Sex hormones and neuromuscular function in relation to non-contact anterior cruciate ligament injury risk in females

Elisa Nédélec

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy

September 2024

Copyright statement

Abstract

The rise in female sports participation and sustained physical activity has highlighted a higher prevalence of non-contact anterior cruciate ligament (ACL) injuries in female compared to males, driven by a complex interplay of physiological, anatomical, biomechanical, and neuromuscular factors, along with gender-specific environmental influences. The first chapters of this thesis investigated the role of sex hormone profiles, including the menstrual cycle (MC), hormonal contraceptive (HC) use, and menstrual irregularities (MI), in contributing to non-contact ACL injuries, while evaluating clinical methodologies for injury risk assessment. Chapter 3 presents a systematic review and meta-analysis on the influence of MC phases and HC use on anterior knee laxity (AKL) and non-contact ACL injury incidence. While no significant effects of hormonal factors were found, the results highlighted high methodological heterogeneity across studies, emphasising the need for robust protocols in hormonal assessment. In Chapter 4, the reliability of the GNRB, an automated knee arthrometer, was assessed, which demonstrated excellent reliability (ICC = 0.94) for AKL evaluation. This has clear potential for broader clinical application in ACL injury diagnosis and AKL monitoring. Chapter 5 identified methodological inconsistencies in common assessments of neuromuscular function (force steadiness [FS]), specifically in calculating the coefficient of variation (CoV) of force output, limiting cross-study comparisons and the development of clinical standards. Building on this, Chapter 6 evaluated knee extensors FS, maximal voluntary isometric strength (MVC), and vastus medialis (VM) and vastus lateralis (VL) muscle activation across the MC, finding no significant hormonal influence on these variables (p = 0.756-0.895; p = 0.393; p = 0.324; and p = 0.775, respectively) in eumenorrheic females. While these results suggested minimal impact of hormonal fluctuations on knee extensor neuromuscular performance, further research is required to explore additional neuromuscular factors, such as motor unit (MU) behaviour, using advanced decomposition techniques from high-density surface electromyography (HDsEMG). Overall, this thesis advances the understanding of non-contact ACL injury mechanisms in females, advocating for standardisation in hormonal assessment and supporting the GNRB's utility in non-contact ACL injury prevention. Moreover, it identifies key areas of further research needed to explore neuromuscular factors that

may contribute to the elevated non-contact ACL injury risk in females, particularly during sport-specific movements.

List of abbreviations and acronyms

ACL – Anterior Cruciate Ligament

AK – Anterior Knee

AKL – Anterior Knee Laxity

AMB – Anteromedial Bundle

ANOVA – Analysis Of Variance

BBT – Basal Body Temperature

BMI – Body Mass Index

CI – Confidence Interval

CMJ – Countermovement Jump

CNS – Central Nervous System

CoV – Coefficient of Variation

CrIs – Credible Intervals

CV – Coefficient of Variation

E – Oestrogen

EF – Early Follicular

ELISA – Enzyme-linked Immunosorbent Assay

EMD – Estimated Mean Difference

FA – Football Association

FS – Force Steadiness

FSH – Follicle Stimulating Hormone

GNRB – Genourob automated knee arthrometer

GnRH – Gonadotrophin-releasing Hormone

HC – Hormonal Contraceptive

HDsEMG – High-density Surface Electromyography

HEPA – Health-enhancing Physically Active

HRT – Hormone Replacement Therapy

ICC – Intraclass Correlation Coefficient

IOC – International Olympic Committee

IPAQ – International Physical Activity Questionnaire

ISRCTN – International Standard Randomised Controlled Trial Number

IUS – Intrauterine System

LH – Luteinising Hormone

LOX – Lysyl Oxidase

MC – Menstrual Cycle

MD – Mean Difference

MDC – Minimum Detectable Change

MI – Menstrual Irregularities

ML – Mid-Luteal

MRI – Magnetic Resonance Imaging

MU – Motor Unit

MUFR – Motor Unit Firing Rate

MVC – Maximum Voluntary Contraction

N – Newton

NOS – Newcastle Ottawa Quality Assessment Scale

NSAIDs – Non-steroidal Anti-inflammatory Drugs

OA – Osteoarthritis

OCP – Oral Contraceptive Pill

OR – Odd Ratio

P – Progesterone

PECOS – Participants, Exposures, Comparators, Outcomes, Study designs

PLB – Posterolateral Bundle

pre-O – Pre-Ovulation

PRESS – Peer Review of Electronic Search Strategies

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PRISMA-S – Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Searching

PROSPERO – International Prospective Register of Systematic Reviews

RED-S – Relative Energy Deficiency in Sport

relRMS – Relative Root Mean Square

RMS – Root Mean Square

SEM – Standard Error of Measurement

sEMG – Surface Electromyography

VL – Vastus Lateralis

VM – Vastus Medialis

Manuscript

This relates to Chapter 2:

Nédélec, E., Foli, E., Shultz, S. J., Swinton, P. A., Dolan, E., Enright, K., Piasecki, J., Matthews, J. J., Sale, C., & Elliott-Sale, K. J. (2021). Effect of menstrual cycle phase, menstrual irregularities and hormonal contraceptive use on anterior knee laxity and non-contact anterior cruciate ligament injury occurrence in women: a protocol for a systematic review and meta-analysis. *BMJ open sport & exercise medicine*, *7*(4), e001170.

Conference abstracts

Nédélec, E., Elliott-Sale, K., Sale, C., Piasecki, J., Enright, K. (2021) The FAIR ACL Project. Sports Tomorrow, Barcelona, Spain.

Nédélec, E., O'Hanlon, M., Inns, T., Hunter, A., Piasecki, M., Piasecki, J. (2024) Methodological considerations for the assessment of force steadiness. UK Sensorimotor, Cambridge, UK.

Nédélec, E., O'Hanlon, M., Inns, T., Hunter, A., Piasecki, M., Piasecki, J. (2024) Vastus medialis activation and knee extensor neuromuscular function across the menstrual cycle. The Physiological Society, Newcastle-upon-Tyne, UK.

Acknowledgements

This work would not have been possible without the support of many people, each of whom played a crucial role in its completion.

First, I am deeply grateful to my Director of Studies, Dr Jessica Piasecki, for her unwavering dedication, patience, and belief in my potential. Her guidance helped me grow as a scientist and explore the world of neuromuscular physiology, which I have come to love. I also extend my sincere thanks to Professor Angus Hunter for his incredible support, kindness, and encouragement over the past two years. I am equally thankful to Dr Mathew Piasecki for his expertise in data and statistical analysis, and for guiding me through the technical challenges of this research. The mentorship I received by this supervisory team provided me with the opportunity to learn new techniques and gave me the confidence to complete this programme. Your passion for neuromuscular physiology was truly contagious.

My gratitude also goes to Nottingham Trent University for supporting this work through the NTU fully-funded PhD studentship, which made this project possible. I would also like to thank Professor Kirsty Elliott-Sale for believing in the research proposal that sparked this journey and for supporting my return to academia. The most important aspect is the journey, not the destination, and this journey has been truly enlightening, teaching me as much about myself as it did about the subject matter.

I am also thankful to the external researchers who enriched my knowledge of female physiology, particularly Professor Sandra Shultz, Mr Elvis Foli, and Dr Paul Ansdell, who closely collaborated on key studies.

To the original (2019) and newer NTU Sports Science PhD crew – Janelle, Rita, Nicola, Joe, and Hannah – thank you for your time, humour, and support, especially during challenging moments. A huge thank you to my lab teammates, Mollie and Tom, who brightened the long days and kept me motivated throughout my experimental studies. Tom, your constant troubleshooting and philosophical conversations were invaluable, and Mollie, your energy and curiosity made the learning process a pleasure.

I would like to extend my genuine thanks to the Sports Science Department and its dedicated technicians, as well as the invaluable assistance of research librarian Victoria Boskett. Their support, along with the contributions of all the participants in these studies, was crucial in advancing research on female physiology.

Lastly, I am deeply thankful for the unwavering encouragement from my family, friends, and the invisible supporters who cheered me on through this PhD journey. To my Mum, Grandma, Uncle, Aunt, Sister, my partner's family, my friends Stéphanie and Marianne, and my former patients in Belgium – thank you for believing in me.

A heartfelt thank you to my co-pilot, Christoph, who has been by my side from day one, steadfastly supporting me through every high and low of this journey. Your constant strength and love have been my anchor, and I am deeply grateful that we shared this adventure together, with the comforting presence of our beloved Satine.

This five-year project was a transformative experience, during which I also faced personal loss, bidding farewell to my Dad and my Grandma, both affected by neurological diseases. I dedicate this thesis to my Grandma, in honour of her values, kindness, and resilience. Thank you for everything.

Table of contents

List of tables

List of figures

Chapter 1. Introduction

1.1 Introduction to thesis topic

Female participation in sports has seen a global rise, both at recreational and professional levels (International Olympic Committee [IOC], 2024; The Football Association [FA], 2020). When females were first allowed to participate in the Olympic Games in Paris in 1900, only 2% of all participants were females. For the first time in Olympic history, during the 2012 edition of the Olympic Games in London, all participating countries had female and male participants, with females comprising 40% of all participants (Hecht & Arendt, 2015). This year (*i.e.*, 2024), the participation rate of registered female athletes for the Olympic Games in Paris reached a record of 50%, therefore achieving the milestone set to attain gender parity at the Olympic Games. The same trend is observed in females' participation in the Winter Olympic Games, with 4,3% of female participants at Chamonix in 1924 and 45% at Beijing in 2022 (IOC, 2024). Similarly, female athlete participation at the Paralympic Games has shown a steady increase since the Sydney Games in 2000, where women made up 25% of the athletes. This upward trend continued in the Tokyo 2020 Paralympic Games, where the percentage of female athletes nearly doubled to 42%. By the Paris 2024 Games, the proportion further increased to 45%, highlighting a progressive rise in female representation over time (Houghton, Pieper, & Smith, 2022; International Paralympic Committee, 2024).

The growth and development of women's sports have led to increasing numbers of reports on the nature and frequency of injuries sustained by female athletes (DiStefano et al., 2018; Hägglund, Waldén, & Ekstrand, 2009; Larruskain et al., 2018). Over the past two decades, data collection and reporting methods have significantly improved which has enabled the development of surveillance and observational or intervention studies in various settings, *i.e.*, including community and elite sports, youth and master's levels, and individual and team sports, involving both able-bodied and disabled athletes (Bahr et al., 2020). Injury rates among females differ from those of their male counterparts depending on factors such as age, sport, and level of participation (Sigward, Pollard, & Powers, 2012). Among the most common musculoskeletal injuries in exercising females is anterior cruciate ligament (ACL) injury, which occurs three to six times more frequently in females than in males during athletic activities (Hewett et al., 2016; Murphy, Connolly, & Beynnon, 2003). Notably, 55 to 75% of ACL injuries are non-contact injuries (Boden et al., 2000; Chia et al.,

2022), meaning there is no direct or indirect interference with the injured player's movement by an external source, such as another player or object (Bahr et al., 2020). Most non-contact ACL injuries occur in high-risk, fast-paced, multidirectional sports involving jumping, landing, and cutting manoeuvres, such as football, rugby, netball, basketball, and field hockey (The UK National Ligament Registry, 2022; Montalvo et al., 2019; Agel et al., 2007). Furthermore, female athletes tend to experience a higher incidence of non-contact ACL injuries during competition compared to training (Chia et al., 2022).

There is a pressing need to support females, given the rise in participation in sport at both elite and recreational levels. This involves ensuring a full understanding of female physiology and its possible relations to injury and injury risk. Therefore, the research conducted as part of this PhD will explore potential contributing risk factors for ACL injuries in females and investigate additional methodologies that may be later used as clinical assessments of ACL injury risk and ligament laxity. The findings from this project will add more robust and conclusive evidence to the existing body of knowledge on the risk of ACL injury amongst exercising females and athletes, which is currently conflicted.

1.2 Injury incidence in female athletes and exercisers

The latest IOC consensus statement defines an injury as any damage to tissues or disruption of normal physical function resulting from rapid or repeated transfer of kinetic energy during sports participation. Injuries can occur suddenly due to acute mechanisms, gradually due to repetitive loads applied to specific body parts, or due to a combination of both. Acute injuries can result from non-contact, indirect contact, or direct contact, with either another player or object. The severity of an injury is mainly, but not exclusively, assessed following three main criteria, such as time loss, *i.e.*, the period an athlete will not be able to train and/or compete, the athlete's self-reported consequences of said injury, *i.e.*, measures of health and sports performance, and the medical extent and societal cost (Bahr et al., 2020).

Although causes of sports injuries remain mostly multifactorial – involving intrinsic and extrinsic factors that may or may not be modifiable – there is moderate evidence that load management is a crucial risk factor for sustaining injuries (Drew & Finch, 2016; Meeuwisse, 1994; Meeuwisse et al., 2007). Inadequate load / recovery

ratio can result in a subclinical tissue damage, clinical symptoms, and potential injury, or more general systemic damage at a later stage (Soligard et al., 2016). Additionally, the risk of injury, for any exercising individual, can be greatly influenced by inter and intra-individual variations that can predispose an athlete or exerciser to a potential injury, *e.g.*, age, sex, body composition, medical background, anatomy, genetic factors, psychological factors, type of sport, sports equipment and environment (Bahr & Krosshaug, 2005). Injuries occurring during training and competition are highly dependent on the type of sport and sex of the participant. For instance, female football players experienced the highest rate of injuries, *i.e.*, 45%, amongst all female athletes, while male athletes in taekwondo suffered most injuries, *i.e.*, 51.6%, amongst all male participants during the 2012 London Summer Olympic Games (Engebretsen et al., 2013). Recreational sports also show sex discrepancies in injury rates with exercising females being more prone to injuries in football and gymnastics while males are more susceptible to get injured in American football and wrestling, in the North American population. Moreover, lower limb injuries are the most common amongst all exercisers (Zumwalt, 2019).

Female exercisers and athletes are specifically at a heightened risk of injury due to physiological, anatomical and biomechanical intrinsic risk factors, such as (i) different body composition, *e.g.*, higher body fat and BMI, higher active and passive flexibility, lower bone mineral density, lower muscle strength, muscular imbalance; (ii) different neuromuscular risk factors, *e.g.*, variation of neuromuscular function and fatigability of the knee extensors across the menstrual cycle (MC); (iii) MC dysfunctions and reproductive hormones changes, *e.g.*, amenorrhea, pre-menstrual syndrome, endometriosis, menopause; (iv) breast health issues; (v) pregnancy management issues; and (vi) risk of Relative Energy Deficiency of Sport (RED-S) (Ansdell et al., 2019; Griffin et al., 2006; Heikura et al., 2018; Martínez-Fortuny et al., 2023; Mountjoy et al., 2014; Nilstad et al., 2014; Pitchers & Elliott-Sale, 2019). Most studies investigating injury risk in female athletes and exercisers focus on football/soccer, as it is the most widely played sport with continuous growth in practice and competition associated with high prevalence of ACL injury. A recent systematic review identified common contributing factors to injury risk in elite female football players and recommended that potential influence of changes in ovarian steroid hormones on injury risk in this population should be explored (Alahmad, Kearney, &

Cahalan, 2020), given the high proportion of recent growing sport-related injury incidences in females. While a few studies have investigated potential impacts of ovarian steroid hormones on injury risk in female football players, their findings are conflicting (Möller-Nielsen & Hammar, 1989; Martin et al., 2021). Möller-Nielsen and Hammar (1989) found that injury incidence was higher during the pre-menstrual phase and during menstruation compared to other MC phases, but their study lacked clear definitions of MC phases and had low numbers of non-hormonal contraceptive (HC) users, *i.e.*, n = 45. In contrast, Martin et al. (2021) reported a greater injury rate in the late follicular phase compared to other MC phases and observed that the MC phases may differentially affect the type of injury or tissue affected in eumenorrheic female athletes. However, Martin et al. acknowledged that their study had a relatively small number of injuries reported, *i.e.*, N = 156, and did not consider the rate of reinjuries among participants. There is a scarcity of research exploring the potential influence of MC phases or HC use on injury risk among female athletes and exercisers, particularly in sports beyond football/soccer. Therefore, it is essential for future studies to address this knowledge gap by increasing the quality of the research methodology, observing larger participant cohorts, and extending the follow-up duration across various sports.

1.3 Anterior cruciate ligament injuries

1.3.1 The anterior cruciate ligament

1.3.1.1 Anatomy

The ACL is a band of dense connective tissue located within the knee joint and outside the synovial membrane. It runs in an oblique direction from the posteromedial edge of the lateral femoral condyle to the anterior intercondylar fossa on the tibial plateau (Figure 1.1). There are disagreements among researchers and surgeons regarding the composition, and therefore the micro function, of the ACL. The seminal study from Arnoczky (1983) reported that the ACL consists of multiple collagen bundles which shape its multifascicular nature, allowing different portions of the ACL to perform distinct functions during a range of motion. In contrast, Amis and Dawkins (1991) and Colombet et al. (2006) suggested that the ACL has two functional parts: the anteromedial bundle (AMB) and the posterolateral bundle (PLB). According to these authors, the AMB tightens during knee flexion, while the PLB tightens during knee extension, assigning specific functions to each bundle. However, this anatomical

and functional description was derived from an experimental study not involving loading conditions, and it was not observed when certain movements are induced, such as anterior tibial loading, anterior translation, and internal tibial rotation (Gardner et al., 2015; Noyes & Barber-Westin, 2018). Gardner et al. (2015) found that both ACL bundles work in synergy to resist subluxations during the Lachman and pivot-shift tests. Similarly, a recent study by Skelley et al. (2016) invalidated the simplified description of the distinct function of the two bundles by demonstrating mechanical and structural properties of the ACL vary in a linear gradient across the ligament, rather than being specific to each bundle. Despite some remaining uncertainties regarding the exact composition and organisation of the ACL, the prevailing depiction maintains its representation as a dual bundle structure.

The ACL has a mean length of 32 mm and a width of 7-17 mm at its midsection when the knee is fully extended (Amis & Dawkins, 1991; Girgis, Marshall, & Monajem, 1975). However, there are anatomical differences between sexes, with females having shorter, thinner ACLs and smaller cross-sectional areas (Chandrashekar, Slauterbeck, & Hashemi, 2005). MRI studies have similarly shown that female individuals have smaller total condylar width, ACL cross-sectional area, and intercondylar notch width (Anderson et al., 2001). A recent consensus also highlighted a relationship between smaller knee joint geometry features and an increased risk of non-contact ACL injuries (Shultz et al, 2015). Biomechanically, evidence suggests that knee joint geometry is associated with higher-risk biomechanics. Females, with more pronounced posterior-inferior lateral tibial slopes, tend to experience greater anterior joint reaction forces (McLean et al., 2010), increased anterior tibial translation relative to the femur (Dejour & Bonnin, 1994; Giffin et al., 2004), and heightened peak anterior tibial acceleration (McLean et al., 2011). When these factors are combined with a smaller ACL cross-sectional area, they contribute to increased peak ACL strain (Lipps et al., 2012).

