

The Influence of Estrogen Exposure on the Relationship Between Mechanical Loading and
Trabecular Bone Mineral Density in Young Female Athletes

by

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Abstract

Mechanical loading through sport, particularly high-impact activity, and estrogen exposure both promote bone mineral density (BMD). However, excessive training combined with insufficient energy availability can disrupt endocrine function, leading to amenorrhea, low bone mass, and increased fracture risk. Estrogen and mechanical loading influence bone through distinct but complementary biological pathways. Hormonal contraceptives containing exogenous estrogen are sometimes considered protective for bone health in athletes experiencing menstrual dysfunction, though evidence of their effects on bone adaptation to mechanical loading remains mixed. Exogenous estrogen may alter bones' adaptive response to mechanical strain, yet few studies have examined how estrogen-containing contraceptive use influences trabecular bone adaptation across different loading regimes. Increased awareness of this relationship is important for understanding how hormonal contraceptive use may influence skeletal health in physically active women, particularly those experiencing menstrual dysfunction. This study assesses whether the benefit of mechanical loading to trabecular BMD among young females is mediated by estrogen supplementation in those with and without a history of amenorrhea. Athletes participating in high-impact (soccer and running) and low-impact (rowing) sports were compared with recreationally active controls. Secondary data from peripheral quantitative computed tomography (pQCT) scans were used to assess trabecular BMD at the distal tibia. Significant differences in trabecular BMD between athletes and controls were observed only among women with a history of estrogen-containing contraceptive use, with the effect primarily driven by lower BMD in controls. These findings have important implications for biological anthropology and for advancing understanding of bone health in physically active women.

Keywords

Trabecular bone, Tibia, Bone Mineral Density, Estrogen-Containing Contraceptives, Hormonal Contraceptives, Amenorrhea, Relative Energy Deficiency in Sport (RED-S), High-Impact Loading, and Low-Impact Loading.

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Introduction

Women face a disproportionate risk of low bone mass and osteoporotic fractures later in life (Lorentzon et al., 2022). As women transition toward menopause, declining estrogen levels disrupt the hormone's regulatory role in bone remodeling and resorption (Charde et al., 2023). Peak bone mass accrued in early life strongly predicts lifelong skeletal health, making it critical to understand the factors that enhance bone mineral accrual (Hereford et al., 2024; Weaver et al., 2016). Mechanical loading, particularly in weight-bearing bones such as the tibia, has a significant influence on BMD (Murray & Erlandson, 2022), with long-term participation in high-impact sports promoting lasting skeletal benefits (Callewaert et al., 2010). However, extensive training and insufficient recovery time in young female athletes can disrupt energy levels and alter endocrine function, leading to Relative Energy Deficiency in Sport (RED-S) (Gallant et al., 2025). Prolonged periods of low energy availability (LEA) can lead to menstrual dysfunction, estrogen deficiency, and impaired health, increasing the risk of stress fractures (Chen et al., 2013). For female athletes, it is essential to optimize the benefits of sport-specific loading to maximize bone mass while preventing the detrimental bone outcomes associated with RED-S.

Mechanical loading and estrogen influence bone through complementary but spatially distinct mechanisms, suggesting that hormonal status can mediate how mechanical loading enhances bone density and structure. Exogenous estrogen from hormonal contraceptives may influence this balance. Evidence regarding the effects of estrogen-containing contraceptives on bone remains inconsistent, particularly in populations exposed to high training demands (Cobb et al., 2007; Weaver et al., 2001). Few studies have examined how hormonal contraceptive use modifies the relationship between mechanical loading and BMD, leaving a gap in our understanding of functional bone adaptation in young female athletes. This study addresses this

gap by examining whether estrogen-based hormonal contraceptive use alters the relationship between mechanical loading and BMD. This research has the potential to contribute to biological anthropology's understanding of trabecular BMD adaptation by examining how it is influenced by estrogen-containing contraceptive use and menstrual dysfunction in young female athletes. In doing so, this research challenges simplified models of bone adaptation and highlights the importance of incorporating hormonal and life history factors. The findings may also help address whether estrogen-containing contraceptives mitigate bone health risks associated with decreased energy availability/RED-S.

This thesis answers the following research questions:

1. How does estrogen-containing contraceptive use modify the relationship between mechanical loading and trabecular bone mineral density in young adult female athletes?
2. In the context of hormonal contraceptive use, how does trabecular bone mineral density differ between athletes with distinct loading regimes (e.g. high-impact versus low-impact)?

Background

Bone Functional Adaptation and the Form-Function Framework

Bone is a dynamic connective tissue that continually adapts its structure in response to habitual mechanical loading, a process described within the framework of bone functional adaptation. Originally articulated as Wolff's Law, this principle holds that bone architecture reflects the mechanical demands placed upon it (Wolff, 1986). Contemporary interpretations emphasize that skeletal adaptation depends not only on load magnitude but also on strain rate, frequency, and directionality, with dynamic and high-impact activities producing the strongest osteogenic stimulus (Ruff et al., 2006; Järvinen et al., 2003). Mechanical adaptation occurs

through the coupled processes of modeling and remodeling. Bone modelling involves the addition of new bone that alters size or shape, particularly through periosteal apposition (bone deposition on the outer surface), whereas remodeling functions to repair micro-damage and maintain the existing structure (Lieberman et al., 2003). During adolescence, modelling predominates and enables long bones to redistribute mass away from neutral axes, increasing resistance to bending and torsion with minimal increases in weight (Kontulainen et al., 2007). In adulthood, remodeling dominates, and skeletal responses to loading are more conservative (Ruff et al., 2006). Bone functional adaptation is situated within the broader form-function framework, which emphasizes a strong relationship between biological structure (form) and its function (Murray, 2022). Understanding bone adaptation through this framework requires interpreting bone morphology as the result of interactions between mechanical loading and broader physiological, hormonal, environmental, and behavioral influences.

Hormonal Regulation of Bone

Hormones play an essential role in regulating skeletal development through the coordination of the timing, rate, and location of bone growth and remodeling. Estrogen regulates bone metabolism in both men and women by promoting epiphyseal closure during puberty, suppressing bone resorption, maintaining trabecular and cortical bone, and reducing bone turnover (Callewaert et al., 2010; Järvinen et al., 2003). Estrogen does not act as the primary driver of bone strength, but as a biological modulator that suppresses excessive bone resorption and regulates skeletal sensitivity to mechanical loading (Järvinen et al., 2003). Estrogen also exerts site-specific effects, preferentially inhibiting bone resorption on endosteal (inner) surfaces and particularly in trabecular bone. During puberty, growth hormone and insulin-like growth factor-1 stimulate longitudinal skeletal growth, while estrogen ensures that bone formation and

resorption remain balanced, allowing mechanically induced gains to be retained (Callewaert et al., 2010). Adolescence therefore represents a critical window for bone mineral accrual, during which endogenous estradiol (estrogen) plays a central role in shaping peak bone mass (Ziglar & Hunter, 2012). Estrogen levels tend to fluctuate across one's lifespan, increasing at the start of puberty, remaining high for roughly the reproductive years (14-39), and then decreasing with the start of menopause (Patel et al., 2025).

