Oregon Undergraduate Research Journal
24.2 (2026) ISSN: 2160-617X (online)
ourj.uoregon.edu

The Influence of Estradiol on Muscle Architecture and Performance Across Hormonal States in Women

Navreet Sandhu*

Abstract

Estrogen, particularly estradiol, plays an important role in maintaining muscle mass and function in women. When estrogen levels decline, whether due to menopause or the use of progestin-only contraceptives, muscle quality may be affected, and the risk of weakness and functional decline may increase. While this relationship has been studied in older women, less is known about how low estrogen influences muscle in younger women. This study compared muscle characteristics across three groups of women with different hormonal profiles: young eumenorrheic women, young women using progestin-only contraceptives, and older ostmenopausal women. Estradiol levels were significantly lower in both the contraceptive-using and postmenopausal groups compared to eumenorrheic women. Significant differences were also found in muscle echogenicity and pennation angle, suggesting that lower estrogen exposure may influence both muscle composition and architecture. These findings suggest that hormonal status can affect structural aspects of skeletal muscle, even when overall muscle size does not differ. Understanding how estrogen influences muscle quality has important implications for both younger women using hormonal contraceptives and older women experiencing long-term estrogen decline.

1. Introduction

Skeletal muscle health is fundamental for maintaining physical function, mobility, and overall quality of life across the female lifespan. Among the many biological factors that influence muscle integrity, estrogen—particularly estradiol (E2), the dominant form of estrogen in premenopausal women—plays a critical regulatory role. Estradiol contributes to the preservation of muscle mass, supports contractile function, and influences the processes involved in muscle repair and recovery (Lowe et al., 2010; Velders & Diel, 2013). The importance of estrogen in muscle physiology is underscored by observations of muscle decline coinciding with reductions in circulating E2, as occurs naturally during menopause or through pharmacological suppression. This decline is associated with decreased muscle strength, impaired neuromuscular function, and an elevated risk of frailty, injury, and chronic diseases such as osteoporosis and sarcopenia (Greising et al., 2009; Collins et al., 2019).

Sarcopenia—the age-related loss of muscle mass and strength—is a major health concern, particularly in women. While sarcopenia affects both sexes, women experience a more abrupt and often earlier onset following the menopause-related drop in estrogen levels

**Navreet Sandhu (navreetsandhu1@yahoo.com) is a senior at the University of Oregon, graduating in the Class of 2025 with a major in Human Physiology with honors, a second major in Sociology, and a minor in Chemistry. He has served as a research assistant in the Muscle Cellular Biology Lab under Dr. Callahan, where he pursued research focused on muscle health and aging. His interdisciplinary background reflects a deep interest in both the biological and social determinants of health. Through his research and academic work, Navreet aims to bridge scientific inquiry with human-centered care.*

(Maltais et al., 2009). Importantly, sarcopenia involves more than a simple reduction in muscle size (e.g., cross-sectional area and mass); it also includes declines in muscle quality, which reflect the muscle's intrinsic force-producing capacity relative to its size and alterations in composition, such as increased fat and connective tissue infiltration. As a result, reductions in strength cannot be attributed solely to the loss of muscle mass. Following the menopause-related decline in estrogen, these combined changes in muscle and quality contribute to measurable impairments in function and an elevated risk of falls among older women (Taaffe et al., 2005). Evidence suggests that estrogen deficiency compromises muscle quality, but hormone replacement therapy (HRT) has demonstrated partial efficacy in mitigating muscle loss and preserving function, suggesting that estrogen has protective effects on musculoskeletal aging (Ronkainen et al., 2009). However, the precise mechanisms through which estrogen exerts these effects are yet to be understood.

Most research examining the relationship between estrogen and muscle function has focused on postmenopausal women or rodent models, leaving a gap in knowledge regarding younger women under conditions of estrogen suppression. Hormonal contraceptive (HC) use, particularly long-acting progestin-only methods such as subdermal implants and hormonal intrauterine devices (IUDs), offers a unique model to study estrogen deficiency in otherwise healthy, young females. These contraceptives suppress ovulation and thereby reduce endogenous estradiol production without providing exogenous estrogen, creating a low-estrogen environment akin to that seen in postmenopausal women (Jin et al., 2022). Despite the widespread use of these methods, their potential effects on musculoskeletal health remain under-investigated (Elliott-Sale et al., 2020). Studying muscle characteristics in young women using progestin-only contraceptives alongside naturally estrogen-replete and postmenopausal women may provide novel insights into estrogen's role in muscle function independent of chronological aging.

Muscle performance depends not only on mass but also on structural and compositional qualities. Key architectural features, such as pennation angle—the angle at which muscle fibers insert into the tendon relative to the line of force generation—affect muscle force production by influencing the alignment and mechanical advantage of muscle fibers (Aagaard et al., 2001). Additionally, tissue composition, including the amount of intramuscular fat as measured by echogenicity—an ultrasound-derived measure of muscle brightness that reflects non-contractile tissue content such as fat and connective tissue—influences contraction efficiency and muscle quality (Young et al., 2015). Functional measures such as torque provide dynamic assessments of muscle responsiveness and quality beyond static size measurements. Integrating these structural and functional assessments across groups with differing estrogen profiles will deepen understanding of how hormones shape skeletal muscle function in women.

This thesis aims to investigate the influence of estrogen status on skeletal muscle architecture and contractile function by comparing three distinct female populations: (1) young eumenorrheic women—those with regular, natural menstrual cycles and no use of hormonal contraceptives—in the mid-luteal phase with high circulating estradiol, (2) young women using long-acting progestin-only hormonal contraceptives, characterized by suppressed endogenous estradiol, and (3) older postmenopausal women with low estradiol levels. The primary outcomes assessed include muscle cross-sectional area (CSA), pennation angle, echogenicity, peak

torque, and specific torque. This multifaceted approach allows for the delineation of estrogen's impact on both muscle structure and performance while controlling for the confounding effects of aging.

We hypothesize that estradiol concentrations will be significantly lower in the hormonal contraceptive and postmenopausal groups compared to eumenorrheic women, and that this hormonal suppression will be associated with reduced specific torque, independent of muscle cross-sectional area. We also expect that lower estradiol levels will be linked to changes in muscle architecture, including altered pennation angle and increased echogenicity, reflecting compromised muscle quality. By examining the relationship between estradiol, specific torque, and structural muscle properties, this study aims to clarify how estrogen status influences the functional capacity of skeletal muscle in females.

2. Methods

2.1. Participant Characteristics

Sixteen healthy adult females participated in this study, divided into three groups based on hormonal status and age: (1) five young women with natural menstrual cycles (eumenorrheic), (2) six young women using progestin-based hormonal contraceptives, and (3) five older women who were at least 10 years past the onset of menopause. All participants provided written informed consent in accordance with institutional ethical guidelines and completed a detailed medical screening questionnaire. Eligibility was determined based on self-reported health status, menstrual history, and medication use.

To qualify for the eumenorrheic group, participants were required to report consistent menstrual cycles lasting between 21 and 35 days, with no extended interruptions over the past year, and no use of hormonal contraceptives in the preceding 12 months. These criteria are consistent with established definitions of menstrual regularity in premenopausal women (Elliott-Sale et al., 2021; Bruinvels et al., 2022). Participants in the hormonal contraceptive group had been using progestin-only contraceptives for a minimum of three months. Acceptable methods included injectable formulations and subdermal implants; intrauterine devices were excluded due to variability in their impact on systemic estrogen levels (Jin et al., 2022). One participant in this group reported concurrent use of a combined estrogen–progestin oral contraceptive and was analyzed separately to acknowledge potential differences in hormonal exposure. The postmenopausal group consisted of women over age 60 who had not menstruated for at least 10 years and were not undergoing hormone replacement therapy. This definition reflects a more stable hypoestrogenic status, minimizing potential hormonal fluctuations that can occur in the early postmenopausal period (Stuenkel et al., 2015).