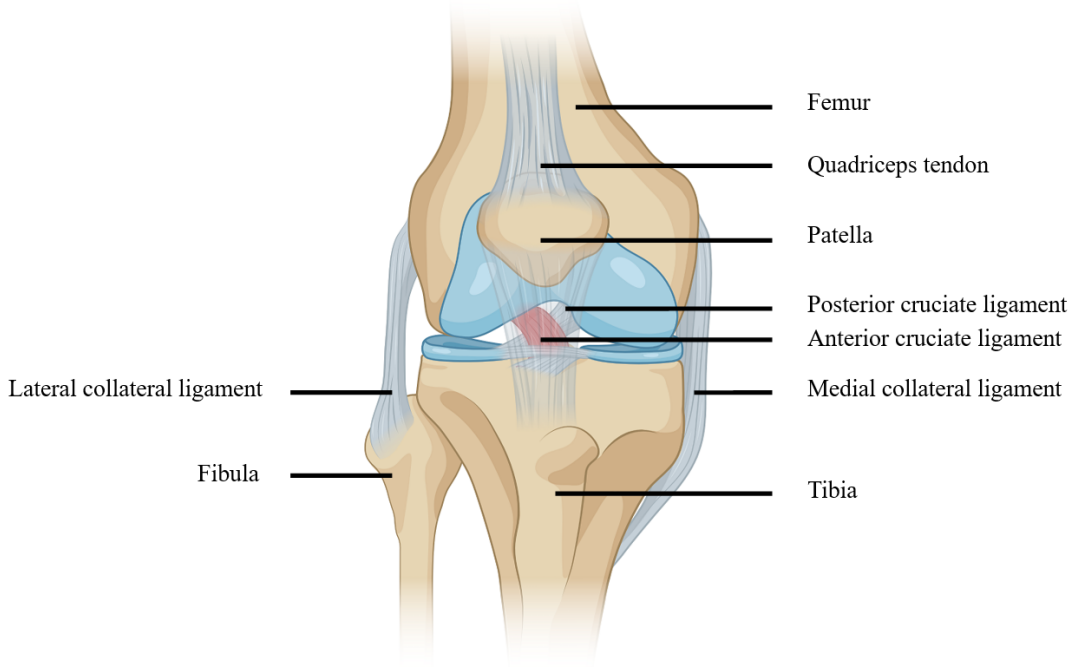

Figure 1.1. Anatomic location of the ACL in a right human knee joint. ACL, anterior cruciate ligament. The ACL is coloured in red for identification purposes. Created with BioRender.com.

1.3.1.2 Microstructure

The ACL can be divided into three distinct zones at the microscopic level. The proximal part is softer and contains a high concentration of cells, collagen type II, and glycoproteins. The middle part is characterised by a dense arrangement of collagen fibres, including an area of cartilage and fibrocartilage. It also possesses elastic components to withstand different types of stresses. The distal part is solid, with a lower density of collagen bundles, and exhibits properties similar to bone-like fibrocartilage (Duthon et al., 2006). In addition to its overall microstructure, different types of collagens are found in specific regions of the ACL: Type I collagen, the primary collagen in ligaments and tendons, provides the ACL with tensile strength; Type II collagen is exceptionally present in the fibrocartilaginous zones of the ACL, offering resistance to pressure; Type III collagen is mainly located near the attachment areas and enhances the pliability of the ACL; Type IV collagen is mainly found in the more vascularised proximal and distal portions of the ACL and; type VI collagen serves as a gliding component between fibrillar units and is predominantly found in the proximal and distal thirds of the ACL. The microstructure of the ACL resembles

that of other soft tissues but exhibits a complex ultrastructural organisation of fibrils with varied orientations of the bundles, and an abundant elastic system (Duthon et al., 2006).

1.3.1.3 Functions

The ACL plays a crucial role in the knee joint as it serves as the primary restraint against anterior tibial translation in relation to the femur (Butler, Noyes, & Grood, 1980). When the ACL is intact, it effectively limits anterior tibial translation in a neutral position. However, in case of ACL injury, the anterior tibial translation increases by four times, which poses a risk of damage to collateral structures (Beynnon et al., 2002). During an anterior load applied at 30° of flexion, the intact ACL restrains approximately 82-89% of the anterior tibial translation. This restraint slightly decreases to 74-85% when the anterior load is applied at 90° of flexion (Beynnon & Johnson, 1994; Woo et al., 1991). Furthermore, the ACL acts as a significant secondary restraint to knee internal rotation, particularly as the knee joint approaches full extension. It also serves as a minor restraint against knee external rotation and varus-valgus movements, especially during weight-bearing situations (Beynnon et al., 1997; Matsumoto et al., 2001). Studies investigating the structural properties of intact ACLs have found that the ultimate load to failure is age- and sex-dependent. In young adults, the ultimate load to failure averages around 2160 ± 157 Newtons (N). However, these values decline with age, as specimens over 60 years exhibit an average ultimate load to failure of 658 ± 129 N (Chandrashekar et al., 2006; Woo et al., 1991).

1.3.2 Sex disparity in ACL injury incidence and outcomes

ACL injuries are regarded as among the most severe injuries athletes can experience during their active years, leading to significant time away from training and competition and creating uncertainty about the future of their athletic endeavours. Important sex disparities have also been observed in the outcomes of these injuries. Larruskain et al. (2018) conducted a study comparing injury rates and types in elite male and female football players over a period of five seasons and found that female football players had a higher incidence of severe joint/ligament injuries, including a nearly five-fold higher risk of suffering an ACL rupture. In addition, injured female football players also required approximately twice the recovery time compared to their

male counterparts, with ACL injuries accounting for 43% of all absences in female football players.

Recent research has highlighted a considerably higher incidence of non-contact ACL injuries in female athletes, particularly those involved in team-ball sports, with female athletes experiencing 0.14 non-contact ACL injuries per 1000 player-hours, compared to 0.05 per 1000 player-hours for males (Chia et al., 2022). A systematic review and meta-analysis by Bruder et al. (2023) further emphasised that female athletes face more significant declines in activity levels and knee-related outcomes within the first ten years post-ACL injury, compared to their male counterparts. Additionally, female athletes have 25% lower chance of returning to their sport within the first five years following an ACL injury, regardless of age. This disparity in recovery outcomes may be due to a complex interplay of knee-related and non-knee related factors, including biological and sociocultural (psychological, rehabilitation, societal roles) influences. Lastly, a systematic review conducted by Hong et al. (2023) revealed that female football players have a secondary ACL injury incidence rate of 27%, whereas male football players exhibited a rate of 10%, underscoring the increased vulnerability of female athletes to recurrent ACL injuries.

1.3.3 Non-contact ACL injury risk factors

The sex disparity in non-contact ACL injuries begins at the onset of puberty and peaks at this stage of life (Beck et al., 2017). In the population of adolescent athletes, it has been observed that female athletes experience nearly 1.5 times more ACL injuries compared to their male counterparts across all sports and all settings. Among adolescent female athletes, football/soccer, gymnastics, lacrosse, and basketball are identified as the sports associated with the highest risk for ACL injuries. Notably, basketball, football/soccer, baseball, and track and field demonstrate a significant relative risk of 4.14, 3.10, 2.68, and 3.20, respectively, for sustaining an ACL injury among adolescent female athletes compared to age-matched males (Bram et al., 2021). In parallel, young females exhibit steadily increasing ACL injury incidences with an annual growth rate of 10,4% in the 5 – 14 years old group, and 6.6% in the 15 – 24 years old group in Australia, for example, in the past two decades (Maniar et al., 2022). Furthermore, an analysis of paediatric ACL injuries in the general population of Finland revealed that females aged 13 −15 years exhibited the

most significant increase, *i.e.*, 143%, in ACL injury incidence over an 18-year study period compared to other age/sex groups (Weitz, Sillanpää, & Mattila, 2020). The risk of primary non-contact ACL injury during adolescence differs between sexes and has been associated with various specific changes that occur in young males and females during physical maturation. These changes include anatomical factors such as body composition and knee joint laxity, genetic factors, physiological factors related to hormones, biomechanical aspects, and neuromuscular recruitment patterns (Griffin et al., 2006). Additionally, gender-related factors in the developmental environment also contribute to this risk (Parsons, Coen, & Bekker, 2021). However, non-contact ACL injuries are mostly not the result of a single factor, but rather the interaction of multiple, often overlapping, factors. The multi-faceted nature of these injuries suggests that a combination of anatomical, hormonal, biomechanical, and neuromuscular components – each influenced by both intrinsic and extrinsic factors – contribute to the injury risk profile (Beynnon et al., 2005; Hewett et al., 2005; Shultz et al., 2004; Uhorchak et al., 2003; Zazulak et al., 2007). Despite this complexity, there remains limited understanding of some of these risk factors and how they interact and converge, complicating efforts to define and mitigate the risk of non-contact ACL injuries in female adolescents and adults (Shultz et al., 2019). Recent studies by Cone et al. (2019b) and Howe et al. (2022) have investigated biomechanical function and size of ACL bundles in a porcine model, revealing age and sex-dependent changes during skeletal growth. While these translational animal studies have provided insights into the structural development of the ACL during growth and offered potential explanations for observed ACL injury rates, it is important to note that knee anatomy and biomechanics in humans may differ slightly from those in animals.

Despite the increasing participation of females in elite-level sports competitions, it remains uncertain whether the onset and progression of menopause have an impact on the incidence of first-time non-contact ACL injuries in female athletes and exercisers experiencing hormonal changes. A thorough search of the literature has yielded no reports specifically addressing this issue. However, a study conducted by Hart and Achari (2010) examined metabolism in knee connective tissues following ovariohysterectomy in a rabbit model. The study observed distinct changes in cell metabolism two months after induced menopause, potentially contributing to an elevated risk of injury and/or degenerative conditions. Gaining a deeper

understanding of each contributing factor and its interaction with others is crucial for effectively addressing the increased risk of primary non-contact ACL injuries in female athletes and exercisers throughout their lifespan.

While it is currently not possible to directly and non-invasively measure the mechanical properties of the ACL, clinical tests of knee laxity, such as the Lachman test, can be conducted either manually or mechanically by trained practitioners. These tests provide valuable qualitative and quantitative information about the structure and integrity of the ACL. As discussed in Section 1.3.1, the main role of the ACL is to preserve the integrity of the knee joint by restricting anterior tibial translation or displacement – commonly referred to as anterior knee laxity (AKL) – as well as internal rotation relative to the femur. Ligament laxity, also known as elasticity – which is the opposite of ligament stiffness – refers to increased passive joint mobility due to reduced stiffness or altered mechanical properties of ligaments, such as the ACL. Elevated laxity can compromise joint stability, reducing the ligament's ability to resist high loads during dynamic movements and increasing injury susceptibility (Beynnon et al., 2005; Shultz et al., 2004). Numerous studies have consistently demonstrated that greater AKL is a well-established risk factor that significantly predicts the likelihood of ACL injury (Kramer et al., 2007; Myer et al., 2008; Scerpella, Stayer, & Makhuli, 2005; Uhorchak et al., 2003; Vacek et al., 2016; Woodford-Rodgers, Cyphert, & Denegar, 1994). As females mature, they tend to exhibit increased levels of AKL (Falciglia et al., 2009; Shultz, Nguyen, & Schmitz, 2008; Ahmad et al., 2006), coinciding with a higher risk of non-contact ACL injuries compared to males (Beck et al., 2017; Stracciolini et al., 2015). Moreover, females are more susceptible to experience sharp increases in AKL while exercising (Shultz et al., 2013) and across the MC (Deie et al., 2002; Eiling et al., 2007; Heitz et al., 1999; Shultz et al., 2004; Shultz et al., 2010; Shultz, Schmitz, & Beynnon, 2011) (see Chapter 3, Section 3.3.2 and Appendix F).

Ovarian steroid hormones have a significant impact on the structure of soft tissues, including muscles, tendons, ligaments, and the ACL. These hormones play a role in determining collagen metabolism and the structural integrity of ligaments in both animal (Liu et al., 1997) and human models (Konopka et al., 2016; Lee et al., 2004; Yu et al., 2001). A series of studies by Liu et al. (1996, 1997) and Hamlet et al.

(1997) have identified receptor sites for oestrogen, progesterone, and androgen receptors in human female ACLs, suggesting that sex hormones can directly influence the structure and composition of the ACL. In vitro studies by Yu et al. (1999, 2001) showed that increasing oestradiol levels reduced type I procollagen synthesis in human ACL fibroblasts, with partial buffering from progesterone. Similarly, Lee et al. (2015) found that physiological oestrogen exposure inhibited lysyl oxidase (LOX) – a key enzyme for collagen cross-linking – leading to reduced ligament stiffness without altering collagen content. These findings suggest that hormonal modulation of fibroblast activity and collagen structure through acute fluctuations in ovarian steroid hormone levels may transiently increase ligament laxity, thereby altering the ACL structure, increasing the risk of ligament failure and elevate the risk of non-contact ACL injury (Lee et al., 2004; Yu et al., 2001). It has been proposed that the female ACL and musculoskeletal system respond to hormonal changes, leading to shifts in tissue properties at various life stages, depending on reproductive hormone profiles (Chidi-Ogbolu & Baar, 2018). It is critical to prevent non-contact ACL injuries, not only to ensure the longevity and stability of female athletes' careers, but also to preserve the integrity of their knee joint and lower the risk of future knee osteoarthritis (OA), leading to a healthier ageing process. The potential role of ovarian steroid hormones in non-contact ACL injury mechanisms warrants greater attention, given the significant differences in reproductive hormone concentrations between biological sexes and across the female lifespan.

1.4 Female endocrinology

1.4.1 The menstrual cycle

The menstrual cycle (MC) is a natural monthly process that occurs from menarche to menopause in females, preparing the human body for pregnancy. A full cycle is measured from the first day of menses to the first day of the next menses, averaging 28 days in length (Mihm, Gangooly, & Muttukrishna, 2011), though it can range from 25 to over 30 days (Bakos et al., 1994). The typical MC is divided into three phases, or hormonal environments: (i) the follicular phase (days 1-14, characterised by low oestrogen and progesterone), (ii) the ovulatory phase (day 14, marked with high oestrogen and low progesterone); and (iii) the luteal phase (day 14-28, with high oestrogen and progesterone during the mid-luteal phase) (Janse de Jonge, Thompson, & Han, 2019; Reed & Carr, 2018). However, recent recommendations for

research in sport and exercise science, particularly those by Elliott-Sale et al. (2021), suggest that dividing the MC into four hormonal profiles, which correspond to distinct changes in oestrogen and progesterone levels, may offer more precision in studying the effects of endogenous ovarian steroids on physiology. These four phases are defined as: (i) phase 1, starts with the onset of menses until day 5, where both oestrogen and progesterone levels are low; (ii) phase 2, occurs in the 14 to 26h prior to ovulation and the luteinising hormone (LH) surge, where oestrogen levels are higher than in other phases; (iii) phase 3, ovulation occurs, marked by a positive urinary ovulation detection test, lasting for 24 to 36h, with oestrogen levels higher than in phase 1 but lower than in phase 2 and 4; and (iv) phase 4, takes place seven days after confirmation of ovulation, where oestrogen levels are higher than phase 1 and 3 but lower than phase 2, and progesterone reaches its peak level (Figure 1.2). These phases reflect the dynamic chronic fluctuations in hormones, primarily oestrogen, progesterone, luteinising hormone (LH), and follicle stimulating hormone (FSH). The hypothalamus releases gonadotrophin-releasing hormone (GnRH), which stimulates the pituitary gland to secrete FSH and LH, regulating the production of the ovarian hormones oestrogen and progesterone.

Historically, females have been underrepresented in medical, sports, and exercise research (Costello, Bieuzen, & Bleakley, 2014; Cowley et al., 2021; Liu & Mager, 2016), largely due to assumptions that female responses mirrored those of males in clinical trials. At the same time, females were viewed as more costly and complex participants because of their hormonal fluctuations (Schiebinger, 2003). This sex and gender imbalance in research has contributed to gaps in both health and sports science. However, growing awareness of these issues (Parekh et al., 2011), along with increased female participation in sports, has spurred a demand for deeper understanding of how hormonal fluctuations affect female athletic performance.

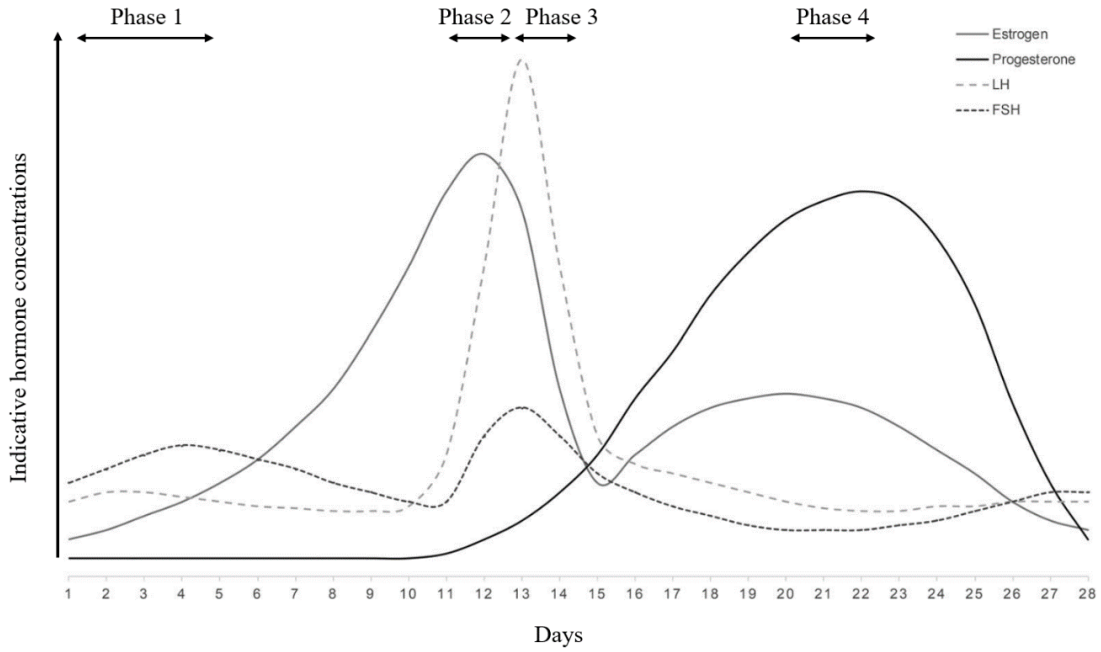

Figure 1.2. Endogenous reproductive hormone fluctuations over a typical 28-day menstrual cycle, with ovulation on day 14, and the four key phases of interest (Elliott-Sale et al., 2021). Adapted from Janse de Jonge, Thompson, and Han (2019). Permission to reproduce this figure has been granted by Wolters Kluwer Health, Inc.

1.4.2 Hormonal contraceptive use

The oral contraceptive pill (OCP) contains synthetic steroid hormones, either progestin alone or a combination of progestin and oestrogen, which regulates oestrogen and progesterone levels throughout the MC (Figure 1.3). While primarily used as a method of birth control, OCPs are also commonly used for MC manipulation, reducing the risk of iron deficiency anaemia by decreasing menstrual blood loss, and managing premenstrual symptoms (Bennell, White, & Crossley, 1999; Schaumberg et al., 2018). These additional benefits may explain the higher prevalence of OCP use among physically active female athletes and exercisers compared to their sedentary or inactive peers (Fisher et al., 2015). The use of HC differs between the general population and elite female athletes in the United Kingdom. Among the general female population, approximately 30% use HCs (Cea-Soriano et al., 2014), while the rate rises to nearly 50% among elite female athletes (Martin et al., 2018). The most commonly used form of HC is the combined OCP, which contains both oestradiol and progestogen. Combined OCPs account for 54% of HC use in the general population (Cea-Soriano et al., 2014) and 68.5% among elite female athletes in the United Kingdom (Martin et al., 2018). Most of these combined OCPs are monophasic, delivering a consistent daily dose of oestradiol and progestogen during the active pill-taking days (Martin et al., 2018). Typically, these pills follow a 21/7-day regimen, with 21 days of fixed doses of oestradiol and progestogen and a 7-day hormone-free interval, during which no exogenous ovarian steroid hormones are taken. There is a range of ethinyloestradiol doses and progestogen types, doses, potency, and androgenicity among different brands of combined monophasic OCP (Burrows & Peters, 2007). The effects of chronic OCP use on athletic performance remain unclear, and large interindividual variability in response to OCP use exists, which necessitates further research (Lebrun, 1994).