Hormonal Contraceptive Use and Bone Health

Hormonal regulation becomes especially relevant when considering the skeletal effects of hormonal contraceptive use. Most oral contraceptives contain a combination of ethinyl estradiol (estrogen) and progestin, although progestin-only formulations are also used. Contraceptives containing both hormones are referred to as combined oral contraceptives and exert systemic effects, compared to intrauterine devices, such as the Mirena, which deliver progestin primarily in the uterus. Combined oral contraceptives suppress endogenous estradiol production via inhibition of the hypothalamic–pituitary–ovarian axis, replacing physiological hormonal fluctuations with exogenous ethinyl estradiol (Ziglar & Hunter, 2012). Skeletal responses to hormonal contraceptives vary by formulation, estrogen dose, and timing of initiation (Cobb et al., 2007; Hartard et al., 2007; Scholes et al., 2010). In adult premenopausal women, combined oral contraceptives generally exhibit neutral effects on bone mineral density (Kuohung et al., 2000; Reed et al., 2003). In contrast, initiation of low-dose formulations during adolescence or shortly after menarche may impair peak bone mass accrual, particularly at trabecular-rich skeletal sites (Scholes et al., 2010; Ziglar & Hunter, 2012). In hypoestrogenic athletes, combined oral contraceptive use may stabilize or modestly increase BMD at the hip and lumbar spine, whereas eumenorrheic athletes typically experience little benefit and, in some cases, small

negative effects (Cobb et al., 2007). Evidence regarding the skeletal effects of oral contraceptives is notably inconsistent. Cross-sectional studies report lower spine, hip, and whole-body bone mineral density (BMD) among long-term low-dose users (Scholes et al., 2010), whereas longitudinal studies report no significant differences in BMD change between users and nonusers (Reed et al., 2003). Methodological differences contribute to this variability, as many studies rely on dual-energy X-ray absorptiometry (DXA) derived areal BMD, which is influenced by bone size and cannot distinguish cortical from trabecular compartments. Peripheral quantitative computed tomography (pQCT), by contrast, provides volumetric measures of BMD and enables separate assessment of trabecular and cortical bone, as well as bone geometry and strength indices. Beyond mineral density, contraceptive use may also influence bone geometry. Hartard et al. (2007) reported smaller bone cross-sectional areas, reduced periosteal circumference, and lower strength indices among current oral contraceptive users compared with former and never users, with effects increasing with duration of use. These findings suggest that hormonal contraceptives may constrain periosteal apposition and limit structural adaptation to mechanical loading. Given the rapid remodeling rate and hormonal sensitivity of trabecular bone, this compartment may be particularly vulnerable to endocrine modulation.

Mechanical Loading in Sport

Bone adaptation to mechanical loading is highly site-specific and depends on the magnitude, rate, frequency, and direction of the applied force. Weight-bearing bones such as the tibia and femur are particularly responsive to mechanical stimulation, as they play a direct role in transmitting ground reaction forces during locomotion. Consequently, these bones are expected to exhibit pronounced functional adaptation in response to substantial compressive, bending, and torsional loads. Research done on athletes demonstrates that high-impact and multidirectional

loading produce the most pronounced skeletal adaptations. Racquet sport athletes who began training at a young age exhibited greater bone mineral content and density in the playing arm compared to controls, whereas those who initiated training later showed benefits primarily in trabecular BMD (Kontulainen et al., 2002). This highlights the importance of early-life loading and the continued responsiveness of trabecular bone into adulthood.

Similarly, studies of Finnish female athletes across a range of sports, including soccer, squash, endurance running, and powerlifting, demonstrated that activities characterized by high ground impacts and multidirectional loading are associated with increased bone strength, though not necessarily changes in bone shape (Niinimäki et al., 2017). Further work done by Niinimäki and colleagues (2019) showed that these adaptations are not solely mediated by increases in muscle mass and strength but also reflect direct effects of mechanical loading. The combined influence of ground reaction forces and loading rates appears to result in increases in bone geometric properties (Niinimäki et al., 2019). Bone mineralization also shows responsiveness to loading. A study of 204 female athletes found that those who participated long-term in high- and odd-impact sports exhibited 15-26% greater bone mineral content at the tibia compared to controls (Nikander et al., 2010). These findings reinforce the principle that bones subjected to frequent and high-impact loads undergo significant functional adaptations.

Trabecular Bone Adaptation to Mechanical Loading and Hormonal Regulation

Trabecular bone develops early in life as a primary, relatively disorganized structure that is progressively remodeled into a more efficient secondary architecture through growth and mechanical loading. Childhood loading plays a critical role in organizing trabecular networks optimized for load transfer, characterized by changes in trabecular orientation, volumetric bone mineral density (vBMD), bone volume fraction (BV/TV), trabecular thickness, number, and

connectivity (Murray & Erlandson, 2022). Due to its porosity and metabolic activity, trabecular bone remodels more rapidly than cortical bone and remains highly responsive to changes in mechanical loading across the lifespan (Kivell, 2016). Mechanical stimuli alter trabecular architecture by modifying micro-structural properties that reflect load distribution and mechanical competence rather than size alone.

Evidence suggests that trabecular bone retains a capacity for meaningful adaptation beyond childhood, particularly in response to novel loading exposures. In young adult females, distal tibial trabecular vBMD is associated with post-menarcheal impact loading regardless of pre-menarcheal loading history, indicating continued responsiveness in adulthood (Murray & Erlandson, 2022). Similarly, female racquet sport athletes who initiated training in adulthood exhibit modest cortical benefits but substantially greater advantages in trabecular vBMD compared to non-athletes (Kontulainen et al., 2002). Sport-specific loading further illustrates this sensitivity; athletes exposed to high-impact and odd-impact activities demonstrate significantly greater trabecular bone mineral content at the distal tibia compared to controls (Nikander et al., 2010). These findings suggest that loading type and directionality may differentially influence trabecular adaptation, particularly at joint-adjacent sites subjected to multidirectional forces.

The regulatory influence of estrogen is particularly pronounced in trabecular bone due to its high surface area and rapid remodeling rate. Declines in estrogen are associated with increased osteoclast activity and accelerated bone resorption, leading to disproportionate bone loss at trabecular-rich sites such as the lumbar spine. This sensitivity is evident in female athletes experiencing menstrual disturbances, where hypoestrogenism is strongly associated with reduced BMD at the lumbar spine, a site rich in trabecular bone, measured using DXA (Drinkwater et al., 1990; Bennell et al., 1997). Amenorrheic athletes consistently exhibit lower lumbar spine BMD

than eumenorrheic peers, with the duration of amenorrhea closely linked to the magnitude of bone loss (Drinkwater et al., 1990). These findings underscore the reliance of trabecular bone integrity on hormonal regulation and its vulnerability to endocrine disruption.

Relative Energy Deficiency in Sport (RED-S) and Menstrual Dysfunction in Athletes

Relative Energy Deficiency in Sport (RED-S) is a syndrome that results from LEA, where insufficient dietary energy remains to support physiological functioning after accounting for the energetic cost of exercise (Logue et al., 2018). LEA can result from several causes, such as high training demands, intentional dietary restrictions, disordered eating, and can even occur in athletes who maintain healthy body mass due to physiological adaptations (Logue et al., 2018). One of the primary consequences of LEA is the disruption of the endocrine system, leading to reduced estrogen production and subsequent menstrual dysfunction. Amenorrhea, defined as the absence of menstruation for three months or longer, is a common clinical manifestation in high-level female athletes and can be classified as primary amenorrhea (failure to initiate menses) or secondary (cessation of menstruation after onset) (Mountjoy et al., 2014). In female athletes, secondary amenorrhea is the most prevalent form, typically resulting from energy deficiency or altered hormonal signaling, and is strongly associated with impaired bone health (Mountjoy et al., 2014). Energy deficiency has a suppressive effect on bone formation, and estrogen deficiency leads to increased regulation of bone resorption (Logue et al., 2018). Together, these processes lead to diminished BMD (Logue et al., 2018; Mountjoy et al., 2014).

Although the mechanical loading from athletic activity is generally beneficial for skeletal adaptation, its protective effects may be negated in the presence of LEA and menstrual dysfunction. Evidence suggests that factors such as irregular menstruation, prolonged participation in endurance sports, and dietary restriction are associated with lower BMD in

female athletes despite high levels of physical activity (Logue et al., 2018). While short-term amenorrhea may allow for recovery of bone density, prolonged menstrual disturbances can result in irreversible deficits (Powell, 2011). In addition to bone health and menstrual dysfunction, disrupted energy levels affect metabolic rate, immunity, susceptibility to injury, protein synthesis, cardiovascular, gastrointestinal, and psychological health (Gallant et al., 2025; Mountjoy et al., 2014). These findings underscore the critical role that energy availability plays in maintaining optimal bone health.

Study Contribution

Despite extensive research on hormonal contraceptive use and bone health, relatively little is known about how estrogen-containing contraceptives influence the relationship between mechanical loading and trabecular bone adaptation in young female athletes. This study investigates how estrogen-containing contraceptive use influences the relationship between trabecular BMD and mechanical loading by comparing tibial vBMD between athletes and non-athlete controls, as well as between high-impact and low-impact athletes.