Participants were excluded if they reported any condition or medication use likely to interfere with skeletal muscle function or hormonal status. This included chronic metabolic or inflammatory illnesses, neurological disorders, or medications that alter neuromuscular signaling. All participants were free from significant physical limitations and reported no history of smoking in the prior year.

2.2. Study Design

This study involved two separate laboratory visits for each participant. The first visit served as an orientation and familiarization session, while the second focused on physiological data collection. During Visit 1, participants were briefed on all

study procedures, provided written informed consent, and completed a health screening questionnaire. They then performed a habituation session using the Biodex dynamometer to become familiar with the testing procedures and reduce potential learning effects during later performance assessments. Women in the eumenorrheic group were additionally provided with ovulation test kits and trained to use them based on manufacturer guidelines. These participants tracked their cycles to schedule Visit 2 during the mid-luteal phase, when estrogen and progesterone levels are expected to be highest (Elliott-Sale et al., 2021). Participants in the hormonal contraceptive and postmenopausal groups were not restricted to a particular cycle phase and were scheduled at their convenience, as their hormonal status is expected to remain stable. Visit 2 took place in the morning to minimize the influence of diurnal (time-of-day related) variation on neuromuscular function (Chtourou & Souissi, 2012). Upon arrival, participants rested before undergoing a venous blood draw for the analysis of circulating estradiol and progesterone concentrations. This was followed by muscle performance testing using the Biodex system, during which maximal voluntary isometric contraction (MVIC) was assessed on the dominant leg to determine peak torque of the quadriceps muscle group. The dominant leg was identified as the leg participants would naturally use to kick a ball, a standard method in neuromuscular research (van Melick et al., 2017).

2.3. Cycle Tracking and Estradiol Analysis

To ensure accurate hormonal profiling, all participants in the eumenorrheic group underwent prospective menstrual cycle monitoring using urine-based ovulation prediction kits (Pregmate®). They were instructed to track two full cycles prior to their experimental testing session. For the first "lead-in" cycle, participants identified day 1 as the onset of menstruation and began daily testing for luteinizing hormone (LH) starting on day 5. LH surges, indicative of imminent ovulation, were confirmed by the research team using photographs submitted by participants. This cycle was used to validate a regular cycle length (21–35 days) and to inform the precise timing of the subsequent study visit.

During the second "experimental" cycle, participants resumed daily LH testing and, upon detection of a surge, were scheduled for their laboratory visit 6 to 9 days later. This timing corresponds with the mid-luteal phase, when both estradiol (E2) and progesterone (P4) levels are elevated (Schnabl et al., 2019), providing a consistent hormonal context for between-group comparisons. At the experimental visit, up to 50 mL of venous blood was collected via standard venipuncture for hormone analysis. Blood samples were processed immediately on-site to isolate serum, which was then divided into aliquots and stored at −80°C until batch analysis.

Quantification of circulating estradiol and progesterone was performed using enzyme-linked immunosorbent assays (ELISA), a sensitive and widely used method for detecting steroid hormones in human serum. Commercially available ELISA kits were used according to manufacturer instructions, allowing for the detection of E2 concentrations within physiological ranges relevant to both premenopausal and postmenopausal populations.

2.4. Ultrasonography and Muscle Strength Assessment

To evaluate muscle morphology and composition, participants underwent B-mode ultrasonographic

imaging of the quadriceps during their initial study visit. A high-resolution diagnostic ultrasound system (Philips EPIQ series) equipped with a linear array transducer (eL18-4; Philips, Andover, MA) was used to obtain both panoramic and longitudinal scans of the thigh musculature. All ultrasonographic imaging was conducted while participants were seated in the dynamometer chair to maintain consistent lower limb positioning and minimize variability in muscle length and tension. This posture facilitated both the muscle imaging and strength testing portions of the protocol. Panoramic scans of the quadriceps were conducted using a lateral-to-medial sweep at five standardized sites along the femur, corresponding to defined percentages of thigh length from the greater trochanter to the lateral epicondyle. However, due to reduced image clarity and alignment challenges at the proximal and distal ends, only the three central mid-thigh scans were selected for analysis. A longitudinal image of the vastus lateralis (VL) was also captured to assess architectural features such as pennation angle.

All images were analyzed using ImageJ software (NIH, Bethesda, MD). For each cross-sectional image, the scale was first calibrated using a visible reference marker or embedded scale bar to ensure accurate and standardized measurements. The VL was then carefully outlined using the polygon selection tool, with superficial and deep aponeuroses serving as consistent anatomical landmarks. These boundaries helped reduce inter-image variability and provided a reliable frame of reference for measuring muscle size. Cross-sectional area (CSA) was calculated based on the traced region. In addition to VL-specific measurements, total quadriceps CSA was also quantified from the same three mid-thigh images using identical tracing methods, ensuring consistent anatomical orientation across measures. This allowed for parallel evaluation of both muscle-specific and group-level morphology. Echogenicity—calculated as the mean grayscale intensity within the outlined VL area—was used as an index of muscle composition, where higher pixel intensity is typically indicative of greater intramuscular fat or connective tissue infiltration (Reimers et al., 1993; Young et al., 2015). Longitudinal images of the VL were used to determine pennation angle, measured using ImageJ's angle tool between the fascicle and the deep aponeurosis. Consistent positioning and orientation during imaging and analysis were prioritized to enhance measurement reproducibility.

Following ultrasonography, participants completed a standardized muscle strength assessment using an isokinetic dynamometer (BioDex System 3™, Biodex Medical Systems, Shirley, NY). Each subject was seated upright with their hip and knee both positioned at 90 degrees of flexion. The axis of the dynamometer was carefully aligned with the lateral femoral epicondyle, and participants secured their upper body by gripping side handles to prevent compensatory movement. To measure maximal voluntary isometric strength, participants performed three maximal voluntary isometric contractions (MVICs) of the knee extensors using their dominant leg. Each contraction lasted approximately five seconds and was separated by 60 seconds of rest to prevent fatigue. The peak torque from each trial was recorded, and the average of the three was used as the participant's maximal torque output. Isometric dynamometry is a validated and reliable method for evaluating neuromuscular performance and force-generating capacity in both clinical and research settings (Cools et al., 2016).

2.5. Statistical Analysis

A one-way ANOVA was used to assess group differences in quadriceps CSA, vastus lateralis CSA, vastus lateralis physiological CSA (PCSA), quadriceps echogenicity, vastus lateralis echogenicity, peak torque, and specific torque of both the vastus lateralis and quadriceps. Where applicable, Bonferroni-corrected independent-samples t-tests were performed to determine differences between individual group pairs. Simple linear regression analysis was used to examine the relationship between circulating estradiol concentrations and muscle-related outcomes, including vastus lateralis and quadriceps specific torque, quadriceps and vastus lateralis CSA, pennation angle, and echogenicity of both the quadriceps and vastus lateralis. These regression analyses were performed across all participants and were repeated using only the EU and HC groups to isolate relationships independent of aging. An alpha level of 0.05 was used to determine statistical significance for all analyses. For Bonferroni-corrected t-tests, the alpha level was adjusted based on the number of comparisons performed. Graphs and scatterplots with trendlines were generated with GraphPad Prism (GraphPad Software, San Diego, CA) to visualize group differences and linear relationships.

3. Results

Table 1. Baseline Physical Characteristics EU, HC, and OF Groups

	n	Height (cm)	Weight (kg)	BMI (kg/m²)	Age (years)
EU	5	163.5 ± 4.6	63.9 ± 6.1	24.0 ± 3.17	19.4 ± 2.6
HC	6	162.0 ± 7.1	56.3 ± 10.3	21.1 ± 2.6#	20.3 ± 0.08
OF	5	164.2 ± 8.2	72.0 ± 13.0	26.7 ± 4.0	73.2 ± 4.00

(*) Indicate a significant main effect of group ($p<0.05$), # different than OF ($p<0.05$). Data are shown as mean ± SD.

