

Negative Spinal Bone Mineral Density Changes and Subclinical Ovulatory Disturbances—Prospective Data in Healthy Premenopausal Women With Regular Menstrual Cycles

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Subclinical ovulatory disturbances (anovulation or short luteal phases within normal-length menstrual cycles) indicate lower progesterone-to-estrogen levels. Given that progesterone plays a bone formation role, subclinical ovulatory disturbances may be associated with bone loss or less than expected bone gain. Our purpose was to perform a meta-analysis of prospective studies in healthy premenopausal women to determine the overall relationship of subclinical ovulatory disturbances to change in bone mineral density. Two reviewers independently identified from serial literature searches 6 studies meeting inclusion criteria: a 2-year study in 114 young adult women, 2006–2009, Vancouver, Canada; a 2-year study in 189 premenopausal women, 2000–2005, Toronto, Canada; a single-cycle study in 14 young women, 1996–1997, Melbourne, Australia; an 18-month study in 53 women, 1990–1995, Santa Clara, California; a 4-year study in 27 women, 1988–1995, Vancouver, Canada; and a 1-year study in 66 women, 1985–1988, Vancouver, Canada. This meta-analysis included a combined sample size of 473 observations in 436 premenopausal women studied over 1–4 years and aged 14–47 years. The percentage of women with ovulatory disturbances varied significantly from 13% to 82%. Women with more frequent ovulatory disturbances had more negative percentage changes in spine bone mineral density (weighted mean difference = -0.86 ; $P = 0.040$) for random-effects analysis. There was significant heterogeneity among these 6 studies ($I^2 = 80\%$). In summary, these data show that regularly menstruating women with more frequent ovulatory disturbances experience more negative changes in bone (approximately -0.9% per year). These cycles with silent estrogen/progesterone imbalance may be clinically important.

bone mineral density; osteoporosis risk; ovulatory disturbances; premenopause; progesterone

Abbreviations: BMD, bone mineral density; DXA, dual energy x-ray absorptiometry; LS-QBT, least-square quantitative basal body temperature; PdG, pregnanediol-3-glucuronide; QCT, quantitative computed tomography.

INTRODUCTION

It is well established that disturbances of menstrual cycle length, such as amenorrhea or oligomenorrhea, result in accelerated bone loss and increased risk of future fractures (1, 2). This is most likely related to lower estrogen levels such as also occur in menopause (3), with premature ovarian failure (4), or in premenopausal women with hypogonadotropic hypogonadism (5, 6). Estrogen plays an important role in bone health by increasing intestinal calcium absorption and suppressing bone resorption through the receptor activator of nuclear factor κ B (RANK) (7).

Like estrogen, progesterone levels also decrease significantly with hypothalamic amenorrhea and are usually low

with oligomenorrhea and irregular cycles; progesterone is increasingly recognized as an estrogen partner in clinical bone metabolism (8). Progesterone has been shown to promote bone formation by increasing osteoblast numbers, maturation, and differentiation in vitro (9–11). However, clinical evidence for increased bone mineral density (BMD) during therapy with progesterone or progestin is sparse (12–14).

Progesterone is produced by the corpus luteum after ovulation and converts the endometrium to its secretory phase to prepare the uterus for egg implantation. It also has the effect of increasing basal temperature. Some women with regular menstrual cycles and adequate estrogen levels experience subclinical ovulatory disturbances (cycles that are

anovulatory or with a short luteal phase), which result in lower levels of progesterone (15). Anovulation is a condition in which no egg is released; thus, there is infertility with usually no production of progesterone. Short luteal phases occur in ovulatory cycles when the temperature plateau is less than 10 days or the length between serum luteinizing hormone peak to onset of menstrual flow is less than 12 days (16). Subclinical ovulatory disturbances are more prevalent in women with cognitive dietary restraint who consciously limit and monitor their food intake to achieve or maintain a desired weight (17), and in women working shifts and in stressful environments (18). They also have been reported to be more common in premenopausal monkeys with subordinate social status (19).

For many years, it has been believed that normal estrogen levels, as indicated by normal menstrual cycle lengths of 21–35 days, are sufficient to maintain premenopausal bone density (20). However, collected data from a number of studies show that the within-cycle downward swings of normal cyclic estradiol levels from the midcycle peak to the next flow are associated with a small net increase in bone resorption (21) and, thus, some bone loss if it is not counterbalanced by progesterone-related bone gain. For this reason and given that progesterone acts specifically through osteoblast receptors to promote bone formation, but does not appear to decrease bone resorption (22), women with adequate estradiol but inadequate progesterone levels related to anovulatory or short luteal phase cycles may experience accelerated bone loss, or, if not yet at peak bone mass, a failure to gain bone at the expected rate.

