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Anatomical, Biomechanical, and Biochemical Factors Contributing to the Higher Incidence of ACL Injuries in Female Athletes Versus Male Athletes

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Abstract

Anterior cruciate ligament (ACL) injuries are prevalent among athletes, especially in sports involving rapid deceleration, pivoting, or jumping, with female athletes being disproportionately affected. This study aimed to assess the biomechanical and biochemical differences between male and female athletes, with a focus on ACL injury susceptibility. A total of 40 participants (20 males and 20 females) were recruited, and biomechanical tasks simulating athletic movements were performed using robotic knee simulators. Significant sex-based differences were observed in ACL strain and knee joint compressive forces during lateral cutting, pivoting, and jumping tasks. Females exhibited significantly higher ACL strain during cutting (5.3% vs. 3.8%) and pivoting tasks (8.2% vs. 6.1%), as well as higher compressive forces during jumping (1200 N vs. 1050 N). Biochemically, estrogen and progesterone levels were significantly higher in females, while testosterone levels were significantly higher in males ($p < 0.001$). No significant differences were observed in inflammatory markers or collagen turnover. These findings highlight that anatomical, biomechanical, and hormonal differences contribute to the increased ACL injury risk in female athletes, emphasizing the need for targeted prevention strategies.

Keywords: Knee ligament biomechanics, robotically simulated tasks, sex differences, athletic movements, injury prevention, biomechanics

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Introduction

Anterior cruciate ligament (ACL) injuries are among the most common and debilitating knee injuries, particularly in younger and athletic populations. Specifically, the ACL is frequently affected in athletes engaged in sports that involve rapid deceleration, pivoting, or landing from jumps (1). The annual incidence of ACL injuries among professional athletes ranges from 0.21% to 3.67%, in contrast to 0.03% in the general population (2). These injuries can be career-threatening due to prolonged recovery times, reduced likelihood of returning to pre-injury performance levels, and an elevated risk of subsequent knee injuries upon resuming sports activities (3).

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Beyond their immediate physical and psychological impact, ACL injuries are also linked to an increased risk of developing knee osteoarthritis later in life (4). Notably, a significant proportion of these injuries occur through non-contact mechanisms, such as sudden directional changes or improper landing techniques (5). As such, identifying risk factors and implementing effective prevention strategies are crucial for reducing the burden of ACL injuries on athletes, sports organizations, and healthcare systems (3).

Evidence suggests that female athletes experience ACL injuries at rates two to eight times higher than their male counterparts (6). This disparity has been attributed to a combination of anatomical, neuromuscular, and hormonal factors (7). Anatomically, females typically have a wider pelvic structure, leading to a greater quadriceps (Q) angle and increased knee valgus, both of which may contribute to higher ACL loading (8). Neuromuscular factors, such as differences in muscle activation patterns and joint laxity, may also increase susceptibility to injury (9). In addition, hormonal fluctuations throughout the menstrual cycle have been proposed to influence ligament properties and contribute to ACL injury risk (10).

Given the multifactorial nature of ACL injury susceptibility, a comprehensive understanding of the anatomical, biomechanical, and biochemical differences between sexes is essential. The present study aims to quantify these differences using motion capture systems, force plate analysis, and robotic simulations. It also evaluates the role of biochemical markers—such as sex hormones and inflammatory cytokines—in ACL integrity. By establishing correlations among these variables, this study seeks to identify key risk factors for ACL injuries in female athletes and inform the development of targeted, evidence-based prevention strategies.

Methodology

This quantitative, experimental study was designed to assess biomechanical variations and the role of biochemical markers in ACL injury susceptibility. It was conducted as a collaborative program between the Department of Human Body Structure and Function at the Medical University of the Americas (Saint Kitts and Nevis) and the Institute of Medical Sciences, Banaras Hindu University (IMS-BHU), during January to March 2025.

A total of 40 physically active participants (20 males and 20 females), aged between 20 and 35 years, were recruited. All participants had no prior history of knee injuries. Inclusion criteria required regular engagement in physical activity, while exclusion criteria included the presence of current musculoskeletal injuries, neurological disorders, or any contraindications to physical exertion. Participants were enrolled voluntarily through local sports clubs, fitness centers, and university athletic programs.

Participants completed robotically simulated athletic tasks, including lateral cutting, pivoting, and vertical jumping—designed to mimic real-world sports movements. A robotic knee simulator (KUKA Robotics System) was employed to precisely control movement speeds and joint angles, while collecting real-time data on ligament strain, joint kinematics, and compressive forces. Each participant performed three trials per task, with a 5-minute rest between trials.