3.1. Group Overview and Descriptive Data

Mean age varied substantially between the groups as expected. The eumenorrheic (EU) participants had an average age of 19.4±2.6 years, while the hormonal contraceptive (HC) group averaged 20.3±0.8 years. In contrast, the postmenopausal (OF) group was significantly older, with a mean age of 73.2±4.0 years. There were no significant differences in height among the groups ($p=0.957$). Although a trend was observed for differences in body weight, the result was not statistically significant ($p=0.073$). Body mass index (BMI), however, was significantly lower in the HC group compared to the OF group ($p=0.041$), with no significant difference between the HC and EU groups ($p=0.483$). These findings indicate that, aside from age and BMI, the groups were generally similar in basic anthropometric characteristics (see Table 1).

Table 2. Circulating 17β-Estradiol (E2) and Progesterone (P4) Levels Across EU, HC, and OF Groups

	n	Serum		Plasma	
		E2* (pg/mL)	P4 (pg/mL)	E2 (pg/mL)	P4 (pg/mL)
Eu	5	72.4 ± 46.7	13.7 ± 11.7#	66.9 ± 46.3	13.9 ± 11.0#
HC	6	15.0 ± 6.7	1.6 ± 1.3	12.6 ± 5.8	1.0 ± 0.5
OF	5	20.2 ± 36.2	0.5 ± 0.6	20.8 ± 38.6	0.3 ± 0.3

Participants*indicate a significant main effect of group ($p<0.05$), # different than other groups ($p<0.05$). Data are shown as mean ± SD.

To ensure accurate hormonal profiling, participants in the eumenorrheic (EU) group monitored two consecutive menstrual cycles prior to data collection. Study visits were deliberately scheduled during the mid-luteal phase, a point in the cycle when endogenous estradiol (E2) and progesterone (P4) are typically at elevated levels. The average duration of the cycle preceding the study visit was 26.8 days, with individual variability ranging from 24 to 31 days. During the

monitored cycle in which testing occurred, the average length was slightly longer at 28.2 days, spanning from 24 to 37 days across participants. Hormonal analyses were conducted to compare circulating estrogen and progesterone concentrations across groups. One participant in the hormonal contraceptive (HC) group did not yet have a completed blood analysis at the time of this report and was excluded from hormonal data analyses.

A significant overall group effect was observed for serum estradiol concentrations ($p = 0.040$). Post hoc comparisons using Bonferroni-corrected independent-samples t-tests revealed that serum E2 levels were higher in the EU group compared to the HC group, though this difference approached but did not reach statistical significance ($p = 0.064$). Serum E2 was also elevated in the EU group compared to the OF group ($p = 0.101$), though this difference also did not meet the corrected significance threshold. Group differences in plasma estradiol concentrations showed a similar trend, with the overall effect nearing statistical significance ($p = 0.063$). In contrast, both serum and plasma progesterone levels were significantly higher in the EU group compared to both the HC and OF groups. Serum P4 was greater in EU than in HC ($p = 0.045$) and OF ($p = 0.028$). Similarly, plasma P4 was significantly elevated in EU relative to HC ($p = 0.022$) and OF ($p = 0.016$). These findings support successful hormonal phase targeting in the EU group and confirm the expected suppression of ovarian hormone production in the HC and OF groups (see Table 2).

3.2. Muscle Morphology Measures

Muscle morphology variables, including muscle size, architecture, and echogenicity, were assessed across the eumenorrheic (EU), hormonal contraceptive (HC), and postmenopausal (OF) groups. Measures included quadriceps and vastus lateralis (VL) cross-sectional area (CSA), VL physiological cross-sectional area (PCSA), VL pennation angle, and echogenicity for both the quadriceps and VL muscles (Figure 1A–1F).

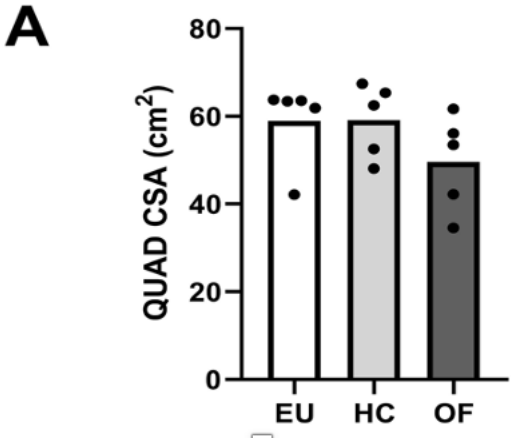

Figure 1A. Individual quadriceps cross-sectional area (CSA) values for younger eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). While CSA values varied across participants, a one-way ANOVA indicated no statistically significant difference between the three groups ($p = 0.2432$). Each dot represents the average QUAD CSA value for an individual within the respective group.

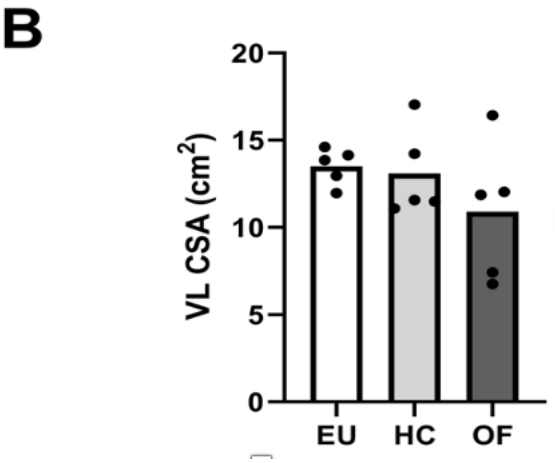

Figure 1B. Individual vastus lateralis (VL) cross-sectional area (CSA) values for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). While some variation was observed across individuals, a one-way ANOVA found no statistically significant differences in VL CSA between groups ($p = 0.3168$). Each dot represents the average VL CSA value for an individual within the respective group.

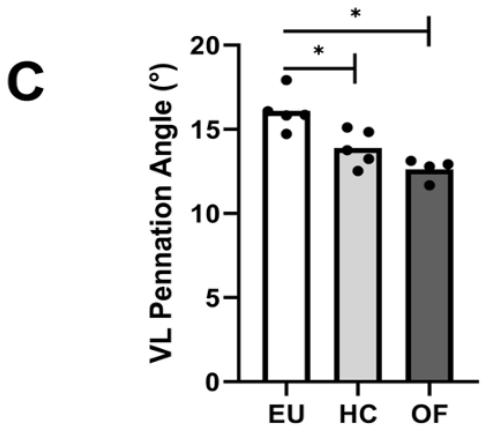

Figure 1C. Individual pennation angle measurements of the vastus lateralis for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). A one-way ANOVA was followed by Bonferroni-corrected pairwise t-tests to assess group differences. Pennation angle was significantly greater in the EU group compared to both the HC group ($p = 0.0149$) and the OF group ($p = 0.0011$). The comparison between the HC and OF groups approached significance ($p = 0.0793$). Asterisk (*) indicates a significance response difference at $p < 0.05$. Each dot represents the average VL pennation angle value for an individual within the respective group.

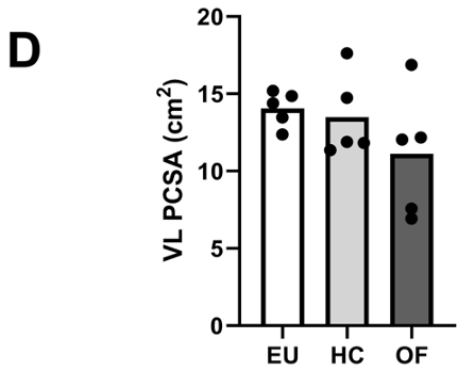

Figure 1D. Individual physiological cross-sectional area (PCSA) values of the vastus lateralis (VL) for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). A one-way ANOVA revealed no statistically significant group differences, though a trend toward significance was observed ($p = 0.0955$). Each dot represents the average VL PCSA value for an individual within the respective group.