The first association between trabecular spinal BMD loss in women with subclinical ovulatory disturbances within normal-length cycles was made by Prior et al. (15) using quantitative computed tomography (QCT). This study showed no differences in estradiol levels between those women with the most ovulatory disturbances (who lost bone) and those with the least ovulatory disturbances (who maintained bone) (15). Because of the multiple and varying unknowable past influences on cross-sectional areal BMD (such as genetics, childhood and adolescent nutritional adequacy/inadequacy, physical activity patterns, reproductive histories, and sociocultural stressors), this ovulation–areal-BMD relationship has never been documented in several small cross-sectional studies (23–25). One cross-sectional study, however, of BMD by dual energy x-ray absorptiometry (DXA) in over 600 women where 65 women had 2 cycles of ovulation and hormonal monitoring was able to show that women having BMD levels within the lowest 10th percentile from a population-based study had significantly lower progesterone and estradiol urinary excretion levels compared with those in women with BMD values between the 50th and 75th percentiles (26). In monkey models, subordinate females compared with dominant females have significantly more subclinical ovulatory disturbances and lower levels of ovarian hormones that are associated with reduced spinal BMD, as well as with abnormal blood vessel histology suggestive of early cardiovascular disease (19). Similarly, a negative relationship between ovulation disturbances and BMD change has been reported in some (27–29), but not all, of the subsequent prospective studies (30, 31). This lack of consistent association may be

secondary to variability in participant populations and in the methodology used in BMD measurements or assessments for evidence of ovulation.

Because of its potential importance to population bone health, we therefore undertook a meta-analysis of currently available evidence to determine whether, overall, there was an association between subclinical ovulatory disturbances and changes in premenopausal spinal BMD.

METHODS

Literature search strategy

After multiple, varying search strategies performed over the last 3 years with Medline and Embase databases, we have refined the strategies into an ultimate search (Medline, from 1949 to the present date) that yielded 50 citations and, excluding 4 duplicates, 46 unique references. From this search, we identified 6 papers that met our inclusion criteria by assessing premenopausal, healthy women and providing prospective data on ovulatory disturbances and BMD change. The search strategy is included in Figure 1, which shows the flow of this literature search and reasons for exclusion. Abstracts from past American Society for Bone and Mineral Research meetings were also screened. Keywords utilized a comprehensive list of controlled vocabulary and natural language terms (Figure 1) (Appendix). Two skilled medical librarians worked with content experts to create, modify, and refine these searches. Articles were selected on the basis of the abstracts before examining the full text. Articles not in English were excluded.

Inclusion and exclusion criteria

Articles were eligible if they were prospective, observational studies that assessed the association between BMD changes and ovulatory disturbances in healthy premenopausal women. We also screened the reference lists of pertinent articles, such as the 1998 population-based study by Sowers et al. (26) and more recent reviews (32–36), for potential additional publications. When prospective research did not report a direct measure of the association between BMD changes and ovulation characteristics, attempts were made to contact the authors for additional information. In addition, when the published data on BMD change were not provided in a common metric (percentage change per year), we used the original data (when available) to calculate percent change and contacted the authors of other articles for this additional information. We excluded animal studies (19) ($n = 1$), review articles ($n = 21$), and commentaries ($n = 1$) that did not contain original data and also cross-sectional studies ($n = 6$). Cross-sectional studies were excluded because 1 single menstrual cycle or a BMD value measured at 1 time point cannot accurately evaluate the association of varying ovulatory function over time (i.e., prevalence of ovulatory disturbances) with *change* in BMD. One population-based, nested case-control study, known from primary reading of the journal and not found in this search, whose results support the outcome of this meta-analysis of prospective studies, was excluded because it was cross-sectional with only a single BMD value (26).