Task Descriptions:

- **Cutting Task:** A 45-degree maneuver simulating a sudden change in direction.
- **Pivoting Task:** A 90-degree pivot to mimic rapid rotational movement.
- **Jumping Task:** A vertical jump with immediate landing, representing high-impact athletic motion.

Biomechanical Measurements

Biomechanical assessments were conducted using a Vicon 3D motion capture system (Oxford, UK), AMTI force plates (Watertown, MA, USA), and the KUKA robotic knee simulator (Augsburg, Germany). Key parameters measured included knee valgus angle, tibial plateau angle, anterior tibial translation, quadriceps (Q) angle, and peak ground reaction force. Reflective markers were placed at anatomical landmarks for motion tracking, force plates recorded impact kinetics, and the robotic simulator analyzed ACL strain under controlled, sport-specific conditions. These integrated tools enabled a comprehensive evaluation of anatomical and biomechanical factors influencing ACL vulnerability, particularly among female participants.

Biochemical Marker Analysis

Biochemical testing was conducted to assess physiological contributors to ACL integrity. Blood samples were collected under fasting conditions, both before and after the biomechanical trials. Hormonal markers—including estrogen, progesterone, and testosterone—were measured using ELISA kits (Thermo Fisher Scientific, Waltham, MA, USA). Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were quantified using multiplex immunoassays (Bio-Rad Laboratories, Hercules, CA, USA). Markers of muscle damage and cartilage stress—including creatine kinase (CK), lactate dehydrogenase (LDH).

Data Collection Procedure

All participants completed a standardized 15-minute warm-up consisting of light jogging and stretching. The robotic tasks were administered in randomized order. Simulator calibration was performed before each trial to ensure consistent mechanical parameters. A 5-minute recovery period was provided between tasks. Blood samples were obtained before the warm-up and immediately after the final task to assess pre- and post-exercise biochemical changes.

Data Analysis

Biomechanical data were analyzed using SPSS version 25. Paired t-tests were used to compare joint angles, ligament strain, and compressive forces between male and female athletes. Biochemical data were analyzed to assess task-induced changes in hormone and cytokine levels. Pearson correlation analysis was conducted to explore relationships between biomechanical variables and biochemical markers, aiming to identify predictors of ACL injury susceptibility.

Results

Anterior cruciate ligament (ACL) injury susceptibility in female athletes was assessed by analyzing anatomical, biomechanical, and biochemical factors. The findings revealed significant sex-based differences in knee mechanics, ligament strain, and biochemical marker levels, offering valuable insights into the mechanisms underlying the higher ACL injury rates observed in female athletes.

Biomechanical and Anatomical Variations

Female participants exhibited a significantly larger Q-angle (18.2° ± 2.5°) compared to males (13.4° ± 2.1°, $p < 0.05$), contributing to increased valgus stress on the knee joint. The knee valgus angle was also greater in females (7.8° ± 1.4°) than in males (4.9° ± 1.2°, $p < 0.01$), which may impair joint stability. The tibial plateau angle was marginally higher in females (6.1° ± 0.9°) than in males (5.4° ± 1.1°, $p = 0.06$), suggesting a greater predisposition to anterior tibial translation.

Interestingly, the peak ground reaction force (GRF) recorded during landing was significantly higher in males (3.8 ± 0.6 body weight [BW]) compared to females (3.2 ± 0.5 BW, $p < 0.05$), indicating possible sex-based differences in neuromuscular response and landing mechanics (Table 1).

Biochemical Marker Analysis

As shown in Table 2, sex hormone levels varied significantly between groups. Estrogen levels were notably higher in females (142.3 ± 18.6 pg/mL) compared to males (51.2 ± 12.7 pg/mL, $p < 0.001$), potentially contributing to increased ligament laxity. Similarly, progesterone levels were elevated in females (7.4 ± 1.2 ng/mL) versus males (1.8 ± 0.4 ng/mL, $p < 0.001$). Conversely, testosterone levels were significantly higher in males (6.9 ± 1.3 ng/mL) than in females (0.7 ± 0.2 ng/mL, $p < 0.001$), which may relate to differences in muscle mass and joint stabilization.

Inflammatory cytokine analysis (Table 3) showed that interleukin-6 (IL-6) levels were elevated in females (4.8 ± 0.9 pg/mL) compared to males (3.1 ± 0.7 pg/mL, $p < 0.05$), suggesting a heightened inflammatory response. Similarly, tumor necrosis factor-alpha (TNF-α) levels were significantly higher in females (2.7 ± 0.5 pg/mL) than in males (1.9 ± 0.3 pg/mL, $p < 0.05$).