Dataset

Participants

This study is a secondary analysis of cross-sectional data previously collected by Alison Murray and colleagues (Macintosh et al., 2017, 2018; Macintosh & Stock, 2019), from a sample of competitive athletes in order to provide a modern comparative sample for bio-archeological analysis of past populations. Individuals were originally recruited from the University of Cambridge between 2016 and 2017 from the Cambridge University Women's Boat Club, Women's Association Football Club, Athletics Club, Hare and Hounds, and Triathlon Club, Cambridge and Coleridge Athletics Club, the Cambridge Triathlon Club, the Beyond the

Ultimate Jungle Ultra 2016, the Everest Trail Race 2016, several University of Cambridge colleges, and the Graduate Union. All the participants provided written informed consent prior to their participation. The sample analyzed for the current study consisted of 102 premenopausal women. Participants were categorized into groups based on their primary athletic activity: rowers (n = 41), soccer players (n = 11), long-distance runners (n = 13), and recreationally active controls (n = 37).

Ethical approval for the original data was provided by the Cambridge University Human Biology Research Ethics Board, and ethical approval for the use of pQCT was obtained from the National Health Service (NHS) Health Research Authority National Research Ethics Service (NRES) Committee of England—Cambridge East (15/EE/0017). Approval for the use of anonymized data in the current study was provided by the University of Victoria's Human Research Ethics Board (25-0602).

Bone Imaging (pQCT)

Peripheral quantitative computed tomography (XCT-3000) scans of the tibia were used to obtain measures of bone density, area, and mass. The tibia was selected as it is a weight-bearing bone that is highly responsive to mechanical loading. pQCT captures multiple X-rays around the limb to create a cross-sectional image of its interior, allowing for differentiation between trabecular and cortical bone, with denser tissues appearing whiter. pQCT provides size-independent volumetric measurements, allowing for more precise assessment of how trabecular bone adapts to mechanical loading (Stagi et al., 2016). As discussed previously, trabecular bone is more metabolically active and responsive to mechanical loading, which makes it an ideal site for examining loading-related questions. The primary site of analysis was the distal tibia (4% slice), where trabecular vBMD (mg/cm^3) was examined. The 4% slice was used because it

contains a high proportion of trabecular bone and distal tibial trabecular vBMD is associated with post- menarcheal impact loading (Murray & Erlandson, 2022).

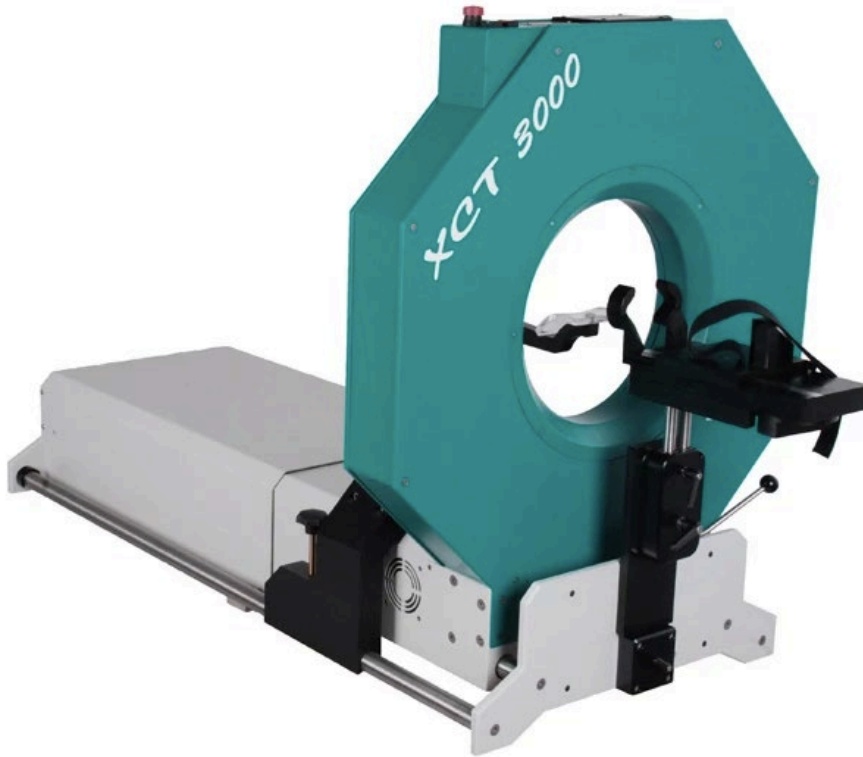


Figure 1. *Peripheral Quantitative Computed Tomography (pQCT) Scanner.* Source: <https://stratec-pqct.com/en/products/xct-3000-2/>

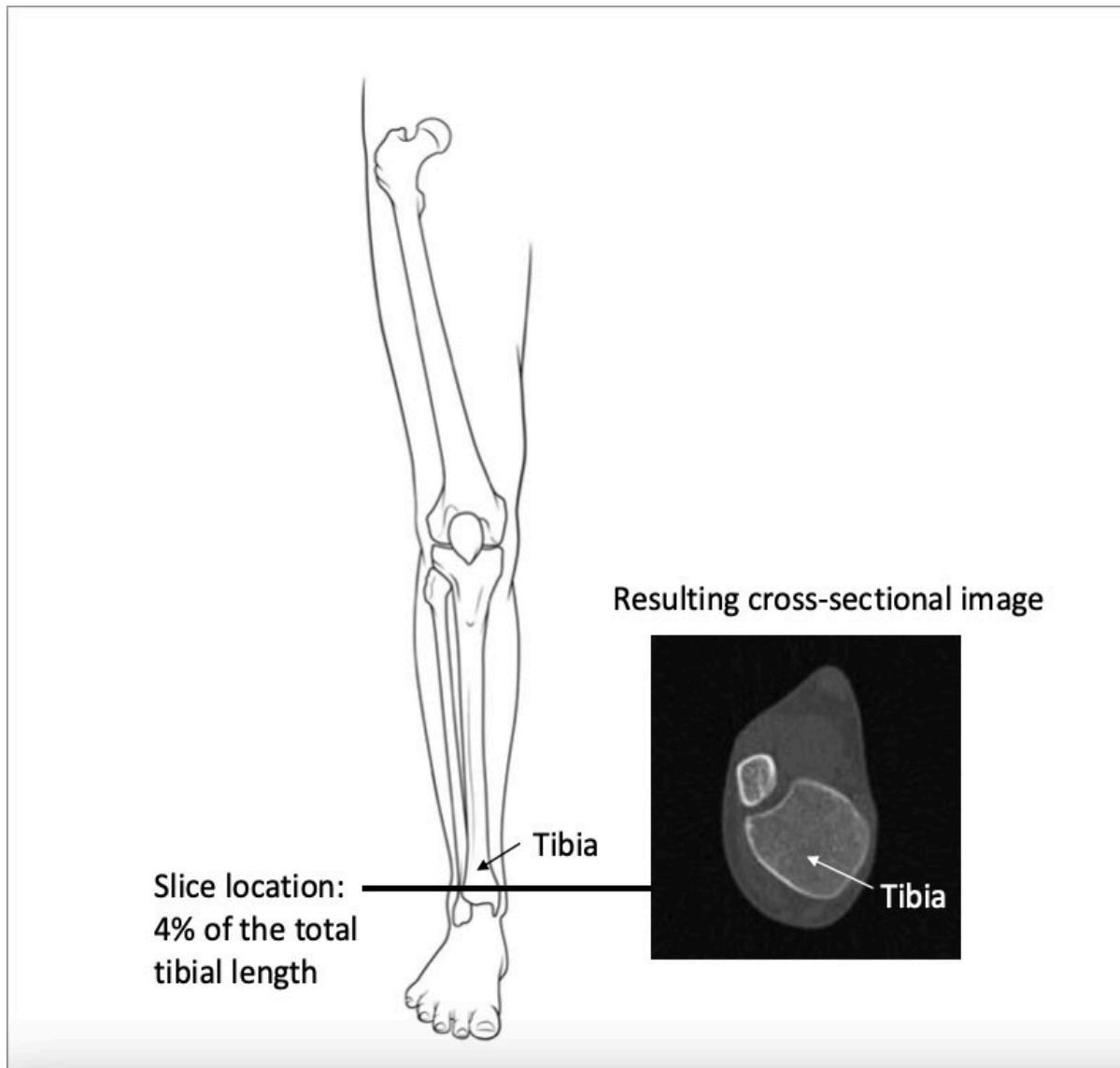


Figure 2. *Distal Tibia Scan at the 4% Slice Location*

Participant Characteristics and Group Classification

In addition to the pQCT scans, participants also completed questionnaires during the original data collection. Survey data included women's menstrual history, sporting history, injury history, and hormonal contraceptive use, with relevant variables presented in Table 1. Self-reported history of amenorrhea was also collected and, when it was not attributable to hormonal contraceptive use, was used as an indicator of disrupted energy availability and hormonal imbalance (Logue et al., 2018). Hormonal contraceptive use was classified into two groups:

Estrogen-containing contraceptive users, including current or past users of combined estrogen-progestin contraceptives, and non-estrogen users, including progestin-only contraceptives and participants who reported never using hormonal contraceptives. Women who reported any current or past estrogen-containing contraceptive use were coded in the first group to reflect their history of exogenous estrogen exposure. Estrogen-containing contraceptives were the focus of this research due to the regulatory role estrogen plays in bone metabolism, as discussed earlier.