There is a significant inter and intraindividual variability in the characteristics of the MC among female individuals who do not use HCs. Even among reproductive-aged females with regular MC, not all of them experience the typical timely predicted monthly hormonal variations, either regarding absolute hormone levels and/or the changes in hormone levels across the MC, as described in Figure 1.2 (Dam et al., 2022; MacNutt et al., 2012). Moreover, female athletes and exercisers commonly experience menstrual disturbances, with a high prevalence of anovulatory cycles, luteal phase

deficiency, and amenorrhea (De Souza et al., 2010; Schaumberg et al., 2017). While most female athletes and exercisers do not experience major MC disturbances, they often report various symptoms affecting their daily life and sports practice. A study conducted in the UK by Martin et al. (2018) revealed that the most common physical MC symptoms experienced by participants occurred in the first two days of menstruation. These symptoms included stomach cramps/abdominal pain (over 40%), unspecified cramps (over 20%), back pain (over 15%), headache/migraine (less than 10%), and bloating, nausea/sickness/vomiting, or tiredness/fatigue/lethargy (less than 15%). Additionally, 4% of the participants stated that they voluntarily refrained from exercise during certain phases of their MC.

In a recent study by Dam et al. (2022), researchers explored the variations in strength and power performance among healthy, relatively untrained eumenorrheic females, and combined OCP users during the MC and HC cycle. The study involved participants to perform physical performance series throughout the MC or HC cycle while also completing a questionnaire about their psychological well-being during each testing session. The results indicated that countermovement jump (CMJ) height and performance in the Wingate bike test were significantly lower at the beginning and end of the MC, specifically during the early follicular and late luteal phases, compared to other phases. Concurrently, physical pain levels were reported to be higher during the early follicular and late luteal phases, while pleasure levels were lower during the early follicular phase relative to other phases of the MC. Furthermore, the study found a positive correlation between physical performance outcomes and various self-reported measures, such as motivation, perception of one's physical performance level, pleasure level, and arousal level. Interestingly, while no significant correlations were observed between variations in ovarian steroid hormones and performance parameters, physical performance did significantly fluctuate with changes in psychological and physical well-being, such as self-reported motivation, perception of own physical performance level, pleasure level, and arousal level, among eumenorrheic participants.

Another study by Bruinvels et al. (2021) examined the prevalence and frequency of MC symptoms in a larger sample of internationally exercising females using an exercise application. The most prevalent symptoms reported included mood

changes or anxiety (90%), tiredness or fatigue (86.2%), stomach cramps (84.2%), and breast pain or tenderness (83.1%). Notably, nearly 50% of the participants stated that they modified or missed training sessions due to these symptoms; however, only 6% reported missing a sporting event or competition as a result of severe symptoms. This suggests that, despite experiencing significant MC symptoms, female exercisers and athletes often continue to compete under sub-optimal physical and psychological conditions (Findlay et al., 2020). Given that injuries arise from a multifactorial system, it is essential to consider as many contributing factors as possible, including physical complaints, during injury surveillance to improve the effectiveness of injury risk reduction strategies (Bolling et al., 2018; Whalan, Lovell, & Sampson, 2020).

The year 2015 marked a significant turning point in MC research. In an interview with BBC Sport, Paula Radcliffe spoke candidly about athletes' experiences with menstruation, stating that sport had "not learned how to deal" with this issue. This discussion coincided with Heather Watson attributing her first-round loss at Wimbledon to "girl things", and Annabel Croft, a former British number one female tennis player, labelling the impact of the MC on female sports performance as "the last taboo in sport" during an interview with BBC Radio 5. This media attention has since spurred increased research in the field, yet findings remain conflicting, underscoring the need for clearer insights into this important topic.

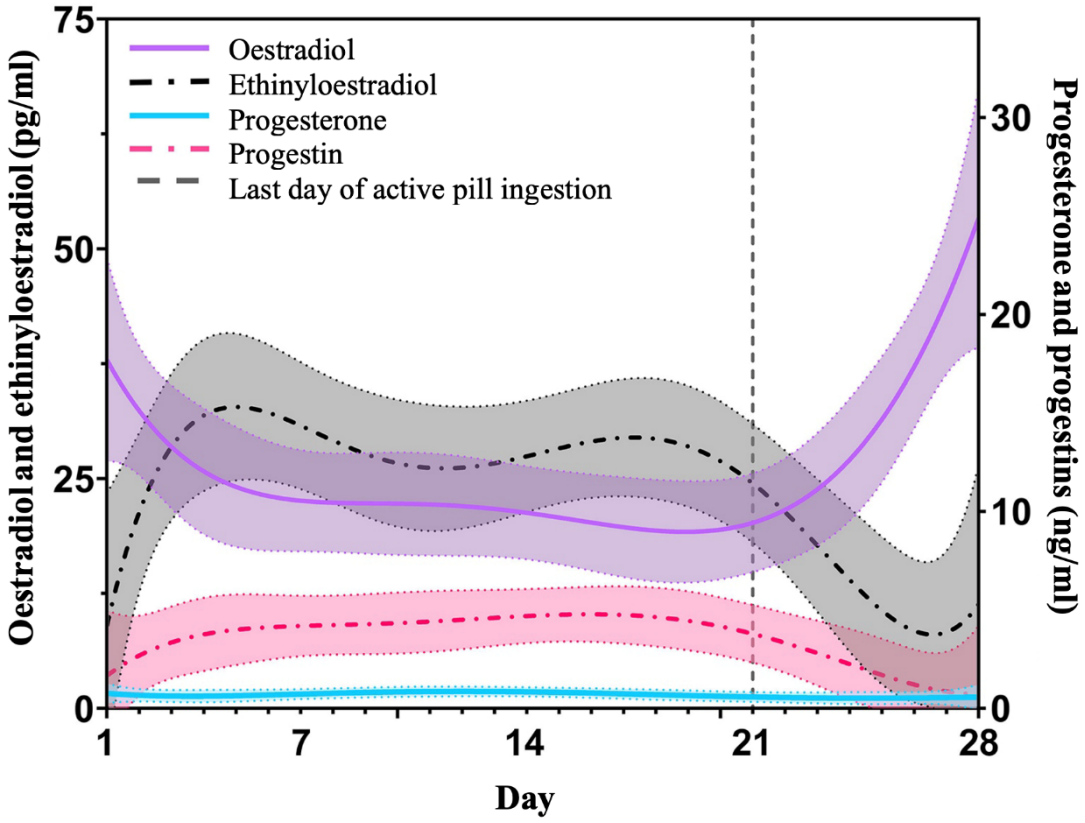

Figure 1.3. Endogenous and exogenous hormone profile across a hormonal contraceptive cycle when taking a combined monophasic oral contraceptive pill. Adapted from Rodriguez et al. (2024). Permission to reproduce this figure has been granted by The American Physiological Society.

1.4.3 Effect of menstrual cycle phase and hormonal contraceptive use on non-contact ACL injury rates

In a study conducted by Myklebust et al. (1998), differences in ACL injury rates were observed among elite male and female handball players over three seasons. Among the injured females, some were using OCPs while others were normally menstruating. Hormonal status was determined through interviews, providing what was deemed a reliable menstrual history for 17 out of the 23 injured females. ACL injuries were accurately classified based on the operating surgeon's diagnosis. Of the 17 females with reliable menstrual history, eight were OCP users and nine were normally menstruating. The researchers adjusted the MC dates to a standard 28-day cycle and categorised injuries into four MC phases – menstrual, follicular, early luteal,

and late luteal. The findings indicated a potential increased risk of ACL injury during the late luteal or menstrual phase, yet no significant difference was found in injury rates between OCP users and normally menstruating participants.

The studies by Lefevre et al. (2013), Ruedl et al. (2009) and Beynnon et al. (2006) shared strong similarities, all investigating the phase of the MC during which ACL injuries occurred in recreational alpine skiers using a pre- and post-ovulation classification, including both normally menstruating participants and those using hormonal therapy. However, it should be noted that Lefevre et al. (2013) and Beynnon et al. (2006) did not specifically assess or report the mechanisms of ACL injury, limiting their conclusions regarding non-contact ACL injuries. The MC phase at the time of injury was determined through interview (Lefevre et al., 2013), questionnaires completed within two days of injury (Ruedl et al., 2009), and questionnaires combined with serum hormone verification (Beynnon et al., 2006). All three studies observed a significantly higher occurrence of ACL injuries pre-ovulation compared to post-ovulation. Additionally, Lefevre et al., (2013) and Ruedl et al., (2009) both found that hormonal therapy provided no protective effect, challenging the earlier theory proposed by Möller-Nielsen and Hammar (1989). While these results partially align with the earlier study by Myklebust et al. (1998), they reaffirm the lack of a protective effect of OCPs against ACL injuries.

Adachi et al. (2008) studied a younger cohort of normally menstruating teenage athletes with ACL injuries. Due to the challenges of obtaining repeated hormone samples, MC phase at the time of injury was established through questionnaires. This study divided the MC into three phases – follicular phase, ovulation and luteal phase – and found significantly more ACL injuries during ovulation, which aligns with the findings of Wojtys et al. (1998), who similarly used questionnaires in adult female athletes and reported a peak in ACL injuries during the ovulatory phase.

Agel, Bershadsky, and Arendt (2006) contributed further by studying a large cohort of 2026 normally menstruating female participants and 1024 on hormonal therapy. Participants sustained either non-contact ACL injuries or ankle sprains during a basketball or soccer season. Although inconsistencies between retrospective injury recall and prospective serum hormonal data collection raised concerned about the reliability of the methods, their findings revealed a peak in ACL injuries among non-

hormonal therapy users between days 7-9 post-menses onset, partly consistent with previous studies. Those using hormonal therapy showed no clear pattern of injury occurrence across the HC, and no significant difference in injury rates between normally menstruating participants and those on hormonal therapy was observed, reinforcing the hypothesis that hormonal therapy does not provide a protective effect against injury in female athletes and exercisers. However, results of ankle sprains and non-contact ACL injury were merged, making it impossible to draw a specific conclusion for each injury category.

Overall, the literature reviewed suggests that females, both athletes and non-athletes, face a higher risk of non-contact ACL injuries in the pre-ovulation phases compared to post-ovulation. More research with powered sample size is required to pinpoint the phase or hormonal timepoint when the risk is highest, as well as the underlying mechanisms. Additional studies on hormonal therapy, examining different types, regimens, brands, and dosages are necessary to confirm the absence of a protective effect. It is also noteworthy that despite the broad focus on "injuries", only one study examined injuries beyond ACL. As other musculoskeletal injuries significantly impact female athletes, future research should explore the incidence of various injuries across the MC to determine if they follow patterns similar to ACL injuries, as found in studies by Adachi et al. (2008), Agel, Bershadsky, and Arendt (2006), Beynnon et al. (2006), Lefevre et al. (2013), Myklebust et al. (1998), and Ruedl et al. (2009). This would provide deeper insights into the mechanisms behind injury risks across the MC.

1.4.4 Effect of menstrual cycle phase and hormonal contraceptive use on anterior knee laxity

In the last two decades, many studies have shown that AKL (Deie et al., 2002; Eiling et al., 2007; Heitz et al., 1999; Khowailed et al., 2015; Lee et al., 2014; Park et al., 2009a, 2009b; Shultz et al., 2005) and the rate of non-contact ACL injuries (Adachi et al., 2008; Agel, Bershadsky, & Arendt, 2006; Beynnon et al., 2006; Lefevre et al., 2013; Myklebust et al.,1998; and Ruedl et al., 2009) vary across the MC in naturally menstruating females, but their conclusions are inconsistent.

In a study conducted by Deie et al. (2002), a small group of naturally menstruating young female volunteers were examined using an arthrometer to measure

anterior tibial displacement at different forces (89N and 134N) two or three times per week over four weeks. The MC phases of the participants were determined based on self-reported daily basal body temperature (BBT) and confirmed through weekly serum sample analysis of oestradiol and progesterone. The authors categorized the MC into three phases – follicular, ovulatory, and luteal. The results indicated that AKL was greater during the luteal phase compared to the ovulatory phase when tested at 89N. Furthermore, AKL was greater during both the luteal and ovulatory phases compared to the follicular phase when tested at 134N. However, it should be noted that serum samples were collected only once a week, and no urinary ovulation detection kit was used to confirm ovulatory cycles. This limited frequency of sample collection and lack of ovulation confirmation may have been insufficient to accurately track and report hormonal fluctuations throughout the MC.

In their study, Eiling et al. (2007) investigated variations in AKL among young female netball players with regular MCs. They used an arthrometer with a loading force of 134N to measure AKL. The participants recorded their MC characteristics for three months before and after the study to ensure they were naturally menstruating. Unlike in Deie et al. (2002)'s study, the authors divided the MC into four phases – menses, mid-follicular, ovulation, and mid-luteal. Blood samples were collected at each visit to analyse hormone levels, *i.e.*, LH, FSH, oestradiol, and progesterone, and confirm the MC phases. Although the study found a higher AKL in the ovulation phase compared to other phases, this difference was not statistically significant. These results were similar to those reported by Karageanes, Blackburn, and Vangelos (2000). However, it is important to note that Karageanes, Blackburn, and Vangelos (2000) did not use a urinary ovulation detection kit, like Eiling et al. (2007), nor did they perform blood sample analyses to confirm the participants' MC phases. Additionally, the force used in their testing was 89N, whereas a minimum force of 133N is recommended for optimal testing of soft tissue involvement, specifically the ACL, in anterior tibial displacement. This higher force creates more tension on the knee joint, as suggested by Highgenboten et al. (1992). The lack of methodological rigor in assessing MC phases in the study by Karageanes, Blackburn, and Vangelos (2000) and the younger age of the participants in both studies may also contribute to the absence of significant AKL variations across the MC.

Heitz et al. (1999) examined AKL in a small sample of active adult females (N = 7) on various days of the MC. They used an arthrometer with a force of 133N to measure AKL. The authors divided the MC into three phases – menstrual, follicular, and luteal. The participants self-reported their MC phase, which was then confirmed by analysing serum samples for oestrogen and progesterone levels at each visit. The study found that AKL values were significantly higher during the follicular and luteal phases compared to the menstrual phase, with highest AKL values observed in the luteal phase. These findings align with the results reported by Deie et al. (2002), although it is important to note that the sample size in Heitz et al. (1999)'s study was relatively small.

In their study, Khowailed et al. (2015) investigated AKL in a small sample of normally menstruating female runners. They measured AKL at two specific points in the MC, on day 1 and day 2 of the MC and within 24 hours of a positive urinary ovulation test, using an arthrometer with a force of 133N. To confirm the MC phase of the participants during both visits, serum samples were analysed for oestradiol levels. The study found that AKL was significantly higher during ovulation compared to the menstrual phase. These findings support previous studies conducted in this area, except for the studies by Eiling et al. (2007) and Karageanes, Blackburn, and Vangelos (2000).

Another research study, Lee et al. (2014), examined AKL in two groups: naturally menstruating female adults and regular OCP users, using an arthrometer with a force of 133N. The MC was divided into four phases – menstrual, follicular, ovulation, and luteal. The OCP users were tested at specific timepoints of their HC cycle, including the withdrawal bleed and three occasions in the OCP active phase that corresponded to distinct timepoints in the naturally menstruating participants' MC. The participants self-reported their MC phase, and oestradiol serum samples were analysed to confirm the phase or timepoint of the MC or HC cycle. The study found that AKL was significantly higher during ovulation compared to the menstrual and follicular phases, and also during the luteal phase compared to the menstrual phase. These findings align with previous studies that have observed variations in AKL across the MC in regularly menstruating participants. Furthermore, the study reported that AKL was significantly lower in OCP users compared to naturally menstruating participants.

Park et al. (2009a, 2009b) investigated AKL in a larger sample of naturally menstruating female participants throughout the MC, using an arthrometer with a force of 89N and at manual maximum force. The MC was divided into three phases – follicular, ovulation, and luteal. The participants were asked to self-report the occurrence of their period and to use urinary ovulation detection kits. Additionally, serum samples were analysed for oestradiol and progesterone to confirm the MC phase during each visit. The study found that AKL was significantly higher during ovulation compared to the luteal phase, supporting findings reported by previous studies in this section. One notable strength of this study was the improved methodological quality, particularly in terms of the larger sample size (N = 26) and the use of urinary ovulation detection kits in combination with the analysis of oestrogen and progesterone serum samples to identify and validate the MC phases.

Perhaps more well known within the literature, Shultz et al. (2005) conducted a comprehensive assessment of AKL in a larger sample of naturally menstruating active female participants throughout the MC, using an arthrometer with applied forces of 46N, 89N, and 133N. To facilitate comparison, the MC was divided into four phases based on daily measurements: menstrual, initial oestrogen rise, early luteal, and late luteal. Participants were asked to self-report the occurrence of their period and to use urinary ovulation detection kits. Serum samples were collected and analysed for oestradiol, progesterone, and testosterone to confirm the phase of the MC during each visit. The study found that AKL significantly increased in the days preceding ovulation and during the early days of the luteal phase when compared to the menstrual or follicular phase. This indicates a potential association between the increase in AKL and the rise in oestradiol levels. These results align with the observations made in previous studies mentioned in this research area. However, what distinguishes this study from others in the field is its high methodological quality. This is reflected in the larger sample size (N = 22) and the rigorous approach of identifying and validating the MC phases using urinary ovulation detection kits in conjunction with daily analysis of oestrogen, progesterone, and testosterone serum samples.

A consistent trend can be observed across studies conducted by Deie et al. (2002), Heitz et al. (1999), Khowailed et al. (2015), Lee et al. (2014), Park et al. (2009), and Shultz et al. (2005). These studies suggest that AKL tends to be higher around

ovulation compared to the early follicular phase. Additionally, there is a potential for AKL to be higher during the luteal phase compared to the early follicular phase. However, it is important to note that these studies varied in their designs and methodological quality. The diverse study designs and variations in methodological rigor among the studies reviewed in this section highlight the need for future studies to address these issues to establish a potential consensus on the relationship between AKL and ovarian steroid hormone fluctuations. By improving study designs and ensuring higher methodological quality, future research can provide more reliable and conclusive evidence in this area.

In contrast to the studies mentioned earlier, several other studies, including those conducted by Beynnon et al. (2005), Carcia et al. (2004), and Van Lunen et al. (2003), did not find any significant changes in AKL across the MC phases in naturally menstruating females. Beynnon et al. (2005) suggested that their stricter inclusion criteria for acceptable levels of oestradiol and progesterone serum concentrations may have contributed to the different observations regarding AKL variations. Carcia et al. (2004) mentioned that their limited hormone measurements throughout the MC might have missed capturing peak oestradiol levels, and their mean oestradiol value was lower than the expected physiological range during the ovulatory phase. They also emphasised the substantial variation in hormone fluctuations across the MC, making it challenging to precisely capture the current hormonal status of participants within narrow testing windows. Similarly, Van Lunen et al. (2003) stated that they might have missed the peak oestradiol levels as their testing was conducted approximately 20 hours after a positive ovulation test, potentially explaining why they did not observe any AKL changes across the MC. Furthermore, Van Lunen et al. (2003) consistently tested participants on day 23 of the MC, representing the assumed luteal phase, rather than after a specific number of days following confirmed ovulation through serum analyses and the use of urinary ovulation detection kits, as recommended in recent methodological guidelines (Janse de Jonge, Thompson, & Han, 2019)

In parallel, some studies have examined the effects of HCs, especially OCPs, on AKL variations (Casey, Hameed, & Dhaher, 2014; Hicks-Little et al., 2007; Lee et al., 2014; Shultz et al., 2012b) and on non-contact ACL injuries (DeFroda et al., 2019, Rahr-Wagner et al., 2014, Wojtys et al., 2002) but their conclusions are also

inconsistent (Herzberg et al., 2017; Konopka, Hsue, & Dragoo, 2019). For instance, Hicks-Little et al. (2007) showed that AKL increased from the third week to the first week of a HC cycle among OCP users, noting that OCP users had a greater AKL compared to their non-OCP counterparts. Conversely, other studies (Casey, Hameed, & Dhaher, 2014; Lee et al., 2014; and Shultz et al., 2012b) found no significant variations in AKL throughout the HC cycle among OCP users. A critical issue in these studies is that authors either failed to specify the type, brand, regimen, and dosage of the OCP used by participants (as seen in Lee et al. [2014] and Hicks-Little et al. [2007]), or, when such information was provided (as in Shultz et al. [2012b] and Casey, Hameed, & Dhaher, [2014]), all OCP users were combined into a single group without conducting further sub-analyses. Therefore, these inconsistencies in findings regarding the MC phase and OCP use may stem from methodological weaknesses, particularly regarding the definition, identification, and verification of MC phases, as well as the heterogeneity of HCs employed in these studies (for a comprehensive overview of methodological issues see Elliott-Sale et al. 2021).

