

Peripubertal estrogen levels and physical activity affect femur geometry in young adult women

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Abstract

Summary The growing skeleton is particularly responsive to exercise around the time of puberty, suggesting a possible role for estrogen in mechanical adaptation in young women. We assessed femoral neck strength index at age 17 in young women with varying adolescent physical activity levels and E2 levels in the first 3 years after menarche. The results indicate that both E2 levels in the first year after menarche and adolescent physical activity

are positively associated with bone strength in young adulthood, such that hormone levels may modify human osteogenic responses to exercise.

Introduction It is well established that physical activity contributes to bone strength in young females, but less is known about how peripubertal estrogen affects skeletal responses to exercise.

Methods We used data from 84 participants in the Penn State Young Women's Health Study to test the prediction that young women who (1) had higher E2 levels during the first year after menarche or (2) were more physically active in adolescence will have greater bone strength at the end of adolescence. Subjects were divided into tertiles of physical activity and of E2 level in the first, second, and third postmenarchal years, and femoral strength was calculated from dual-energy X-ray absorptiometry scans of the proximal femur using hip structure analysis.

Results At age 17, subjects with the highest E2 levels in year 1 after menarche had 5–14% greater strength in the narrow neck and intertrochanteric region, and the most active subjects had 10–11% greater strength in the femoral narrow neck vs. less active girls.

Conclusions This study suggests that both physical activity and peripubertal estrogen have important influences on young adult bone strength and that hormone levels may be mediators of human osteogenic responses to exercise.

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Introduction

Bone is a dynamic tissue that adapts to its mechanical environment throughout life, and the growing skeleton is particularly responsive to exercise prior to the end of

puberty (reviewed in [1]). Girls who begin strenuous exercise prior to menarche gain twice as much BMC as do girls who begin after puberty [2–4], with the interval around Tanner stages II–III (typically just before or around the time of menarche) having the strongest association with increased bone strength [1, 5]. Similarly, self-reported activity in peripubertal boys and girls is correlated with increased bone density and strength from ages 9 to 13, but from ages 13 to 16 has little additional effect in either sex [6]. The heightened mechanosensitivity of peripuberty suggests a potential role for the sex steroids in mechanical adaptation. The major human estrogen, estradiol, is a primary mediator of periosteal bone growth in females, as well as in males through aromatization of testosterone [7–9]. Several recent studies established that variations in estradiol or estrogen receptor bioavailability affect osteogenic responses to mechanical loading [10–13], although a similar experiment in rats found no such effect [14]. Overall, these studies suggest that estradiol may influence bone strength, as well as overall bone growth. If so, then individuals with relatively higher peripubertal estradiol levels are predicted to have greater responses to mechanical loading and consequently stronger adult bones [8, 15]. However, few studies in humans include the necessary longitudinal data to address the relationships between hormone levels, integrated physical activity measurements (as one form of mechanical loading), and development of bone strength. In this report, we focus on the relationship between estradiol levels in the first, second, and third postmenarchal years, adolescent exercise, and bone cross-sectional geometry, an indirect measurement of bone strength.

Many studies of bone health during adolescence have used areal bone mineral density (aBMD) as the primary proxy for bone strength. However, bone geometric properties may change in response to mechanical loading in ways that are not apparent in aBMD measured by dual-energy X-ray absorptiometry (DXA). Bone cross-sectional geometry is important because it expresses in engineering terms the amount of bone tissue and how it is distributed. Stresses (force concentrations) generated in loading are determined by the geometry and the force applied. As shown in Fig. 1, the cross-sectional area of the bone surface (not including marrow) determines axial compressive strength, while the section modulus, which determines resistance to bending loads, varies with the radial distribution of bone tissue. Widening of the periosteal envelope, as what occurs during skeletal growth and in response to exercise, displaces bone mass further from the bending axis, thus increasing bending strength. However, BMD measurements using DXA are not well suited to capture these structurally important changes.