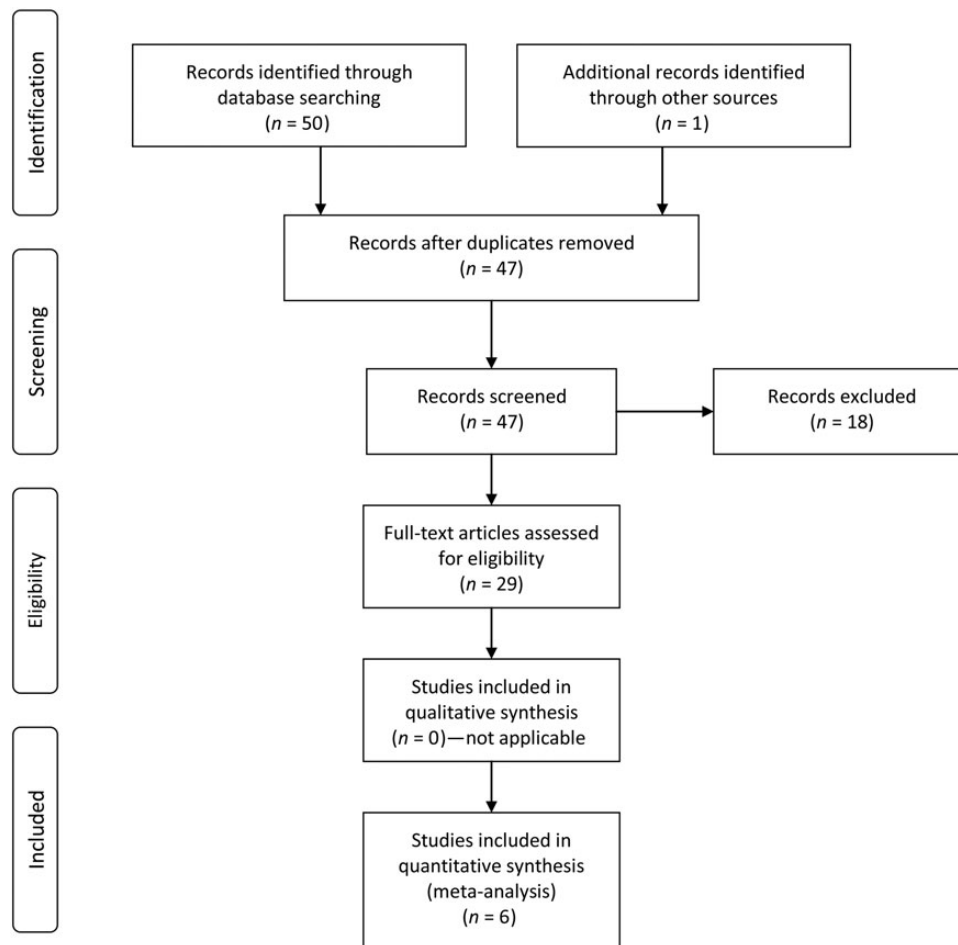


Figure 1. This diagram shows the final, refined literature search results in PRISMA formatting for the citation data represented in this meta-analysis. Of 50 different papers brought up by the literature search strategy (refer to the Appendix), after exclusion of 4 duplicates, there were 46 unique citations from which 6 eligible publications were analyzed. PRISMA, preferred reporting items for systematic reviews and meta-analyses.

Data collection

Two reviewers (D. L., J. C. P.) worked independently to screen for potential eligible studies based on the search strategy and inclusion criteria described above and shown in Figure 1. Disagreements were resolved by consensus.

Data extraction was performed by 1 reviewer using a pre-defined extraction sheet. These data included study characteristics (author, publication year, number of subjects, length of follow-up, number of cycles analyzed per year); sample characteristics (age, body mass index, cycle length, percentage of women with ovulatory disturbances, methodology used to define evidence of ovulation and luteal phase lengths (various hormonal sampling methods vs. least-square quantitative basal body temperature (LS-QBT))); and BMD methodology (QCT vs. DXA).

Finally, we reanalyzed the entire bone outcome data set as annualized percentage change in BMD from baseline in groups with more versus less prevalent ovulatory disturbances defined as anovulatory or short luteal phases within regular menstrual cycles.

The criteria for short luteal phase length depend on the method of detecting ovulation. The luteinizing hormone surge precedes ovulation, but basal temperature rises only in response to corpus luteum-produced progesterone 2–3 days following the estradiol and luteinizing hormone peaks and egg release. Accordingly, a short luteal phase defined by the serum or urine luteinizing hormone peak occurs when the duration is 12 or fewer days to the next flow (16, 37). With the quantitative basal temperature method (38), because of the delay in temperature rise, a short luteal phase is fewer than 10 days in length (39).

For studies that had greater than 50% of women with subclinical ovulatory disturbances, we analyzed the study's original data using the method of median split to divide the participants into those with more than or less than the median percentage of cycles with ovulatory disturbances (if this had not already been reported by the authors). For studies that had less than 50% of women with subclinical ovulatory disturbances, groups were defined on the basis of the authors' specified thresholds.

Statistical analysis

We used fixed-effect and random-effects meta-analysis (40) to compute the weighted mean difference of the percent change in BMD between women with fewer and more prevalent ovulatory disturbances. The I^2 statistic was used to assess heterogeneity; an I^2 value of greater than 50% represents substantial inconsistency among studies. We assessed studies as having higher quality the more menstrual cycles were monitored for ovulatory characteristics per year. Studies with 5 or more cycles per year in which ovulatory characteristics were assessed were considered of higher quality, as they were more likely to accurately reflect a woman's ovulatory experience during that year than those with fewer monitored cycles each year. Publication bias was assessed by visually inspecting the funnel plot to detect asymmetry in data given the small number of qualified studies. Statistical analysis was conducted by using the main meta-analysis command *metan* in Stata, version 12.1, statistical software (StataCorp LP, College Station, Texas).

RESULTS

The results of the meta-analysis will be presented after describing the choice of the studies for inclusion and the methods within each of these 6 studies for documenting and describing ovulatory disturbances and for measurement of changes in bone mineral density.

Search results

As shown in Figure 1 (a preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram), our final, refined search strategy (Appendix) yielded a total of 50 citations plus an additional citation from another source. Four were excluded as duplicates, thus yielding 47 unique references. After appropriate exclusions, a total of 6 were eligible because they were observational, in healthy premenopausal women with prospective bone change, and had identified ovulatory characteristics. We excluded articles that were reviews and did not provide original data (21, 33, 41–44); presented therapy, ill women, or were controlled trial data (12, 45–49); and finally 1 study that was not conducted in humans (19). We also reviewed the full text of 29 studies, of which 23 were excluded (Figure 1) for various reasons including lack of prospective data (35, 50, 51) and 3 were excluded for having studied human populations that were perimenopausal rather than premenopausal (25, 52, 53). The six studies that were included were all prospective BMD studies in healthy premenopausal women that both reported spinal BMD changes and assessed ovulatory disturbances (15, 27–31).