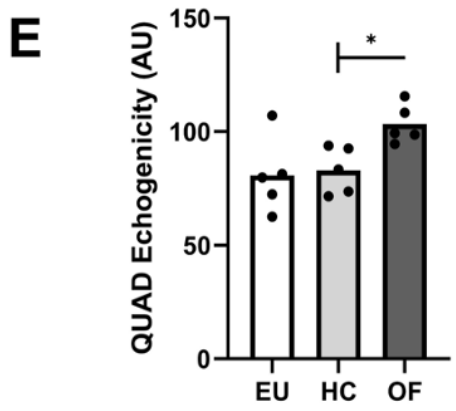

Figure 1E. QUAD echogenicity was measured in young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). One-way ANOVA with Bonferroni corrected t-tests showed QUAD echogenicity was significantly higher in the OF group compared to HC ($p = 0.0094$). Asterisk (*) indicates a significant response difference at $p < 0.05$. Each dot represents the average QUAD echogenicity value for an individual within the respective group.

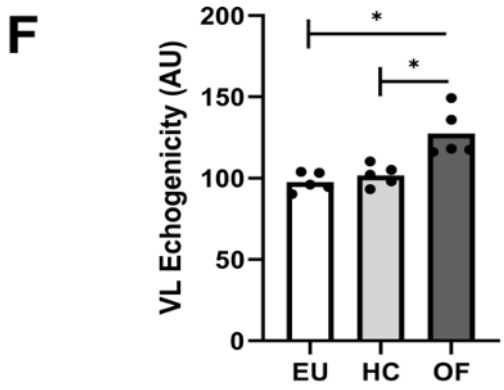

Figure 1F. Vastus lateralis (VL) echogenicity was measured in young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). One-way ANOVA with Bonferroni corrected t-tests showed VL echogenicity was significantly higher in the OF group compared to EU ($p = 0.003$) and HC ($p = 0.0074$), with no difference between EU and HC ($p = 0.3228$). Asterisk (*) indicates a significant response difference at $p < 0.05$. Each dot represents the average VL CSA value for an individual within the respective group.

Quadriceps CSA values (Figure 1A) were not significantly different between the three groups, as determined by one-way ANOVA ($p = 0.2432$). Similarly, VL CSA values (Figure 1B) did not differ significantly by group ($p = 0.3168$). In contrast, VL pennation angle (Figure 1C) showed a significant main effect of group ($p < 0.05$). Bonferroni-corrected t-tests indicated significantly higher pennation angles in the EU group compared to both the HC group ($p = 0.0149$) and the OF group ($p = 0.0011$). The difference between HC and OF approached significance ($p = 0.0793$). For VL PCSA (Figure 1D), the one-way ANOVA did not reveal a significant difference between groups, though the result approached the threshold for significance ($p = 0.0955$). Analysis of quadriceps echogenicity (Figure 1E) showed a significant group effect. Bonferroni-corrected post hoc testing revealed significantly greater echogenicity in the OF group compared to the HC group ($p = 0.0094$). For VL echogenicity (Figure 1F), one-way ANOVA also revealed significant differences, with the OF group exhibiting higher values than both the EU group ($p = 0.003$) and the HC group ($p = 0.0074$). Taken together, these findings partially support the hypothesis that differences in estrogen status would be reflected more in muscle architecture and composition than in overall muscle size. Although CSA did not differ significantly between groups, significant differences in pennation angle and echogenicity highlight structural and compositional variations that align more closely with hormonal status than with gross muscle mass alone.

3.3. Muscle Function Measures

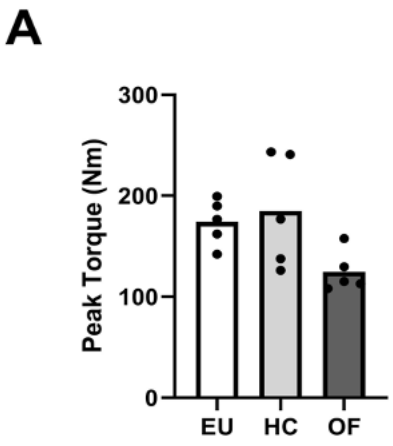

Figure 2A. Individual peak torque values for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). A one-way ANOVA revealed a trend toward a group difference in torque output, though it did not reach statistical significance ($p = 0.0502$). Each dot represents the peak torque value for an individual within the respective group.

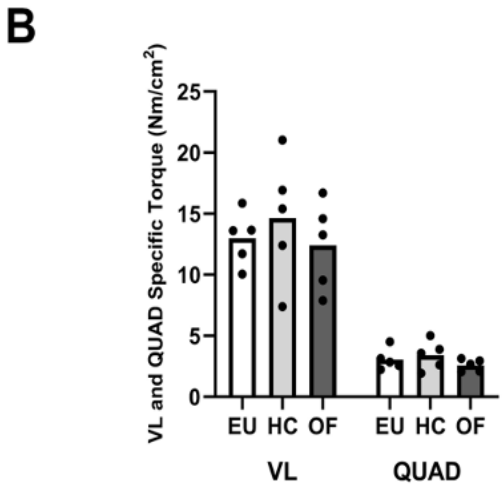

Figure 2B. Individual specific torque values of the vastus lateralis (VL) and whole quadriceps muscle group for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). One-way ANOVAs were conducted to assess group differences. VL torque did not differ significantly between groups ($p = 0.6473$), nor did quadriceps specific torque ($p = 0.3764$). Each dot represents the average VL and QUAD specific torque value for an individual within the respective group.

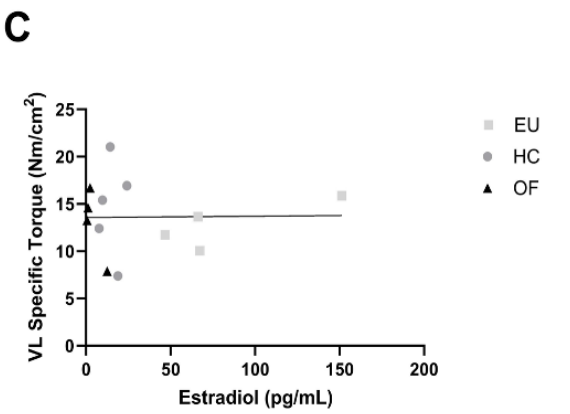

Figure 2C. Relationship between circulating estradiol levels and vastus lateralis (VL) specific torque for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). A linear regression analysis was performed to assess the association between estradiol and VL specific torque. The analysis revealed no significant relationship (R^2= 0.0024, p = 0.9598).

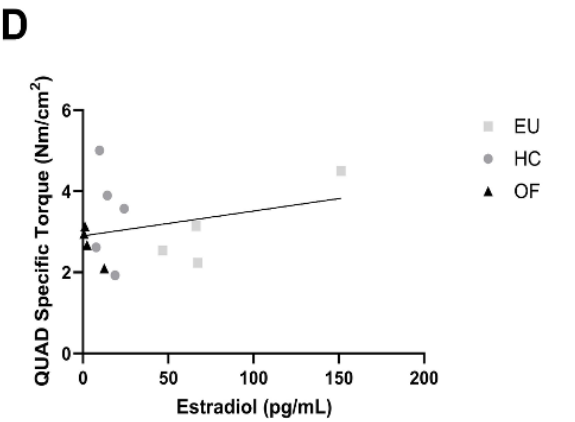

Figure 2D. Relationship between circulating estradiol levels and quadriceps specific torque for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). A linear regression analysis was performed to assess the association between estradiol and VL specific torque. The analysis revealed no significant relationship (R^2= 0.07891, p = 0.3525).