In this report, we used data from the Penn State Young Women's Health Study to examine the relationships of

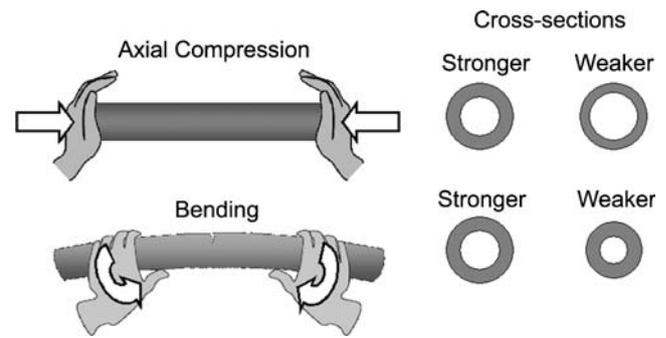


Fig. 1 Cross-sectional area determines axial compressive strength, while the section modulus determines resistance to bending loads

peripubertal estradiol and activity levels on young adult bone strength using the Hip Structure Analysis (HSA) program [16, 17]. The Penn State Young Women's Health Study is a longitudinal study of over 80 women enrolled prior to menarche and followed through young adulthood. The data include hormone levels and physical activity from ages 12 to 22, as well as bone strength measurements from ages 17 to 22. Previous analyses of data from this study [18] demonstrated positive associations between physical activity, integrated adolescent estradiol levels, and increases in bone strength from ages 17 to 22. Given evidence that skeletal mechanosensitivity is particularly high around the time of puberty and that E2 may play a role in skeletal adaptations to loading, we sought to expand upon these previous observations. Here, we test a very specific prediction about the relationship between E2, exercise, and bone, that higher E2 levels *in the year immediately following menarche* will be associated with greater young adult bone strength, particularly in physically active subjects.

Methods

Subjects and data

The subjects were participants in the Penn State Young Women's Health Study, which began with 112 healthy, premenarchal girls of European origin, aged 11.9 ± 0.5 (SD) years at enrollment. This longitudinal study was approved by the Pennsylvania State University College of Medicine Institutional Review Board and required informed consent by both subjects and their parents. Participants were recruited from public schools near the Milton S. Hershey Medical Center, where the study was based. At baseline, all participants were within $\pm 20\%$ of ideal weight for height, with mean BMI of 18.2 ± 2.4 kg/m² and mean body fat of $27.7 \pm 3.7\%$ [19]. Participants did not take ongoing prescription medications and did not have any known abnormalities of bone development, nor any known dietary

or eating disorders. Study visits were biannual for the first 4 years (ages 12–16) and annual thereafter (ages 17–22), for a total of 15 study visits from ages 12 to 22. This analysis includes data for the 84 subjects who remained in the study through age 17 (visit 10). Previous studies revealed no significant differences between those subjects who dropped out and those who continued [18]. One clinical research coordinator performed all study visits.

Bone measurements

All bone measurements were made using a single Hologic QDR-2000 W DXA bone densitometer, with daily scans using the manufacturer's lumbar spine phantom to maintain quality control and to correct for instrument drift. The day-to-day coefficient of variation was <0.7%. Beginning at approximately age 17 (visit 10), we obtained yearly bilateral proximal femur scans in the array mode, using an Osteodyne hip positioner (Osteodyne, Research Triangle Park, NC, USA). We report the average of left and right proximal femur scans.

Hip structure analysis

Indices of bone strength in the proximal femur were analyzed using the Hip Structure Analysis software program [16]. As has been detailed elsewhere, the HSA program measures bone mineral density and cross-sectional geometry based on the distribution of bone mineral mass across the bone axis [18]. Here, we focus on the femoral neck at its narrowest point (NN) and in the intertrochanteric region (IT). These locations are relevant clinically as they are frequent sites of osteoporotic fractures [20]. At each location, we assessed bone bending strength using the section modulus (cubic centimeter), which measures resistance to bending in the plane of the section. Section modulus was calculated as the cross-sectional moment of inertia (bisquare centimeter), which measures the distribution of bone about a given axis, divided by the distance from the center of mass to the medial or lateral cortex, whichever is greater [18]. Given that mechanical loads in the lower limb are proportional to body mass and to bone length [21, 22], we calculated a bending strength index (SI, squares centimeter per kilogram) by dividing section modulus by subjects' weight and by height as a proxy for femur length (which was not directly measured in this study).

