspapers.

(c) Plans for completing the study: We believe that once we are able to augment our recruitment methods in the Los Angeles area to include runners who are identified through road races, websites, and advertisements and who also happen to attend college, we will reach our goal of 150 participants. However, this means that the study is being extended two and a half years beyond its scheduled termination date. Although we still have funds for bone densitometry, for which we are charged only when study subjects are measured, we realized that we would have insufficient funds to support the project coordinator and study physician at Stanford, both of whom are essential for the duration of the study. The Army has kindly agreed to provide us with modest supplemental funds, mainly for the support of these two positions. We thus believe that, given yet more time and these additional funds, we should be able to complete the trial, albeit long after we had originally planned.

(7) KEY RESEARCH ACCOMPLISHMENTS:

- Attached is a manuscript describing some baseline results from the first 91 subjects. We found that (a) disordered eating is strongly related to menstrual irregularity, (b) menstrual irregularity is associated with low bone mineral density independent of body weight and body composition, and (c) disordered eating is associated with low bone mineral density in the absence of menstrual irregularity.
- Since the submission of this manuscript for publication, we have analyzed baseline data from an additional 30 subjects. The trends that were observed for the first 91 participants were maintained when the additional 30 participants were added to the data set. Additionally, differences between the amenorrheic and oligomenorrheic groups are now

becoming apparent. Disordered eating and low bone mineral density are more strongly associated with amenorrhea than with oligomenorrhea.

- In another manuscript currently in preparation, we report our finding from baseline data that dietary protein intake is inversely associated with bone mineral density in the runners, independent of menstrual irregularities and disorder eating (draft abstract attached).

(8) REPORTABLE OUTCOMES: None to date.

(9) CONCLUSIONS: We will have no firm conclusions to report on the primary hypothesis of the study until the end of the trial. However, in preparing interim data for our Medical Monitor, we can report that oral contraceptive use may be beneficial to spine bone mineral density in oligo/amenorrheic runners. We can also report that resumption of regular menses among the oligo/amenorrheic runners (either spontaneous resumption or due to oral contraceptives) is associated with gains in spine bone mineral density and, to a lesser extent, with gains in hip and whole body bone mineral density. In addition, we observed that oral contraceptive use appears to bring about small gains in both lean and fat mass in young women runners.

(10) REFERENCES: None

(11) APPENDICES: Please see the attached manuscript and abstract.

Title Page

Title: Disordered Eating, Menstrual Irregularity, and Bone Mineral Density in Female Runners

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Justification for >6 authors: This study is part of a multi-site study, and therefore involved five co-Principal Investigators in addition to the many researchers at the Coordinating Center (Stanford) who contributed substantially to data collection, analysis, and preparation of the manuscript.

Abstract

Purpose: To examine the relationships between disordered eating, menstrual irregularity, and low bone mineral density (BMD) in young female runners.

Methods: Subjects were 91 competitive female distance runners aged 18-26 years. Disordered eating was measured by the Eating Disorder Inventory (EDI). Menstrual irregularity was defined as oligo/amenorrhea (0-9 menses per year). BMD was measured by dual X-ray absorptiometry (DXA).

Results: An elevated score on the EDI (highest quartile) was associated with oligo/amenorrhea, after adjusting for percent body fat, age, miles run per week, age at menarche, and dietary fat, (OR [95% CI]: 4.6 [1.1-18.6]). Oligo/amenorrheic runners had lower BMD than eumenorrheic runners at the spine (-5%), hip (-6%), and whole body (-3%), even after accounting for weight, percent body fat, EDI score, and age at menarche. Eumenorrheic runners with elevated EDI scores had lower BMD than eumenorrheic runners with normal EDI scores at the spine (-11%), with trends at the hip (-5%), and whole body (-5%), after adjusting for differences in weight and percent body fat. Runners with both an elevated EDI score and oligo/amenorrhea had no further reduction in BMD than runners with only one of these risk factors.

Conclusion: In young competitive female distance runners, (i) disordered eating is strongly related to menstrual irregularity, (ii) menstrual irregularity is associated with low BMD, and (iii) disordered eating is associated with low BMD in the absence of menstrual irregularity.

Key Words: female athletes, long-distance, osteopenia, osteoporosis, amenorrhea, oligomenorrhea, eating attitudes, eating disorder inventory, female athlete triad

Introduction

Paragraph Number 1 The "female athlete triad" (34) is the combination of disordered eating, menstrual irregularity and osteoporosis/osteopenia seen in young female athletes. Disordered eating, which affects as many as two-thirds of young female athletes (34), consists of restrictive eating behaviors that do not necessarily reach the level of a clinical eating disorder (2). Women athletes with disordered eating may limit their caloric and/or fat intakes but maintain high training levels, often resulting in a state of chronic energy deficit. Among other adverse consequences, energy imbalance has been linked to depressed estrogen levels, metabolic disturbances, and amenorrhea or oligomenorrhea (2,7,27,35,51-52). Amenorrheic/oligomenorrheic athletes on average have lower bone mineral density (BMD) than eumenorrheic controls (6-7,9,21,23-25,27,29,30,33,35,38-40, 47, 49-50). This bone deficit may be related to an increased incidence of stress fractures (1,10,31) and may be only partially reversible (17,19,22) putting women at risk for life-long health consequences.