Muscle function was evaluated by measuring peak torque output and calculating specific torque for both the vastus lateralis (VL) and the quadriceps muscle group. Peak torque was obtained during maximal voluntary isometric contraction using an isokinetic dynamometer. Specific torque was calculated by dividing peak torque by the corresponding cross-sectional area (CSA) of the VL and quadriceps to assess torque output relative to muscle size (Figure 2A–2B).

A one-way ANOVA was performed to compare peak torque across groups (Figure 2A). While the analysis revealed a trend toward group differences, the result did not reach statistical significance (p = 0.0502). For VL-specific torque and quadriceps-specific torque (Figure 2B), one-way ANOVAs revealed no significant group differences. VL-specific torque yielded a p-value of 0.6473, and quadriceps-specific torque had a p-value of 0.3764. To evaluate the relationship between estradiol levels and specific torque, linear regressions were conducted for both the VL and quadriceps across all participants (Figure 2C–2D). One participant from the EU group and one from the OF group were excluded from these analyses due to inconsistent estradiol values. The regression between estradiol and VL-specific torque yielded no significant relationship (R^2= 0.0024, p = 0.9598). Similarly, the regression between estradiol and quadriceps-specific torque did not show a significant association (R^2= 0.07891, p = 0.3525). Taken together, these findings do not support the hypothesis that higher estrogen status would be associated with enhanced muscle function or greater torque production relative to muscle size. Despite a trend toward group differences in peak torque, neither specific torque nor its relationship with estradiol levels reached significance, suggesting that variations in estrogen status were not strongly reflected in functional output in this sample.

3.4. Estrogen and Muscle Structure

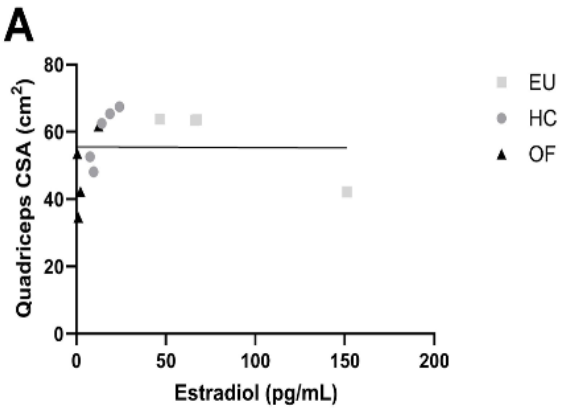

Figure 3A. Relationship between circulating estradiol levels and quadriceps cross-sectional area (CSA) for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). A linear regression analysis revealed no significant association (R² = 6.463e-005, p = 0.9792).

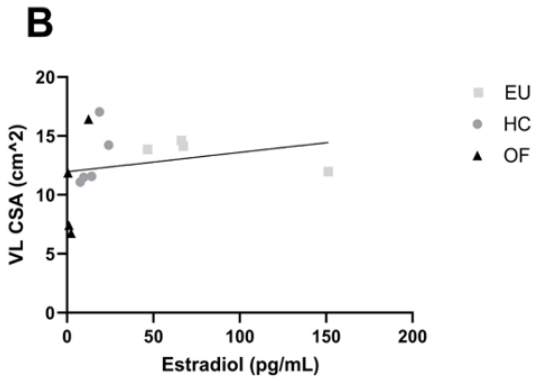

Figure 3B. Relationship between circulating estradiol levels and vastus lateralis (VL) cross-sectional area (CSA) for young eumenorrheic (EU), young hormonal contraceptive (HC), and older postmenopausal females (OF). A linear regression analysis revealed no significant association (R² = 0.05259, p = 0.4510).

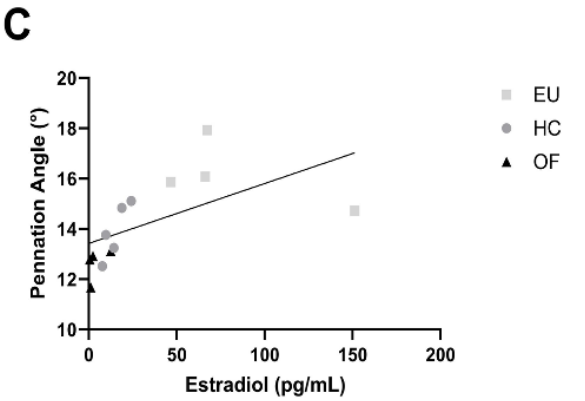

Figure 3C. Relationship between circulating estradiol levels and vastus lateralis pennation angle across participants. A linear regression analysis revealed a significant positive association (R² = 0.3344, p = 0.0384).

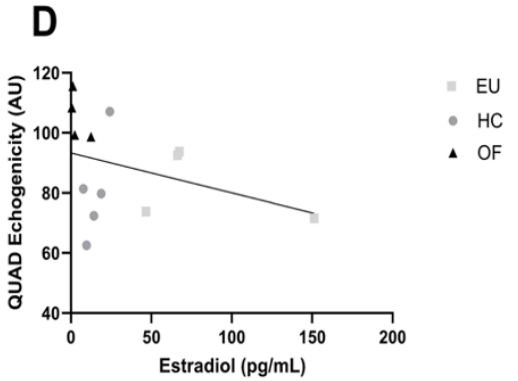

Figure 3D. Relationship between circulating estradiol levels and quadricep echogenicity across participants. A linear regression analysis revealed no significant association (R² = 0.1163, p = 0.2541).

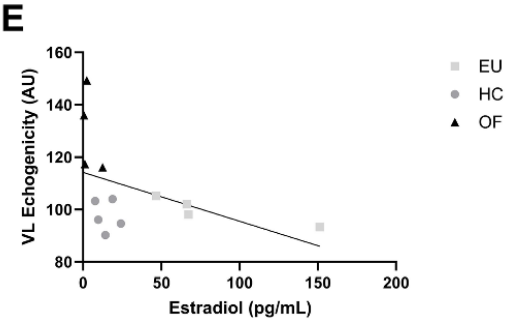

Figure 3E. Relationship between circulating estradiol levels and vastus lateralis echogenicity across participants. A linear regression analysis revealed no significant association (R² = 0.2058, p = 0.1194).

To assess the relationship between circulating estradiol levels and muscle morphology, a series of linear regression analyses was conducted using estradiol concentration as the independent variable. Structural outcomes included quadriceps CSA, vastus lateralis (VL) CSA, VL pennation angle, and echogenicity measures for both the quadriceps and VL (Figure 3A–3E). In Figure 3A, the regression between estradiol and quadriceps CSA yielded an R² value of 6.43×10^{-5} and a non-significant p-value of 0.9792, indicating no meaningful association. Similarly, the relationship between estradiol and VL CSA (Figure 3B) was not significant ($R^2 = 0.0526$, $p = 0.4510$). A significant relationship was found in Figure 3C, where estradiol levels were associated with VL pennation angle, producing an R² value of 0.3344 and a p-value of 0.0384. For muscle composition, quadriceps echogenicity vs. estradiol (Figure 3D) resulted in an R² value of 0.1163 and a p-value of 0.2541, indicating no statistically significant relationship. Similarly, VL echogenicity vs. estradiol (Figure 3E) was not significant ($R^2 = 0.2058$, $p = 0.1194$). Overall, among the structural outcomes examined, only the pennation angle demonstrated a statistically significant relationship with circulating estradiol levels, providing partial support for the hypothesis that estrogen status would be more closely associated with muscle architectural features than with overall muscle size or composition.