Participants were categorized into groups based on their primary athletic activity and the associated loading regime. Athletes who participated in rowing were classified as the low-impact group, as rowing involves repetitive loading with minimal ground-reaction forces. Athletes who participated in soccer and long-distance running were classified as high-impact groups because of the substantial ground-reaction forces and impact loading associated with these activities. Group allocation was determined based on the participant's self-reported primary sport and training history. Women were excluded if they had medical conditions or used medications known to affect bone health, were currently or recently pregnant, younger than 18, or post-menopausal. Controls (recreationally active participants) had no history of competitive sport and engaged in less than 3 hours a week of weight-bearing intensive activity. Competitive athletes were required to have trained and competed intensively for at least the past three years.

Methods

Data Coding

Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS). Prior to analysis, qualitative survey variables were coded into quantitative values. For example, responses to binary questions (e.g., whether participants were recreationally active

prior to menarche) were coded numerically (1 = yes, 2 = no) (Antonius, 2013). This coding procedure was applied to all categorical variables. For variables reported as ranges, a single value was found by calculating the midpoint. For instance, a reported weekly training volume of 180-200km was coded as 190km. Once the data were entered into SPSS, descriptive statistics were generated to give an overview of participant characteristics. Normality was assessed by dividing the skewness statistic by its standard error (SE). If the result exceeded ± 1.96 , it was treated as skewed, whereas values within ± 1.96 were treated as approximately normally distributed.

Statistical Analyses

To test differences between athletes and controls in categorical variables (incidence of stress fracture, history of amenorrhea, and contraceptive use), Pearson's chi-square tests were used. Spearman's rank-order (non-parametric) correlations were conducted to examine relationships between trabecular vBMD with training history, menstrual history, contraceptive use, body size, and life history variables. Independent samples t-tests were used to compare the trabecular vBMD between athletes and controls within subgroups defined by their history of contraceptive use, regardless of menstrual status. Analysis of variance (ANOVA) was conducted to assess mean differences in trabecular vBMD across loading groups, looking at participants with a history of estrogen-containing contraceptive use. ANOVA was used again to test mean differences in trabecular vBMD across loading groups, this time looking at participants with a history of amenorrhea. Within each impact group, independent samples t-tests were used to compare mean vBMD between participants with and without a history of amenorrhea. A univariate general linear model was used to examine differences in trabecular vBMD between athletes and controls, with group, estrogen-containing contraceptive use, and history of

amenorrhea specified as fixed factors, and body mass and age as covariates. Estimated marginal means were subsequently examined. A second general linear model was conducted for impact loading type (high or low) with age, body mass, age at onset of training, years of cumulative training, and training volume (km/week) included as covariates.

Results

Table 1 presents the descriptive statistics for participant characteristics, including variables such as vBMD, menstrual history, and training history. Overall, 69% of athletes and 76% of controls reported ever using contraceptives, with 56% of athletes and 53% of controls reporting current use. 65% of athletes and 59% of controls reported using estrogen-containing contraceptives. There was no significant difference between athletes and controls in the frequencies of 'Ever' or 'Current' contraceptive use or estrogen-containing contraceptive use more specifically. Twenty-seven percent of the athletes had a history of amenorrhea compared to eleven percent of the controls, while this was not statistically significant ($p = 0.065$), it should not be understood as the absence of evidence, but as an observed difference that did not meet the conventional threshold for statistical significance and therefore should be interpreted cautiously (Andrade, 2019). Looking at injury history, 23% of athletes and 3% of controls reported a history of stress fracture. Athletes had a significantly higher incidence of stress fractures ($p = 0.007$) than controls. This result is driven specifically by the high incidence of rib stress fractures in the low-impact athletes (rowers), with ~30% of rowers having a history of rib stress fracture.

Table 1

Descriptive Statistics for Participant Characteristics and Bone Mineral Density by Loading Group

Variables	Competitive Athletes			Controls N = 37
	All Pooled N = 65	High-Impact Athletes N = 24	Low-Impact Athletes N = 41	
Age (years)	23 (3.54) [19-33]	25 (4.15) [19-33]	21.5 (2.31) [19-28]	23 (3.51) [19-32]
Body Mass (kg)	65.61 (9.60) [47.80-90.00]	59.01 (6.72) [47.80-74.30]	69.47 (8.95) [51.80-90.00]	61.26 (10.88) [40.00-92.10]
Total Bone Mineral Density (mg/cm ³) *	311.96 (34.07) [227.42-397.57]	317.11 (36.12) [253.23-384.06]	308.94 (32.89) [227.42-397.57]	281.27 (31.49) [203.50-341.26]
Trabecular Bone Mineral Density (mg/cm ³) *	278.80 (29.38) [209.64-363.56]	283.38 (30.76) [228.33-353.36]	276.12 (28.58) [209.64-363.56]	250.67 (26.95) [182.73-303.79]
Average Distance Rowed or Run per Week (km)	102.29 (34.37) [15.00-190.00]	64.81 (24.29) [15.00-100.00]	114.17 (28.05) [55.00-190.00]	
Age at Menarche (years)	13 (1.38) [10.00-16.00]	13 (1.60) [10.00-16.00]	13 (1.25) [11.00-16.00]	13 (1.78) [10.00-17.00]
Age at Start of Active Training (years)	16 (4.47) [5.00-26.00]	13.5 (5.75) [5.00-26.00]	17 (2.93) [10.00-24.00]	
Years of Active Training	6.53 (4.80) [1.00-18.00]	10.27 (5.03) [1.50-18.00]	4.34 (3.30) [1.00-13.00]	
Years Post- Menarche at Sport Initiation	2.58 (4.82) [-7.00-13.50]	.33 (6.11) [-7.00-13.50]	3.90 (3.30) [-4.00-12.00]	
Age at First Contraceptive Use (years)	18 (2.38) [13-24]	18 (2.47) [15-24]	18 (2.34) [13-24]	17.5 (3.07) [10-24]
Menarche Status at Training Initiation				
Pre/Peri- Menarche	21 (32.3%)	15 (62.5%)	6 (14.6%)	
Post- Menarche	44 (67.7%)	9 (37.5%)	35 (85.4%)	

Stress Fracture				
Yes	15 (23.1%)	3 (12.5%)	12 (29.3%)	1 (2.7%)
No	50 (76.9%)	21 (87.5%)	29 (70.7%)	36 (96.3%)
Estrogen-Based Contraceptive Use				
Yes	39 (65.0%)	20 (83.3%)	19 (52.8%)	19 (59.4%)
No	21 (35.0%)	4 (16.7%)	17 (47.2%)	13 (40.6%)
Contraceptive Use				
Yes	45 (69.2%)	21 (87.5%)	24 (58.5%)	28 (75.7%)
No	20 (30.8%)	3 (12.5%)	17 (41.5%)	9 (24.3%)
Current Contraceptive Use				
Yes	27 (56.3%)	13 (59.1%)	14 (53.8%)	19 (52.8%)
No	21 (43.8%)	9 (40.9%)	12 (46.2%)	17 (47.2%)
History of Amenorrhea				
Yes	17 (26.2%)	6 (25.0%)	11 (26.8%)	4 (10.8%)
No	48 (73.8%)	18 (75.0%)	30 (73.2%)	33 (89.2%)

Data presented as mean (*SD*) [range] for continuous variables and as n (%) for categorical variables.