Study descriptions

The characteristics of women described in the eligible publications are shown in Table 1. Pooling of studies resulted in a combined sample size of 473 observations in 436 women, as some of the women in the 1990 study by Prior et al. (15) were later followed over the subsequent 4 years in the 1996 study by Prior et al. (31). The length of follow-up ranged from 1 to

Table 1. Characteristics of Participants and Design and Measurement Modalities of Studies^a Included in This Meta-analysis

First Author, Year (Reference No.)	No. of Women	Duration, Years	Age, Years	Mean Body Mass Index ^b	No. of Cycles/Year ^c	Cycle Length, Days	% with ≥ 1 Ovulatory Disturbance(s) ^d	Excluded PCOS	ODM	Sensitivity of ODM, %	Specificity of ODM, %	Bone Mineral Density Modalities
Bedford, 2010 (28)	114	2	22	22	7	31	82	Yes	LS-QBT	97	25	DXA (L1–L4)
Wagh, 2007 (27)	189	2	32	24	6	29	33		Urinary LH surge	53	100	DXA (L1–L4)
Morris, 1999 (29)	14	1.5	15	22	1	30	36		E1G and PdG			DXA (L2–L4)
Waller, 1996 (30)	53	1.5	33		3	30	13		E1C/(PdG + 1)			DXA (L2–L4)
Prior, 1996 (31)	27	4	36	22	2	28	66	Yes	LS-QBT	97	25	QCT (T12–L3)
Prior, 1990 (15)	66	1	34	22	10	28	62	Yes	LS-QBT	97	25	QCT (T12–L3)

Abbreviations: DXA, dual-energy x-ray absorptiometry; E1C, estrone conjugate; E1G, estrone conjugate; L1–L4, first through fourth lumbar vertebrae (measurement); L2–L4, second through fourth lumbar vertebrae (measurement); L3, third lumbar vertebra (measurement); LH, luteinizing hormone; LS-QBT, least-square quantitative basal body temperature; ODM, ovulation detection method; PCOS, polycystic ovarian syndrome; PdG, pregnanediol-3-glucuronide; QCT, quantitative computed tomography; T12, 12th thoracic vertebra (measurement).

^a Arranged in reverse chronological order from 2010 to 1990.

^b Body mass index: weight (kg)/height (m)².

^c Those with ovulatory characteristics documented.

^d Percentage of women experiencing one or more regular, normal-length menstrual cycles with anovulation or short luteal phases within the duration of the study.

4 years. On average, 5 cycles were available per woman per year in which data assessing ovulatory status were available. The age of participants ranged from 14 to 47 years. In the 4 studies reporting body mass index, mean values were in the normal weight range. The ethnicity of women participants was mainly Caucasian, except that 63% of those in the 2010 study by Bedford et al. (28) were Asian. The percentage of women with ovulatory disturbances in monitored cycles varied significantly among studies, from 13% in the study by Waller et al. (30) to 82% in the study by Bedford et al. (28).

Because there is no universally accepted, “gold standard” method for prospectively documenting ovulation and luteal phase lengths in ambulatory women, there was considerable variability in the methods used; all studies that assessed luteal phase length considered 10 days to be the shortest normal luteal phase length. These methods are summarized in Table 1. Briefly, 3 studies (15, 28, 31) used the LS-QBT method, which has been validated against the serum luteinizing hormone peak and urinary pregnandiol-3-glucuronide (PdG) standards, respectively (38, 54). One study (27) used the urinary luteinizing hormone surge and salivary progesterone threshold method (55). Waller et al. (30) used urinary PdG and highest estrone conjugate/(PdG + 1) ratio to detect ovulation and luteal phase length (56), and Morris et al. (29) classified cycles as ovulatory if high urinary estrone glucuronide levels (100–400 nmol/24 hours) were followed by a rapid rise in urinary PdG to above 9.0 $\mu\text{mol}/24$ hours.

BMD was measured volumetrically in trabecular bone by using single-energy spinal QCT of the 12th thoracic vertebra (T12)–third lumbar vertebra (L3) in 2 studies (15, 31) and by DXA of L2–L4 in 2 studies (27, 28) and L1–L4 in 2 studies (29, 30) (Table 1). Lumbar spine DXA provides a 2-dimensional areal estimate of BMD consisting of both cortical and trabecular bone; this measure is dependent on 2-dimensional bone size (57). QCT determines volumetric trabecular BMD without the inaccuracies caused by soft tissue, extraosseous calcifications, and hyperostosis that may confound DXA (57), although QCT may be altered by increased marrow adiposity (58).