3.5. Muscle Function and Structure in the Hormonal Contraceptive and Eumenorrheic Groups

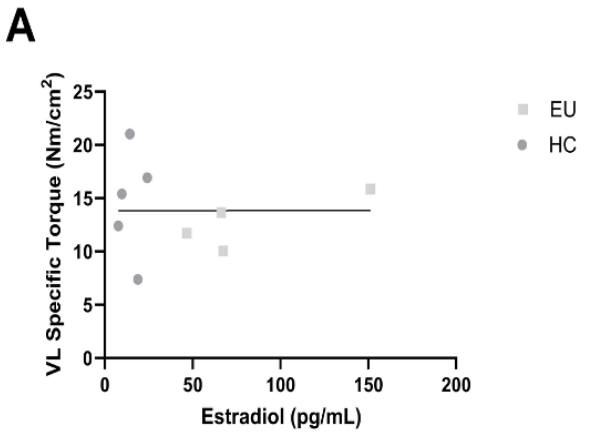

Figure 4A. Relationship between circulating estradiol levels and vastus lateralis (VL) specific torque for young eumenorrheic (EU) and young hormonal contraceptive (HC) females. A linear regression analysis was performed to assess the association between estradiol and VL specific torque. The analysis revealed no significant relationship ($R^2 = 7.903e\text{-}006$, $p = 0.9943$).

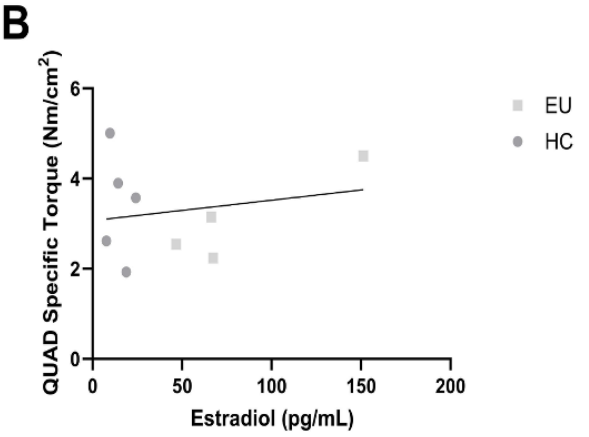

Figure 4B. Relationship between circulating estradiol levels and quadricep specific torque for young eumenorrheic (EU) and young hormonal contraceptive (HC) females. A linear regression analysis was performed to assess the association between estradiol and VL specific torque. The analysis revealed no significant relationship ($R^2 = 0.03909$, $p = 0.6101$).

To further investigate the role of estrogen on muscle function in younger individuals, linear regression analyses were conducted between circulating estradiol levels and specific torque values in only the eumenorrheic (EU) and hormonal contraceptive (HC) groups. These analyses focused on the vastus lateralis (VL) and the whole quadriceps muscle group (Figure 4A–4B). The regression analysis between estradiol and VL specific torque (Figure 4A) showed no significant relationship, with an R² value of 7.903×10^{-6} and a p-value of 0.9943. Similarly, the regression between estradiol and quadriceps-specific torque (Figure 4B) did not reveal a significant association (R²= 0.0391, p = 0.6101).

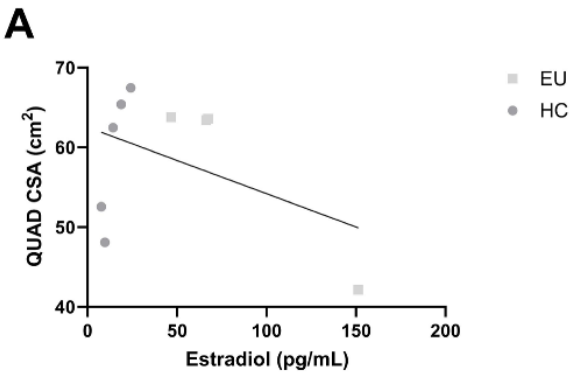

Figure 5A. Relationship between circulating estradiol levels and quadricep cross-sectional area (CSA) for young eumenorrheic (EU) and young hormonal contraceptive (HC) females. A linear regression analysis revealed no significant association (R²= .1863, p = 0.2460).

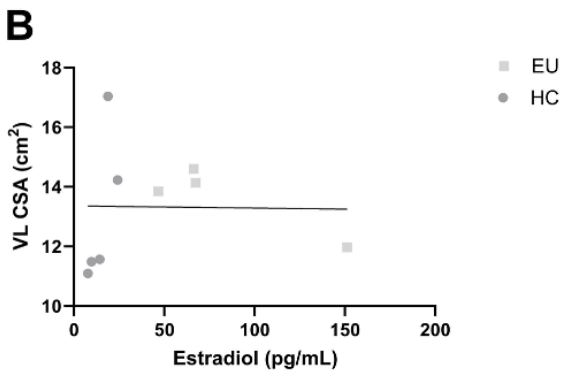

Figure 5B. Relationship between circulating estradiol levels and vastus lateralis (VL) cross-sectional area (CSA) for young eumenorrheic (EU) and young hormonal contraceptive (HC) females. A linear regression analysis revealed no significant association (R²= 0.0002790, p = 0.9660).

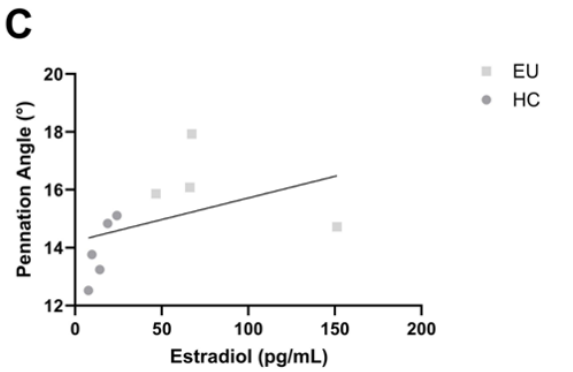

Figure 5C. Relationship between circulating estradiol levels and vastus lateralis pennation angle across young participants. A linear regression analysis did not reveal a significant association (R²= 0.1778, p = 0.2582).

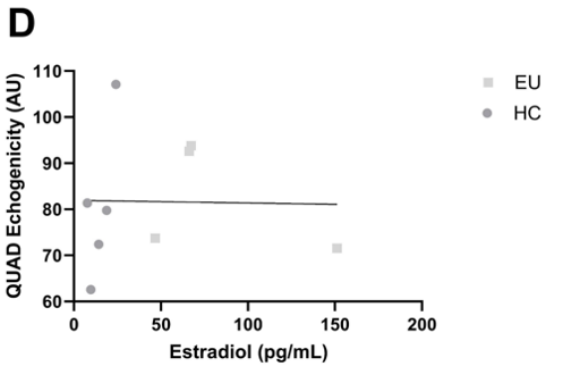

Figure 5D. Relationship between circulating estradiol levels and quadricep echogenicity across young participants. A linear regression analysis revealed no significant association (R^2 = 0.0003521, p = 0.9618).

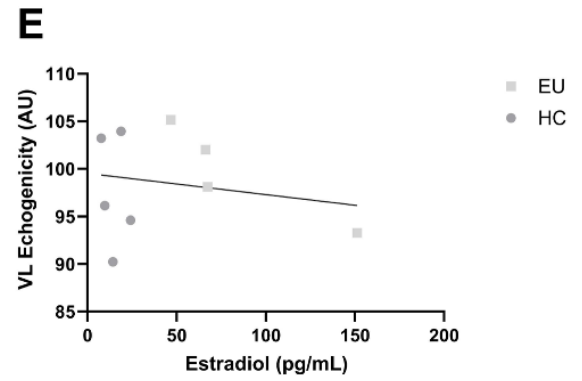

Figure 5E. Relationship between circulating estradiol levels and vastus lateralis echogenicity across young participants. A linear regression analysis revealed no significant association (R^2 = 0.03765, p = 0.6169).