Hormonal data and age at menarche

Age at menarche was taken from interview questionnaires administered by the research coordinator. Twenty-four-hour urine specimens were obtained every 6 months during the first 4 years of the study, independent of menstrual cycle day, and analyzed for total estradiol using standard

radioimmunoassay techniques and calcium levels via spectrophotometry [23–25].

To characterize E2 levels, we calculated mean E2 measurements for all study visits occurring in years 1, 2, and 3 following menarche. Subjects were then divided into E2 tertiles for each year (lowest, middle, and highest) in order to test for differences in bone strength index among women with relatively higher or lower E2 levels. There are no significant differences in physical activity scores among E2 tertiles, as shown in Table 1.

Given that calcium and vitamin D consumption are also critical determinants of bone health, we calculated mean urinary calcium levels and reported vitamin D intake in years 1, 2, and 3 following menarche and compared these values to bone SI in the same manner.

Physical activity

As an index of adolescent exercise behavior, we used the integrated physical activity score (low activity, $n=23$; moderate activity, $n=26$; high activity, $n=29$) assigned to each subject by the study coordinator at age 17. The physical activity (PA) score integrates subjects' responses to a sports-exercise questionnaire [26] and their objective fitness assessments between ages 12 and 17. There are no significant differences in E2 among PA tertiles, as shown in Table 1.

Statistics and hypothesis testing

Size adjustment As mechanical loads in the lower limb are proportional to body mass and to bone length [21, 22], section modulus data were adjusted for body size by dividing section modulus by subjects' weight and by height as a proxy for femur length (which was not directly measured in this study) to obtain a bending SI (square centimeter per kilogram).

Hypothesis testing We used analyses of variance (ANOVA) in Statistica (StatSoft, Tulsa, OK, USA) to test for intergroup differences in E2, vitamin D, and calcium, adolescent physical activity, and age at menarche. To test for interactions between hormone/nutrient levels, physical activity, and bone strength, we used main effects ANOVA, with physical activity and E2 tertile as independent factors, to determine overall significance, and post hoc type 3 tests of fixed effects to examine intergroup differences in least squares means.

Results

To examine the relationships among estradiol, exercise, and femoral geometry, we compared geometric properties among subjects divided into tertiles of PA and of mean

Table 1 Variability in height, weight, and menarchal age across physical activity (PA) tertiles and across E2 tertiles in year1 postmenarche

| N | Physical activity tertiles (1 = least active, 3 = most active) | | | | | | E2 tertiles, year1 postmenarche (A = lowest, C = highest) | | | | | | |
|------------------------------------|--|----------|-------|--------|-------|----------|---|-----------|-------|-------------|-------|------------|-------|
| | PA 1 | ±1 SD | PA 2 | ±1 SD | PA 3 | ±1 SD | E2 A | ±1 SD | E2 B | ±1 SD | E2 C | ±1 SD | |
| Height (cm) | 84 | 164.13 a | 5.44 | 165.00 | 5.95 | 167.66 a | 5.95 | 164.21 | 5.12 | 167.11 | 6.10 | 165.86 | 6.34 |
| Weight (kg) | 84 | 56.69 a | 8.93 | 57.73 | 7.54 | 61.38 a | 7.08 | 58.14 | 8.25 | 60.14 | 8.26 | 58.00 | 6.88 |
| Days postmenarche (at E2 measures) | 84 | 195.83 | 58.62 | 182.67 | 59.61 | 188.00 | 61.59 | 168.38 a | 62.04 | 219.71 a, b | 57.17 | 179.54 b | 47.01 |
| Age at menarche (years) | 84 | 13.27 | 0.65 | 13.04 | 0.83 | 13.50 | 0.84 | 13.51 | 0.88 | 13.30 | 0.80 | 13.07 | 0.69 |
| E2 (µg/day) | | 10.91 | 5.33 | 9.80 | 6.25 | 9.51 | 3.95 | 4.99 a, b | 1.56 | 9.16 a, c | 1.27 | 15.79 b, c | 3.70 |
| PA score | | | | | | | 2.20 | | 0.76 | 2.04 | 0.86 | 2.00 | 0.87 |