Paragraph Number 2 The existence of the female athlete triad is implicit in studies that established a relationship between eating behaviors and menstrual irregularity (2,7,27,35,40,51-52) and those that established a relationship between menstrual irregularity and low BMD (6-7,9,21,23-25,27,29,30,33,35,38-40,47,49-50). However, few studies have actually measured menstruation, diet, and BMD simultaneously (7,24,27,35,40) and these studies were conducted, largely, before the female athlete triad was recognized as a distinct syndrome. Therefore, the female athlete triad has yet to be explored as a triad, and the complex relationships among all three components have yet to be established.

Paragraph Number 3 In this paper, we examine eating attitudes and patterns, menstrual status, and BMD in a group of 91 competitive female distance runners, using data collected at the baseline examination of a randomized controlled trial. We examine the etiology of menstrual irregularity in this population, specifically as it relates to diet and eating behaviors. We address the question of whether low body weight can explain the differences in BMD between eumenorrheic and oligo/amenorrheic athletes, as several researchers have suggested (6,33,47,50) or if menstrual irregularity is associated with BMD independently of low weight. Finally, we examine the relationship between disordered eating and BMD independent from menstrual irregularity, a link that has not been well studied in female athletes.

Materials/Methods

Paragraph Number 4 We analyzed the baseline cross-sectional data from 91 competitive female long-distance runners, aged 18-25 years, who enrolled in a randomized controlled trial to examine the effect of oral contraceptives on BMD in female runners.

Subjects

Paragraph Number 5 Women were recruited from inter-collegiate cross country teams, post-collegiate running clubs, and road race participants in the geographic areas of Palo Alto, CA, Los Angeles, CA, Ann Arbor, MI, West Haverstraw, NY, and Boston, MA. To be eligible, women had to run at least 40 miles/week during peak training times, and they had to compete in running races. Additionally, because the women were

recruited as part of a randomized trial of oral contraceptives, they could not have used oral contraceptives or other hormonal contraception within 6 months prior to entering the study; they had to be willing to be randomized to take oral contraceptives or not to take them; and they could have no medical contraindications to oral contraceptive use. All women were required to visit a study physician or student health service staff member prior to enrollment in the study. Details of the study and testing procedures were explained to each subject, and a written, informed consent was obtained. The experimental protocol was approved by the Institutional Review Boards of Stanford University, the University of California, Los Angeles, the University of Michigan, the Helen Hayes Hospital, and Massachusetts General Hospital.

Questionnaire

Paragraph Number 6 A self-administered questionnaire was used to assess training regimen and menstrual history. Women were asked to record the number of miles they ran per week during each competitive season (fall cross country, winter track, spring track) and the off-season (summer) in the past 12 months. From this information, an average number of miles run per week was calculated for the year prior to study enrollment.

Paragraph Number 7 Women reported the number of menses in the previous 12 months, and were classified, accordingly, as eumenorrheic (10 or more cycles in the past year), oligomenorrheic (4-9 menstrual cycles per year), or amenorrheic (fewer than 4 cycles in the past year) (41). Menstrual irregularity has been defined as 0-9 menses per year in previous studies of young women runners (1,29,31,40) and we used that definition

here. Both oligomenorrheic and amenorrheic athletes have previously been found to have lower serum estradiol concentrations (25,40,43) and to have lower BMD than eumenorrheic athletes (6,25,39,40,43). In our study population, amenorrheic and oligomennorheic athletes were similar in BMD, EDI scores, and past menstrual irregularity, justifying their combination into a single group. Women recorded their age at menarche and indicated whether they had had 0, 1-3, 4-9, or 10-13 menses during each year after menarche. Total lifetime menses was calculated using the midpoint of each of these categories. The total number of past years of amenorrhea was calculated by summing the number of years for which women checked "0" or "1-3" periods, excluding the year of menarche and the current year. Past oligomenorrheic years was calculated similarly, except using the category "4-9" periods.

Diet and Eating Behaviors

Paragraph Number 8 An expanded version of the 97-item National Cancer Institute Health Habits and History food frequency questionnaire (4) was used to estimate usual nutrient intake during the prior 6 months. We modified the questionnaire to accommodate the special diets of college-aged female athletes by adding low-fat and non-fat versions of certain foods, vegetarian and vegan foods, ethnic foods, and sports nutrition products (such as Gatorade and Power Bars). The nutrient contents of the added foods were obtained from the U.S. Department of Agriculture Nutrient Database for Standard Reference, release 14 (44), and from food labels. Total intakes of energy, protein, fat, carbohydrates, calcium, phosphorous, iron, fiber, and vitamin C were calculated.

Paragraph Number 9 Three subscales (drive for thinness, bulimic tendencies, and body dissatisfaction) of the Eating Disorder Inventory (EDI) were used to screen for subclinical eating disorders (2,12-13). Athletes with subclinical eating disorders have previously been shown to have significantly elevated scores on these three subscales of the EDI (2, 11, 13, 36). Responses on each EDI subscale were scored separately and also totaled.

Physical and Bone Measurements

Paragraph Number 10 At each of the five clinical assessment sites, height and weight were measured using standard stadiometers and balance-beam scales, respectively. Body mass index was calculated as kg/m^2.

Paragraph Number 11 Bone mineral density (BMD, g/cm^2) at the left proximal femur, spine, and whole body, and body composition (lean body mass and fat mass) were measured by dual energy x-ray absorptiometry (DXA; QDR 4500A, Hologic). The coefficient of variation for these machines is less than 1.0% for all bone sites (15). Machines were cross-calibrated using a circulating Hologic anthropomorphic spine phantom. Each site maintained a standard quality assurance program. All women were asked to refrain from heavy physical activity twenty-four hours prior to screening in order to minimize the effect of fluctuations in hydration status on body composition measurements.