Prospective BMD data were originally published as percentage DXA change by Bedford et al. (28), Morris et al. (29), and Waller et al. (30). The original QCT data were accessed for the 2 studies by Prior et al. (15, 31), and a new variable “percentage BMD change” was created for use in the meta-analysis statistics. Waugh et al. (27) provided the percentage change for the women with ≥ 2 cycles of ovulatory disturbance/year and those with < 2 (E. J. Waugh, University of Toronto, personal communication, 2013). Thus, all data in this meta-analysis are reported as percentage annual change in BMD.

The different criteria for women with more versus fewer ovulatory disturbances in the 6 studies are summarized in Table 2, which also presents data on mean annual percentage BMD changes for 2 groups of women within each study who experienced more and less frequent ovulatory disturbances. BMD changes in women with a lower than, versus greater than, median percentage of ovulatory disturbances were recalculated from the original data in the studies by Prior et al. (15, 31) using the method of median split. The median split documented 33% of cycles with ovulatory disturbances for the 1990 data (15) and 31% for the 1996 data (31). In the study by Bedford et al. (28), data were reported by median split; the median proportion of cycles with ovulatory disturbances was 38%. In the study by Waller et al. (30), those with ≥ 1 ovulatory disturbances were contrasted with those without ovulatory change while disregarding cycle length. The report by Morris et al. (29) provided information on the rate of BMD change in teenaged light-weight competitive rowers classified as having an ovulatory cycle or an anovulatory cycle. Initially, additional ovulation-related results were obtained directly from the authors in the 2007 study by Waugh et al. (27) (E. J. Waugh, University of Toronto, personal communication, 2010). Women with more than 2 cycles with ovulatory disturbances were contrasted with those with 1 or no anovulatory and/or short luteal phase cycles (27). Additional primary information was also requested related to the study by Waller et al. (30), but these data are apparently no longer available.

Table 2. Annual Percent Changes in Bone Mineral Density in Women With Fewer or More Frequent Ovulatory Disturbances Defined by Either a Median Split or Preset Criteria of Studies^a Included in This Meta-analysis

First Author, Year (Reference No.)	Fewer Ovulatory Disturbances		More Ovulatory Disturbances		Criteria for Women With More Ovulatory Disturbances
	Mean (SD)	No.	Mean (SD)	No.	
Bedford, 2010 (28)	1.9 (3.0)	57	0.7 (3.0)	57	Median split: $>38.8\%$ of cycles abnormal ^b
Waugh, 2007 (27)	0.83 (1.4)	157	0.22 (1.6)	32	≥ 2 cycles abnormal ^b
Morris, 1999 (29)	4.1 (1.2)	9	2.6 (0.7)	5	Ovulatory vs. anovulatory
Waller, 1996 (30)	-0.04 (1.52)	46	0.55 (1.63)	7	≥ 1 cycle abnormal ^b
Prior, 1996 (31) ^c	-0.98 (0.82)	14	-0.87 (0.67)	13	Median split: $>33\%$ of cycles abnormal ^b
Prior, 1990 (15)	-0.83 (3.30)	33	-3.90 (2.59)	32	Median split: $>31\%$ of cycles abnormal ^b

Abbreviation: SD, standard deviation.

^a Arranged in reverse chronological order from 2010 to 1990.

^b Abnormal cycles comprised cycles that were anovulatory and/or had a short luteal phase length.

^c These women also participated in the 1990 study by Prior, but with nonoverlapping time intervals.

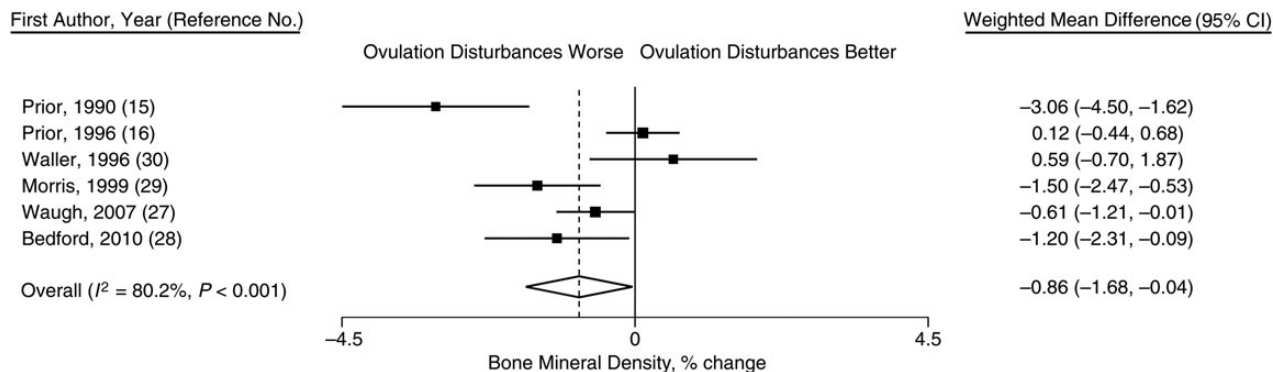


Figure 2. Forest plot showing the weighted mean difference in percent annual change comparing changes in spinal bone mineral density (by either quantitative computed tomography or dual-energy x-ray absorptiometry) between premenopausal women with a more or less frequent percentage of regular normal-length menstrual cycles with ovulatory disturbances per year. The size of the square reflects the study-specific statistical weight. The horizontal line shows the 95% confidence interval (CI) of the weighted mean difference. The diamond shows the pooled weighted mean difference and 95% confidence interval based on random-effects modeling of all studies. The I^2 assesses heterogeneity among studies.