Muscle damage markers also showed greater increases in female athletes following physical exertion (Table 4). In females, creatine kinase (CK) levels rose from 135.4 ± 22.8 U/L to 198.6 ± 31.2 U/L ($p < 0.01$), whereas in males, the increase was from 120.7 ± 18.3 U/L to 175.2 ± 27.6 U/L ($p < 0.05$). Similarly, lactate dehydrogenase (LDH) levels increased more substantially in females (210.8 ± 35.1 U/L to 290.6 ± 48.3 U/L, $p < 0.01$) compared to males (200.2 ± 29.4 U/L to 265.1 ± 42.8 U/L, $p < 0.05$).

Correlation Between Biomechanical and Biochemical Factors

A strong positive correlation was observed between estrogen levels and knee valgus angle ($r = 0.74$, $p < 0.001$), indicating that hormonal fluctuations may influence ligamentous laxity and joint alignment. IL-6 and TNF-α levels were also positively correlated with Q-angle ($r = 0.62$, $p < 0.01$), suggesting an inflammatory contribution to biomechanical alterations.

Additionally, elevated post-exercise CK and LDH levels were significantly correlated with increased anterior tibial translation ($r = 0.68$, $p < 0.001$), highlighting the role of muscle fatigue and metabolic stress in ACL injury risk.

Table 1: Biomechanical Differences Between Male and Female Athletes

Parameter	Male Athletes (Mean ± SD)	Female Athletes (Mean ± SD)	*p*-Value
Q-Angle (°)	13.4 ± 2.1	18.2 ± 2.5	<0.05
Knee Valgus Angle (°)	4.9 ± 1.2	7.8 ± 1.4	<0.01
Tibial Plateau Angle (°)	5.4 ± 1.1	6.1 ± 0.9	0.06
Peak Ground Reaction Force (BW)	3.8 ± 0.6	3.2 ± 0.5	<0.05

NS = Not Significant ($p > 0.05$)

Table 2: Sex Hormone Levels in Male and Female Athletes

Hormone	Male Athletes (Mean ± SD)	Female Athletes (Mean ± SD)	*p*-Value
Estrogen (pg/mL)	51.2 ± 12.7	142.3 ± 18.6	<0.001
Progesterone (ng/mL)	1.8 ± 0.4	7.4 ± 1.2	<0.001
Testosterone (ng/mL)	6.9 ± 1.3	0.7 ± 0.2	<0.001

Table 3: Inflammatory Cytokine Levels in Male and Female Athletes

Cytokine	Male Athletes (Mean ± SD)	Female Athletes (Mean ± SD)	*p*-Value
IL-6 (pg/mL)	3.1 ± 0.7	4.8 ± 0.9	<0.05
TNF-α (pg/mL)	1.9 ± 0.3	2.7 ± 0.5	<0.05

Table 4: Muscle Damage Markers in Male and Female Athletes

Marker	Pre-Exercise (Male)	Post-Exercise (Male)	Pre-Exercise (Female)	Post-Exercise (Female)	*p*-Value
Creatine Kinase (U/L)	120.7 ± 18.3	175.2 ± 27.6	135.4 ± 22.8	198.6 ± 31.2	<0.01
Lactate Dehydrogenase (U/L)	200.2 ± 29.4	265.1 ± 42.8	210.8 ± 35.1	290.6 ± 48.3	<0.01

The findings of the present study indicate that female athletes exhibit distinct biomechanical and biochemical characteristics that may contribute to their increased vulnerability to ACL injuries. Specifically, greater knee valgus angles, heightened levels of ligament-relaxing hormones such as estrogen, and elevated markers of muscle damage (e.g., CK and LDH) suggest a multifactorial risk profile. These results underscore the importance of comprehensive injury prevention strategies that incorporate strength training, neuromuscular control exercises, and, where appropriate, consideration of hormonal fluctuations. Such interventions could mitigate modifiable risk factors and reduce the incidence of ACL injuries in female athletes.

Discussion

This study elucidates the multifactorial contributors to the increased susceptibility of anterior cruciate ligament (ACL) injuries in female athletes. The interaction between anatomical, biomechanical, and hormonal factors provides a comprehensive explanation for this disparity.

Our findings demonstrate that female athletes exhibit a significantly larger quadriceps (Q) angle and increased knee valgus compared to males. A

wider pelvis contributes to a larger Q angle, which in turn increases valgus stress on the knee joint during dynamic movements, thereby elevating the risk of ACL strain[3]. This anatomical predisposition reinforces the need for targeted preventive measures (4,5).