Statistical Analyses

Paragraph Number 12 Statistical analyses were performed using the SAS

statistical package, version 6.12 (SAS Institute, Cary, NC, U.S.A.). Means were compared between groups using t-tests for normally distributed variables and the Wilcoxon sign-rank test for non-normally distributed variables. Tukey's multiple comparisons test was used to compare mean BMD across more than two groups. Analysis of covariance was used to control for age, weight, and body composition.

Paragraph Number 13 The relationships between oligo/amenorrhea and training, diet, and physical characteristics were assessed by multiple logistic regression. Multiple linear regression was used to examine the effects of menstrual group and EDI score on BMD when considering EDI score as a continuous variable.

Results

Paragraph Number 14 Thirty-six percent of the study sample met criteria for abnormal menses; 26% were oligomenorrheic and 10% were amenorrheic during the past year. Oligo/amenorrheic women were similar to eumenorrheic women in age, weight, height, and body composition (**Table 1**). The oligo/amenorrheic women had menarche a mean of 1.2 years later and had had an average of 45% fewer menstrual periods in their lifetime than eumenorrheic women. They also ran an average of 18% more miles per week than eumenorrheic women.

Disordered Eating and Menstrual Irregularity

Paragraph Number 15 The women were divided into two groups (normal EDI/elevated EDI) by their total scores on three subscales of the eating disorder inventory (EDI). Women in the highest quartile of total EDI were classified as having elevated EDI scores compared to women in the lowest three quartiles. Women in the elevated EDI group had EDI values comparable to those previously published for patients with anorexia nervosa (12) on the drive for thinness and body dissatisfaction subscales (**Table 2**). Athletes with elevated EDI scores reported 19% lower daily caloric intakes compared to women with normal EDI scores (**Table 2**), and reported that they obtained 25% fewer of those calories from fat. The groups were similar in consumption of other nutrients. While the elevated EDI group had a somewhat lower daily calcium intake, this was proportional to their lower energy intake. Both groups, on average, consumed greater than 1,200 mg of calcium per day, which is the U.S. recommended daily allowance (RDA) for this age group.

Paragraph Number 16 Of 23 women with elevated EDI scores, 65% had oligo/amenorrhea, whereas only 25% of 67 women with normal EDI scores did. Though each of the three EDI subscales scores was higher in the oligo/amenorrheic group, the drive for thinness EDI subscale had the strongest association with oligo/amenorrhea (**Table 3**). Oligo/amenorrheic athletes and eumenorrheic athletes were similar in daily nutrient profiles, though oligo/amenorrheic athletes reported a lower percentage of their calories from fat (**Table 3**).

Paragraph Number 17 **Table 4** shows odds ratios for several factors associated with oligo/amenorrhea. Being in the top quartile of EDI score conferred a four-fold increased odds of oligo/amenorrhea. Every one-year increase in age at menarche was associated with a more than two-fold increase in the odds of oligo/amenorrhea. Odds of oligo/amenorrhea were also increased with greater miles run per week and was decreased with a higher percent body fat and with a higher percent fat intake, but the confidence intervals for these associations included one. Total energy intake was not associated with menstrual disturbances.

Paragraph Number 18 EDI score and percent fat intake were modestly negatively correlated (Spearman rank correlation coefficient: r=-.34), and reduced fat intake may lie in the causal pathway between elevated EDI and oligo/amenorrhea. If dietary fat is removed from the logistic regression model, the OR for elevated EDI score increases from 4.6 to 6.7 (1.8, 25.6), suggesting that low fat intake accounts for some of the association between elevated EDI and menstrual irregularity. EDI score was not correlated with miles run per week (Spearman rank correlation coefficient: r=.01), so

increased training, though related to oligo/amenorrhea, does not mediate the relationship between elevated EDI scores and oligo/amenorrhea.

Menstrual Irregularity and Bone Mineral Density

Paragraph Number 19 BMD was 5%, 6%, and 3% lower at the lumbar spine, total hip and whole body, respectively, in oligo/amenorrheic women compared to eumenorrheic women, after adjustment for weight, percent body fat, EDI score, and age at menarche **(Table 5)**. Adjusted and unadjusted BMD values were similar (**Table 5**); thus, although weight was strongly correlated with BMD at all skeletal sites (the Pearson correlation coefficients were: whole body: r=.43; hip: r=.40; and spine: r=.38), lower weight did not account for the association between menstrual irregularity and low BMD in this study population.

Disordered Eating and Bone Mineral Density

Paragraph Number 20 There were no differences in BMD between women with elevated EDI scores and women with normal EDI scores before adjusting for body size. However, women with elevated EDI scores were heavier (138.5 ± 3.2 lbs) and had a higher percent body fat (25.7 ± 1.1 %) than those with normal EDI scores (125.8 ± 1.7 lbs; 22.8 ± 0.6 %). Based on multiple linear regression, we would expect the women with elevated EDI to have .038 g/cm^2 greater BMD at the spine and hip and .028 g/cm^2 greater BMD at the whole body due to their higher weight (correcting for their higher percent body fat). Once we adjusted for body weight and composition, women with

elevated EDI scores had significantly lower BMD compared to women with normal EDI scores at the spine (-6%), with trends at the hip (-3%) and whole body (-4%).

Paragraph Number 21 Menstrual status modified the effect of EDI score on adjusted BMD (**Table 6**). Among eumenorrheic women, those with elevated EDI scores had significantly lower spine BMD and non-significant trends for lower hip and whole body BMD compared to women with normal EDI scores (**Table 6**). These differences were not attributable to past menstrual history, which was similar in the two groups. Among oligo/amenorrheic women, however, there were no trends for lower BMD among women with elevated EDI compared to women with normal EDI.