Meta-analysis

A meta-analysis using the random-effects model from the 6 eligible studies showing an association between ovulatory disturbances and changes in lumbar spine BMD is illustrated by a forest plot in Figure 2. Both the random-effects model (weighted mean difference = -0.86 , 95% confidence interval: -1.68 , -0.04) ($P = 0.040$) and the fixed-effects model (weighted mean difference = -0.56 , 95% confidence interval: -0.90 , -0.23) ($P < 0.001$) showed significant associations between ovulatory disturbances and less favorable changes in BMD (meaning greater bone loss in studies in women older than 25 but less positive bone gain in younger women). There was significant heterogeneity among the 6 studies ($I^2 = 80\%$), but the number of studies was too small to further assess this. Examination of the funnel plot provided no indication of publication bias.

DISCUSSION

This meta-analysis revealed a clinically important association between spinal BMD loss, or more negative bone change, in healthy, regularly menstruating premenopausal women with more prevalent versus less prevalent subclinical ovulatory disturbances. The weighted mean difference reflects a weighted mean loss of approximately 0.9% per year (range, -1.7% to -0.04% per year) in those with more versus fewer cycles with ovulatory disturbances per year. Although it is not currently known how consistent ovulatory disturbances are within-woman over time, recent cross-sectional population-based data suggest that 26%–38% of over 3,000 women with spontaneous, regular, normal length menstruation were anovulatory in the tested cycle (59). Should ovulatory disturbances persist, with a similar prevalence as shown in these 6 studies, throughout the 30–40 years of premenopausal life, this could contribute to a substantial deficit in the BMD that women have in late perimenopause and thus a greater risk for postmenopausal osteoporosis and fracture.

The strengths of this meta-analysis are the extensive nature of our literature searches, the selection of prospective studies with similar research questions, and the inclusion of quality assessment. That the 3 studies that documented ovulatory characteristics in 5 or more cycles/year/woman all showed a significant negative association of ovulatory disturbances with BMD change further suggests the validity of the data (Table 1). Using a median split of the ovulation-related data also is a stronger method than simply reporting a percentage of the monitored cycles with ovulatory disturbances.

It is also an analytical strength that this meta-analysis applied 2 different statistical models: fixed- and random-effects models. To our knowledge, there are no clear criteria for the appropriate model in analysis of ovulatory disturbances and bone change. The fixed-effects model assumes that there is 1 true effect size that underlies all the studies in the analysis, and that all differences in the observed effects are due to sampling (60). Both the random- and the fixed-effects models showed statistically significant associations between more frequent ovulatory disturbances and more negative bone density changes. With the random-effects model, the effect size is assumed to vary in different studies, as different studies may have different participant characteristics and use various research methods and, therefore, different effect sizes (60). Given the significant results we found using a random-effects meta-analysis model, our ultimate result is a mean of all effect sizes that can be generalized to an infinite number of studies (60) and thus can be used to reflect population data.

One major limitation of this meta-analysis is the considerable heterogeneity among these studies. This heterogeneity is a consequence of the various differences in the studies as summarized here and described below: 1) methods to assess evidence of ovulation and to define short luteal phase lengths; 2) number of cycles monitored for each woman per year; 3) composition of the participant populations (some excluded obesity and polycystic ovary syndrome and some did not); and, 4) BMD measurement modalities (QCT and DXA). Nevertheless this meta-analysis compares women *within*

each population with more or fewer ovulatory disturbances by using percentage annual change in their common BMD method's rate of change. Despite these substantial study differences, it is notable that 4 of the 6 studies, as well as those 3 studies having the highest quality, were consistent in showing associations between more negative BMD change and ovulatory disturbances.