It is clear, despite the growing body of evidence, there is large disparity in methodology used between research groups in relation to ACL injury incidence and the influence of fluctuating ovarian steroid hormones on AKL. Therefore, inconsistent findings are apparent, which ultimately make it difficult to draw firm conclusions to then use within clinical and applied settings.

The aim of the PhD is to explore the potential contributing mechanisms of ACL injuries in female athletes and exercisers; and add, with detail, gold standard methodologies, and firm findings pertaining to risk factors and screening for ACL injury.

The aim will be achieved by the following objectives:

1. Summarise and critical appraise of the existing literature with a systematic review and meta-analysis;
2. Screen a new device for the assessment of AKL in an active population of male participants and female OCP users;
3. Consider methodological issues in the assessment of muscle force output and propose improvements;

4. Explore neuromuscular factors that may contribute to the risk of non-contact ACL injury in eumenorrheic females.

The focus of this thesis is to add to current knowledge an understanding of the interaction between endogenous and exogenous ovarian steroid hormones and a physiological marker of ACL injury risk, *i.e.*, variations of AKL, and some neuromuscular factors which might contribute to non-contact ACL injury incidence in females. The findings from this project add more robust and conclusive evidence to the body of knowledge, which is currently conflicted.

Chapter 2. Effect of menstrual cycle phase, menstrual irregularities, and hormonal contraceptive use on anterior knee laxity and non-contact anterior cruciate ligament injury occurrence in women: a protocol for a systematic review and meta-analysis

Authors: **Elisa Nédélec**, Elvis Foli, Sandra J Shultz, Paul A Swinton, Eimear Dolan, Kevin Enright, Jessica Piasecki, Joseph J Matthews, Craig Sale, Kirsty Jayne Elliott-Sale

Keywords: Anterior Cruciate Ligament, Menstrual Cycle, Menstrual Irregularities, Hormonal Contraceptives, Oral Contraceptive Pill, Laxity, Injury; Anterior Knee

This protocol for a systematic review and meta-analysis was published in *BMJ Open Sport & Exercise Medicine* in October 2021. Please note that this chapter is presented in the journal format, but has been numbered [section headings, tables and figures] in line with this thesis and some additions have been made to the introduction and the discussion for the purposes of the thesis submission.

2.1. Abstract

Exercising women report three to six times more ACL tears than men, which happen, in the majority of cases, with a non-contact mechanism. This sex disparity has, in part, been attributed to the differences in reproductive hormone profiles between men and women. Many studies have shown that anterior knee (AK) laxity and the rate of non-contact ACL injuries vary across the menstrual cycle, but these data are inconsistent. Similarly, several studies have investigated the potential protective effect of hormonal contraceptives on non-contact ACL injuries, but their conclusions are also variable. The purpose of this systematic review and meta-analysis is to, identify, evaluate and summarise the effects of endogenous and exogenous ovarian hormones on AK laxity (primary outcome) and the occurrence of non-contact ACL injuries (secondary outcome) in women. We will perform a systematic search for all observational studies conducted on this topic. Studies will be retrieved by searching electronic databases, clinical trial registers, author's personal files and cross-referencing selected studies. Risk of bias will be assessed using the Newcastle Ottawa Quality Assessment Scale for Cohort and Case–Control Studies. Certainty in the cumulative evidence will be assessed using the Grading of Recommendations Assessment, Development and Evaluation approach. The meta-analyses will use a Bayesian approach to address specific research questions in a more intuitive and probabilistic manner. This review is registered on the international database of prospectively registered systematic reviews (PROSPERO; CRD42021252365).

2.2 Key Messages

What is already known?

- Female athletes and exercisers report three to six times more anterior cruciate ligament (ACL) injuries than men. Most of them happen with a non-contact mechanism.
- The differences in reproductive hormone profiles between men and women have been identified as a risk factor for non-contact ACL injuries.
- Despite many studies on variations of anterior knee (AK) laxity and the rate of non-contact ACL injuries amongst normally menstruating participants and hormonal contraceptive users, the inconsistency of the literature makes it difficult to evaluate the effects of endogenous and exogenous ovarian hormones on AK laxity and the occurrence of non-contact ACL injuries.

What this study could add:

- By including both anterior knee (AK) laxity and the occurrence of non-contact ACL injuries in naturally menstruating women, women with menstrual irregularities and hormonal contraceptive users, we will provide a detailed summary and interpretation of the current state of the art of this topic, following a theoretical biological pathway from mechanism to outcome.
- This study will be the most comprehensive review to date of AK laxity and the occurrence of non-contact ACL injuries, due to the diversity of included participants.
- The findings of this review will make the available evidence more accessible to practitioners and will therefore have practical implications for exercising women.

2.3 Background

The participation of girls and women in sport has increased worldwide, both in recreational and professional practice (International Olympic Committee, 2020; The Football Association, 2020). This growth in and development of women's sport has resulted in a growing number of reports regarding the nature and rate of injuries sustained by sportswomen (DiStefano et al., 2018). Depending on the age-group, the sport and the level of practice, women report different rates of musculoskeletal, sports-related injuries than their male counterparts (Sigward, Pollard, & Powers, 2012). As one of the most prominent musculoskeletal injuries, exercising women report three to

six times more ACL tears than men (Agel, Rockwood, & Klossner, 2016), which occur, in the majority of cases, via a non-contact mechanism (Olsen et al., 2004). Most non-contact ACL injuries happen during fast-paced multidirectional activities (*e.g.*, snow skiing, netball, football, rugby, gymnastics; Agel et al., 2007). The sex disparity for non-contact ACL injuries starts at the adolescent growth spurt and peaks during adolescence (Beck et al., 2017). This sex difference has been attributed to several factors that also emerge at this time, namely: anatomical (*e.g.*, laxity, body composition), physiological (especially hormonal), biomechanical, neuromuscular recruitment patterns (Griffin et al., 2006) and gendered factors present in the developmental environment (Parsons, Coen, & Bekker, 2021). The potential impact of hormones on the mechanisms underpinning non-contact ACL injuries deserves greater attention given the numerous differences in the concentration of reproductive hormones between sexes and the time course of reproductive endocrinology, especially in women.

Ovarian hormone profiles vary between and within women and are not stable over a women's lifespan (*e.g.*, they change across phases of the menstrual cycle, as a result of hormonal contraceptive use, during pregnancy and following menopause). Ovarian hormones influence the structure of all soft tissues (*i.e.*, muscles, tendons, and ligaments) by determining their collagen metabolism (Liu et al., 1997: data from rabbits; Yu et al., 2001, and Konopka et al., 2016: data from human ACL cells), and structural integrity (Konopka et al., 2016: data from human ACL cells; Lee et al., 2015: data from engineered ligaments). Alterations of the ACL structure, caused by fluctuations in ovarian hormone levels, may increase the risk for potential ligament failure (Lee et al., 2015: data from engineered ligaments; Yu et al., 2001: data from human ACL cells). Indeed, it has been suggested that women's ACLs and musculoskeletal systems react to changes in the reproductive hormone milieu, thus changing their properties at certain points of the lifespan corresponding to different hormonal profiles (Chidi-Ogbolu & Baar, 2018).

In the last two decades, many studies have shown that anterior knee (AK) laxity (Shultz et al., 2005) and the rate of non-contact ACL injuries (Adachi et al., 2008) change during different phases of the menstrual cycle in eumenorrheic women, although the findings from studies in this area are inconsistent. Several studies have

also been conducted on the potential protective effect of hormonal contraceptives, especially oral contraceptive pills (OCPs), on non-contact ACL injuries, due to their users having a consistently downregulated endogenous ovarian hormone profile, although these data are also inconsistent (Herzberg et al., 2017). These inconsistencies in findings (*i.e.*, menstrual cycle phase and OCP use) might be due to poor methodological quality, especially with regards to the definition and confirmation of menstrual cycle phases and the heterogeneity of hormonal contraceptives used in these studies (for a comprehensive overview of methodological issues see Elliott-Sale et al., 2021).

A systematic review and meta-analysis published in 2017 (Herzberg et al., 2017) concluded that the quality of evidence (*i.e.*, data published up to August 2016), on the effect of the menstrual cycle and hormonal contraceptives on the laxity of the ACL and the occurrence of non-contact injuries to the ACL, was 'very low', due to numerous methodological shortcomings affecting the eligibility of the participants. Our systematic review and meta-analysis will expand the review by Herzberg et al. (2017) by: (i) including studies published up to and since August 2016, (ii) performing a meta-analysis on the injury data and not just the laxity data, (iii) employing different inclusion/exclusion criteria and (iv) including women with menstrual irregularities. In addition, our review will adopt a different statistical method (*i.e.*, a Bayesian approach) to allow for a more intuitive and probabilistic synthesis and interpretation of existing data. Therefore, the purpose of this systematic review is to identify, evaluate and summarise the effects of endogenous and exogenous ovarian hormones on knee joint laxity and occurrence of non-contact injuries of the ACL in women. We hypothesise that: (i) AK laxity will differ in response to the fluctuations in endogenous ovarian hormones that occur at different phases of the menstrual cycle, leading to an increased occurrence of non-contact ACL injury, (ii) AK laxity and the occurrence of non-contact ACL injury would be greater in non-hormonal contraceptive users.

2.4 Methods

The protocol for this aetiology systematic review and meta-analysis follows the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (Moher et al., 2015) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for Searching (PRISMA-S) (Rethlefsen et al., 2021) and is

registered with the International Prospective Register of Systematic Reviews (PROSPERO) registration number CRD42021252365.

2.4.1 Eligibility criteria

Studies will be selected according to the PECOS (*i.e.*, participants, exposures, comparators, outcomes, study designs) criteria (Table 2.1). There will be no restrictions on the time frame or setting of the studies. Studies reported in English, French, Spanish, Portuguese, and German languages will be considered. A list of possibly relevant titles in other languages will be provided as an appendix if relevant.

Table 2.1. Overview of PECOS eligibility criteria

Participants	Human female athletes (defined as one who takes part in an individual or organised team sport wherein: (i) they compete regularly against others; (ii) excellence and achievement are emphasised and (iii) systematic intensive training is required; Maron et al., 2007) and female exercisers (defined as one who engages in physical activity with the will to: (i) augment their fitness level; (ii) improve their health; (iii) ameliorate their physique and (iv) acquire or improve skills; MacMahon & Parrington, 2017) of reproductive age (*i.e.*, post-menarche and premenopausal) will be included. Specifically, eumenorrheic, naturally menstruating women, women with menstrual irregularities (*e.g.*, oligomenorrhoea, polymenorrhoea, amenorrhoea, anovulatory and luteal phase deficient cycles) and hormonal contraceptive users (*e.g.*, combined and progestogen-only OCPs, injections, implants, patches, intra-uterine systems) will be included; with pregnant and perimenopausal women excluded. Participants must not be using any form of medication known to affect ovarian hormone profiles (with the exception of hormonal contraceptives) or the musculoskeletal system.
Exposures	Of interest will be habitual exposures affecting the endogenous ovarian hormone status of the participants; that is, menstrual cycle and associated disturbances and hormonal contraceptives.
Comparators	Where relevant, hormonal contraceptive users will be compared with non-hormonal contraceptive users.
Outcomes	Outcomes relating to the physical assessment of AK laxity (primary outcomes) and the occurrence of non-contact ACL injuries (secondary outcomes). The primary outcomes are focused on micro changes (*i.e.*, physiological changes to the AK laxity that potentially occur due to changes in ovarian hormone concentrations) and the secondary outcomes are focused on macro changes (*i.e.*, number of non-contact ACL injuries that may potentially

	occur due to micro changes). AK laxity refers to the degree of tightness/looseness of the AK in a sagittal plan; in the knee, ligaments are present to connect and stabilise the various bones that are present by keeping the knee joint flexible enough to move but also firm enough to provide support. It is measured using (i) clinical examination (*e.g.*, Lachman test—manual test to assess the AK laxity; subjective measure) and (ii) equipment designed to evaluate the AK laxity by quantifying the anterior displacement of the anterior tibial tubercle relative to the femur when a predefined anteriorly directed force is applied, from the upper calf (*e.g.*, arthrometers; objective measure). Within this systematic review and meta-analysis, we will exclusively focus on studies reporting primary non-contact ACL injuries (defined as sudden-onset injuries resulting from a non-contact mechanism showing no evidence of direct or indirect physical disruption or perturbation of the player's movement pattern by an external source; Bahr et al., 2020).
Study designs	Observational studies will be considered for inclusion if they meet the following inclusion criteria: (i) published, in full, in a peer-reviewed journal; (ii) have the objective of assessing changes in AK laxity in response to phases of the menstrual cycle, menstrual irregularities and/or hormonal contraceptive use; and (iii) report the incidence of ACL injuries aligned with phases of the menstrual cycle, menstrual irregularities and/or hormonal contraceptive usage. Cohort studies and case-control studies will be included when reporting primary outcomes (*i.e.*, the physical assessment of AK laxity). Cross-sectional studies, cohort studies and case–control studies will be included when reporting secondary outcomes (*i.e.*, the occurrence of non-contact ACL injuries). Case studies, review articles, protocol papers, editorials, conference abstracts and commentaries will be excluded.

AK, anterior knee; ACL, Anterior cruciate ligament; OCPs, Oral contraceptive pills

2.4.2 Information sources

Search strategies will be developed using text words related to the population, exposures, and outcomes. Five electronic databases will be searched from their inception onwards: PubMed Central (includes MEDLINE), SPORTDiscus (via EBSCOhost interface), Scopus, the Cochrane Central Register of Controlled Trials and ProQuest Central: Health and Medical Collection; Nursing and Allied Health; Research Library: Health and Medicine. The electronic database search will be supplemented by searching for trial protocols through three registers: Clinical Trials (www.clinicaltrials.gov), EU Clinical Trials Register (www.clinicaltrialsregister.eu) and International Standard Randomised Controlled Trial Number (ISRCTN) (www.isrctn.com). To ensure literature saturation, the reference lists of included studies or relevant reviews identified, which may have been identified through the initial search strategy, will also be hand searched. All authors will search their personal files to make sure that all relevant material has been identified.

2.4.3 Search strategy

The PubMed Central search strategy will be developed with input from all authors using the Peer Review of Electronic Search Strategies standard (McGowan et al., 2016). In addition, the search strategy will be peer-reviewed by a research librarian who has expertise in systematic review searching and is not otherwise associated with the project. A draft search strategy for PubMed Central is included in Appendix B. Once the PubMed Central search strategy is finalised, the search strategy will be adapted to the syntax and subject headings of the other databases. The search will be updated toward the end of the review, prior to publication, to retrieve any articles published during the interim period.

2.4.4 Study record

All search results will be uploaded and stored in a systematic review management platform (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia), which will be accessible to all reviewers. Covidence will automatically remove duplicates by checking the following fields: titles, year, volume, authorship. Two reviewers will independently check the duplicates removed by Covidence and verify their accuracy.

Titles and abstracts will be independently screened by two reviewers, guided by the inclusion and exclusion criteria. Disagreements will be resolved with a consensus-based discussion, and, when in doubt, articles will be carried forward to full-text review. The full text of eligible papers, based on the titles and abstracts, will be downloaded, and independently screened. If the reviewers are not in agreement, a third reviewer will be consulted and will provide recommendations. The reviewers will use the annotation facility on the decision dashboard to explain their decision and inform further discussions. If a study is reported in more than one publication, the multiple reports will be collated. When in doubt regarding the eligibility criteria of a study, the reviewers will contact the authors; with a maximum of three attempts, two emails and one phone call (if possible), over a 4-week period. Any ongoing trials, which have not yet been reported, will be recorded, so that they can be added to the ongoing studies table. A PRISMA flowchart detailing the search and selection process will be included (see Appendix B for a draft template); as well as a list of all full-text studies excluded, detailing the specific reason for exclusion.

A data extraction template will be created based on those used in similar meta-analyses (Elliott-Sale et al., 2020). Data will be extracted by two reviewers. To ensure consistency across reviewers, calibration exercises will be conducted before starting the data collection process (*i.e.*, the data extraction form will be pilot tested by each reviewer on five randomly selected studies). When outcome data are not reported in a usable format (*i.e.*, in a figure instead of a numerical format) specialist software will be used to extract the data from the figure (*e.g.*, WebPlotDigitizer Version 4.4).

In order to avoid double-counting data, records will be scrupulously compared, for example, juxtaposing author names, treatment comparisons, sample sizes and/or outcomes. If the same study data are reported in more than one publication, all publications will be treated as one dataset rather than multiple datasets. When we extract data, we will prioritise the following criteria: greatest number of participants, longest follow-up and primary reports where the primary outcome assessed is most relevant to our research questions. If the data differ across publications, it will be noted, investigated, and the authors contacted for more information; with a maximum of three attempts, two emails and one phone call, over a 4-week period.

Disagreements will be recorded and resolved with a consensus-based discussion between the two reviewers. Any disagreement that cannot be resolved will be referred to a third reviewer who will provide a recommendation. Study authors will be contacted if there are any doubts about the extracted data, with a maximum of three attempts, two emails and one phone call, over a 4-week period. If any disagreement cannot be resolved (*i.e.*, either through discussion between the reviewers or with the authors) the disagreement will be reported in the review.

2.4.5 Data items

Reviewers will extract data on the following: (i) study characteristics (*i.e.*, design, location, sources of funding, study aim), (ii) participant characteristics (*i.e.*, eligibility criteria, age, height, body mass, body mass index, training status, etc), (iii) exposure and comparison characteristics (*i.e.*, type, dosage, and duration of hormonal contraceptive use, menstrual cycle phase, type of menstrual irregularity, methods of determining participants' ovarian hormonal status, etc), (iv) outcome characteristics for AK (*i.e.*, method of assessment, assessment characteristics, etc) and occurrence of ACL injuries (*i.e.*, method[s] used to confirm the injury, profile of the injury [injury mechanism, context of the injury, primary or recurrent injury, isolated ACL injury or other collateral structures injured], etc).

2.4.6 Outcomes and prioritisation

The primary outcomes are the physical assessment of AK laxity, and the secondary outcomes are the occurrence of non-contact ACL injuries.

2.4.7 Risk of bias assessment

Risk of bias will be initially evaluated at the individual study level, using the Newcastle Ottawa Quality Assessment Scale for Cohort or Case–Control Studies (Wells et al., 2013). The Newcastle Ottawa Quality Assessment Scale is a domain-based risk of bias tool that comprises eight items within three categories to assess the key bias domains: (i) selection; (ii) comparability and (iii) outcome/exposure. We have developed coding systems, that are very similar to formerly published work in our research area (Ekås et al., 2020), according to our outcomes (*i.e.*, anterior knee laxity and ACL injury occurrence) and ensured that the assessment is specific to each outcome. We have opted for using the Newcastle Ottawa Quality Assessment Scale

without the star-rating system, as the PRISMA explanation and elaboration (Page et al., 2021b) states that presenting assessments for each domain in the tool is preferable to reporting an overall 'quality score' because it enables users to understand the specific domains that are at risk of bias in each study. Accordingly, we will separate the key bias domains covered by the Newcastle Ottawa Quality Assessment Scale when assessing bias and we will present the results in a table.