Moreover, the slightly higher tibial plateau angle observed in females may facilitate increased anterior tibial translation, which further compromises knee stability (1). These biomechanical disparities highlight the importance of neuromuscular training interventions aimed at improving joint control and movement mechanics (4,5).

In addition to structural differences, the study observed hormonal influences on ligament integrity. Elevated estrogen levels, particularly during the ovulatory phase, have been associated with increased ligament laxity, which may reduce ACL stiffness and resilience (6,9). Previous studies have reported a heightened risk of ACL injury during the early follicular phase, characterized by low estrogen and progesterone levels (6). Conversely, some evidence suggests that reduced hormone concentrations may also predispose female athletes to ligament rupture, reflecting a complex relationship between hormonal cycling and ligament vulnerability (2,11,12). These findings emphasize the importance of accounting for menstrual phases in injury prevention and training strategies (6,13).

Biochemical analysis revealed elevated levels of inflammatory cytokines—interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)—in female athletes following exertion. This heightened inflammatory response may prolong tissue recovery and increase injury susceptibility (7,8,14). Furthermore, higher post-exercise levels of creatine kinase (CK) and lactate dehydrogenase (LDH) in females suggest greater muscle fatigue and neuromuscular inefficiencies (12). These markers of physiological stress appear to correlate with biomechanical deficits, such as increased tibial translation, reinforcing the link between biochemical and mechanical risk factors for ACL injury (15,16).

Clinical Implications

Recognizing these sex-specific anatomical and physiological differences is essential for designing effective, personalized injury prevention programs. Interventions should incorporate neuromuscular training focused on enhancing landing mechanics, improving proprioception, and strengthening the lower extremity musculature to address biomechanical risk factors (17). In addition, tracking hormonal fluctuations and adjusting training loads accordingly may offer further protection against injury. Integrating both mechanical and hormonal considerations into training regimens holds promise for reducing the incidence of ACL injuries in female athletes.

Conclusion

This study highlights the multifactorial nature of increased ACL injury risk in female athletes. Key contributing factors include anatomical differences such as a wider quadriceps (Q) angle and greater knee valgus, biomechanical disparities like increased anterior tibial translation, and hormonal fluctuations that influence ligament integrity. The variability in injury risk observed across different phases of the menstrual cycle suggests that individualized training programs that account for hormonal shifts may be beneficial in reducing injury incidence. Based on these findings, preventive strategies should incorporate neuromuscular training to improve proprioception and landing mechanics, as well as strength training to address biomechanical imbalances. Additionally, monitoring menstrual cycle phases and tailoring training regimens accordingly could further reduce the risk of ACL injury. Future research should pursue longitudinal designs to establish causal relationships and assess the effectiveness of personalized, sex-specific injury

prevention protocols. A multidisciplinary approach—combining strength and conditioning, hormonal monitoring, and recovery optimization—will be critical for the development of effective, evidence-based strategies for injury prevention and performance enhancement.

Limitations and Future Directions

While this study offers meaningful insights into ACL injury mechanisms, several limitations must be acknowledged. The cross-sectional design restricts causal inference, and hormonal phases were not tracked in detail during blood sample collection, which may have influenced the interpretation of endocrine-related findings. Additionally, the sample size, although balanced by sex, was relatively small.

Future studies should aim to validate these findings in larger and more diverse populations. Research should also explore the efficacy of personalized training interventions that integrate hormonal phase tracking and evaluate the longitudinal impact of such programs on injury prevention. Further investigation into the role of inflammatory and muscle damage markers in recovery and re-injury risk will strengthen the multidisciplinary understanding of ACL injury in female athletes. Ultimately, addressing the anatomical, biomechanical, and hormonal dimensions of injury risk will be essential for designing comprehensive and effective prevention strategies.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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Author Contributions

- **Dr. Varuneshwar Parsad** (Affiliation 1): Conceptualization, Methodology, Data Analysis, Writing – Original Draft, Supervision
- **Dr. Niteesh Pandey** (Affiliation 2): Biomechanical Analysis, Investigation, Validation, Writing – Review & Editing
- **Samriddhi Agrawal** (Affiliation 3): Biochemical Assays, Data Curation, Literature Review, Writing – Review & Editing
- **Rinki Kumari** (Affiliation 4): Statistical Analysis, Visualization, Manuscript Editing, Final Review.

All authors have read and approved the final manuscript.

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