To further explain the heterogeneity among the studies, we used different methods to detect *evidence of ovulation*, as there is no universally accepted gold standard. In the study by Waugh et al. (27), midcycle urinary luteinizing hormone peak data were used to assess evidence of ovulation, which had a sensitivity of 53% and specificity of 100% validated against *serum* luteinizing hormone (61), although a luteinizing hormone peak does not inevitably mean an ovulatory cycle (62). In addition, instead of using 12 days as the cutoff for short luteal phase detected by the urinary luteinizing hormone peak (as the luteinizing hormone surge precedes ovulation), this study erred in using 10 days, which is the cutoff number used by the LS-QBT method based on the fact that basal body temperature rises 2–3 days following the luteinizing hormone peak (38). Altogether, the low sensitivity of the luteinizing hormone peak detection method (53%) and the shorter definition of luteal phase length (10 vs. 12 days) contributed to the smaller percentage of women (33%) who had ovulatory disturbances in the study by Waugh et al. (27). Waller et al. (30) adopted the modified method described by Kassam et al. (56) for detecting ovulation from urinary PdG, but they used an exceptionally low ovulatory threshold for urinary PdG (1 + the square root of the baseline level), compared with 3 times the baseline levels described by Kassam et al. (56). This likely resulted in misclassification of some anovulatory cycles as ovulatory. It is worth noting that the 13% rate of ovulatory disturbances in the study by Waller et al. (30) is much lower than the rate in all of the other studies. The LS-QBT method used in the studies by Prior et al. (15, 31) and Bedford et al. (28) has been shown to correlate well with the serum midcycle luteinizing hormone peak (38). However, compared with the method of Kassam et al. (56), LS-QBT has a sensitivity of 97% and specificity of 25% for detecting anovulation (54). It is possible that this low specificity may contribute to the higher percentage of women with ovulatory disturbances, from 62% in the 1990 study by Prior et al. (15) to 82% in the study by Bedford et al. (28). Overall, because all of the above methods that are suitable for frequent measurement over long durations have limited specificity or sensitivity, it is possible that ovulation may have been misclassified as anovulation and vice versa. However, classification was consistent within each study, and the primary outcome of this analysis was within-study differences in bone change.

Another possible explanation for varied rates of ovulatory disturbances is differences among study populations. Ovulatory disturbances are more prevalent in very young women and increase again in perimenopause (39). Women who are less than 10 years post menarche are known to have a higher percentage of ovulatory disturbances than women in their mid-30s (39). In the studies by Bedford et al. (28) and Morris et al. (29), the participants were much younger (average ages, 22 and 15 years, respectively) compared with the other studies (average ages, 32–36 years), thus likely contributing to

the greater percentage of women with ovulatory disturbances. It is also known that psychological stress is associated with decreased levels of estrogen, as well as ovulatory disturbances with decreased levels of progesterone (19). In a study of student nurses, 64.9% were anovulatory during the school term, but some recovered to more consistent ovulatory cycles during the spring and summer holidays (18). Similar academic stressors may have been reflected in the study by Bedford et al. (28), in which a majority of the participants were full-time university students; these data included 2 full years and did not differentiate between school terms and breaks, however.

Studies also differed in whether researchers screened participants on the basis of body size and, in particular, whether women with polycystic ovarian syndrome were included or excluded (Table 1). In women with polycystic ovarian syndrome, the relationship between ovulation and bone may be confounded by androgen excess, chronically higher estradiol levels, and often a greater prevalence of insulin resistance and obesity (63). Three of the studies (15, 28, 31) specifically or indirectly excluded women with ovulatory disturbances related to polycystic ovarian syndrome. On the other hand, in the investigation by Waugh et al. (27), the study population had a higher body mass index (mean = 24.3), and no upper limit of body mass index was described in the recruitment criteria. In the study by Waller et al. (30), body mass index was not provided, but the text reported that women with luteal phase abnormalities were heavier (82.5 kg vs. 65.5 kg) and had more body fat (36.4% vs. 29.4%). Women with menstrual dysfunction related to polycystic ovarian syndrome seem to have increased areal BMD and possibly also higher bone material quality (63). Moreover, differences in regional body composition (increased central adiposity and truncal mass) may contribute to site-specific BMD increases in participants with polycystic ovarian syndrome (64). The heavier population in the study by Waller et al. (30) and screening criteria may account for the decreased ability of this study to show differences in bone change by the presence or absence of ovulatory disturbances.

Heterogeneity within this meta-analysis also arises in the number of cycles with ovulatory characterization per year. This varied from 10 cycles/year in the 1990 study by Prior et al. (15) to a single cycle in the study by Morris et al. (29). Those studies that did not show an association between ovulatory disturbances and BMD change tended to have had a below average number of monitored cycles per year (25, 27), but here the study by Morris et al. (29) is an exception.

These 6 studies also differed in BMD methodology. Trabecular bone makes up 50% of vertebral bone volume and has a higher rate of bone turnover than cortical bone. As QCT measures trabecular bone separately from cortical bone, it is therefore more sensitive in measuring BMD change than DXA is, which combines trabecular and cortical bone measurements. This, in turn, may lead to the larger difference by ovulatory disturbances seen in the 1990 study by Prior et al. (15). It is also of note that all of these studies except that by Waller et al. (30) assessed menstrual cycles and ovulation during the time period over which BMD was measured; in that study, DXA was initially measured more than a year after the conclusion of the menstrual cycle/ovulation assessments (30).