2.4.8 Certainty in cumulative evidence

Certainty will be assessed by two independent reviewers using a strategy based on the recommendations of the Grading of Recommendations Assessment Development and Evaluation working group (Guyatt et al., 2011). Any differences between reviewers will be resolved by discussion and, if needed, in consultation with a third reviewer. Certainty in cumulative evidence will be based on consideration of five domains, namely risk of bias (assessed using the NOS as described above), indirectness, inconsistency, imprecision, or evidence of publication bias. Directness will be ascertained based on the methods used to identify and confirm menstrual cycle phase, along with injury confirmation. This information is considered essential, given that if unconfirmed, any result observed cannot be directly attributed to the phase under investigation. This will be evaluated based on the response to two questions:

(Q1) Was the ovarian hormone profile confirmed?

If the authors provide a definition for the sampled population and report using blood samples to confirm ovarian hormone status, the a priori rating will be maintained, if not the study will be downgraded a level (*e.g.*, a study that is classified as 'high' quality, would be downgraded to 'moderate' quality).

(Q2) Was the injury medically diagnosed either as part of the study or prior to the study?

If the authors report a medical diagnosis, the a-priori rating will be maintained, if not (*i.e.*, self-reported/unsubstantiated injury) the study will be downgraded a level (*e.g.*, a study that is classified as "high" quality, would be downgraded to "moderate" quality).

These questions are based on methodological conclusions made in previous studies (Herzberg et al., 2017; Elliott-Sale et al., 2020).

Consistency will be ascertained using the meta-analysis results and will be based on visual inspection of effect size and variance estimates across the different levels (*e.g.*, within study variation, between study variation and between outcome variation). Precision will be judged based on the number of outcomes available (with outcomes based on < 3 data points downgraded) and on interpretation of width of the credible intervals (CrIs). Small-study effects (*i.e.*, publication bias) will be visually inspected with funnel plots and quantified with a multi-level extension of Egger's regression-intercept test (Fernández-Castilla et al., 2021). Collectively, these procedures will result in a final level of certainty for each outcome (Table 2.2): namely of 'high', 'moderate', 'low' or 'very low'. This certainty appraisal strategy will not be used to exclude any study.

Table 2.2. Significance of the four certainty of evidence categories (Balshem et al., 2011).

High	Confident that the true effect lies close to that of the estimate of the effect
Moderate	Moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low	Confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect
Very low	Little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect

2.4.9 Data synthesis

Data will be presented in summary tables, which will describe the study characteristics and outcomes. A Bayesian framework was chosen over a frequentist approach as it provides a more flexible modelling approach that will enable results to be interpreted intuitively through reporting of subjective probabilities rather than null hypothesis tests or frequentist CIs (Kruschke & Liddell, 2018). For the primary outcomes comprising assessment of menstrual cycle phase, menstrual irregularities,

and hormonal contraceptive use on AK laxity, both repeated measures data and independent group data will be used to create standardised mean difference effect sizes. Standard distributional assumptions will be used to estimate within study sampling error (Morris, 2008). For repeated measures data where a correlation value is required, a standard value of 0.7 will be used to generate an informative prior with variance included to account for correlations ranging from 0.5 to 0.9. For the secondary outcome comprising the occurrence of ACL injuries, count data will be used to calculate ORs and within study sampling error. All meta-analyses will comprise a three-level hierarchical model to account for random variation across studies and covariance between multiple outcomes reported from the same study. Inferences from all analyses will be performed on posterior samples generated by Hamiltonian Markov Chain Monte Carlo with Bayesian 95% CrIs. Interpretations will be based on visual inspection of the posterior sample, the median pooled effect size value (ES0.5: 0.5-quantile) and 95% CrIs for location parameters and 75% CrIs for variance parameters. Heterogeneity will be quantified using the posterior distribution of the between study variance parameter. Where possible, meta-regressions will be used to explore sources of variance including the type of hormonal contraceptive (*e.g.*, OCPs, implants, injections, etc). Meta-regression will be performed when there is sufficient data including a minimum of four data points per category level or ten data points for continuous variables (Fu et al., 2011). Sensitivity analyses will also be conducted to assess the influence of research quality (inclusion of 'moderate' and 'high' quality studies only) on overall conclusions and effect size magnitudes. Results of meta-analyses will be presented in tables and visually through forest and funnel plots. Where quantitative pooling is not possible due to insufficient data, narrative synthesis will be conducted.

2.5 Discussion

This systematic review and meta-analysis will synthetise evidence to evaluate the effects of various levels of endogenous and exogenous ovarian hormones on AK laxity and the occurrence of non-contact ACL injuries in women. By including both AK laxity and the occurrence of non-contact ACL injuries in naturally menstruating women, women with menstrual irregularities and hormonal contraceptive users, we will provide an up-to-date, detailed summary and interpretation of the current state of the art of this topic. Furthermore, this meta-analysis will examine the strength of the

outcomes and indicate methodological considerations for future research. The findings of this review will have practical implications for female athletes (elite to recreational) and for those working with active women. As more females engage in exercise and continue to do so over a longer span of their lives, it is essential to establish a solid understanding on non-contact ACL injury risks and develop effective prevention strategies. This will ensure that female athletes and exercisers are equipped with the most accurate and up-to-date information to support their performance and health.

Chapter 3. Effect of menstrual cycle phase, menstrual irregularities, and hormonal contraceptive use on anterior knee laxity and non-contact anterior cruciate ligament injury occurrence in women: a systematic review and meta-analysis

3.1 Introduction

As mentioned earlier in Chapter 1, Section 1.1, and Chapter 2, Section 2.2.3, the growing number of physically active females in sports has led to an increase in reports about the most common injuries experienced in women's sports (DiStefano et al., 2018; Hägglund, Waldén, & Ekstrand, 2009; Larruskain et al., 2018). These reports have highlighted the existence of disparities between male and female exercisers with regards to the type and frequency injuries that is experienced. Among these injuries, one of the most serious is the non-contact anterior cruciate ligament (ACL) injury, in which the prevalence among male and female exercisers or athletes differs distinctly. Numerous factors contribute to non-contact ACL injury risk, but the focus herein is on one particular risk factor that remains globally misunderstood: the potential effect of endogenous and exogenous ovarian steroid hormones on anterior knee laxity (AKL) and ACL injury rates in female athletes and exercisers.

According to the latest systematic review and meta-analysis on this topic conducted by Herzberg et al. (2017) with a registered protocol on PROSPERO, the literature suggests that AKL and the risk of sustaining an ACL injury may be increased in the ovulatory phase of the menstrual cycle (MC). However, as highlighted by Herzberg et al. (2017), it is important to note that the existing evidence regarding the influence of the MC and hormonal contraceptive (HC) use on AKL and ACL injury risk is of notably low quality. This low quality can primarily be attributed to various methodological flaws such as the composition of the cohort and inclusion criteria. Additionally, there have been issues with outcome measurements, such as a lack of blinding of outcome assessors to the hormonal status of the participants and insufficient confirmation of ACL injuries using MRI. Furthermore, relevant analytic factors were overlooked, such as the absence of records on the level of physical activity, body mass index (BMI), or current medication usage of the participants. Moreover, the substantial variability in the methods used to assess the hormonal status of the participants significantly impacts the overall quality of the studies and hinders the comparative ability. Within previous meta-analysis it has been noted that, frequently, studies omit to confirm participants' ovulation, resulting in an inaccurate hormonal profile for the intended purpose of the study. More than a third of naturally menstruating females do not ovulate during their MC, which can subsequently affect outcome measurements and lead to flawed study conclusions if the control for

ovulation is not accurately accounted for (Herzberg et al., 2017; Prior et al., 2015). Lastly, over the last two decades, various types of tests, devices, and forces have been used to assess AKL. This diversity further complicates the comparison between studies, unless these factors are thoughtfully considered in the analyses.

There have been two recent systematic reviews of note within this topic area. Moriceau et al. (2022), investigated the influence of the MC and oral contraceptive pills (OCPs) on knee laxity or ACL injury risk. Their results aligned with those of Herzberg et al. (2017), suggesting that AKL was greater in the ovulatory phase of the MC. However, Moriceau et al. (2022) did not differentiate between contact and non-contact ACL injuries in their qualitative analyses, nor did they perform a meta-analysis of their results. The second review, conducted by Dos'Santos et al (2023), aimed to assess the effects of MC phases on ACL neuromuscular and biomechanical injury risk surrogates in eumenorrheic and naturally menstruating females. In their conclusion, Dos'Santos et al. (2023) stated that it remains uncertain whether a specific MC phase exposes eumenorrheic and naturally menstruating females to a higher non-contact ACL injury risk. However, Dos'Santos et al. (2023) did not conduct a quantitative analysis of their findings and solely focused on MC phases, without examining the potential influence of HC use. In summary, these systematic reviews (Dos'Santos et al., 2023; Herzberg et al., 2017; Moriceau et al., 2022) provide valuable insights into the potential relationship between the MC, hormonal contraceptive use, ACL injury risk, and AKL, but they are still limited in terms of the analyses performed and the aspects they considered in their investigations.

The systematic review and meta-analysis herein aims to expand the work of Herzberg et al. (2017), complement the qualitative reviews performed by Moriceau et al. (2022) and Dos'Santos et al. (2023), and offer a more thorough qualitative and quantitative analysis of the existing literature by having stricter screening criteria, which meet the complexity of the research topic. Additionally, this review addresses a gap in the previous studies by examining the potential effects of menstrual irregularities, and other forms of hormonal contraception than OCPs, on AKL and the occurrence of non-contact ACL injuries. Therefore, the aim of this chapter is to perform a systematic review and meta-analysis to identify, assess, and summarise the effects of both endogenous and exogenous ovarian steroid hormones on AKL and the

occurrence of non-contact ACL injuries in exercising females and athletes. The information presented in this chapter can be used to inform practical recommendations for female athletes, female exercisers, practitioners, and researchers who are interested in preventing the occurrence of primary non-contact ACL injuries in females.

3.2 Methods

The aetiology systematic review and meta-analysis conforms to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines (see Appendix A; Moher et al., 2015; Shamseer et al., 2015), the PRISMA-S (Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Searching) (Rethlefsen et al., 2021) and follows the previously published review protocol (Nédélec et al., 2021). The utilised protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (registration number CRD42021252365).

3.2.1 Eligibility criteria

As mentioned in the previously published protocol (Nédélec et al., 2021) the systematic review and meta-analysis incorporated studies based on the PECOS framework, encompassing participants, exposures, comparators, outcomes, study designs (Table 2.1). To be eligible for inclusion, studies had to (i) include female athletes (Maron et al., 2007) or female exercisers (Macmahon & Parrington, 2017) of reproductive age, *i.e.*, post menarche and premenopausal, who were either eumenorrheic, naturally menstruating, having menstrual irregularities, or HC users, and who did not use any form of medication affecting ovarian steroid hormone profiles or the musculoskeletal system, (ii) assess and report the endogenous ovarian steroid hormone status or HC use by the participants, and (iii) report either AKL with a quantitative measure in millimetres (mm), or the occurrence of primary non-contact ACL injuries (see Bahr et al., 2020; Nédélec et al., 2021; and Chapter 2, Table 2.1 for the definition of primary non-contact ACL injuries used in the systematic review herein). Studies that were published in full in peer-reviewed journals were considered for inclusion. Both cohort studies and case-control studies were included when reporting AKL in mm, while for reporting the occurrence of primary non-contact ACL injuries, cross-sectional studies, cohort studies, and case-control studies were considered for inclusion. Case studies, review articles, protocol reports, editorials,

conference abstracts, and commentaries were excluded. There were no restrictions on the time frame or setting of the studies. Studies reported in English, French, German, Portuguese, and Spanish languages were eligible for inclusion (Nédélec et al., 2021; Chapter 2, Section 2.4.1). Overall, the selected studies met these rigorous criteria to ensure a comprehensive and accurate analysis of the topic.

3.2.2 Information sources

The search strategies employed were developed using a combination of MeSH terms and text words relevant to the population, exposures, and outcomes of interest. The search was conducted across five electronic databases from their inception onwards: PubMed Central (including MEDLINE), SPORTDiscus (via EBSCOhost interface), Scopus, the Cochrane Central Register of Controlled Trials and ProQuest Central, specifically the Health and Medical Collection, Nursing and Allied Health, and Research Library: Health and Medicine. To enhance the comprehensiveness of the search, trial protocols were also sought through three registers: Clinical Trials (www.clinicaltrials.gov), EU Clinical Trials Register (www.clinicaltrialsregister.eu), and International Standard Randomised Controlled Trial Number (ISRCTN) (www.isrctn.com). In order to ensure thoroughness, the search process was supplemented by manually examining the reference lists of included studies and relevant reviews identified during the initial search and performing citation searches when applicable. Additionally, all designated researchers searched through their personal files to ensure that all pertinent material had been identified and included (Nédélec et al., 2021; Chapter 2, Section 2.4.2).

3.2.3 Search strategy

The PubMed Central search strategy was collaboratively developed by all authors using the Peer Review of Electronic Search Strategies (PRESS) standard (McGowan et al., 2016). To ensure the quality and robustness of the full search strategy, it was peer-reviewed by an independent research librarian. The initial full search strategy was developed for PubMed Central and is detailed in Appendix B. Once this strategy was finalised, it was adapted to the syntax and subject headings of the other selected databases to suit their requirements. To ensure consistency and accuracy in the search results, two reviewers (EF and EN) independently conducted searches across all selected databases and clinical trial registers. This approach was

implemented to verify that the full search strategies yielded identical results for each reviewer, minimising any potential discrepancies. A comprehensive literature search was initially performed in June 2021 and then rerun in October 2022. To account for any newly published literature during the review process, a PubMed email alert was set up using the full search strategy. This alert provided weekly updates on new publications relevant to the study topic. These measures were taken to ensure that the systematic review and meta-analysis remained current and incorporated the most recent and up-to-date literature available on the subject.

3.2.4 Article selection and study record

All search results were uploaded, stored, and managed in a systematic review management platform (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia), which was accessible to all reviewers. Covidence automatically identified and removed most duplicates by comparing fields such as titles, year, volume, and authorship. The duplicates identified by Covidence were then independently checked by two reviewers (EF and EN) to ensure the accuracy of the removal process. However, a few duplicates remained within the pool of selected studies. To address this issue, all the search results were exported to a Mendeley library, an academic reference management software (Mendeley Reference Manager, Elsevier, Amsterdam, the Netherlands), where the search results were examined for duplicates once again, and any remaining duplicates were manually removed.

Two reviewers (EF and EN) independently conducted the initial screening of titles and abstracts – guided by the predefined inclusion and exclusion criteria (Nédélec et al., 2021; Chapter 2, Section 2.4.4). In cases where disagreements arose between the reviewers, a consensus-based discussion was initiated to resolve the discrepancies. If necessary, a third reviewer (JJM) was consulted to provide additional input and assist in reaching an agreement. Reports that remain uncertain or ambiguous at titles and abstract screening level were retained for a full-text review. The full text of eligible reports was downloaded, and independently reviewed by EF and EN. In instances where the reviewers did not reach a unanimous decision, the third reviewer (JJM) was consulted to provide recommendations and help with the final decision. In cases where a study was reported in multiple publications, all the relevant reports were gathered and collated. If there were uncertainties regarding the eligibility criteria of a

particular study, the reviewers attempted to contact the authors up to three times – by email – over a four-week period for clarification. To ensure that data extraction stage runs smoothly and that only studies that met all inclusion criteria were analysed, EN performed a second round of screening at full-text level and found that a pool of articles (n = 26) had to be discarded.

3.2.5 Data extraction

A data extraction template was created based on templates used in similar meta-analyses conducted by Elliott-Sale et al. (2020) and McNulty et al. (2020). This template was then customised to specifically suit the requirements of the current systematic review and meta-analysis. Concurrently, a comprehensive data extraction codebook was developed, taking into consideration recommendations from the report by Elliott-Sale et al. (2021) and the latest textbook of endocrinology by Melmed et al. (2019). The aim of this codebook was to ensure accuracy and maintain consistency throughout the data collection process. Data extraction for this systematic review and meta-analysis was performed by a single reviewer (EN). The primary outcome of interest in this study is the physical assessment of AKL, while the occurrence of non-contact ACL injuries serves as the secondary outcome. In cases where outcome data were not presented in a usable numerical format but instead provided in figures or graphs, a semi-automated specialist software called WebPlotDigitizer (version 4.6) was used to extract the necessary numerical data from the figures.

To prevent duplication of data, a rigorous comparison of records was conducted, which involved examining author names, hormonal status comparisons, sample sizes, and reported outcomes. In cases where identical study data were reported in multiple publications, all relevant publications were treated as a single dataset to prevent redundancy. During the data extraction process for these studies, specific criteria were prioritised. These included selecting data from reports with the highest number of participants, the longest follow-up duration, and primary reports that aligned with our research questions. If there were discrepancies in the date across publications, these were noted and investigated. In order to obtain further information, the authors of the studies were contacted via email up to three times within a four-week period. Similarly, when data were incomplete or required complementary information, the same approach was followed by contacting the authors for

clarification. When authors were unable to provide clarification or did not respond after four weeks, the study was excluded if no relevant data could be extracted from the published version of the study.

The extracted data encompassed the following key elements: (i) study characteristics, such as study design, location, sources of funding, and study aim; (ii) participant characteristics, including age, eligibility criteria, height, body mass, body mass index, and training status; (iii) details related to exposures and comparisons, such as type, dosage, and duration of hormonal contraceptive use, menstrual cycle phase, type of menstrual irregularity, and methods employed to determine participants' ovarian hormonal status; and (iv) outcome characteristics for AKL, including the method of assessment and specific assessment characteristics, as well as the occurrence of non-contact ACL injuries, encompassing the method(s) used to confirm the injury, and details about the injury profile, such as the mechanism, context, and whether it was a primary or recurrent injury.

To facilitate the identification of studies suitable for the meta-analysis, an additional category was incorporated into the data collection spreadsheet. The data extracted from the studies were then evaluated within the 'exposure and comparison' section, using a classification grid recommended by Elliott-Sale et al. (2021).

3.2.6 Risk of bias assessment

The risk of bias assessment was conducted at the individual study level using the Newcastle Ottawa Quality Assessment Scale (NOS) for Cohort or Case–Control Studies as outlined by Wells et al. (2013) (Appendix C). This domain-based tool consists of eight items (domains) grouped into three categories, evaluating key bias domains including selection, comparability, and outcome/exposure. Codebooks and NOS quality assessment scales were developed, aligning with previously published works in the field, such as Ekås et al. (2020), ensuring the specificity of the assessment for each outcome (Appendix D and E).

In this review, the NOS was employed without using the star-rating system. The decision to omit the overall 'quality score' was based on the PRISMA explanation and elaboration by Page et al. (2021b), which emphasises the importance of presenting assessments for each domain individually. This approach allows users to identify

specific domains that are at risk of bias in each study. Therefore, the key bias domains covered by the NOS were assessed separately for each study, without providing an overall score. For each domain, the risk of bias was rated as either low or high based on predefined decision rules (Appendix D and E). These ratings were applied to all eight domains and to overall bias across the studies. The risk of bias assessment was independently performed by two reviewers (EN and JP) and any disagreements were resolved through discussion and consensus between the reviewers.