To explore the relationship between circulating estradiol and muscle morphology in younger participants, linear regressions were also conducted within the eumenorrheic (EU) and hormonal contraceptive (HC) groups. Structural measures included quadriceps and vastus lateralis (VL) cross-sectional area (CSA), VL pennation angle, and echogenicity for both the quadriceps and VL (Figure 5A–5E). In Figure 5A, the regression between estradiol and quadriceps CSA produced an R^2 value of 0.1863 and a p-value of 0.2460, indicating a non-significant association. The relationship between estradiol and VL CSA (Figure 5B) was also not significant (R^2 = 0.0002790, p = 0.9660). Similarly, pennation angle vs. estradiol (Figure 5C) showed no significant association, with an R^2 of 0.1778 and a p-value of 0.2582. For quadriceps echogenicity (Figure 5D), the regression yielded an R^2 of 0.0003521 and a p-value of 0.9618. VL echogenicity vs. estradiol (Figure 5E) also showed no significant relationship (R^2 = 0.0377, p = 0.6169). Collectively, these findings do not support the hypothesis that variations in circulating estradiol within younger women are meaningfully associated with differences in muscle function or morphology, as no significant relationships were observed across functional or structural measures in the EU and HC groups.

4. Discussion

4.1. Overview of Key Findings

This study examined the influence of circulating estrogen levels on muscle structure and function in young eumenorrheic (EU), young hormonal contraceptive users (HC), and older postmenopausal females (OF). While structural measures such as muscle cross-sectional area did not significantly differ between groups, pennation angle and echogenicity were affected by hormonal status. No significant differences were found in muscle function after normalizing CSA, and linear regression analyses revealed limited associations between estradiol and muscle outcomes. Together, these findings highlight the complex and variable relationship between estrogen and musculoskeletal characteristics in females.

4.2. Muscle Morphology Across Hormonal Groups

Although there were no statistically significant differences in quadriceps or vastus lateralis CSA between groups, trends in VL physiological CSA (p = 0.0955) and consistent group differences in pennation angle and echogenicity suggest that

hormone status may affect muscle quality more than gross muscle size. Increased echogenicity observed in the OF group aligns with previous research showing that aging is associated with higher intramuscular fat and connective tissue, as reflected in greater muscle echogenicity (Pillen et al., 2009; Reimers et al., 1993). In contrast, the pennation angle was significantly greater in the EU group compared to both the HC and OF groups. This finding is consistent with research indicating that muscle architecture, including fascicle orientation, can be influenced by anabolic hormonal environments and training status (Narici et al., 2006). These differences may reflect structural adaptations that occur with aging and hormonal suppression.

4.3. Muscle Function and Normalization

Despite a trend toward reduced absolute torque in the OF group ($p = 0.0502$), normalization of torque output to muscle size (specific torque) revealed no significant differences across groups for either the VL or quadriceps. This suggests that when accounting for muscle size, intrinsic force production capacity remains similar across the hormonal groups studied. Previous work has shown that aging may not always reduce specific torque unless accompanied by substantial neuromuscular degradation (Delmonico et al., 2009) and that force output relative to muscle area can be preserved in older adults under certain conditions (Trappe et al., 2000). These findings underscore the importance of normalizing strength measures to better isolate functional deficits beyond atrophy.

4.4. Hormonal Influence on Structural Characteristics

Across all participants, linear regression analyses showed no significant associations between estradiol and most structural outcomes, except for pennation angle, which was modestly correlated ($R^2 = 0.3344$, $p = 0.0384$). The relationship between estrogen and architectural features like pennation has not been extensively studied in humans, though animal models suggest estrogen may play a role in modulating structural proteins and muscle fiber alignment (Tiidus, 2005). In contrast, no associations were observed between estradiol and CSA or echogenicity. When limiting the analysis to the EU and HC groups, all relationships between estradiol and structure were non-significant, possibly due to greater variability in hormone levels among younger participants or insufficient statistical power.

4.5. Hormonal Influence on Strength-Related Metrics

No significant associations were found between estradiol concentrations and specific torque values for either the VL or quadriceps, regardless of whether all participants were included or only the young groups. These results contrast with animal studies demonstrating a role for estrogen in enhancing myosin regulatory light chain phosphorylation—a molecular modification that increases the sensitivity of the contractile apparatus to calcium and enhances force production—and improving twitch potentiation, the transient increase in muscle force output following a prior contraction (Lai et al., 2016). However, human studies have reported mixed findings on the effects of estrogen on muscle function, with some showing minor improvements in strength or fatigability during higher estrogen phases (Petrofsky & Lee, 2015), while others report minimal or inconsistent effects (Sung et al., 2014). The lack of significant findings in this study may be due to differences in contraceptive types, variability in hormone

exposure, or the timing of hormonal measurements.

4.6. Methodological Considerations and Limitations

Several limitations should be acknowledged. First, estradiol was measured at a single time point, which may not fully represent an individual's hormonal profile due to natural daily or cycle-related fluctuations. Second, while an effort was made to schedule EU participants during the mid-luteal phase, variability in ovulation timing and cycle length may have influenced circulating hormone levels. Additionally, one participant from each of the EU and OF groups was excluded from hormonal analyses due to inconsistent measurements, potentially reducing statistical power. Sample sizes were modest, particularly within subgroup analyses, limiting the ability to detect small or moderate effects. Finally, imaging-based assessments such as CSA and pennation angle depend on operator consistency and may be sensitive to probe placement or tissue boundaries.

5. Conclusions and Future Directions

In summary, this study found that estrogen status did not significantly affect muscle size or strength when normalized for CSA, though structural parameters such as pennation angle and echogenicity differed between groups. Specifically, the hypothesis that estrogen status would be associated with differences in muscle architecture and composition was partially supported, as pennation angle varied by group and demonstrated a modest association with circulating estradiol. In contrast, the hypothesis that higher estrogen levels would correspond with greater muscle size or superior force production relative to muscle size was not supported.

Across the three populations studied—young eumenorrheic women, young hormonal contraceptive users, and older postmenopausal women—these findings suggest that estrogen-related differences may be more evident in muscle quality and architectural organization than in gross muscle mass or torque output. This distinction has practical implications for understanding age-related muscle changes in postmenopausal women and for interpreting functional performance in younger women with differing hormonal profiles. Overall, these findings contribute to a growing body of literature suggesting that the relationship between sex hormones and muscle health in females is complex and may depend on additional factors such as age, training, and long-term hormone exposure. Future research should consider longitudinal designs, repeated hormone sampling, and mechanistic outcomes, such as muscle protein signaling to further clarify estrogen's role in musculoskeletal physiology.

Acknowledgements

I would like to sincerely thank Dr. Callahan for providing me with the opportunity to engage in research through the Muscle Cellular Biology Lab. His support and guidance have been instrumental in introducing me to the world of scientific inquiry and helping me grow as a student and researcher. I am also deeply grateful to Austin and Julissa for their mentorship over the past few years. Their patience, encouragement, and willingness to answer questions have made the lab a welcoming and enjoyable space to learn and explore. I've truly valued their guidance and the positive environment they've fostered throughout my time in the lab.