Different letters indicate significant differences between pairs, $p < 0.05$, ANOVA, Fishers' PLSD

estradiol (E2) values in years 1, 2, and 3 following menarche. This approach raises the possibility that PA or E2 tertiles might differ in other variables that influence bone, so we first tested for differences in height, weight, E2 level, and age at menarche (Table 1). Comparing PA tertiles, the most active women tended to be taller and heavier than their counterparts ($p < 0.04$, Table 1). Therefore, all bone measurements were size-adjusted before comparison (see above). Across physical activity tertiles, there were no differences in age at menarche or in E2 levels in years 1, 2, or 3 postmenarche (Fig. 2 and Table 1). We also considered the effect of variation in the timing of estrogen measurements. As noted above, study visits occurred at 6-month intervals, independent of onset of menarche or menstrual cycle day, so differences in estradiol level among subjects may be due to nonrandom distribution of time of menarche or menstrual cycle day, rather than true variation in E2. Although menstrual cycle data are not available, for year 1, we calculated the number of days after menarche on which each subject's E2 measurements occurred (Table 1). The timing of E2 measurements vs. menarche did not differ among PA tertiles in year 1. However, E2 measurements for women in the middle E2 tertile occurred significantly later vs. menarche than for in the low and high tertiles ($p < 0.01$, Table 1).

Physical activity

We then compared skeletal SI in the femoral narrow neck and intertrochanteric region to PA tertiles. As predicted, adolescent physical activity was significantly positively associated with young adult femoral bending strength as measured by SI (Table 2). Young women who were most active during puberty had 10–11% greater SI in the femoral narrow neck ($p < 0.02$ for PA group 3 vs. groups 1–2) and intertrochanteric region ($p < 0.05$ for PA group 3 vs. groups 1–2) compared to less active young women. There was no difference in SI between young women with low vs. moderate activity levels.

E2, vitamin D, and calcium

We also compared SI values among E2 tertiles in years 1, 2, and 3 postmenarche, controlling for physical activity. In year 1 only, there was a significant relationship between E2 levels and young adult SI (Fig. 3 and Table 2). Compared to women with lower year 1 E2 levels (groups A–B), women with the highest E2 levels (group C) had 5–14% greater SI in the femoral narrow neck ($p < 0.05$ for group B vs. C, $p < 0.07$ for group A vs. C) and intertrochanteric region (overall $p < 0.07$; $p < 0.03$ for group B vs. C). However, in years 2 and 3, there were no associations of E2 level and bone strength index at age 17.

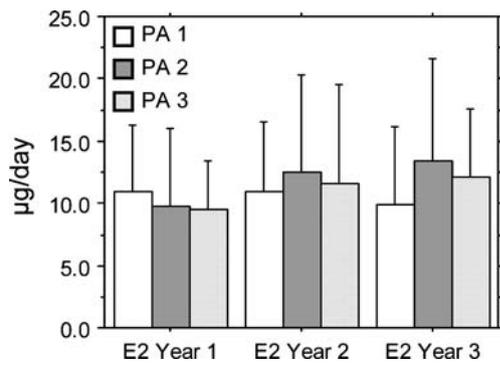


Fig. 2 Mean 24-h urinary estradiol (E2, µg/day, ± 1 SD) in year 1 (N=75), year 2 (N=71), and year 3 (N=57) postmenarche did not vary across physical activity (PA) tertiles

A further question is whether these differences in bone SI are maintained over time. Thus, we compared percent change in narrow neck SI from age 17 to 22 across E2 tertiles. Women with the lowest year 1 E2 levels (group A) gained less bone (0.93+5.99%) vs. those in groups B–C (3.66+5.62% and 3.89+6.35%). Although the differences were not statistically significant, these data suggest that women with low E2 levels just after menarche may accrue less bone in young adulthood than do women who had higher E2 levels around the time of menarche.