Paragraph Number 22 Multiple linear regression analysis confirmed the significant interactions between menstrual irregularity and total EDI score (0-69) on BMD at all skeletal sites (**Figure 1**). Among eumenorrheic runners, EDI score is inversely related to BMD. However, among oligo/amenorrheic women, BMD is not related to EDI score. Similarly, among women with low EDI scores, oligo/amenorrheic women had lower BMD than eumenorrheic women, but, among women with high EDI scores, menstrual irregularity was not related to BMD.

Discussion

Paragraph Number 23 This study confirms the existence and significance of the "female athlete triad," a syndrome composed of three interrelated conditions: disordered eating, menstrual irregularity, and osteopenia/osteoporosis (34). (i) We confirm that disordered eating in female runners is correlated with oligo/amenorrhea; (ii) we demonstrate that the association between oligo/amenorrhea and low BMD in female

runners is independent of body weight and body composition; and (iii) we provide novel evidence that disordered eating is associated with low BMD in eumenorrheic women runners.

Paragraph Number 24 The women in our study who were in the highest quartile of total eating disorder inventory (EDI) score had similar values on 2 EDI sub-scales to patients with diagnosed anorexia nervosa (12); they also had similar or slightly higher EDI scores than women athletes with established subclinical eating disorders (2,11,13,36). The EDI measures only attitudes about food and body size. However, we verified that elevated scores on the EDI translated to actual eating practices; women with elevated EDI scores reported lower total energy intakes (by about 19% per day) and lower percent fat intakes (by about 25% per day) than women with normal EDI scores. None of the 91 women in our study indicated that she was dieting to lose weight (data not shown), suggesting that this observed dietary restriction represents long-term, chronic restriction, rather than temporary attempts to lose weight.

Paragraph Number 25 Women with elevated EDI scores had a four-fold increase in risk for oligo/amenorrhea, when controlling for other factors. Chronic energy deficit has previously been implicated in the etiology of athletic amenorrhea (2,7-8,20,26-27,32,35,46,48,51-52). Menstruation requires a small amount of energy, and halting menstruation may be an adaptive energy-conservation mechanism. In our study, the caloric restriction of the elevated EDI group did not appear to explain their excess oligo/amenorrhea. Rather, our data suggest that the development of oligo/amenorrhea in these women may have been mediated in part by a reduction of dietary fat intake. Though dietary fat, independent of total energy intake, has previously been shown to

influence the menstrual cycle in non-athletic women (16,28), this association has not previously been demonstrated in female athletes and needs verification in further studies. We speculate that women with disordered eating may have more aberrant patterns of eating, such as binging and fasting cycles; although total energy intake may not be altered, these patterns have potential to alter metabolic pathways, hormone levels, and, ultimately, menstruation (3,5,14).

Paragraph Number 26. We found that oligo/amenorrheic runners ran more miles per week than eumenorrheic runners. Therefore, although energy intake was not associated with menstrual irregularity, oligo/amenorrheic runners may have had greater energy imbalance due to a higher energy expenditure. Energy imbalance may cause hypothalmic dysfunction which disrupts both menses and bone remodelling (51).

Paragraph Number 27 We confirm previous research that shows that delayed menarche is a strong predictor of later menstrual irregularity (6, 9, 30, 35). Delayed menarche was correlated with menstrual irregularity in both women who initiated training prior to menarche (n=22) and women who started training after menarche (n=69); thus, prior training does not explain the delay in menarche in the oligo/amenorrheic runners. This finding suggests that some women, such as those with a natural lanky "runner's build," may be predisposed to menstrual irregularity, which would account for the existence of a subset of women with low total EDI scores (6.8 ± 1.8) and putatively sufficient caloric intake (2443 ± 210) who still lost their periods. Alternatively, disordered eating patterns may have developed pre-menarche and pre-training in certain women which caused a delay in the onset of menarche and has

subsequently continued to disrupt the menses. Our data were insufficient to evaluate this hypothesis.

Paragraph Number 28 We confirm numerous reports of reduced BMD in oligo/amenorrheic female athletes, with the largest and most consistent effects having been demonstrated at the lumbar spine. The BMD differences between oligo/amenorrheic and eumenorrheic women that we observed were not attributable to differences in body weight, body composition, or EDI score. The magnitude of the difference was important; six percent of the oligo/amenorrheic young women had spine BMD values that would be considered osteoporotic, that is, a BMD value less than 2.5 SDs below young adult BMD (42) (<.772 g/cm^2 as measured with the Hologic densitometer). Forty-eight percent were osteopenic at the spine, a BMD between -1 SD and -2.5 SDs below the young adult value (.772-.937 g/cm^2). In contrast, none of the eumenorrheic athletes were classified as being osteoporotic and only 26% were classified as being osteopenic based on spine BMD values.

Paragraph Number 29 Women with elevated EDI scores had low BMD for their weight. We attempted to determine if low BMD among women with elevated EDI scores was due to oligo/amenorrhea or if the disordered eating had an independent effect on bone. Eight women with high EDI scores were currently eumenorrheic and had no history of amenorrhea or delayed menarche. BMD was significantly lower at the spine and was lower at the hip and whole body in this subgroup compared to eumenorrheic women with normal EDI, after adjusting for weight, body composition, age, and age at menarche. Eumenorrheic women with elevated EDI were heavier and had more body fat than all other subgroups; they also started running at a later age (18.3 ± 1.3 yrs).