*Bone mineral density measured at the distal tibia (4% slice)

Group Differences without Covariate Adjustment

Among all females pooled, trabecular vBMD was significantly negatively correlated with Sport Group (Athlete or Control; $\rho = -0.412$, $p < 0.001$) and with Impact Group (High-Impact, Low-Impact, or Control; $\rho = -0.430$, $p < 0.001$), indicating that participation in sports and in high-impact loading in particular was associated with higher trabecular vBMD. Among the entire sample pooled, not controlling for physical activity, trabecular vBMD had a tendency to be lower among older (age; $\rho = -0.180$, $p = 0.07$) or lighter females (body mass; $\rho = 0.19$, $p = 0.056$), and those with a history of estrogen-containing contraceptive use ($\rho = 0.191$, $p = 0.056$), but not significantly so. Among athletes specifically, trabecular vBMD had a significant negative correlation with age at initiation of training ($\rho = -0.275$, $p = 0.027$). This indicates that individuals who began training at a later age tended to have lower trabecular BMD. History of

amenorrhea was significantly correlated with a history of contraceptive use ($\rho = -0.388$, $p < 0.001$) and estrogen-containing contraceptive use ($\rho = -0.485$, $p < 0.001$). This result indicates that individuals reporting contraceptive use, estrogen-containing or not, were less likely to report a history of amenorrhea unrelated to contraceptive use.

Among women with a history of estrogen-containing contraceptive use, regardless of menstrual status, trabecular vBMD was significantly higher among athletes than controls ($p < 0.001$; Figure 3). This pattern appeared to be driven primarily by differences within the control group, as controls with a history of estrogen-containing contraceptive use had significantly lower trabecular vBMD compared to controls without a history of use ($p = 0.02$). There was no significant difference in the trabecular vBMD between athletes and controls who never used estrogen-containing contraceptives.

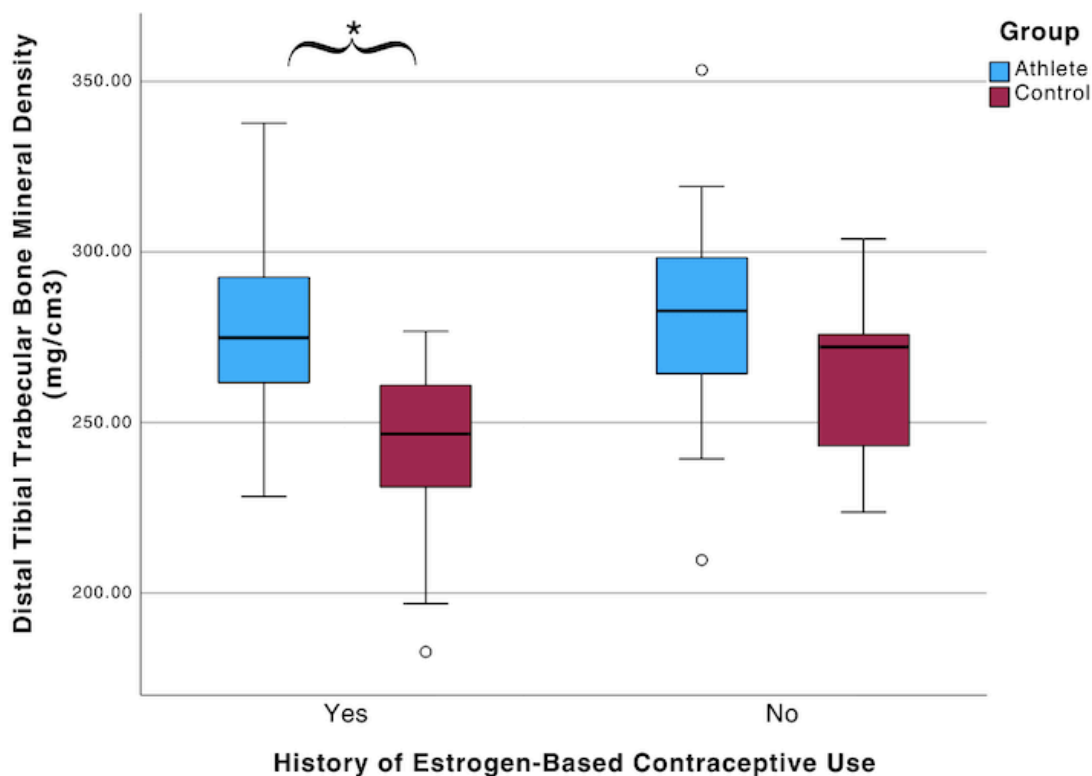


Figure 3. *Trabecular Bone Mineral Density in Athletes and Controls by Estrogen-Containing Contraceptive Use.* * $p < 0.001$.

Analysis of impact loading did not alter these trends: controls had significantly lower trabecular vBMD than both high- and low-impact loading groups ($p < 0.001$), but there was no significant difference between high- and low-impact loading groups (Figure 4). There was no significant difference between the three groups when looking at the women who never used estrogen-containing contraceptives.

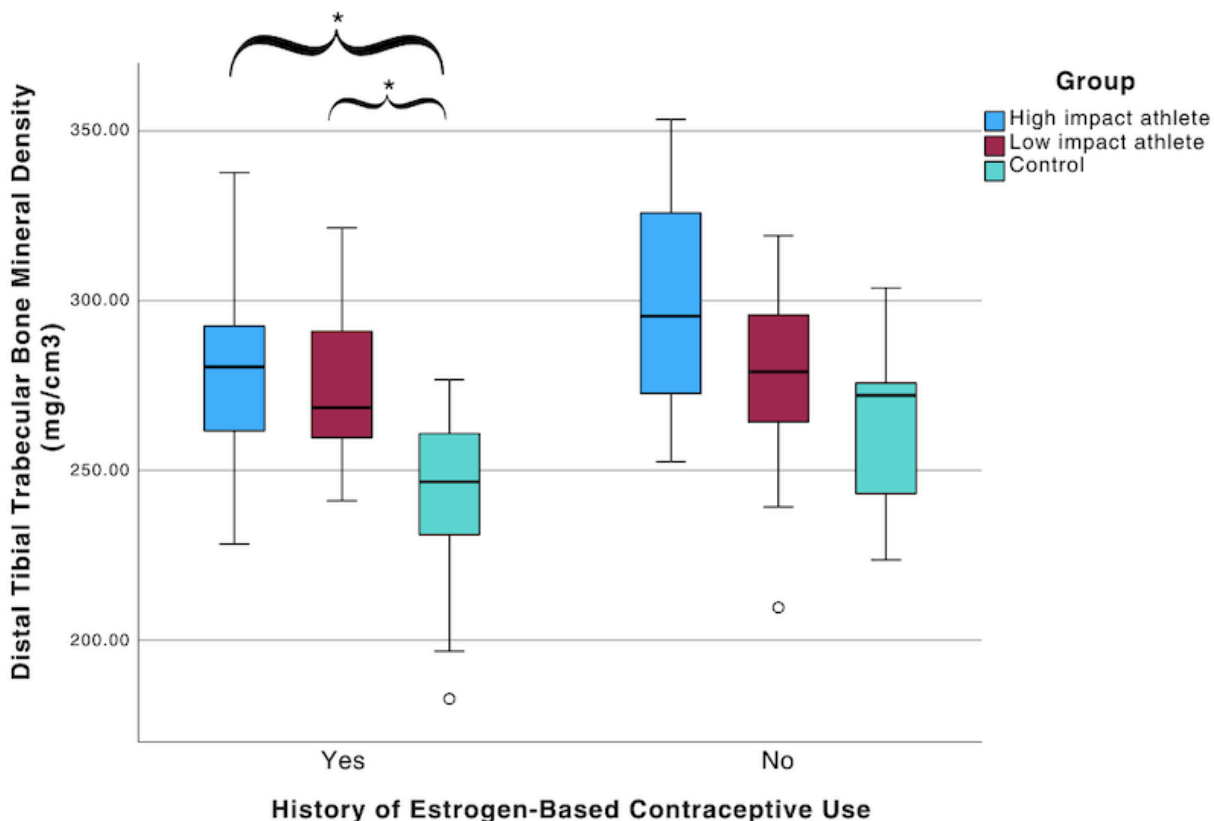


Figure 4. *Trabecular Bone Mineral Density in High-Impact Athletes, Low-Impact Athletes, and Controls by Estrogen-Containing Contraceptive Use. *p < 0.001*

Among women with a history of amenorrhea, regardless of contraceptive use, there was no significant difference in trabecular vBMD across loading groups. However, among women with no history of amenorrhea there was a significant difference in trabecular vBMD: controls without a history of amenorrhea had significantly lower trabecular vBMD compared to both high-impact and low-impact athletes without a history of amenorrhea ($p < 0.001$), while the two athlete groups did not differ from each other (Figure 5). Finally, within each loading group, trabecular vBMD did not differ significantly by history of amenorrhea. In the high-impact athletes, mean trabecular vBMD was lower in those with a history of amenorrhea

(263.15mg/cm³) compared to those without (290.12mg/cm³), although the difference was not statistically significant. No meaningful differences were observed in the low-impact or control groups.