3.2.7 Statistical analysis

An inverse variance, random effects meta-analysis was performed on the AKL data, with calculations of mean difference (MD) using Review Manager Software (RevMan, Version 5.3.5, Cochrane Collaboration, Oxford, United Kingdom). Meta-analyses were conducted when two or more studies measured AKL using the same methods and units. Effect sizes were categorised based on the magnitude of AKL changes between hormonal statuses, such as MC phase 1, to another, such as MC phase 2. These classifications followed criteria from Cohen (1998), Turner and Bernard (2006), and Sawilowsky (2009), with ranges defined as very small (0.01 – 0.19), small (0.20 – 0.49), moderate (0.50 – 0.79), large (0.80 – 1.19), very large (1.20 – 1.99), and huge (> 2). Statistical heterogeneity among studies was assessed using the standard Cochran chi-squared test (Higgins et al., 2003), and the I^2 statistic was used to quantify the heterogeneity magnitude. Values ranging from 0% to 40% indicated low or non-important heterogeneity, 40% to 60% indicated moderate heterogeneity, 50% to 75% suggested substantial heterogeneity, and 75% to 100% indicated considerable heterogeneity, according to Cochrane guidelines (Deeks, Higgins, & Altman, 2021). Furthermore, additional sub-group analyses, using the same tests as stated above, were performed on AKL data measured at similar loading forces, specifically at 130N, 133N, or 134N.

3.3 Results

3.3.1 Literature search

The literature search and selection of studies are presented in Figure 3.1.

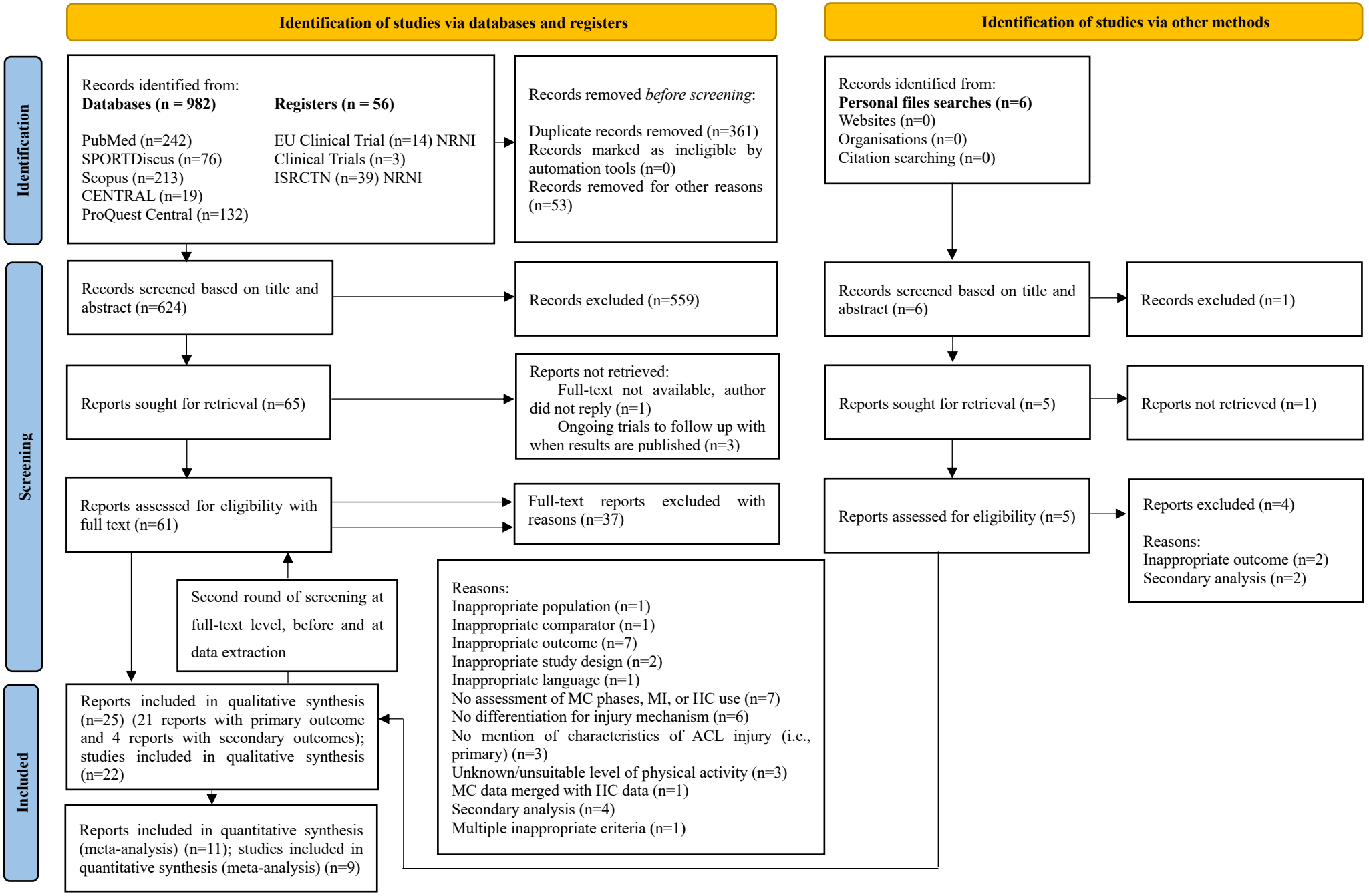

Figure 3.1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart for literature search and study selection. ACL, anterior cruciate ligament; MC, menstrual cycle; HC, hormonal contraceptive; MI, menstrual irregularities; NRNI, not relevant not imported.

3.3.2 Study characteristics

In total 25 reports, *i.e.*, 22 studies (Adachi et al., 2008; Agel et al., 2006; Belanger et al., 2004; Beynnon et al., 2005; Carcia et al., 2004; Eiling et al., 2007; Heitz et al., 1999; Hertel et al., 2006; Hicks-Little et al., 2007; Karageanes et al., 2000; Khowailed et al., 2015; Landram & Halligan, 2020; Lee et al., 2013, 2014; Maruyama et al., 2021; Park et al., 2009a, 2009b; Pollard, Braun, & Hamill, 2006; Ruedl et al., 2009, 2011; Shagawa et al., 2021; Shultz et al., 2005; Shultz et al., 2010; Shultz et al., 2012b; Van Lunen et al., 2003), with a total of 3653 participants, were included.

Twenty-one reports, *i.e.*, 19 studies (Belanger et al., 2004; Beynnon et al., 2005; Carcia et al., 2004; Eiling et al., 2007; Heitz et al., 1999; Hertel et al., 2006; Hicks-Little et al., 2007; Karageanes et al., 2000; Khowailed et al., 2015; Landram & Halligan, 2020; Lee et al., 2013, 2014; Maruyama et al., 2021; Park et al., 2009a, 2009b; Pollard, Braun, & Hamill, 2006; Shagawa et al., 2021; Shultz et al., 2005; Shultz et al., 2010; Shultz et al., 2012b; Van Lunen et al., 2003), with a total of 392 participants with AKL as primary outcome were included. All studies with AKL as primary outcome were prospective observational cohort studies (100%). Sixteen studies assessed AKL variations across the MC in eumenorrheic or naturally menstruating females (84%) (Belanger et al., 2004; Beynnon et al., 2005; Carcia et al., 2004; Eiling et al., 2007; Heitz et al., 1999; Hertel et al., 2006; Karageanes et al., 2000; Khowailed et al., 2015; Landram & Halligan, 2020; Maruyama et al., 2021; Park et al., 2009a, 2009b; Pollard, Braun, & Hamill, 2006; Shagawa et al., 2021; Shultz et al., 2005; Shultz et al., 2010; Van Lunen et al., 2003). Three studies (16%) assessed AKL variations across the MC and the HC cycle in naturally menstruating females, and in HC users (Hicks-Little et al., 2007) who were either low-dose combined OCP users (< 50 μg-ethinyloestradiol) (Lee et al., 2013, 2014) or combined monophasic and triphasic OCP users (Shultz et al., 2012b).

Four reports, *i.e.*, three studies (Adachi et al., 2008; Agel et al., 2006; Ruedl et al., 2009, 2011) with a total of 3261 participants with primary non-contact ACL injury as secondary outcome were included. Two studies with primary non-contact ACL injury as secondary outcome were retrospective observational cohort studies (67%) (Adachi et al., 2008; Agel et al., 2006) and one study was a case-control study (33%) (Ruedl et al., 2009, 2011). One study observed the primary non-contact ACL injury

rate across the MC in naturally menstruating females (33%) (Adachi et al., 2008). Two studies (67%) observed the primary non-contact ACL injury rate across the MC and the OCP cycle in naturally menstruating females, and in monophasic and triphasic combined OCP users (Agel et al., 2006), OCP users (Ruedl et al., 2009, 2011). Details of all included studies are shown in Appendix F and G.

3.3.3 Risk of bias assessment

3.3.3.1 Risk of bias assessment within domains for individual studies

All results of the risk of bias assessment are presented in Tables 3.1, 3.2, 3.3, and 3.4. Three of the included AKL studies (16%) had no limitations in any bias domains and were, therefore, judged to be at an overall low risk of bias (Carcia et al., 2004; Eiling et al., 2007; Shultz et al., 2005) (Table 3.2). Nine and 13 studies (47% and 68% of the 19 studies) were judged at high risk of bias in the respective domains: *ascertainment of exposure (ascertainment bias)* and *assessment of the outcome (detection bias)* (Table 3.4). Ten studies identified and verified participants' hormonal status using gold-standard methods, *i.e.*, the use of urinary ovulation detection kit and serum sample analysis. Additionally, six studies assessed the outcome (AKL) with appropriate measures, by an operator blinded to the hormonal status of the participants (Table 3.4). Furthermore, all 19 studies (100%) adequately demonstrated that the outcome of interest was not present at the start of the study. They had sufficient follow-up time, lasting at least one MC, and a follow-up rate of at least 80%. Moreover, participants were confirmed to have no prior ACL or knee injuries in either knee and no known medical conditions affecting connective tissue.

All the primary non-contact ACL injury studies included in this analysis had limitations in at least two domains. The three included studies were all judged to be at high risk of bias for the domain of *ascertainment of exposure* as none of the studies accurately identified and verified participants' hormonal status using gold-standard methods, such as the use of urinary ovulation detection kit and serum sample analysis (Table 3.3). Two studies either assessed the outcome (*i.e.*, ACL injury) using appropriate measures, specifically MRI, which was conducted by an independent operator blinded to the hormonal status of the participants (Adachi et al., 2008), or adequately defined the study cases (ACL injury) with independent validation determined by MRI (Ruedl et al., 2009, 2011) (Table 3.3). In terms of follow-up, the

two studies assessed in this bias domain had adequate follow-up time. However, the follow-up rate was deemed adequate in two out of three studies (defined as at least 80%) (Table 3.5).

3.3.3.2 Menstrual cycle and hormonal contraceptive cycle phase identification and verification

Of the 22 studies included in this analysis, one study (Shultz et al., 2012b) used data from a prior study (Shultz et al., 2005) regarding MC phases. To prevent data duplication, this study excluded from the subsequent statistical analysis for MC phase identification and verification. In the remaining 21 studies evaluated for the quality of MC phase identification and verification, one study (5%) did not provide any details on how the MC phases were identified. However, the remaining studies employed a variety of methods to identify MC phases, as follows:

1) A combination of methods including day counting, basal body temperature (BBT), participant self-report of MC phase, MC history, and urinary ovulation detection kits (11 studies, 52%);
2) A combination of methods including day counting, BBT, participant self-report of MC phase, and MC history, but without the use of urinary ovulation detection kits (five studies, 24%);
3) Participant self-report of MC phase (four studies; 19%).

Of the 22 studies included in the review, 33% (seven studies) retrospectively verified the MC phase using both serum oestrogen and progesterone, 14% (three studies) used only serum oestrogen for retrospective verification, 10% (two studies) employed saliva oestrogen and progesterone, and 5% (one study) used urine samples for verification. However, 38% (eight studies) did not specify their method for MC phase verification. Additionally, one study (5%) monitored participants' MC characteristics over two MCs prior to testing using a calendar-based approach, and another (5%) repeated outcomes across a second MC.

Among the 22 studies included in this analysis, five studies (23%) included a group of HC users with various profiles. Out of these, four studies (80%) identified the type of HC used by the participants through self-report as the identification

method. In one study (20%), detailed information was provided about the type, brand, formulation, and dosage of the OCPs used by the participants, *i.e.*, a list of seven monophasic and triphasic OCPs. Another study (20%) did not provide further information about the brand or dosage of the OCPs; it only mentioned that participants used combined monophasic and triphasic OCPs. In one study (20%), information was given about the upper threshold dosage of ethinyloestradiol in the OCPs used by the participants, *i.e.*, low-dose monophasic combined OCP (< 50 µg ethinyloestradiol). However, this study did not provide further details about the brand or specific dosage. One study (20%) mentioned the type of HC used by the participants as OCPs but did not report any further information about the type, brand, formulation, or dosage. Lastly, one study (20%) did not identify the type of HC used by the participants.

Regarding the verification of the HC phase, among the five studies that included a HC group in their study design, two studies (40%) conducted a retrospective verification using either serum oestrogen (one study, 20%) or both serum oestrogen and progesterone (one study, 20%). However, three studies (60%) did not retrospectively verify the HC phase. Additionally, the two studies (40%) that performed verification strictly included participants who had been using HC for more than three months. In contrast, the three studies (60%) that did not verify the HC phase did not assess the duration of HC use among participants.

Table 3.1. Risk of bias at study individual level of included AKL studies by Newcastle-Ottawa Scale domains (dichotomised to low/high/unclear).

Study	*Selection/representativeness*				*Comparability*	*Outcome*		
	Cohort	Controls	Exposure	Outcome	Of cohorts	Assessment	Follow-up time	Follow-up rate
Belanger	b	na	b	a	na	a	a	b
Beynnon	d	c	a	a	a	a	a	b
Carcia	b	na	a	a	na	a	a	a
Eiling	a	na	a	a	na	a	a	a
Heitz	d	na	b	a	na	b	a	a
Hertel	b	na	a	a	na	b	a	b
Hicks-Little	a	na	c	a	na	b	a	a
Karageanes	a	na	c	a	na	b	a	a
Khowailed	a	na	a	a	na	b	a	b
Landram	b	na	b	a	na	a	a	a
Lee (2013, 2014)	c	na	b	a	na	b	a	b
Maruyama	c	na	b	a	na	b	a	b
Park (2009a, 2009b)	b	na	a	a	na	b	a	a
Pollard	b	a	a	a	a, b	b	a	a
Shagawa	c	na	b	a	na	b	a	b
Shultz (2005)	b	a	a	a	a, b	a	a	a
Shultz (2010)	b	na	b	a	na	b	a	b
Shultz (2012)	b	a	a	a	a, b	b	a	a
Van Lunen	b	na	a	a	na	b	a	a

Orange colour, high risk of bias; green colour, low risk of bias; yellow colour, unclear risk of bias (not described). Studies also included for quantitative analysis are marked in bold letters.

Table 3.2. Risk of bias at study individual level of included primary non-contact ACL injury studies by Newcastle-Ottawa Scale domains (dichotomised to low/high/unclear).

		Selection/representativeness				*Comparability*	*Outcome/Exposure*		
Study	Design	Cohort/case definition	Controls/representativeness of the cases	Exposure/ selection of controls	Outcome/ Definition of controls	Of cohorts/of cases and controls	Assessment/ ascertainment	Follow-up time/same method for cases and controls	Follow-up rate/non-response rate
Adachi	cohort	a	na	c	b	na	a	a	a
Agel	cohort	a	na	c	a	na	b	a	a
Ruedl (2009, 2011)	case-control	a	a	a	a	a,b	d	a	b

Orange colour, high risk of bias; green colour, low risk of bias; yellow colour, unclear risk of bias (not described)

Table 3.3. Overall risk of bias assessment for AKL studies (n = 19).

Newcastle-Ottawa scale domain	Evaluation of risk of bias in included studies			
	Not appropriate, n (%)	**High risk, n (%)**	**Unclear (not described), n (%)**	**Low risk, n (%)**
Selection/Representativeness				
Representativeness of exposed cohort		3 (16)	2 (10)	14 (74)
Representativeness of the controls	15 (79)		1 (5)	3 (16)
Ascertainment of the exposure (MC or HC)		9 (47)		10 (53)
Demonstration that outcome of interest was not present at start of study (no previous ACL injury)				19 (100)
Comparability				
Comparability of cohorts	15 (79)			4 (21)
Outcome				
Assessment of outcome (AKL)		13 (68)		6 (32)
Was follow-up long enough for outcomes to occur (minimum over one MC or HC cycle)				19 (100)
Adequacy of follow-up of cohorts (minimum 80%)				19 (100)

ACL, anterior cruciate ligament; AKL, anterior knee laxity; HC, hormonal contraceptive; MC, menstrual cycle

Table 3.4. Overall risk of bias assessment for primary non-contact ACL injury studies (n = 3).

Newcastle-Ottawa scale domain	Evaluation of risk of bias in included studies			
	Not appropriate, n (%)	**High risk, n (%)**	**Unclear (not described), n (%)**	**Low risk, n (%)**
Selection/Representativeness				
Representativeness of exposed cohort/case definition				3 (100)
Representativeness of the controls/cases	2 (66.7)			1 (33.3)
Ascertainment of the exposure (MC or HC)/selection of controls		2 (66.7)		1 (33.3)
Demonstration that outcome of interest was not present at start of study (no previous ACL injury)/definition of controls		1 (33.3)		2 (66.7)
Comparability				
Comparability of cohorts/of cases and controls	2 (66.7)			1 (33.3)
Outcome/Exposure				
Assessment of outcome (primary non-contact ACL injury)/ascertainment of exposure (MC or HC)		2 (66.7)		1 (33.3)
Was follow-up long enough for outcomes to occur (minimum of 1 year of practice, training, or competition)/same method of ascertainment for cases and controls				3 (100)
Adequacy of follow-up of cohorts (minimum 80%)/non-response rate		1 (33.3)		2 (66.7)

ACL, anterior cruciate ligament; HC, hormonal contraceptive; MC, menstrual cycle

3.3.4 Meta-analysis

3.3.4.1 Anterior knee laxity across the menstrual cycle

Of the nine included studies, seven assessed AKL across the MC. However, not all provided data for each of the four MC phases according to the MC phase classification used in this review. Consequently, meta-analyses were limited to comparisons between MC phases 1 and 2, 1 and 3, and 1 and 4.

The comparison between exposure to MC phase 2 and MC phase 1 revealed no significant alteration in AKL (Z = 0.69, [p = 0.49], d = -0.28, 95% CI [-1.07, 0.54]; Figure 3.2) when the influence of different loads was not considered across the studies. Furthermore, there was no evidence of heterogeneity among the studies (Chi^2 = 0.15, df = 1 [p = 0.70], I^2 = 0%).

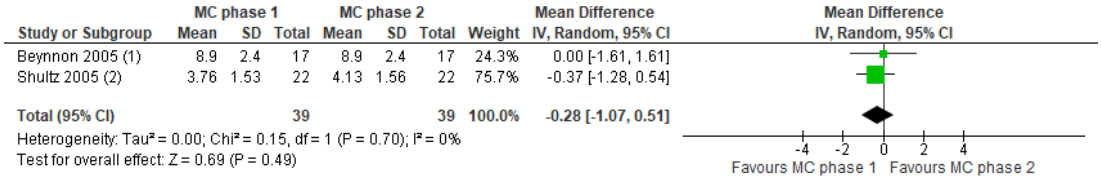

Figure 3.2. Effect of exposure to MC phase 2 on AKL in comparison to MC phase 1, measured by KT-1000 at 130N or KT-2000 at 46, 89, and 133N.

The comparison between exposure to MC phase 3 and MC phase 1 revealed no significant alteration in AKL (Z = 1.36, [p = 0.17], d = -0.69, 95% CI [-1.69, 0.30]; Figure 3.3) when the influence of different loads was not considered across the studies. Additionally, there was considerable heterogeneity among the studies (Chi^2 = 18.87, df = 3 [p = 0.0003], I^2 = 84%).