Possibly, this group was resistant to loss of menses despite their aberrant eating because their menstrual cycles were established before they started running and/or because they were not as thin (42). It is also possible that these women have subclinical menstrual abnormalities, such as anovulatory cycles and shortened luteal phase, which have been associated with spinal bone density losses (37).

Paragraph Number 30 In our study population, having both disordered eating and oligo/amenorrhea was no more detrimental for bone than having either disorder alone. The numbers in some of our groups were small, however, and this observation should be verified in further studies. That there was no excess risk suggests that the two disorders share causal pathways. Both oligo/amenorrhea and disordered eating have been associated with low serum estrogen concentrations (51-52,26), which would be expected to have an adverse effect on BMD. Accordingly, disordered eating may result in estrogen deficiency or other sex hormone changes, which then may lead both to bone loss and menstrual irregularity. Menstrual irregularity and disordered eating may also contribute to bone loss, or lack of bone formation, through metabolic changes (51).

Paragraph Number 31**Figure 2** summarizes risk factors for low BMD and menstrual irregularity, as well as possible pathways connecting elements of the female athlete triad. Disordered eating may decrease menstruation and BMD through estrogen deficiency and through alterations of other metabolic pathways (45). Low weight is an established independent risk factor for low BMD; in this study population, women weighing less than 115 pounds had a 5-fold increased odds of being osteopenic at any skeletal site (OR [95% CI]: 5.3 [1.6-17.0]). Some previous studies also found an association between low

weight and oligo/amenorrhea (27,6,33,47), though this study did not. Menstrual irregularity may be related to low BMD through mechanisms other than reduced estrogen (37, 51). Training factors and delayed menarche have direct influences on the menstrual cycle and on BMD.

Paragraph Number 32 It is difficult to explain why the athletes with elevated EDI scores were heavier than the women with low EDI scores even though they reported lower energy and fat intakes. We would expect women with subclinical eating disorders to have lower weight and body fat, but this was not the case in our study. Possibly, heavier women are more prone to eating disorders because they are more dissatisfied with their natural body type. Some of these athletes with higher EDI scores may have had bulimic behaviors which could have explained the higher weights . Alternatively, the EDI scale may identify women in the early stages of an eating disorder, but may miss women in the later stages, when they have already lost weight. We speculate that some of the women in the thinnest subgroup, the oligo/amenorrheic women with low EDI scores, may have had eating disorders, but may be in denial and/or may currently be satisfied with their bodies because they have succeeded in reaching a low weight. We further recognize that the division of the population into normal EDI/elevated EDI is simplistic. There is a continuum of disordered eating behavior, but we have artificially imposed a division on that continuum. However, multiple linear regression analysis, in which we treat EDI as a continuous variable, confirms our categorical data results.

Paragraph Number 33 Our results are limited by the fact that menstrual status, training history, and diet were assessed by subject recall. We recognize that recall menstrual histories cannot be as accurate as those obtained by prospective record

keeping. However, we believe that these menstrual histories were reasonably accurate, as the subjects were young, had short histories to recall, and, as competitive athletes, tend to be aware of their overall health. Many competitive runners keep detailed logs of their training and their miles run per week, which may have helped to minimize recall errors on the training section of our questionnaire. Finally, we recognize the limitations of food frequency questionnaires, but note that the questionnaire that was employed was specifically modified to accommodate the special diets of college-aged female athletes. Prospective studies are needed confirm and further explore our findings.

Paragraph Number 34 Additionally, we may have missed women with subclinical menstrual abnormalities, such as anovulatory cycles and shortened luteal phase, because we assessed menstrual irregularity by questionnaire rather than laboratory testing. Measurement of serum hormone levels would have provided additional information about the role of sex hormones. Accurate measurements of energy expenditure using doubly labeled water would have helped us to assess the role of energy balance in menstrual irregularity and low BMD. However, such measurements were outside of the scope and resources of the present study.

Paragraph Number 35 A further limitation of our findings is that eating attitudes and body image perception may influence the reporting of food intake (8). We cannot rule out the possibility that women with aberrant attitudes about body and food systematically underreport intake. As they are hyperconscious about their food intake, they may report what they think they should be eating rather than what they actually eat. Food frequency questionnaires, despite other limitations, may help minimize this tendency, as the total amounts of daily food, calories, and fat being reported are not

readily quantifiable to the athlete.

Paragraph Number 36 In conclusion, we provide evidence that confirms the female athlete triad. We also conclude that the female athlete triad may be more hidden than previously realized. The women in this study were not excessively lean; indeed, amenorrheic women averaged more than 22% body fat and women with elevated EDI scores averaged more than 25% body fat. Thus, those with the triad may not be readily discernible to a coach or a physician. However, both amenorrhea and disordered eating significantly affect bone, even in the absence of the other. Because there is a high prevalence of osteopenia in this population that may have serious life-long consequences, we recommend that all competitive women endurance athletes, particularly those in sanctioned collegiate programs, receive screening for eating disorders and menstrual irregularity and education about the female athlete triad.

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Table 1. Mean ± one standard error of the mean for selected physical and reproductive characteristics, and training variables, by menstrual group.