Despite all of this heterogeneity and these limitations, this meta-analysis showed that, in premenopausal women with regular cycles, those with more frequent ovulatory disturbances had significantly more negative change in spinal bone mineral density. This is confirmed by the population-based, nested case-control study by Sowers et al. (26), demonstrating that women with the lowest BMD had lower progesterone and estradiol levels and tended to have more ovulatory disturbances. Overall, these data have significant population health implications, as well as relevance for clinical practice and future research. As the primary hormonal change in ovulatory disturbances is in progesterone levels (its absence, too few days of high levels, or lower than normal levels), it is plausible that cyclic progesterone supplementation would prevent bone loss in premenopausal, normal weight women with ovulatory disturbances. This has been demonstrated by cyclic medroxyprogesterone and/or calcium therapy in a single-center, 1-year, randomized 2 × 2 factorial controlled trial in 61 healthy, active, normal-weight premenopausal women in their mid-30s with hypothalamic ovulatory disturbances (64%), oligomenorrhea (20%), or amenorrhea (16%) who were stratified into active therapies or placebo therapies (12). Spinal BMD by DXA increased over 1 year in women treated with cyclic medroxyprogesterone (10 mg/day for 10 days per month), did not change in women treated only with calcium (1,000 mg/day), and significantly decreased in women on placebos for both calcium and medroxyprogesterone (12). In another randomized controlled 1-year clinical trial, adolescent participants with hypothalamic cycle disturbances were treated with oral contraceptives, medroxyprogesterone, or placebo (65). Treatment with oral contraceptives increased lumbar spine DXA measurements, whereas medroxyprogesterone or placebo had no effect. This study, however, had only 5 women per group and included adolescent women with undernutrition related to eating disorders and anorexia (65).

Decreasing estrogen levels cause an accelerated bone loss at the onset of amenorrhea, in perimenopause when estradiol levels are labile (66), and with the surgical removal of ovaries (3, 22). As progesterone acts primarily through osteoblasts to increase bone formation and has no effect to decrease bone resorption, it is unlikely to prevent bone loss in participants whose bone resorption is increased. This has also been demonstrated in a controlled trial in which progesterone alone was not an effective therapy for BMD in early postmenopausal women (67); however, in other controlled trials, estrogen-progestin combination therapy was more effective at increasing BMD than was estrogen alone (13, 14). Theoretically, the additive benefit of increasing bone formation by physiological progesterone levels and luteal phase lengths in women with normal bone resorption translates into a 1% greater annual bone gain. The literature suggests that this would be associated with a concomitant 8% decrease in nonvertebral fractures (68).

The important heterogeneity-limiting interpretation of this meta-analysis may not be solely because of study population, methods, and BMD measurements but simply because of the normal variability of ovulatory characteristics, either within 1 woman over a year or in a given woman across her life cycle (69). It is noteworthy that there was a significant and likely

clinically important BMD difference by ovulatory characteristics within seemingly normal menstrual cycles. We encourage all future prospective studies of ovulatory disturbances and bone change to recruit participants of a specified age range, with documented body mass index characteristics (normal or overweight but not obese or undernourished), to exclude those with polycystic ovarian syndrome, androgen excess, or insulin resistance/diabetes and to apply a common method (or cross-validated methods) for evaluation of ovulatory characteristics. Future studies also need to record follicular and luteal phase estradiol levels and the ratio of estradiol to progesterone in each documented cycle. Because development of osteoporosis appears dependent on peak bone mass and is also likely related to the premenopausal change in BMD when perimenopausal bone loss begins, prevention of bone loss during the premenopausal years may result in the reduction of osteoporosis and fragility fractures in postmenopausal women. This potential long-term benefit of detecting and treating ovulatory disturbances needs to be explored further.

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(Appendix follows)

APPENDIX

Literature Search Strategy as Performed
in OvidSP Medline

The numbered list to the right explains the search strategy used in this meta-analysis of currently available evidence to determine whether, overall, there was an association between subclinical ovulatory disturbances and changes in premenopausal spinal BMD. The symbols and notations in the search statements, that is, the slash and the asterisks and also the OR statements, have a particular meaning in this search language. The slash at the end of a term, for example, means it is a Medical Subject Headings (MESH) term or a subject heading that is being searched rather than a keyword. The Boolean operator “OR” is used in line 6 to combine the results for searches 1–5 and in line 13 to combine the results for searches 7–12. Then the 2 sets of results are combined with the Boolean operator “AND” (in line 14) to retrieve the results about both

BMD change and ovulatory disturbances in premenopausal women.

1. Bone Density/ (*n* = 39,391)
2. bone mineral dens*.mp. (*n* = 26,360)
3. BMD.mp. (*n* = 19,051)
4. bone change*.mp. (*n* = 2,432)
5. (bone adj5 change*).mp. (*n* = 15,403)
6. 1 OR 2 OR 3 OR 4 OR 5 (*n* = 57,924)
7. Anovulation/ (*n* = 1,948)
8. anovulat*.mp. (*n* = 5,226)
9. ovulatory disturbance*.mp. (*n* = 73)
10. (ovulat* adj5 disturb*).mp. (*n* = 412)
11. Luteal Phase/ (*n* = 4,503)
12. (short* adj5 luteal).mp. (*n* = 418)
13. 7 OR 8 OR 9 OR 10 OR 11 OR 12 (*n* = 10,032)
14. 6 AND 13 (*n* = 53)
15. Limit of 14 to English language (*n* = 50)