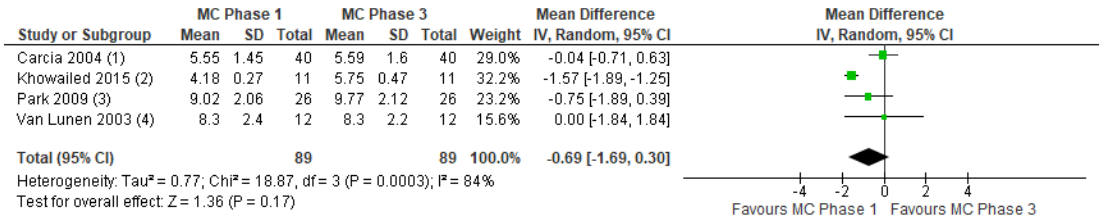

Figure 3.3. Effect of exposure to MC phase 3 on AKL in comparison to MC phase 1, measured by KT-2000 at 134N, at 133N, at 89N and manual maximal force, or KT-2000 at 133N.

The comparison between exposure to MC phase 4 and MC phase 1 revealed no significant alteration in AKL (Z = 0.24, [p = 0.81], d = -0.07, 95% CI [-0.64, 0.50]; Figure 3.4) when the influence of different loads was not considered across the studies. Furthermore, there was no evidence of heterogeneity among the studies (Chi² = 0.50, df = 2 [p = 0.78], I² = 0%).

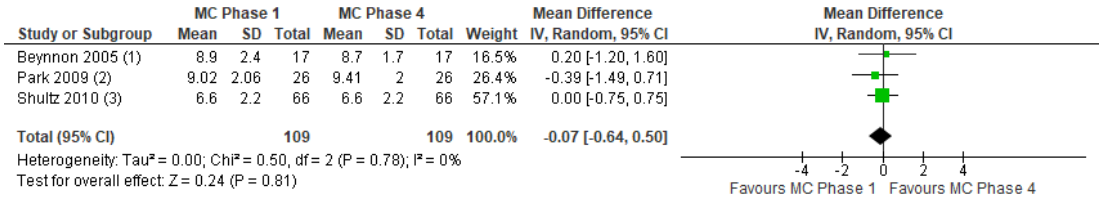

Figure 3.4. Effect of exposure to MC phase 4 on AKL in comparison to MC phase 1, measured by KT-1000 at 130N, KT-2000 at 89N and manual maximal force, or KT-2000 at 133N.

The comparison between exposure to MC phase 2 and MC phase 1 revealed no significant alteration in AKL (Z = 0.66, [p = 0.51], d = -0.29, 95% CI [-1.14, 0.56]; Figure 3.5) when the influence of similar loads was accounted for across studies. There was no heterogeneity among the studies (Chi² = 0.17, df = 1 [p = 0.68], I² = 0%).

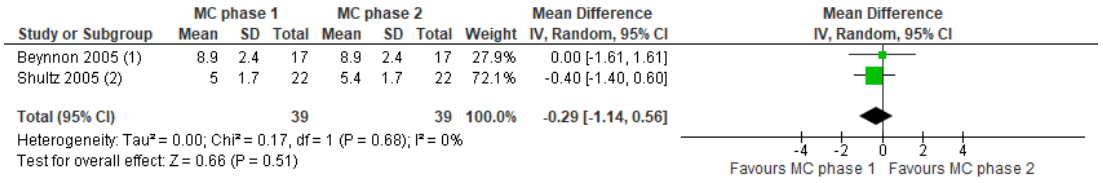

Figure 3.5. Effect of exposure to MC phase 2 on AKL in comparison to MC phase 1, measured by KT-1000 at 130N or KT-2000 at 133N.

The comparison between exposure to MC phase 3 and MC phase 1 revealed no significant alteration in AKL (Z = 1.00, [p = 0.32], d = -0.65, 95% CI [-1.93, 0.63]; Figure 3.6) when the influence of similar loads was accounted for across studies. In addition, there was considerable heterogeneity among the studies (Chi² = 18.16, df = 2 [p = 0.0001], I² = 89%).

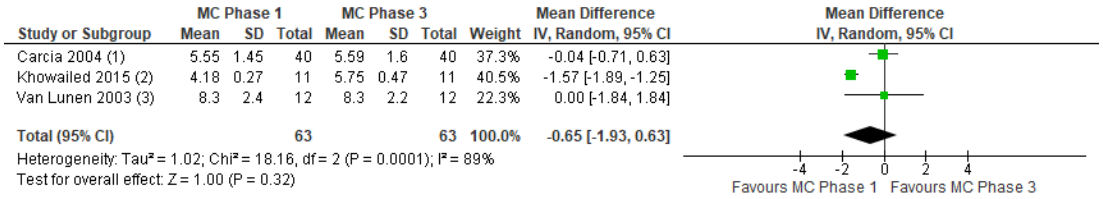

Figure 3.6. Effect of exposure to MC phase 3 on AKL in comparison to MC phase 1, measured by KT-2000 at 134N or at 133N.

The comparison between exposure to MC phase 4 and MC phase 1 revealed no significant alteration in AKL (Z = 0.13, [p = 0.89], d = 0.04, 95% CI [-0.62, 0.71]; Figure 3.7) when the influence of similar loads was accounted for across studies. There was no heterogeneity among the studies (Chi^2 = 0.06, df = 1 [p = 0.80], I^2 = 0%).

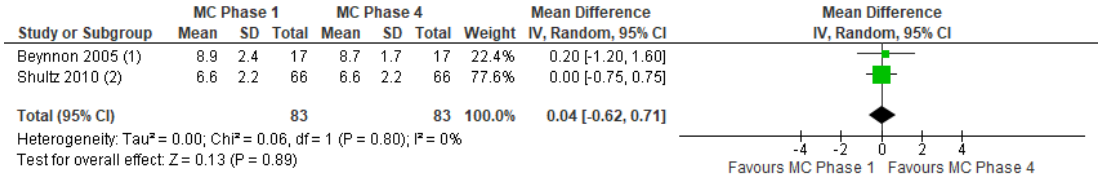

Figure 3.7. Effect of exposure to MC phase 4 on AKL in comparison to MC phase 1, measured by KT-1000 at 130N or KT-2000 at 133N.

3.3.4.2 Anterior knee laxity across the hormonal contraceptive cycle

Of the nine included studies, two assessed AKL throughout the HC cycle in OCP users, allowing for a meta-analysis comparing the active and inactive phases of OCP use.

The comparison between exposure to combined OCP inactive phase and combined OCP active phase revealed no significant alteration in AKL (Z = 0.09, [p = 0.93], d = -0.03, 95% CI [-0.68, 0.62]; Figure 3.8). There was no heterogeneity among the studies (Chi^2 = 0.00, df = 1 [p = 0.96], I^2 = 0%).

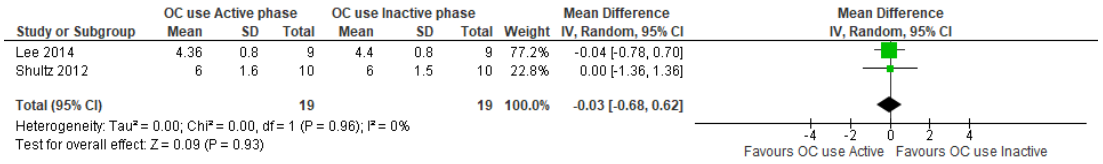

Figure 3.8. Effect of exposure to combined OCP inactive phase on AKL in comparison to combined OCP active phase, measured by KT-2000 at 133N.

3.3.4.3 Anterior knee laxity between oral contraceptive pill users and non-oral contraceptive pill users

Of the nine included studies, two assessed AKL in both combined and non-combined OCP users, enabling a meta-analysis comparing these two groups.

The comparison between exposure to combined OCP and non-exposure to combined OCP revealed no significant alteration in AKL (Z = 0.41, [p = 0.68], d = -0.23, 95% CI [-1.36, 0.90]; Figure 3.9). There was low to moderate heterogeneity among the studies (Chi^2 = 1.66, df = 1 [p = 0.20], I^2 = 40%).

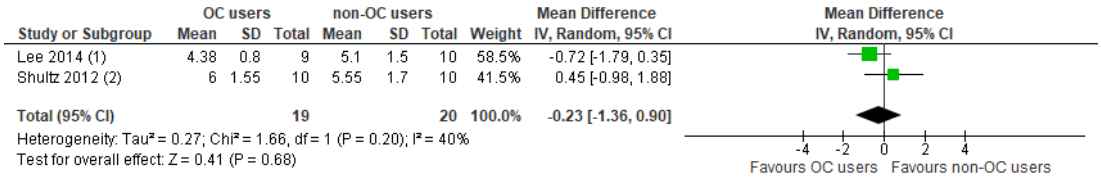

Figure 3.9. Effect of exposure to combined OCP use on AKL in comparison to non-exposure to combined OCP use, measured by KT-2000 at 133N.

3.3.5 Synthesis without meta-analysis

The list of studies, along with their characteristics, which were included in the systematic review but not in the meta-analysis can be found in Appendix F and G.

3.3.5.1 Variation in anterior knee laxity

Out of the 19 AKL studies included in the review herein, ten did not provide quantitative measures suitable for inclusion in the meta-analysis due to discrepancies in their MC phase classification and/or HC cycle testing timepoints as compared to the review's classification criteria. Among the studies, seven (70%) with participants numbers ranging from 11 to 26 reported no significant alterations in AKL throughout the MC (Belanger et al., 2004; Eiling et al., 2007; Hertel et al., 2006; Karageanes et al., 2000; Maruyama et al., 2021; Pollard, Braun, & Hamill, 2006; Shagawa et al., 2021).

Conversely, two studies (20%) involving seven to ten participants indicated significant fluctuations in AKL across the MC (Heitz et al., 1999; Landram &

Halligan, 2020). Heitz et al. (1999) observed increased AKL during the follicular (day 10, 11, 12, and 13) and luteal (day 20, 21, 22, and 23) phases in comparison to the menstrual phase (day 1), while Landram and Halligan (2020) noted higher antero-posterior knee laxity in the luteal phase compared to the follicular phase of the MC.

Lastly, the study conducted by Hicks-Little et al. (2007), encompassing 53 participants (28 naturally menstruating female athletes and 25 HC users), reported significant increases in AKL during ovulation and the luteal phase as opposed to the follicular phase of the MC. Additionally, this study found that AKL was significantly greater in HC users than in non-HC users.

3.3.5.2 Anterior cruciate ligament injury rate

The three studies that reported data on non-contact ACL injury rates across the MC and/or with HC use were excluded from the meta-analysis due to disparities in their MC phase classification and/or HC cycle testing timepoints as compared to the review's classification criteria. Among these studies, two (67%; Agel et al., 2006; Ruedl et al., 2009, 2011) included naturally menstruating females and OCP users as participants, while one study (33%; Adachi et al., 2008) observed the rate of non-contact ACL injuries in a sample of naturally menstruating female athletes.

Adachi et al. (2008) – which included 18 participants – reported a significantly higher rate of non-contact ACL injuries during the ovulatory phase of the MC when compared with other phases. In contrast, Ruedl et al. (2009, 2011), who included 93 ACL injured participants and 93 controls, found a notable increase in the rate of non-contact ACL injuries during the preovulatory phase compared to the postovulatory phase of the MC. In addition, Ruedl et al. (2009, 2011) noted that the non-contact ACL injury rates showed no disparity between OCP users and non-OCP users.

Similarly, Agel et al. (2006), with a substantial sample size of 3150 participants, concluded that there was no difference in non-contact ACL injury rates between OCP users and non-OCP users.

3.4 Discussion

3.4.1 Overview

The aim of this chapter was to investigate the potential influence of both endogenous and exogenous ovarian steroid hormones on AKL and non-contact ACL injuries rates among female athletes and exercising females. The outcomes of the meta-analysis reveal that neither endogenous nor exogenous ovarian steroid hormones exhibit a significant impact on AKL (i) throughout the MC in eumenorrheic or naturally menstruating females; (ii) throughout the HC cycle in OCP users; and (iii) when comparing OCP users with non-OCP users. In addition to the observed absence of effects from endogenous and exogenous ovarian steroid hormones on AKL, the results of random effects meta-analyses suggest notable heterogeneity among studies that provided suitable AKL data either during phases 1 and 3 of the MC or in OCP users and non-OCP users. This suggests that diverse methodological aspects, such as sample sizes, participant characteristics, or assessment of the measured outcome could potentially contribute to any effect. Moreover, most studies included in this meta-analysis were classified as 'high risk' of bias in the specific domain of 'assessment of outcome', primarily due to the lack of blinding of measurement operators to the hormonal status of the participants. Notably, this meta-analysis was conducted with a limited number of studies, specifically nine, and each individual random effects meta-analysis comprised a range of two to four studies. The small sample size was due to a lack of consistent definitions, identification, and verification methods for the hormonal status of the participants. Given the lack of evidence of a significant effect from endogenous and exogenous ovarian steroid hormones on AKL, the substantial heterogeneity or absence of consistent definitions, and identification and verification methods regarding participants' hormonal status in existing literature, along with the limited number of studies included in this review, it is not yet possible to formulate comprehensive guidelines for prevention strategies against primary non-contact ACL injury based on the hormonal status of females. Instead, it is recommended that further research of higher methodological quality be conducted in this domain.

3.4.2 Anterior knee laxity at different phases of the menstrual cycle

The outcomes of this meta-analysis show that there is no significant alteration in AKL across the MC for eumenorrheic or naturally menstruating exercising females

and athletes. However, a trend emerges from individual random effects meta-analyses focused on AKL changes in the four phases of the MC. Although statistically insignificant, it appears that phase 1 of the MC tends to exhibit the lowest AKL levels across most included studies, implying potentially reduced ACL elasticity during this phase. Additionally, phase 3 of the MC appears to show the highest AKL levels in 50% of the included studies (non-significant, Figure 3.3). Nevertheless, when a sub-analysis was conducted, accounting for consistent similar loads applied by the testing device, *i.e.*, 130N, 133N, or 134N, across studies (Figure 3.6), only one study showed a distinct outcome where AKL was notably higher in phase 3 of the MC compared to phase 1. It is essential to note that this meta-analysis was performed with a limited number of studies, thus requiring cautious interpretation of the conclusions.

Of the findings from the synthesis without meta-analysis a noteworthy 70% of studies align with the meta-analysis results, indicating that AKL remains relatively constant throughout the MC. However, among the seven studies not observing any AKL changes, four (57%) were classified as 'high risk' of bias in the 'ascertainment of exposure' domain, indicating inadequate assessment of participants hormonal status based on MC phase classification criteria. The three studies demonstrating significant AKL differences across the MC reported higher AKL either in the ovulatory or luteal phases compared to the early follicular phase, aligning with the non-significant trend in the meta-analysis herein. Despite this, two of these three studies had small sample sizes (seven to ten participants), all studies were rated as 'high risk' of bias in 'ascertainment of exposure', and two studies were classified as 'high risk' of bias in 'assessment of outcome'. Overall, out of the ten studies excluded from the meta-analysis, seven were rated as 'high risk' of bias in 'ascertainment of exposure'; as such no firm conclusions can be drawn from these studies,

The results of this systematic review and meta-analysis partially align with prior research findings in this field, which have also identified some variation but note very low to low study quality and quality of evidence due to methodological limitations, aligning with this chapter's observations. For instance, Dos'Santos et al. (2023) concluded that there is no consistent effect of the MC phases on knee laxity, while Moriceau et al. (2022) suggested higher AKL during the ovulatory and luteal

phases. Similarly, Herzberg et al. (2017) highlighted increased AKL in the ovulatory phase compared to the follicular phase.

Overall, based on existing literature and a meta-analysis involving a restricted number of studies, the extent of AKL variation across the MC remains uncertain, as does whether AKL is notably higher in phase 3 of the MC. However, consensus exists regarding the generally low quality of studies included in existing reviews and the current review. This is particularly apparent in the accurate ascertainment of participants' hormonal status and the assessment of AKL, where operators often lack blinding to participants' hormonal status during testing. Furthermore, this review indicates that all studies exclusively focused on naturally menstruating or eumenorrheic females, while neglecting females experiencing menstrual irregularities, such as oligomenorrhoea, polymenorrhoea, amenorrhoea, anovulatory and luteal phase deficient cycles. Given the likelihood of these menstrual irregularities being more prevalent within the physically active population, it would be pertinent for future research studies to incorporate these hormonal profiles into their designs and align the quantification of hormonal status with methodological recommendations (Janse de Jonge, Thompson, & Han, 2019).

3.4.3 Anterior knee laxity during hormonal contraceptive use

The outcome of this meta-analysis shows no significant alteration in AKL across the HC cycle for exercising females and athletes who use combined OCPs. The phase of the HC cycle, whether active or inactive, does not appear to influence AKL. However, it is important to note that, of the studies included for analysis, one study was rated as 'high risk' of bias in 'representativeness of the exposed cohort' and 'ascertainment of exposure', while both studies were assessed as 'high risk' of bias in 'assessment of outcome'. In addition, while one study provided a comprehensive list of OCPs used by participants, the other only reported the upper threshold of ethinyloestradiol of combined OCPs accepted by its inclusion criteria. The lack of transparent and precise information regarding the exogenous hormonal profile of participants limits drawing meaningful conclusions about the impact of OCPs on AKL changes throughout the HC cycle (Burrows & Peters, 2007). It is worth highlighting that this meta-analysis was solely based on two studies (Fig. 3.8) with small sample sizes, highlighting the need for cautious interpretation of this result.

This meta-analysis marks the first instance of a comparison of AKL across the HC cycle in OCP users. This review highlights the absence of studies conducted with participants using HCs other than combined OCPs, despite the use of various other types of HCs, such as progestogen-only pill, contraceptive injection, implant, patch, vaginal ring, or intrauterine system (IUS), among the population of exercising females and athletes (Baumgartner et al., 2023; Doohan et al., 2023; Martin et al., 2018). Overall, it is recommended that further research of higher quality be carried out among OCP users, and users of different types of HCs to yield more comprehensive quantitative and qualitative insights into the potential effects of various HCs on AKL. In addition, it should be noted that studies utilising OCP users should differentiate between the type of OCP, given the wide variation in concentration of exogenous hormones amongst the different type/brands of OCPs.

3.4.4 Anterior knee laxity between hormonal contraceptive users and non-hormonal contraceptive users

The outcome of this meta-analysis indicates that there is no significant disparity in AKL between exercising females and athletes who use combined OCPs *versus* those who are naturally menstruating or eumenorrheic. Limitations of the studies encompassed within this meta-analysis, as well as of the meta-analysis itself, are discussed in the preceding section.

In contrast to the results of the meta-analysis, the findings gleaned from the synthesis without meta-analysis suggest that AKL might be higher among HC users in comparison to non-HC users. However, this synthesis without meta-analysis relies solely on one study that does not provide any information regarding the type, brand, formulation, or dosage of HC used by the participants. Furthermore, this study has been appraised as ‘high risk’ of bias in the domains of ‘ascertainment of exposure’ and ‘assessment of outcome’.

Studies adopting this specific approach to comparing potential AKL variations between HC users and non-HC users are scarce, and they fail to provide sufficient information of acceptable quality to draw meaningful conclusions. Previous reviews on this research topic have also refrained from formulating definitive conclusions, thereby suggesting the necessity for further research of higher quality among

exercising females and athletes who are either naturally menstruating, eumenorrheic, or HC users.

3.4.5 Anterior cruciate ligament injury risk rate

The findings from the synthesis without meta-analysis suggest that there could be a specific phase of the MC in which exercising females and athletes who are either normally menstruating or eumenorrheic might face an elevated risk of experiencing a primary non-contact ACL injury. However, this conclusion relies on two studies that do not reach a consensus on the precise MC phase associated with this heightened risk. These studies reported differing results, indicating participants were at significantly increased risk of non-contact ACL injury either during the ovulatory phase or in the preovulatory phase of the MC. Due to the varying classification of MC phases in these studies, a conclusion cannot be drawn from this synthesis. Furthermore, both of these studies were evaluated as 'high risk' of bias in the 'ascertainment of exposure' domain, which restricts the confidence in their findings since participants' hormonal status was based solely on self-reported and was not corroborated.