Characteristic	Menstrual Group	
	eumenorrheic (*n=58*)	oligo/amenorrheic* (*n=33*)
Age (yrs)	21.7 ± .3	21.8 ± .5
Weight (lbs)	129.1 ± 1.9	128.1 ± 2.7
Height (inches)	65.1 ± .3	65.4 ± .5
BMI (kg/m^2)	21.5 ± .2	21.1 ± .3
Body fat (%)	23.9 ± .6	22.7 ± 1.0
Menses in past year (no. cycles)	11.5 ± .1	5.0 ± .5†
Menarche (age in years)	12.6 ± .2	13.8 ± .2‡
Total lifetime menstrual periods (no. cycles)	89.5 ± 4.4	49.5 ± 4.0‡
Started running (age in years)	14.5 ± .5	14.7 ± .7
Amount of running (miles/wk in past 12 months)	33.0 ± 1.2	39.0 ± 2.2§

*Oligo/amenorrhea was defined as 0-9 menses over the past 12 months.
†$p<.0001$, Wilcoxon sign-rank test.
‡$p<.0001$, t-test.
§$p<.05$, t-test.

Table 2. Mean ± one standard error of the mean for selected diet and nutrition characteristics by eating disorder inventory (EDI) group and, for comparison, a previously published anorectic group.

Characteristic	EDI Group (this study)		anorectics (previously published)[†]
	normal EDI (*n=67*)	elevated EDI[*] (*n=23*)	(*n=155*)
EDI scores[*]			
Drive for thinness subscale (0-21)	1.6 ± 0.3	16.3 ± 0.8[‡]	13.8 ± 0.5
Bulimia subscale (0-21)	0.8 ± 0.2	3.2 ± 0.7[‡]	8.1 ± 0.5
Body dissatisfaction subscale (0-27)	3.6 ± 0.5	16.0 ± 1.2[‡]	15.5 ± 0.6
total (0-69)	6.0 ± 0.8	35.6 ± 1.8[‡]	37.4 ± 0.9
Daily Nutrient Intake			
Calories (kcal/d)	2346 ± 112	1904 ± 148[§]	
Fat (% of total calories)	18.7 ± .8	14.0 ± 1.0[|]	
Protein (% of total calories)	16.4 ± .3	16.0 ± .8	
Calcium, mg	1467 ± 96	1300 ± 147	
Fiber, g	30.6 ± 2.5	26.4 ± 2.2	
Vitamin C, mg	291 ± 23	247 ± 23	
Iron, mg	23.6 ± 2.3	20.0 ± 1.9	

[*]EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead.[33] Elevated scores are defined as the highest quartile (≥23). One subject
is missing EDI scores; therefore she is removed from all analyses involving EDI.
[†]Average scores for anorexia nervosa patients as published by Garner and Olmstead.[33]
[‡]elevated EDI group vs. normal EDI group, p<.0001, Wilcoxon sign-rank test.
[§] elevated EDI group vs. normal EDI group, p<.05, t-test.
[|]elevated EDI group vs. normal EDI group, p<.01, t-test.

Table 3. Mean ± one standard error of the mean for selected diet and nutrition characteristics by menstrual group.

	Menstrual Group	
Characteristic	eumenorrheic (*n=58*)	oligo/amenorrheic (*n=33*)
EDI scores*		
Drive for thinness subscale (0-21)	3.3 ± 0.7	9.3 ± 1.4†
Bulimia subscale (0-21)	0.9 ± 0.2	2.3 ± 0.5‡
Body dissatisfaction subscale (0-27)	5.4 ± 0.8	9.3 ± 1.5‡
total (0-69)	9.6 ± 1.5	20.9 ± 3.0†
Daily Nutrient Intake		
Calories (kcal/d)	2241 ± 121	2219 ± 147
Fat (% of total calories)	18.7 ± .9	15.3 ± 1.0§
Protein (% of total calories)	16.3 ± .4	16.3 ± .5
Calcium, mg	1418 ± 106	1437 ± 123
Fiber, g	28.1 ± 2.2	32.0 ± 3.7
Vitamin C, mg	283 ± 23	274 ± 28
Iron, mg	22.2 ± 2.6	23.6 ± 2.1

*EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead.[38]

†oligo/amenorrheic vs. eumenorrheic, p<.005, Wilcoxon sign-rank test

‡oligo/amenorrheic vs. eumenorrheic, p<.05, Wilcoxon sign-rank test

§eumenorrheic vs. oligo/amenorrheic, p<.05, ttest

Table 4. Odds ratios (and 95% confidence intervals) for the association between selected characteristics and oligomenorrhea/amenenorrhea.*

Characteristic	Odds Ratios (95% CI)
Elevated EDI score (≥ 23 vs <23)	4.56 (1.12, 18.61)
Menarche (each 1 year later)	2.45 (1.46, 4.11)
Miles/wk (every 10 miles)	1.64 (0.96, 2.79)
Dietary fat (every 5% of total calories)	.61 (0.36, 1.03)
Body fat (every 5%)	.56 (0.30, 1.07)

*Adjusted for age and each of the other variables in the table by multiple logistic regression.

Table 5. Observed and adjusted* spine, hip, and whole body bone mineral density (BMD, $g/cm^2 \pm$ one standard error of the mean), by menstrual group.

	Menstrual Group	
	eumenorrheic (*n=58*)	oligo/amenorrheic† (*n=33*)
spine BMD		
observed	1.01 ± .013	.94 ± .018‡
adjusted*	1.00 ± .013	.95 ± .019§
total hip BMD		
observed	1.00 ± .015	.95 ± .020§
adjusted*	1.00 ± .014	.94 ± .020§
whole body BMD		
observed	1.12 ± .011	1.08 ± .015§
adjusted*	1.11 ± .010	1.08 ± .015

*Adjusted for age, body weight, percent body fat, EDI score and age at menarche by analysis of covariance.

† Oligo/amenorrhea was defined as 0-9 menses over the past 12 months.