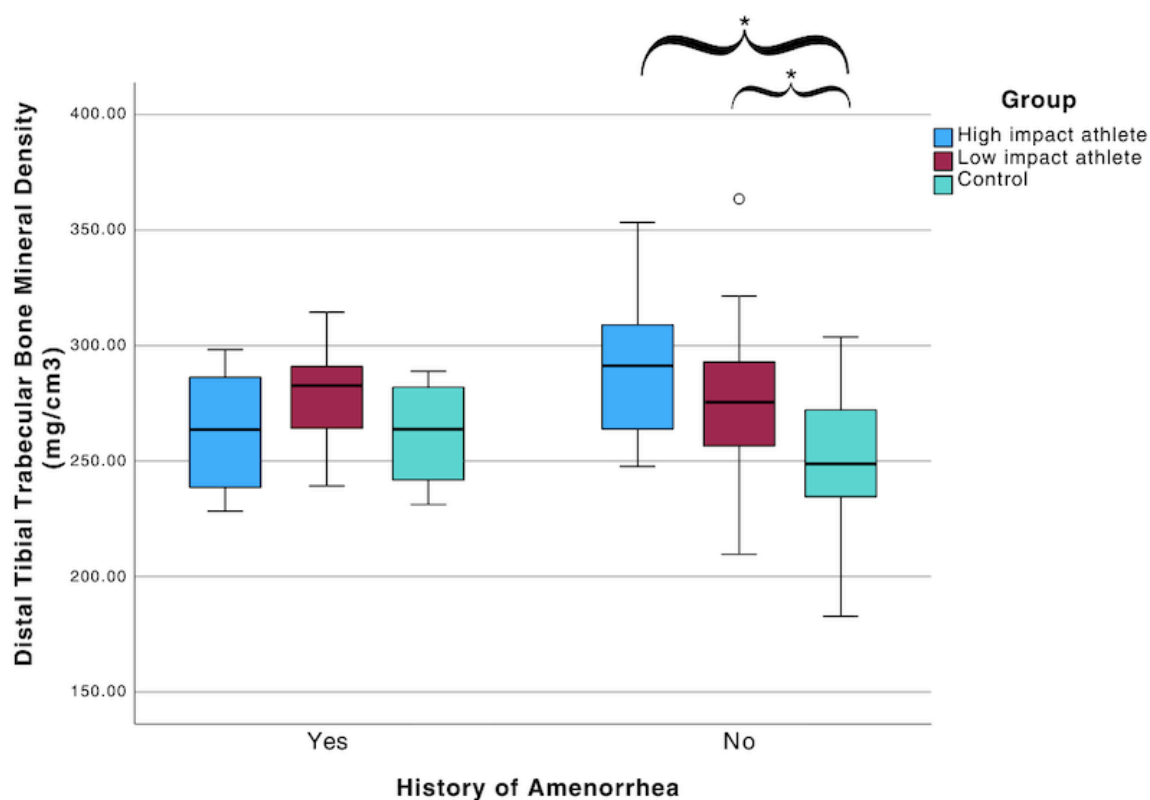


Figure 5. Trabecular Bone Mineral Density in High-Impact Athletes, Low-Impact Athletes, and Controls by History of Amenorrhea. * $p < 0.001$.

Unadjusted mean values for trabecular vBMD by sport participation, menstrual history, and contraceptive use are presented in Figure 6. Relative to controls, athletes consistently exhibited higher mean trabecular vBMD regardless of contraceptive use or menstrual history. However, two groups stand out: first, among athletes, those specifically with both a history of amenorrhea *and* of estrogen-containing contraceptive use averaged ~20 mg/cm³ lower vBMD than the others (consistent mean of ~280 mg/cm³). Second, among controls, those with a history

of amenorrhea and no supplemental estrogen from contraceptives, vBMD was relatively high (272 mg/cm^3), and actually exceeded that of the athlete group mentioned above.

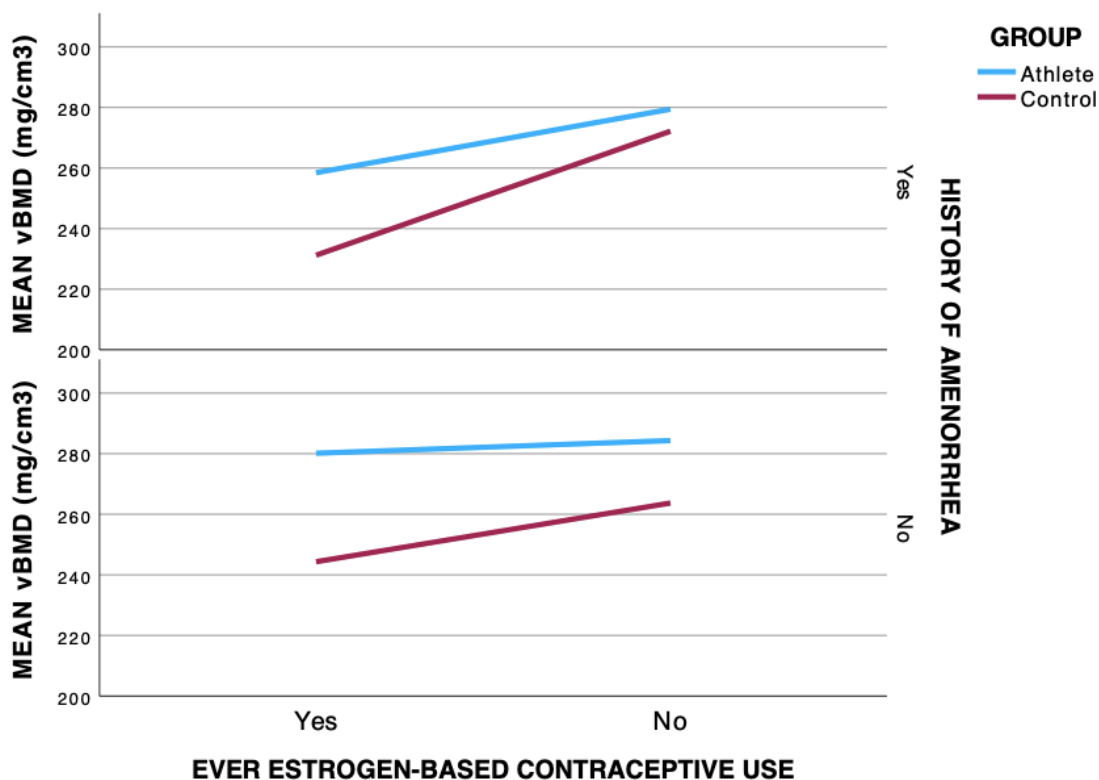


Figure 6: Mean Unadjusted Tibial vBMD by Group, Contraceptive Use, and Menstrual History
Influence of Estrogen Exposure on the Skeletal Benefits of Mechanical Loading

After adjusting for the influence of all covariates in a univariate GLM (Table 2), the effect of sport participation on vBMD was significant ($p = 0.019$). Similarly, after adjusting for all other covariates, the effect of estrogen-containing contraceptive use on vBMD was significant ($p < 0.05$). However, neither contraceptive use nor history of amenorrhea significantly interacted with sport participation to better explain variation in vBMD, after adjustment for the other covariates. This indicates that the relationship between loading and trabecular vBMD did not

differ significantly by menstrual history or contraceptive use, and the association between contraceptive use and trabecular BMD was consistent across loading groups.