References

Aagaard, P., Andersen, J.L., Dyhre-Poulsen, P., Leffers, A.M., Wagner, A., Magnusson, S.P., Halkjaer-Kristensen, J., Simonsen, E.B. (2001). A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. *J Physiol*. 534(2):613–623. https://doi.org/10.1111/j.1469-7793.2001.00613.x

Baltgalvis, K.A., Greising, S.M., Warren, G.L., Lowe, D.A. (2010). Estrogen regulates estrogen receptors and antioxidant gene expression in mouse skeletal muscle. *PLoS One*. 5(4):e10164. https://doi.org/10.1371/journal.pone.0010164

Bruinvels, G., Burden, R.J., Brown, N., Richards, T., Pedlar, C.R. (2022). The prevalence and impact of heavy menstrual bleeding (menorrhagia) in elite and non-elite athletes. *PLoS One*. 17(1):e0261862. https://doi.org/10.1371/journal.pone.0261862

Chtourou, H., Souissi, N. (2012). The effect of training at a specific time of day: a review. *J Strength Cond Res*. 26(7):1984–2005. https://doi.org/10.1519/JSC.0b013e31825770a7

Collins, B.C., Arpke, R.W., Larson, A.A., Baumann, C.W., Xie, N., Cabelka, CA., Nash, N.L., Spangenburg, E.E., Lowe, D.A. (2019). Estrogen regulates the satellite cell compartment in females. *Cell Rep*. 28(2):368–381.e6. https://doi.org/10.1016/j.celrep.2019.06.037

Cools, A.M., Johansson, F.R., Borms, D., Maenhout, A. (2016). Prevention of shoulder injuries in overhead athletes: a science-based approach. *Braz J Phys Ther*. 19(5):331–339. https://doi.org/10.1590/bjpt-rbf.2014.0109

Delmonico, M.J., Harris, T.B., Visser, M., Park, S.W., Conroy, M.B., Velasquez-Mieyer, P., Boudreau, R., Manini, T.M., Nevitt, M., Newman, A.B. (2009). Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr*. 90(6):1579–1585. https://doi.org/10.3945/ajcn.2009.28047

Elliott-Sale, K.J., McNulty, K.L., Ansdell, P., Goodall, S., Hicks, K.M., Thomas, K., Brooker, H., Sanderson, M., Dolan, E., Arnold, J. (2020). The effects of oral contraceptives on exercise performance in women: a systematic review and meta-analysis. *Sports Med*. 50(10):1785–1812. https://doi.org/10.1007/s40279-020-01317-5

Elliott-Sale, K.J., McNulty, K.L., Ansdell, P., Goodall, S., Hicks, K.M., Thomas, K., Brooker, H., Sanderson, M., Dolan, E., Arnold, J. (2021). Methodological considerations for studies in sport and exercise science with women as participants: a working guide for standards of practice for research on women. *Sports Med*. 51(5):843–861. https://doi.org/10.1007/s40279-021-01435-8

Fitts, R.H., Widrick, J.J. (1996). Muscle mechanics: adaptations with exercise-training. *Exerc Sport Sci Rev*. 24:427–473. https://doi.org/10.1249/00003677-199600240-00014

Greising, S.M., Baltgalvis, K.A., Lowe, D.A., Warren, G.L. (2009). Hormone therapy and skeletal muscle strength: a meta-analysis. *J Gerontol A Biol Sci Med Sci*. 64(10):1071–1081. https://doi.org/10.1093/gerona/glp089

Jin, M., Brar, A., Bui, T., Dinh, T., Nguyen, N., Nguyen, T. (2022). The effects of progestin-only contraceptives on bone mineral density and fracture risk: a systematic review. *Contraception*. 106:1–9. https://doi.org/10.1016/j.contraception.2021.12.003

Lai, A.M., Lichtwark, G.A., Schache, A.G., Lin, Y.C., Brown, N.A., Pandy, M.G. (2016). In vivo behavior of the human soleus muscle with increasing walking and running speeds. *J Appl Physiol*. 120(12):1483–1490. https://doi.org/10.1152/japplphysiol.01041.2015

Lowe, D.A., Baltgalvis, K.A., Greising, S.M. (2010). Mechanisms behind estrogen's beneficial effect on muscle strength in females. *Exerc Sport Sci Rev*. 38(2):61–67. https://doi.org/10.1097/JES.0b013e3181d496bc

Maltais, M.L., Desroches, J., Dionne, I.J. (2009). Changes in muscle mass and strength after menopause. *J Musculoskelet Neuronal Interact*. 9(4):186–197. https://pubmed.ncbi.nlm.nih.gov/20090399/

Narici, M.V., Maganaris, C.N. (2006). Adaptability of elderly human muscles and tendons to increased loading. *J Anat*. 208(4):433–443. https://doi.org/10.1111/j.1469-7580.2006.00548.x

Petrofsky, J.S., Lee, S. (2015). Greater reduction of fatigue during the menstrual cycle in women taking oral contraceptives. *Eur J Appl Physiol*. 115(7):1417–1424. https://doi.org/10.1007/s00421-015-3115-3

Pillen, S., Tak, R.O., Zwarts, M.J., Lammens, M.M., Verrijp, K.N., Arts, I.M., van der Laak, J.A., Hoogerbrugge, P.M., van Engelen, B.G. (2009). Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. *Ultrasound Med Biol*. 35(3):443–446. https://doi.org/10.1016/j.ultrasmedbio.2008.09.016

Reimers, C.D., Harder, T., Schedel, H. (1993). Age-related changes in the motor unit. *Muscle Nerve*. 16(6):578–585. https://doi.org/10.1002/mus.880160605

Ronkainen, P.H., Kovanen, V., Alén, M., Pollanen, E., Palonen, E.M., Ankarberg-Lindgren, C., Hämäläinen, E., Turpeinen, U., Kujala, U.M., Puolakka, J. (2009). Postmenopausal hormone replacement therapy modifies skeletal muscle composition and function: a study with monozygotic twin pairs. *J Appl Physiol*. 107(1):25–33. https://doi.org/10.1152/japplphysiol.00448.2009

Schnabl, K.L., Vanstone, C.A., Clandinin, M.T., Weiler, H.A. (2019). Estrogen and progesterone modulate bone turnover markers and bone mineral density in young women. *J Clin Endocrinol Metab*. 104(4):1420–1429. https://doi.org/10.1210/jc.2018-01994

Smith, G.I., Atherton, P., Reeds, D.N., Mohammed, B.S., Rankin, D., Rennie, M.J., Mittendorfer, B. (2014). Estrogen replacement therapy increases skeletal muscle protein synthesis in healthy postmenopausal women. *Am J Physiol Endocrinol*

Metab. 306(5):E535–E544. https://doi.org/10.1152/ajpendo.00502.2013

Stuenkel, C.A., Davis, S.R., Gompel, A., Lumsden, M.A., Murad, M.H., Pinkerton, J.V., Santen, R.J. (2015). Treatment of symptoms of the menopause: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 100(11):3975–4011. https://doi.org/10.1210/jc.2015-2236

Sung, E., Han, A., Hinrichs, T., Vorgerd, M., Manchado, C., & Platen, P. (2014). Effects of follicular versus luteal phase-based strength training in young women. *SpringerPlus, 3*, 668. https://doi.org/10.1186/2193-1801-3-668

Taaffe, D.R., Newman, A.B., Haggerty, C.L., Colbert, L.H., de Rekeneire, N., Visser, M., Goodpaster, B.H., Nevitt, M.C., Tylavsky, F.A., Harris, T.B. (2005). Estrogen replacement, muscle composition, and physical function: The Health ABC Study. *Med Sci Sports Exerc*. 37(10):1741–1747. https://doi.org/10.1249/01.mss.0000181678.28092.31

Tiidus, P.M. (2005). Can oestrogen influence skeletal muscle damage, inflammation, and repair? *Can J Appl Physiol*. 30(3):329–341. https://doi.org/10.1139/h05-119

Trappe, S., Williamson, D., Godard, M., Porter, D., Rowden, G., Costill, D. (2000). Effect of resistance training on single muscle fiber contractile function in older men. *J Appl Physiol*. 89(1):143–152. https://doi.org/10.1152/jappl.2000.89.1.143

van Melick, N., Meddeler, B.M., Hoogeboom, T.J., Nijhuis-van der Sanden, M.W., van Cingel, R.E. (2017). How to determine leg dominance: The agreement between self-reported and observed performance in healthy adults. PLoS One. 12(12):e0189876. https://pubmed.ncbi.nlm.nih.gov/29287067/

Velders, M., Diel, P. (2013). How sex hormones promote skeletal muscle regeneration. *Sports Med*. 43(11):1089–1100. https://doi.org/10.1007/s40279-013-0081-6