‡eumenorrheic vs. oligo/amenorrheic, $p < .005$, t-test.

§eumenorrheic vs. oligo/amenorrheic, $p < .05$, t-test.

Table 6. Observed and adjusted* spine, hip, and whole body bone mineral density ($g/cm^2 \pm$ one standard error of the mean) by combined menstrual and eating disorder inventory (EDI) groups.

	Group 1	2	3	4
EDI score group†	normal	normal	elevated	elevated
Menstruation	eumenorrhea	oligo/amenorrhea‡	eumenorrhea	oligo/amenorrhea
N	50	17	8	15
Mean weight (pounds ± SE)	126.3 ± 1.8	123.5 ± 4.3	146.4 ± 5.7	133.4 ± 3.2
spine BMD ($g/cm^2 \pm$ SE)				
observed	1.02 ± .015	.90 ± .024§	.97 ± .027	.97 ± .025
adjusted*	1.02 ± .014	.93 ± .024§	.91 ± .036|	.96 ± .025**
total hip BMD ($g/cm^2 \pm$ SE)				
observed	1.00 ± .016	.91 ± .038|	1.00 ± .023	.98 ± .032
adjusted*	1.01 ± .015	.93 ± .027|	.96 ± .040	.96 ± .027
whole body BMD ($g/cm^2 \pm$ SE)				
observed	1.12 ± .013	1.07 ± .018	1.12 ± .029	1.09 ± .016
adjusted*	1.13 ± .010	1.08 ± .019	1.07 ± .028	1.08 ± .020

*Adjusted for body weight, percent body fat, age, and age at menarche by analysis of covariance.

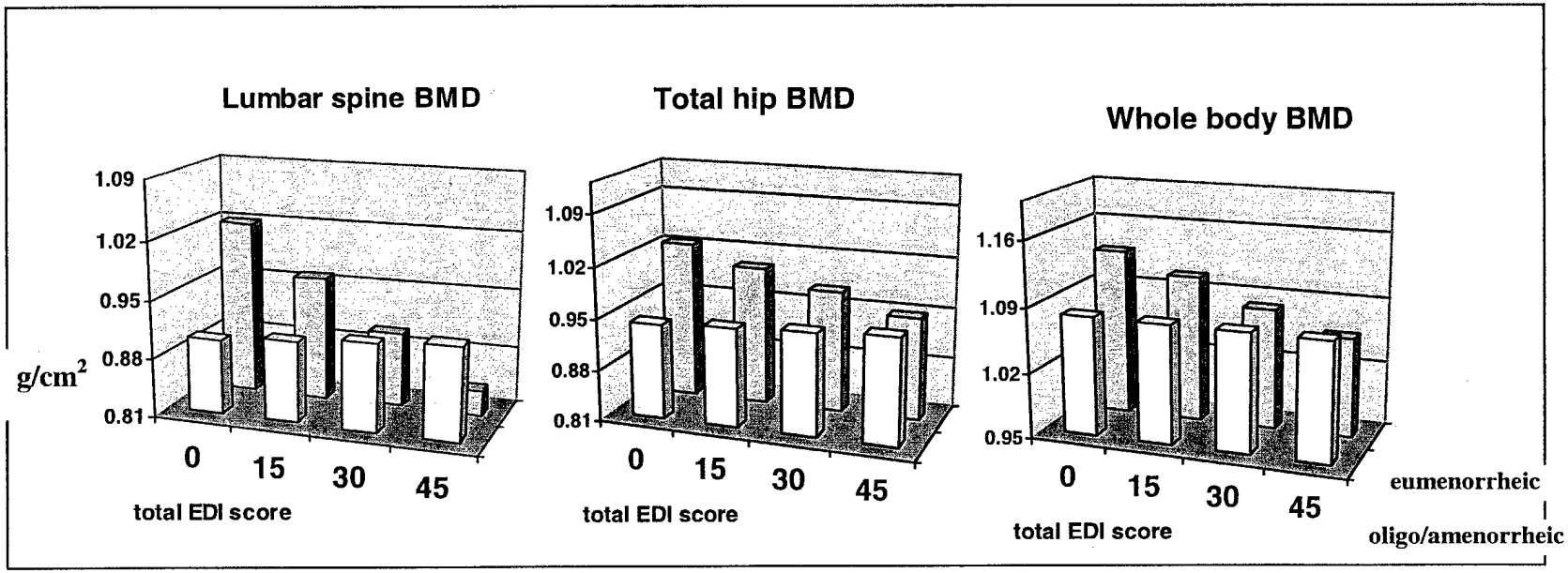
†EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead.[33] Elevated scores are defined as the highest quartile (≥23). One subject is missing EDI score; therefore she is removed from all analyses involving EDI.

‡Oligo/amenorrhea was defined as 0-9 menses over the past 12 months.

§Group1 vs. Group 2, $p<.005$, Tukey's test for comparing multiple group means.

|Group1 vs. Group 2; Group1 vs. Group 3, $p<.05$, Tukey's.