Table 2

Estimated Marginal Means of Trabecular Bone Mineral Density by Estrogen-Containing Contraceptive Use, Amenorrhea History, and Athletic Status

Estrogen-Containing Contraceptive Use	History of Amenorrhea	Athlete Type	Mean (mg/cm ³)	Standard Error	Confidence Interval	
					Lower Bound	Upper Bound
Yes	Yes	Athlete	260.18	12.32	235.66	284.69
		Control	232.30	27.42	177.76	286.84
Yes	No	Athlete	280.24	4.62	271.06	289.42
		Control	245.04	6.42	232.27	257.81
No	Yes	Athlete	278.41	7.97	262.55	294.26
		Control	271.90	15.54	240.99	302.80
No	No	Athlete	283.05	9.21	264.73	301.37
		Control	263.50	8.54	246.51	280.48

Covariates were held constant at the following values: Age = 22.98 and body mass (kg) = 63.66.

Influence of Loading Magnitude on Athlete's vBMD

Among athletes, the influence of impact loading type (high or low) on vBMD was assessed after adjustment for age at training, years of cumulative training, training volume (kms/week), age, and body mass, in a second univariate general linear model (Table 3). Both age at scanning and age at initiation of training were negatively but non-significantly associated with trabecular vBMD in the model ($B = -1.486$, $p = 0.67$; $B = -0.608$, $p = 0.85$), while body mass, duration of training (years), and weekly volume of training were positively but not significantly associated with trabecular vBMD. Figure 7 supports these findings by comparing tibial vBMD between an athlete and a control scan. However, after controlling all other variables, the model revealed subtle influences of age, timing, and duration of loading on vBMD. For example, each additional year of age at the time of bone scanning was associated with a 1.5 mg/cm³ decrease in trabecular

vBMD, and each additional year delay in initiation of sport-specific training was associated with a 0.6 mg/cm³ decrease in trabecular vBMD. In contrast, each additional year of training was associated with a 0.8 mg/cm³ increase in trabecular BMD, after controlling for all other variables. Ultimately, after adjusting for the influence of all the covariates, the additional effect of impact type, amenorrhea, or contraceptive use on trabecular vBMD among athletes was non-significant.

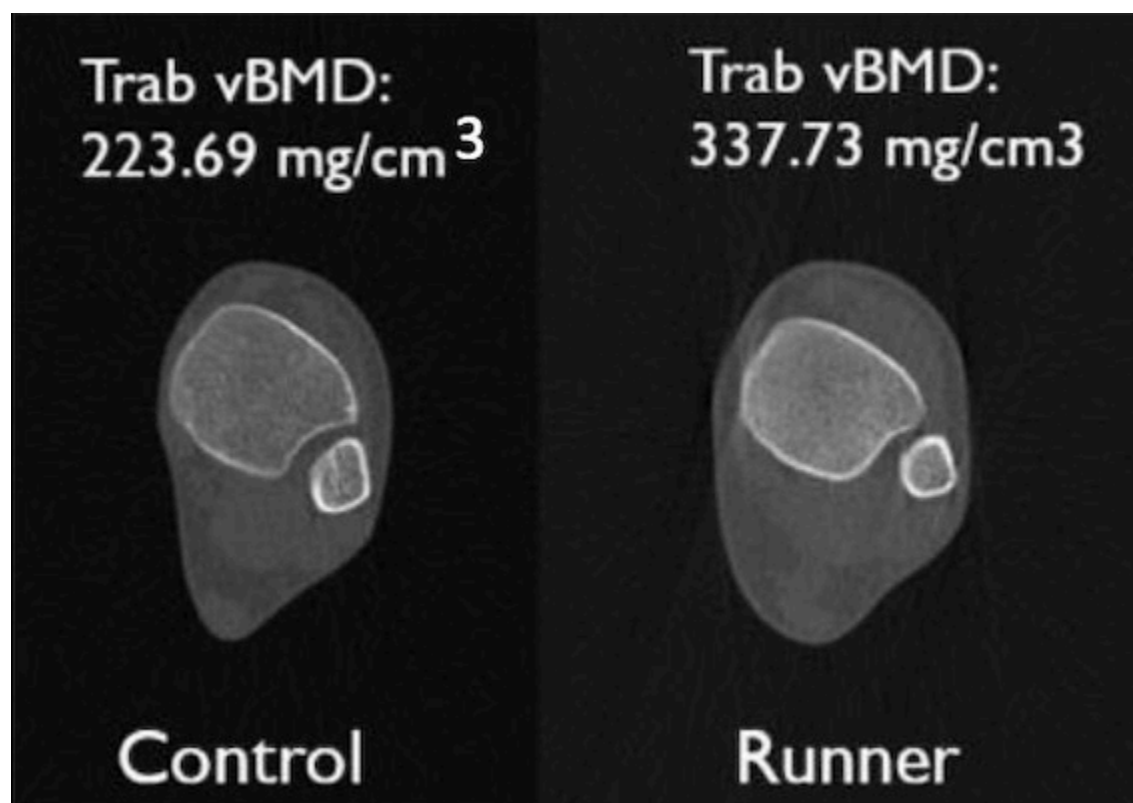


Figure 7. Comparison of distal tibial pQCT scans from an athlete and a control.

Table 3

Estimated Marginal Means of Trabecular Bone Mineral Density by Estrogen-Containing Contraceptive Use, Amenorrhea History, and Impact-Based Athlete Type

Estrogen-Containing Contraceptive Use	History of Amenorrhea	Athlete Type	Mean (mg/cm ³)	Standard Error	Confidence Interval	
					Lower Bound	Upper Bound
Yes	Yes	High impact	263.48	19.57	223.83	303.12
		Low impact	261.46	29.45	201.78	321.13
Yes	No	High impact	286.59	17.16	251.81	321.36
		Low impact	273.54	7.88	257.56	289.51
No	Yes	High impact	250.52 ^a	30.99	187.72	313.31
		Low impact	275.99	9.58	256.59	295.39
No	No	High impact	—	—	—	—
		Low impact	269.82	12.40	244.70	294.94

Dashes indicate that the mean could not be estimated because this combination of factors was not observed. Covariates were held constant at the following values: Age at start of active training = 17.04, years of training = 5.32, Average weekly distance (km) = 100.44, age = 22.90, and body mass (kg) = 65.3. ^aThe estimated marginal mean for this cell is derived from only one participant (n = 1)

Discussion

This study examined the effects of exogenous estrogen on the relationship between trabecular vBMD and mechanical loading, and whether this relationship varies across loading regimes. This research found that estrogen exposure and a history of amenorrhea appear to modify the relationship between broadly defined mechanical loading and trabecular vBMD. Among controls, estrogen-containing contraceptive use was significantly associated with lower trabecular vBMD, while among athletes, estrogen-containing contraceptive use combined with a history of amenorrhea was associated with reduced trabecular vBMD. While athletic participation is associated with increased trabecular vBMD in the literature (Kontulainen et al., 2002; Nikander et al., 2010), and in this study, the interaction between estrogen-containing contraceptive use and amenorrhea may alter the skeletal benefits of sports-related loading. This may reflect a situation where endocrine disruption and/or low energy availability limits bones' capacity to fully adapt to mechanical strain.

Mechanical loading from sports participation was associated with higher trabecular tibial vBMD in the sample, consistent with the framework of functional bone adaptation and previous published work using this dataset (Macintosh & Stock, 2019). A substantial body of research has demonstrated that bone responds to mechanical strain, a principle commonly described by Wolff's law (Kivell, 2016; Ruff, 2006). This relationship reflects expected patterns in functional adaptation, where greater exposure to athletic loading leads to increased bone density. Establishing this relationship provides a foundation for interpreting the broader findings, confirming that the sample behaves in accordance with established bone functional adaptation principles. The type and magnitude of loading also appear to influence trabecular vBMD. Higher-impact activities were associated with greater trabecular vBMD. This aligns with the previously presented evidence that osteogenic responses are particularly sensitive to high magnitude loading (Niinimäki et al., 2017). However, the athletes engaged in high-impact sports also exhibited higher prevalence of menstrual disturbance, indicating a potential trade-off between the benefits of mechanical loading and physiological stress.