For vitamin D and calcium, controlling for physical activity level, there was no relationship between bone strength at age 17 and either reported vitamin D intake or urinary calcium levels in years 1, 2, or 3 following menarche (see also [27, 28]).

Table 2 Strength index varies with physical activity and with E2 in years 1–3 postmenarche

| | N | Strength index ^a (cm ² /kg) | | | |
|-------------------------|----|---|-------|--------------------------|-------|
| | | Narrow neck | | Intertrochanteric region | |
| | | Mean | ±1 SD | Mean | ±1 SD |
| Physical activity | 74 | | | | |
| PA 1 (least active) | 22 | 0.116 a | 0.015 | 0.322 a | 0.050 |
| PA 2 | 24 | 0.114 b | 0.016 | 0.321 b | 0.055 |
| PA 3 (most active) | 28 | 0.127 a, b | 0.020 | 0.355 a, b | 0.065 |
| E2, year 1 postmenarche | 74 | | | | |
| E2 A (lowest) | 25 | 0.117 ^b | 0.016 | 0.338 | 0.049 |
| E2 B | 24 | 0.115 a | 0.018 | 0.311 a | 0.049 |
| E2 C (highest) | 26 | 0.125 ^b a | 0.019 | 0.349 a | 0.069 |
| E2, year 2 postmenarche | 71 | | | | |
| E2 A (lowest) | 24 | 0.121 | 0.018 | 0.336 | 0.047 |
| E2 B | 24 | 0.119 | 0.020 | 0.347 | 0.149 |
| E2 C (highest) | 23 | 0.117 | 0.016 | 0.338 | 0.056 |
| E2, year 3 postmenarche | 57 | | | | |
| E2 A (lowest) | 19 | 0.118 | 0.014 | 0.336 | 0.046 |
| E2 B | 19 | 0.124 | 0.024 | 0.369 | 0.164 |
| E2 C (highest) | 19 | 0.114 | 0.017 | 0.317 | 0.055 |

Different letters indicate significant differences between pairs, $p < 0.05$, mixed model ANOVA

^a ((Section modulus/(ht × wt)) × 1,000)

^b $p < 0.07$

Interactions of E2 and physical activity

Given the above data demonstrating that both exercise and perimenarchal estrogen are both associated with bone strength, we investigated the possibility of synergistic effects of estrogen and exercise on bone strength index. We noted that in subjects with the highest year 1 E2 levels, the most active women (PA 3) had 12% greater SI vs. moderately active women (PA 2), while in subjects with the lowest E2 values, the most active women had only 3% greater SI vs. moderately active women (Fig. 4). This interaction is not statistically significant, reflecting our small sample size. When subjects were divided into tertiles of estradiol and physical activity, sample sizes decreased to five to seven individuals/group, and statistical power declined to 0.2–0.3 (ANOVA). A power test using our own data (narrow neck strength index, 0.121+0.017 for low E2/high active, 0.036+0.021 for high E2/high active) suggested that 12 individuals/group would provide 80% power for E2 × PA comparisons (α two-tailed=0.05, effect size 0.75). Therefore, larger sample sizes would be needed to determine whether E2 and exercise have additive or synergistic effects on bone.