**Group1 vs. Group 4, $p<.10$, Tukey's.


Lumbar spine BMD
Total hip BMD
Whole body BMD
g/cm²
1.09
1.02
0.95
0.88
0.81
1.16
0
15
30
45
total EDI score
eumenorrheic
oligo/amenorrheic

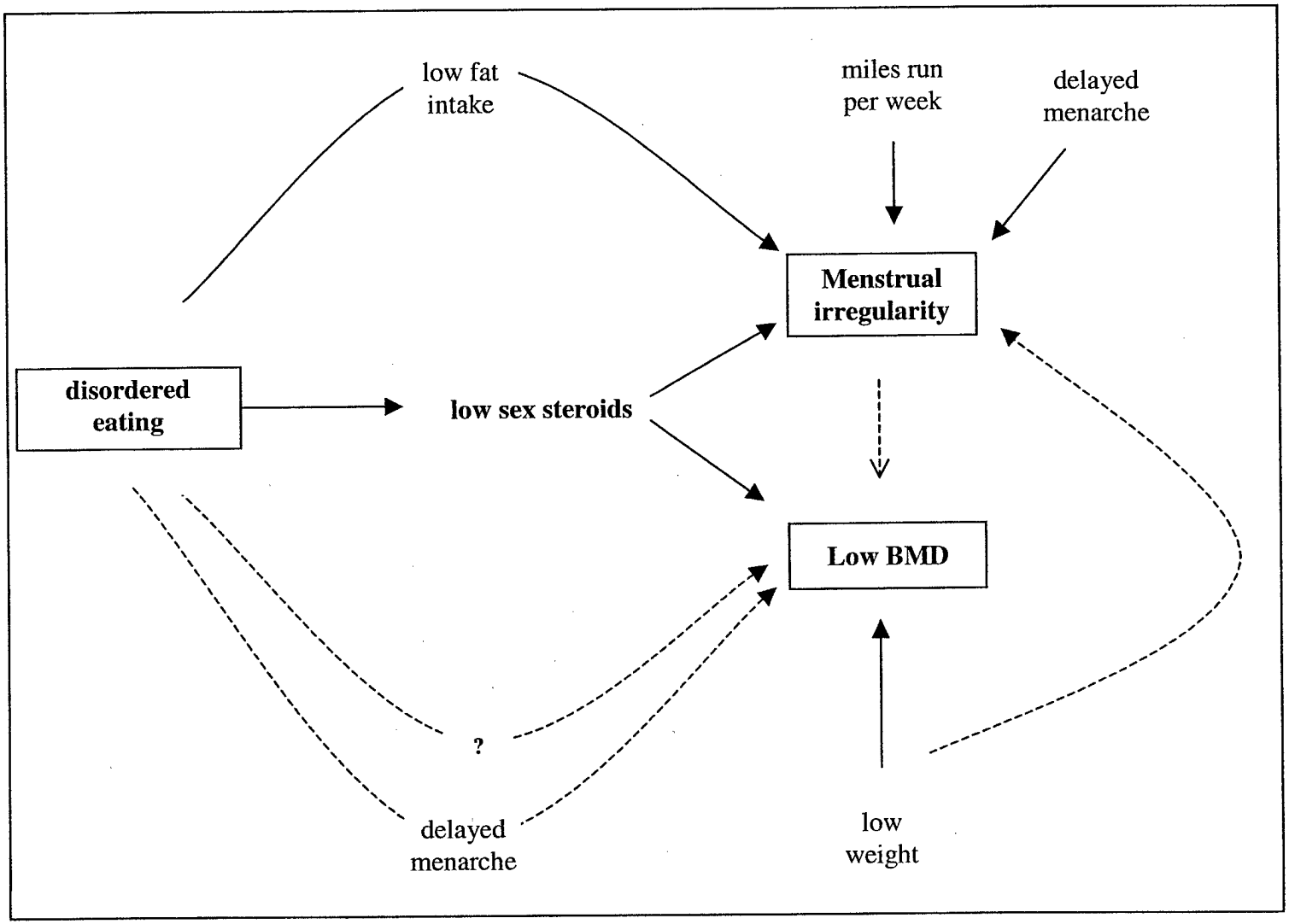

low fat intake
miles run per week
delayed menarche
Menstrual irregularity
disordered eating
low sex steroids
Low BMD
?
delayed menarche
low weight

Figure 1. Mean BMD (g/cm^2) at the spine, hip and whole body by menstrual status and varying levels of total EDI score (from multiple linear regression*).

*The means are based on the following multiple linear regression results (adjusted for age, weight, and percent body fat):

Regression coefficients (standard error)		
menstrual status†	EDI score (0-69)	interaction: menstrual status x EDI score
-.12 (.033)	-.004 (.001)	+.0043 (.002)
-.089 (.036)	-.002 (.001)	+.002 (.002)
-.060 (.025)	-.002 (.001)	+.002 (.001)

† Menstrual status equals 1 if the woman is oligo/amenorrheic and 0 if the woman is eumenorrheic.

Figure 2: Proposed pathways among disordered eating, menstrual irregularity, and low BMD. Solid lines represent associations suggested by the current study; dashed lines represent associations suggested by previous studies.

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Dietary Protein Intake and Bone Mineral Density in Young Female Distance Runners

Abstract

Female athletes in endurance and appearance-related sports may adopt weight loss strategies. These athletes are at risk for the female athlete triad of disordered eating, menstrual irregularities, and low bone mineral density (BMD). In this susceptible population, little is known about specific eating practices that may enhance bone loss. Many popular weight loss strategies involve a high-protein diet. The relationships between dietary protein intake and bone mineral density (BMD) were investigated in 117 competitive female distance runners aged 18-25. Dietary protein was inversely associated with BMD at the hip, spine, and whole body. Both animal and vegetable protein appeared to contribute to the overall inverse association. When controlling for menstrual irregularity, body weight, percentage body fat, and age in multivariate models, the magnitude of the inverse associations became slightly greater. These findings support the possibility of a negative role for dietary protein in the skeletal health of young female distance runners.