The timing of training emerged as an important determinant of bone outcomes. Earlier initiation of participation in sports was associated with greater trabecular vBMD among athletes. This relationship likely reflects the heightened skeletal plasticity during childhood and adolescence, when bone modelling is most responsive to mechanical stimuli (Weaver et al., 2016). Results from the general linear model showed a similar relationship between age at onset of training and trabecular vBMD; however, this association was not statistically significant. When holding all other variables constant, for each additional year delay in initiation of training, trabecular vBMD at the distal tibia declined by 0.6 mg/cm^3 . This relationship was driven by the soccer players who exhibited the highest trabecular vBMD and typically began training at

younger ages, suggesting that early exposure to high-impact loading may contribute to greater trabecular vBMD. These findings stress the importance of accruing bone mass during critical developmental periods (Hereford et al., 2024). High-impact activities such as soccer expose the skeleton to substantial ground forces, which are known to stimulate bone formation and skeletal adaptation (Beck & Snow, 2003). Early and sustained exposure to high-impact loading regimes plays an essential role in optimizing trabecular vBMD.

Hormonal contraceptive use appears to influence bone density differently in athletes and non-athletes, complicating the relationship between mechanical loading and vBMD. Among women with a history of estrogen-containing contraceptive use, trabecular vBMD was significantly higher in athletes compared to controls. However, this difference was primarily driven by variation within the control group rather than the athletes themselves. In controls, estrogen-containing contraceptive use was associated with reduced distal tibial vBMD. This reduction in BMD is not a direct effect of contraceptive use per se, but likely reflects underlying factors associated with contraceptive use, such as menstrual irregularities or amenorrhea. In contrast, consistently higher vBMD among athletes may suggest a protective effect of mechanical loading, although differences in energy availability, hormonal status, and training history may also contribute.

Menstrual dysfunction and energy deficiency may attenuate the skeletal benefits of athletic activity. A higher proportion of athletes reported a history of amenorrhea compared to controls, which suggests that more of the athletes are likely experiencing low energy availability related to training demands. Although group differences were not statistically strong, the pattern is consistent with known associations between high training loads, energy deficiency, and menstrual disturbance (Logue et al., 2018). History of amenorrhea was also strongly associated

with contraceptive use and estrogen-containing contraceptive use, suggesting complex interactions between menstrual function and introduced estrogen. While the general linear model demonstrated no significant interactions between loading groups, history of amenorrhea, and estrogen-containing contraceptive use, interesting trends related to amenorrhea were discovered. Within the athlete group, trabecular vBMD was relatively consistent across contraceptive use and menstrual history, with one notable exception. Athletes with both a history of amenorrhea and use of estrogen-containing contraceptives exhibited lower mean vBMD than other athlete subgroups. Similarly, the lowest estimated marginal means across both athletes and controls were observed in individuals with a history of amenorrhea and contraceptive use, suggesting that this combined profile may reflect the most compromised bone health status, potentially due to prolonged disruption of both energy balance and hypothalamic-pituitary-ovarian function (Logue et al., 2018).

Several mechanisms may explain this pattern. Oral contraceptives may blunt endogenous estrogen production and suppress natural hormonal fluctuations that are essential for bone remodeling (Burrows & Peters, 2007; Hereford et al., 2024). Additionally, exogenous estrogen does not address the underlying hypothalamic suppression that is characteristic of functional hypothalamic amenorrhea, which may continue to disrupt other hormonal pathways influencing bone health, including growth hormone and parathyroid hormone signaling (De Souza et al., 2014; Ackerman & Misra, 2018). There is also concern that combined oral contraceptives may blunt physiological hormonal fluctuations that are important for normal bone turnover and adaptation in athletes with RED-S. Romano and Sass (2025) conclude that combined oral contraceptive pills should not be used in adolescent and young adult athletes with RED-S to improve bone health, as they may mask ongoing energy deficiency and potentially impair bone

accrual through effects such as reduced IGF-1 and increased sex hormone-binding globulin, which lowers bioavailable estradiol. Instead, they recommend transdermal estradiol with cyclic oral progesterone for athletes who fail to improve energy availability after about a year and who continue to have low bone mineral density or recurrent bone stress injuries and it should be used alongside nutritional and training interventions. Supporting this, Ackerman et al. (2019, 2020) showed that transdermal estrogen therapy improves bone mineral density and bone micro-architecture more effectively than combined oral contraceptives or no treatment in oligo-amenorrhoeic athletes.

Limitations

Several limitations should be considered when interpreting the results of this research. First, the sample size became increasingly small when subdivided by specific sport and contraceptive subgroups, which likely reduced statistical power and limited the generalizability of subgroup analyses. This researcher acknowledges that coding complex, multidimensional experiences, such as training intensity, menstrual history, and contraceptive use, into numerical variables represents an inherent oversimplification. This approach may obscure important nuances in the participants lived experiences and physiological variability. Additionally, using self-reported survey data introduces the potential for recall bias, particularly for variables such as past menstrual irregularities or duration of contraceptive use. Some survey categories also lacked sufficient specificity, such as the absence of detailed information on when the participants started using contraceptives, the duration of use, and how long they had been off them, which may have influenced the precision of the analysis. Finally, the cross-sectional design of the study limits the ability to draw causal inferences between contraceptive use, training variables, and bone outcomes.

Future Research

Future research would aim to address the limitations by incorporating more comprehensive data on contraceptive use and menstrual history. Understanding the reasons participants started using contraceptives and the underlying causes of their amenorrhea would provide important contextual information for interpreting the combined effect of estrogen-containing contraceptives and amenorrhea on trabecular vBMD. Collecting specific information on contraceptive history and duration of use would allow for a precise evaluation of their potential effects. Examining the long-term effects of contraceptive use on vBMD for athletes and controls could provide important information on how contraceptive use influences bone health over time. Further investigation of multiple tibial sites, including assessments of cortical bone, would also strengthen the understanding of site-specific skeletal adaptations.

Increased sample sizes within specific sport and contraceptive subgroups would improve the statistical power and subgroup analyses. For the purposes of this study, soccer players and runners were pooled together, as both are associated with high-impact loading regimes. However, there are notable differences in the way these sports load the skeleton, with runners primarily experiencing repetitive unidirectional loading, whereas soccer players are exposed to more multidirectional and variable impact forces associated with rapid changes in direction and jumping. Additionally, these athletes may experience differing energy availabilities due to variations in training volume, competition demands, and sport-specific body composition pressures, which may influence menstrual function and bone health (Moss et al., 2021). An increased sample size would allow for the examination of sport-specific differences in the effects of estrogen-containing contraceptive use on trabecular vBMD. Similarly, with a larger sample

size, heavyweight and lightweight rowers could be analyzed separately, providing greater insight into the influence of training history and dietary factors.

Conclusion

This research investigated how estrogen-containing contraceptive use and menstrual dysfunction in young female athletes influence trabecular bone outcomes. Exogenous estrogen use appears to modify the relationship between mechanical loading and trabecular BMD; however, the observed effects are likely influenced by underlying factors such as amenorrhea and energy availability, rather than representing a direct effect of contraceptive use. Although sport-related mechanical loading typically benefits bone density, the use of estrogen-based contraceptives alongside a history of menstrual dysfunction may attenuate the adaptive skeletal response to loading. No substantial differences in trabecular BMD were observed between athletes in the high- and low-impact loading groups. These findings suggest that hormonal and energetic factors may interact with mechanical loading to influence trabecular bone adaptation in young female athletes.

These results also highlight the importance of considering broader physiological contexts, including energy availability and menstrual function, when interpreting skeletal responses to physical activity. Within the framework of Relative Energy Deficiency in Sport (RED-S), chronic low energy availability may disrupt hormonal regulation and impair bone remodeling, potentially limiting the benefits of mechanical loading on bone health. In this context, the use of estrogen-containing contraceptives may mask underlying menstrual dysfunction without fully addressing the energetic and endocrine factors that contribute to altered bone metabolism.

Age at initiation of athletic training may also play an important role in shaping skeletal outcomes. Participation in high-impact sports during adolescence has been associated with

greater bone mass accrual and structural adaptation during the critical period of peak bone mass development. However, if intensive training coincides with menstrual dysfunction or inadequate energy availability, the expected osteogenic benefits of early sport participation may be reduced. Together, these findings emphasize the need to consider hormonal status, energetic balance, and training history when evaluating bone adaptation in athletic populations.

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