Discussion

This study examines the relationships among estradiol levels in years 1, 2, and 3 postmenarche, adolescent physical activity, and bone strength in women studied from

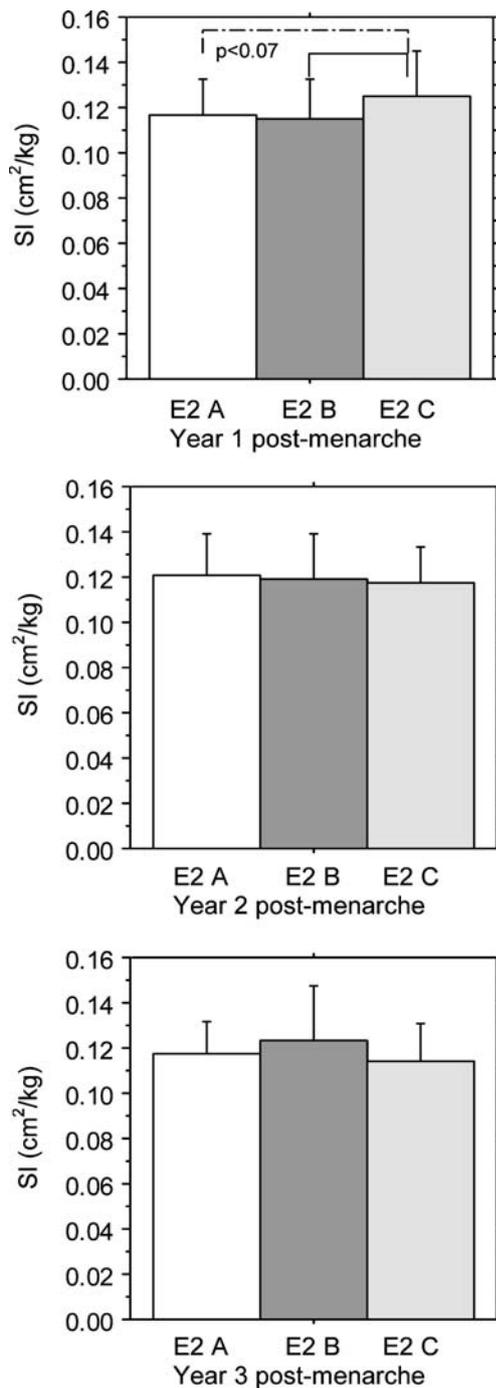


Fig. 3 Femoral narrow neck strength index (SI , cm^2/kg , ± 1 SD) vs. E2 tertile (1 = lowest, 3 = highest) in year1 ($N=75$), year2 ($N=71$), and year3 ($N=57$) postmenarche. Significant differences ($p < 0.05$) as indicated

age 12 years. The results support the hypothesis that both physical activity levels during menarche and adolescence and higher E2 in the first year postmenarche are associated with significantly stronger bones at skeletal maturity in young women. We found that subjects who reported high physical activity during menarche and adolescence had

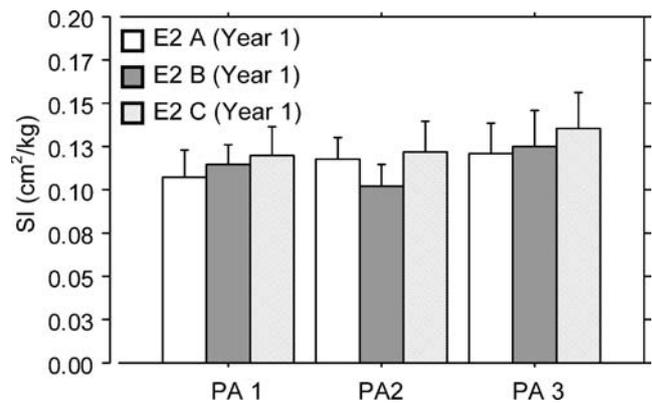


Fig. 4 Femoral narrow neck strength index (SI , cm^2/kg , ± 1 SD) across both PA and E2 (year 1) tertiles ($N=5-9/\text{group}$). Both E2 in the first postmenarchal year and PA are associated with higher strength index; these effects may be additive or synergistic

greater femoral bone strength indices than did women who reported being less physically active, in agreement with other studies showing that adolescent exercise is positively associated with adult BMD [5, 29, 30].

More provocatively, our data show that women who had the highest E2 levels *in the first year after menarche* had greater SI in the femoral narrow neck and the intertrochanteric region, compared to women with lower postmenarchal E2 levels. However, we found no such association between bone SI and E2 levels in year 2 or 3 after menarche, which was the year just before bone measurement occurred. Thus, the positive correlation between E2 and bone SI was limited to the first calendar year after menarche. To our knowledge, this is the first study to demonstrate differential associations of peri- vs. postmenarchal E2 levels with bone strength. Interestingly, only 20–35% of subjects remain in the same E2 tertile from years 1 to 2 or years 2 to 3, so the women with the highest E2 levels immediately following menarche tended not to maintain those high levels in subsequent years. This trend further supports an estrogen effect on bone strength index specifically around the time of menarche.

These results expand upon previous evidence that osteogenic responses to exercise are greatest around the time of puberty [2, 31, 32] in several important ways. First, our analysis demonstrates that in addition to exercise, estradiol variation in the first postmenarchal year (but not in subsequent years) also influences young adult bone cross-sectional geometry and strength. This finding is not surprising given the well-known role of estrogen in mediating longitudinal bone growth and acquisition of bone mineral density. Numerous studies have shown that early maturers and children with precocious puberty tend to have higher bone mineral density and peak bone mass than later maturers and children with hypogonadism [33–41]. Second, while many prior studies have demonstrated that exercise during skeletal acquisition improves bone strength

(reviewed in [42]), the longitudinal design of the Penn State Young Women's Health Study allows us to show that these benefits extend into young adulthood (see also [43–46]). Finally, our findings suggest a possible effect of E2 on osteogenic responses to loading in women studied from age 12 through adulthood, although this must be tested in a larger sample size. In keeping with our findings, a recent study in men [47] found a positive association between E2 levels and BMD at the radius and tibia, as well as evidence for positive interactions between E2 (but not testosterone) and physical activity on tibial bone size. Thus, the role of E2 during skeletal acquisition may extend beyond mediation of longitudinal growth and bone mineral density to include possible effects on periosteal bone apposition and cross-sectional geometry.

There are several limitations of this study. Our bone strength measurements were derived from DXA data, and the HSA algorithm measures bending only in the frontal plane of the proximal femur [16, 17]. Further, geometry is measured from projected dimensions of the femur in the DXA scan, which may affect precision [48]. These limitations should not affect the overall patterns on which our results are based [49], but may make it more difficult to detect significant differences in bone strength on among groups, particularly at smaller sample sizes.

In conclusion, physical activity and peripubertal E2 level are both important influences on the development of young adult cross-sectional geometry and therefore of bone strength. Exercise is a well-known modifiable determinant of bone strength, but estrogen bioavailability is not. If low peripubertal E2 levels reduce osteogenic responses to exercise, specific populations may be at risk for skeletal fragility, such as adolescents with amenorrhea, anorexia, or hypogonadism [50–52]. The results of this study also expand our understanding of human evolutionary biology. If estrogen alters osteogenic responses to loading in human bone, as it does in animals, it follows that the skeleton's ability to adapt to its mechanical environment is greatest when estrogen levels are high, around the time of puberty, and declines thereafter [15, 53]. It is worth noting that until recently, puberty occurred in late adolescence, and many individuals experienced mechanical loading through physical labor. Today, puberty occurs in early adolescence, accompanied by decreased physical activity and thus reduced mechanical loading [54], which may help to explain historic decreases in human bone strength [55]. Our results indicate the need for further study of how interactions between perimenarchal E2 and exercise affect maximum bone strength at the end of adolescence.

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Conflicts of interest The Hip Structure Analysis Program developed by Dr. Beck has been licensed to Hologic Corporation by the Johns Hopkins Medical Institutions. All other authors have no conflicts of interest.

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