Lastly, the synthesis without meta-analysis indicated no significant disparity in non-contact ACL injury rates between exercising females and athletes who either use OCPs or who are naturally menstruating or eumenorrheic. Although the studies contributing to this synthesis share a consistent conclusion, it is essential to note that this synthesis is built upon only two studies, both of which were assessed as 'high risk' of bias in the 'ascertainment of exposure' domain. This limitation, again, reduces the confidence in their findings due to the reliance on self-reported, unverified hormonal status of the participants.

3.4.6 Strengths and limitations

3.4.6.1 Included studies

Overall, the methodology of the studies encompassed in this systematic review and meta-analysis was poor. While the cornerstone of methodological rigor lies in the accurate identification and verification of participants' hormonal status to ensure reliable conclusions, merely seven studies (33%) used urinary ovulation detection kits and serum oestrogen and progesterone analysis for this purpose. In addition, only one study (5%) repeated the measurement of outcome (*i.e.*, AKL) over a second MC.

A similar deficiency in methodological quality was evident in studies involving HC users, where only one study (20%) provided comprehensive information about the type, brand, formulation, and dosage of the OCPs used by the participants, *i.e.*, a list of 7 monophasic and triphasic OCPs. Remarkably, this same study was also the sole one that verified participants' hormonal status through serum oestrogen and progesterone analysis.

Turning to studies that addressed the secondary outcome (*i.e.*, ACL injury), it is notable that two studies (67%), which had the smallest participant pool, accurately evaluated the outcome through a medical diagnosis determined by MRI, demonstrating a higher methodological quality in this aspect.

3.4.6.2 Review process

The application of the predefined strict inclusion criteria to the search results identified 25 reports (comprising 22 studies) eligible for inclusion in this review, and 11 reports (comprising 9 studies) were included in the subsequent meta-analysis. This relatively small number may be surprising, especially considering the extensive research conducted in this field over the past three decades and the substantial clinical impact that such investigations could have on the domain of sports injury prevention, particularly within the context of women's sports, which is a burgeoning field. However, the efficacy of the comprehensive search strategy was validated through pilot testing, and it was further bolstered by supplementing electronic findings with manual searches of reference lists, along with a supplementary search within the personal records of the review's researchers. This approach helped mitigate the risk of inadvertently overlooking crucial studies or data, thereby ensuring that conclusions derived from this chapter could be grounded in a synthesis of all available evidence.

Moreover, the classification of MC phases used at data extraction level deviated from the conventional reporting of participants' ovarian hormonal status as phases and instead employed specific testing timepoints that corresponded to distinct hormonal statuses in the participants. This augmented classification framework bolstered the meta-analysis by enhancing the scrutiny of methodological quality in the included studies and facilitating more reliable inter-study comparisons. In addition, this approach aligns with recommended methodologies with regards to the quantification and confirmation of hormonal status in studies involving female cohorts

(Janse de Jonge, Thompson, & Han, 2019). Lastly, the deliberate inclusion of studies exclusively focusing on primary non-contact ACL injuries effectively limited the final number of selected studies for this review. Indeed, it is worth noting that sustaining an ACL injury increases the risk to experience subsequent ipsilateral or contralateral ACL injuries over one's lifetime (Barber-Westin & Noyes, 2020; Hong et al., 2023). This intricate scenario requires an investigation into supplementary risk factors that is beyond the scope of this chapter. Furthermore, in cases where a primary ACL injury occurred through a contact mechanism, considering the hormonal status of the injured female athlete or exerciser as a risk factor might not be pertinent. This is because an acute traumatic knee injury resulting from an extreme impact is highly likely to lead to injury, regardless of the hormonal profile of the individual sustaining it.

However, it is important to acknowledge a few limitations within this systematic review and meta-analysis process. Firstly, the review only included published studies from peer-reviewed journals, potentially introducing publication bias into the outcomes. Nonetheless, the impact of this bias seems minimal, as only one study originally published as an abstract was encountered during the search, and its inclusion would not have altered the results. Additionally, a second round of screening of reports during the data extraction stage was conducted, prompted by the little experience of the review researchers who had managed the selection process prior to data extraction. This cautious approach was deliberate, intended to prevent the inadvertent omission of relevant data. Lastly, the data extraction process diverged slightly from the established protocol, as data extraction was executed by a single researcher instead of the prescribed two. However, given the rigorous protocol followed it was deemed that this omission would not have any profound effect on the conclusions.

3.5 Conclusion

In this systematic review and meta-analysis, the aim was to explore the potential impact of endogenous and exogenous ovarian steroid hormones on AKL and non-contact ACL injury rates among female athletes and exercisers. The collective findings of the meta-analysis reveal that neither endogenous nor exogenous ovarian steroid hormones exert a significant influence on AKL in the following situations:

1) Throughout the MC: Across the various phases of the MC, no alteration in AKL was observed for eumenorrheic or naturally menstruating female athletes or exercisers;
2) During HC use: The HC cycle, whether in the active phase or in the inactive phase does not appear to have a significant impact on AKL in female athletes and exercisers using OCPs;
3) Comparison between HC users and non-HC users: No significant difference was found in AKL between female athletes and exercisers who use OCPs and those who are naturally menstruating or eumenorrheic.

Despite the limitations associated with the number of available studies and the complexity of methodological disparities, this review highlights the absence of robust evidence supporting a substantial effect of the hormonal profile of ovarian steroids on AKL variations or non-contact ACL injury rates. Notably, the included studies exhibited heterogeneity, possibly due to factors such as sample size, participant characteristics, the methodology used to assess exposure, and the way outcomes were measured.

This review argues strongly in favour of improving the quality of the research in this area. Accurate identification and verification of hormonal profile and status is essential to the formulation of comprehensive prevention strategies against primary non-contact ACL injuries. Inconsistent conclusions in the existing literature, compounded by the absence of studies addressing menstrual irregularities, highlight the need to refine methodologies encompassing a broader range of hormonal profiles among female athletes and exercisers.

Furthermore, it is essential to acknowledge the challenges that researchers face when planning and conducting these studies. Limited resources and funding constraints undoubtedly influence the scale and scope of research investigations. However, it is crucial not to discourage further much-needed research efforts. Incremental methodological improvements, such as the inclusion of participants' hormonal profile tracking for at least a month ahead of data collection, the use of urinary ovulation detection kits, and confirmation of the hormonal status by analysis of oestrogen and progesterone serum sample, can significantly enhance the reliability

and rigor of studies in this domain. Notably, these improvements can be implemented at relatively minimal to no additional cost.

In conclusion, the intricate interplay between ovarian steroid hormones and AKL variations, as well as non-contact ACL injury rates, requires rigorous research using enhanced methodological standards. The current state of evidence does not yet provide a solid foundation for developing concrete guidelines for injury prevention strategies solely based on hormonal profiles and statuses. This chapter highlights the need for a comprehensive approach and rigorous future investigations.

Note: Acknowledgements to all reviewers who helped in this review and who were not part of the supervisory team (*i.e.*, Elvis Foli, Professor Sandra Shultz, Dr Joe John Matthews, Dr Eimear Dolan, and Dr Paul Swinton) and to NTU's research librarian, Victoria Boskett, for her crucial assistance in developing relevant search strategies across all included databases.

Chapter 4. Assessing the reliability of the Genourob (GNRB) Rotab automated knee arthrometer

4.1 Introduction

4.1.1 Endogenous ovarian steroid hormone fluctuations and the ACL

As mentioned in Chapter 1, Section 1.3.4, numerous studies have identified greater anterior knee laxity (AKL), also known as anterior tibial translation, as a significant risk factor for predicting the likelihood of primary non-contact anterior cruciate ligament (ACL) injuries (Kramer et al., 2007; Myer et al., 2008; Scerpella, Stayer, & Makhuli, 2005; Uhorchak et al., 2003; Vacek et al., 2016; Woodford-Rodgers, Cyphert, & Denegar, 1994), secondary knee injuries, and knee osteoarthritis (OA) (Neuman et al., 2012). As females mature, they tend to exhibit increased AKL values (Falciglia et al., 2009; Shultz, Nguyen, & Schmitz, 2008; Ahmad et al., 2006) which is associated with a higher risk of non-contact ACL injuries, comparatively to their male counterparts (Beck et al., 2017; Stracciolini et al., 2015).

The neuromusculoskeletal system in females, including the ACL, responds to fluctuations in reproductive hormone levels, resulting in systemic changes at various life stages, each characterised by distinct hormonal profiles (Chidi-Ogbolu & Baar, 2018). Several in vitro studies have identified receptor sites for oestrogen, progesterone, and relaxin in human female ACLs harvested from surgeries (Dragoo et al., 2003; Liu et al., 1996) and in animal models (Liu et al., 1997), suggesting that reproductive hormones may influence ACL's structure and composition, purportedly by altering collagen metabolism.

More recent studies in both animal (Liu et al., 1997; Seneviratne et al., 2004) and human models (Yu et al., 1999, 2001) have further investigated oestrogen and progesterone effects on ACL collagen metabolism. However, these findings are conflicting. For instance, in rabbits, Liu et al. (1997) reported a decrease in collagen synthesis and fibroblast proliferation with increasing concentrations of oestradiol when ACLs were exposed for two weeks to a minimum physiological level of 25 pg/mL. Conversely, Seneviratne et al. (2004) observed no difference in collagen synthesis and fibroblast proliferation when sheep ACLs were exposed for 4 to 6 days to both physiological and supra-physiological levels of oestradiol (ranging from 2.2 to 2500 pg/mL).

In human models, Yu et al. (1999, 2001) made two notable observations in human ACL cells; (i) type I procollagen synthesis decreased with increasing oestradiol levels (ranging from 2.9 pg/mL to 2500 pg/mL), stagnating at supra-physiologic levels (*i.e.*, 2500 pg/mL); and (ii), progesterone had a buffering effect on inhibition caused by oestradiol. The effects of these hormones were temporary, lasting 1 to 3 days, with a significant decrease noted between 3 to 7 days. Complementing these findings, Lee et al. (2015) found that exposure to physiological levels of oestrogen (from 5 pg/mL to 500 pg/mL) for 24 to 48h inhibited lysyl oxidase (LOX) activity in human engineered ACL. LOX, a primary enzyme produced by fibroblasts, generates collagen cross-links (Kagan & Li, 2003), and its inhibition reduced ligament stiffness without affecting collagen levels.

These findings in human studies suggest that temporary and high fluctuations in ovarian steroid hormone levels across the MC could alter ACL collagen synthesis and structure by affecting fibroblast proliferation and collagen cross-linking thus weakening the ACL, thereby increasing the risk of ligament failure (Yu et al., 2001; Lee et al., 2015). Although findings from animal models are conflicting, there is a need for more research on human models. Given the likely influence of ovarian steroid hormone fluctuations on ACL structure, along with the rising number of female participants in sports (IOC, 2024; The FA, 2020) and the increasing incidence of non-contact ACL injuries among females (Chia et al., 2022; Maniar et al., 2022), it is crucial to ensure that clinical practices and assessment devices are optimally suited to provide bespoke prevention regimes whilst considering the female hormonal environments. Aligning applied practice with the literature on human models to date is essential to effectively contribute to the prevention and management of the risk of non-contact ACL injury.

4.1.2 Clinical assessment of anterior knee laxity

The current clinical approach to non-invasive assessment of ACL integrity, or increased AKL, typically involves performing either a manual or mechanical Lachman test. In this test, the patient lies in a supine position with the knee joint slightly flexed between 20 to 30° (Figure 4.1). The examiner stabilises the patient's thigh with one hand while grasping the lower leg near the tibiofemoral joint with the other. The tibia is then moved forward to evaluate the amount of anterior translation of the tibia

relative to the femur and to assess the perceived end-feel (Torg, Conrad, & Kalen, 1976). According to Torg, Conrad, and Kalen (1976), a positive Lachman test is identified by visible anterior translation of the tibia and a soft end-feel, and this test has been a staple in clinical practice since its inception. However, the manual Lachman test lacks quantifiable outcomes, and studies have reported relatively poor intra- and inter-rater reliability (Cooperman, Riddle, & Rothstein, 1990). To address these limitations, several mechanical devices, such as the KT-1000, KT-2000, Rolimeter, GNRB, have been progressively developed to provide objective and standardised AKL assessments using various levels of loading forces (Mouton, Theisen, & Seil, 2016).

Currently, there are various tests (*i.e.*, antero-posterior or anterior knee laxity tests), devices (*i.e.*, KT-1000, KT-2000, KS Measure), and loading forces (*e.g*, manual maximum force, 89N, 120N, 134N, 150N) used to assess AKL. This wide range of methodologies has resulted in numerous approaches in studies investigating the impact of MC phases and HC use on AKL. However, this diversity complicates the comparison of outcomes and hinders the formulation of consistent conclusions that could enhance the prevention and management of non-contact ACL injuries in females. In Chapter 3's meta-analysis, all included studies used either the hand-held KT-1000 or KT-2000, which have been the most frequently used devices for measuring AKL over the past two decades (Beynnon et al., 2005; Carcia et al., 2004; Khowailed et al., 2015; Lee et al., 2013, 2014; Park et al., 2009a, 2009b; Shultz et al., 2005, 2010, 2012b; Van Lunen et al., 2003). However, factors such as examiner experience (Ballantyne et al., 1995), gender (Klasan et al., 2019), and hand dominance (Sernert et al., 2007) have also been shown to influence AKL measurements.

4.1.3 Current development of automated anterior knee laxity assessment

To address these issues, the Genourob (GRNB) Rotab knee arthrometer has been designed with an automated motorised and controlled application of the loading force on the calf up to 250N and pressure control of the patella (Figure 4.1), providing better intra- and inter-reproducibility than the KT-1000 (Collette et al., 2012; Robert et al., 2009), which production was discontinued in 2015. Studies on the reliability of the GNRB for AKL measurements have emerged with various designs, protocols, populations, loading forces, and degrees of standardisation in participant positioning. These studies include healthy knees (Magdič, Dahmane & Vauhnik, 2023; Mouarbes

et al., 2018; Smith, Miller, & Laslovich, 2022; Vauhnik et al., 2013) and ACL-injured participants with partial or total tears (Robert et al., 2009). However, most of these reliability studies did not control for the reproductive hormone status of female participants and lacked sex-specific data analysis (Magdič, Dahmane & Vauhnik, 2023; Mouarbes et al., 2018; Robert et al., 2009; Smith, Miller, & Laslovich, 2022). Although one study did report the reproductive hormone status of female participants (Vauhnik et al., 2013), it did not specify the type of hormonal contraceptive (HC) used where applicable or the MC phase during testing. Furthermore, no sub-analysis for each reproductive hormone status was performed due to the small sample size of only 13 participants.

4.1.4 Selection criteria for study population

To explore the intra-rater reliability of the GNRB for AKL measurements, it is essential to include populations without cyclic fluctuations of endogenous ovarian steroid hormones such as oestrogen and progesterone. These hormones can affect female ACLs and musculoskeletal systems (Chidi-Ogbolu & Baar, 2018), thus influencing AKL, as reported in various studies meta-analysed in Chapter 3 (Beynnon et al., 2005; Carcia et al., 2004; Khowailed et al., 2015; Lee et al., 2013, 2014; Park et al., 2009a, 2009b; Shultz et al., 2005, 2010, 2012; Van Lunen et al., 2003). Males and most HC users meet the criteria for such a reliability study design.

4.1.5 Combined monophasic oral contraceptives

Combined HC is the most common form of HC used by females, accounting for 54% of HC use in the general population (Cea-Soriano et al., 2014) and 68.5% among elite female athletes in the United Kingdom (Martin et al., 2018). The vast majority of these are combined monophasic oral contraceptive pills (OCP) (99.3%) (Martin et al., 2018), which deliver the same amount of oestradiol and progestogen daily during the active pill days/pill taking days, providing a regular hormonal environment comparatively to that of a eumenorrheic cycle with acute changes in hormones across approximately 28 days. Most combined monophasic OCPs follow a 21/7-day regimen (*i.e.*, 21 days with fixed doses of oestradiol and progestogen, and 7 days of a hormone-free interval) with various doses of ethinyloestradiol and different types, doses, potency, and androgenicity of progestogens (Burrows and Peters, 2007).

4.1.6 Impact of exogenous hormones on endogenous levels

During the 21 active pill days, exogenous hormones are more potent than endogenous ovarian steroids. The intake of fixed doses of oestradiol and progestogen in the active pill downregulates the production of endogenous ovarian steroid hormones, with a daily surge of exogenous ovarian steroid hormones occurring about an hour after active pill ingestion. During the hormone-free interval, the withdrawal of exogenous hormones causes a sharp rise in endogenous oestradiol serum level, peaking between day 26 and 28 of the HC cycle (Rechichi, Dawson, & Goodman, 2008; Rodriguez et al., 2024) (Figure 1.3).

To date, no reliability studies have exclusively focused on two key aspects: (i) assessing AKL during the most stable phase of exogenous hormonal influence, (specifically, between day 7 and 21 of the pill taking phase) to exclude the effects of endogenous ovarian steroid hormones on AKL; and (ii) including participants who represent the majority of OCP users (*i.e.*, those using combined monophasic OCPs). To ensure reliable results that accurately reflect the GNRB intra-rater reliability and eliminate potential measurement variations due to hormonal fluctuations between visits, it was essential to incorporate these restrictive inclusion criteria into the study design. Therefore, this study aims to assess the intra-rater reliability of the GNRB in a mixed-sex cohort of participants who do not experience chronic ovarian steroid hormone fluctuations.

4.2 Methods

4.2.1 Participants

This was a mixed-sex cohort study in which participants were (i) males; and (ii) females using combined monophasic OCP – in the active phase, between day 7 and day 21 of the HC cycle. Sixty-five potential participants were screened, 48 were not eligible, and 11 participants (seven males and four females aged 24 – 38 years) took part in the study. All participants were recruited via poster, word of mouth, and email from January to March 2023, and completed an informed consent form, health screen, menstrual history questionnaire, and COVID-19 questionnaire (Appendix I, J, K, L) prior to participating. The study was approved by the Nottingham Trent University Research Ethics Committee (Humans – Invasive, approval ID:1595733) and participants were free to withdraw from the study at any point.

Female participants were excluded from the study if they had chronic ovarian steroid hormone fluctuations, menstrual irregularities (MI) (*e.g.*, amenorrhea, oligomenorrhea, polymenorrhea, luteal phase deficiency), if they were pregnant, or have been pregnant in the 12 months prior enrolling in the study, if they were breastfeeding, or have breastfed in the 12 months prior to enrolling in the study, and if they took combined monophasic OCP, combined HC, or cyclical combined hormone replacement therapy (HRT) for less than 3 months prior to the beginning of the trial period. Combined HC users were excluded in case of incorrect use of their HC during the trial period (*e.g.*, if the participant missed a pill, or more than one pill, in the HC cycle of the testing period) as this might have an impact on the stability of their ovarian steroid hormone levels.

Participants who had any history of a medically diagnosed knee joint injury or surgery were excluded from the research study. Participants with a BMI below 15 or above 30 kg/m^2 were also excluded due to the potential impact on the reproductive steroid hormone levels and on the composition of soft tissues. Participants who needed to use non-steroidal anti-inflammatory drugs (NSAIDs) in the 24 hours prior to a testing session were not accepted due to the promotion of sodium and water retention (for an overview of electrolyte and acid-base disturbances associated with NSAIDs, see Kim & Joo, 2007).

The level of physical activity of participants was also assessed, following the participant classification framework proposed by McKay et al. (2022). Participants classified in Tier 1 and Tier 2 (respectively recreationally active and trained/developmental) were recruited to provide representation of most of the global population. In addition, the ACL structure has less risk to be affected by these physical activity levels in the Tier 1 and Tier 2 categories than in higher Tiers.

4.2.2 Research design

This study followed an intra-rater test-retest reliability study design with one experienced examiner (EN) performing all measurements using the GNRB on three separate days. The examiner underwent training with a GNRB representative as well as several months of practice sessions prior to testing. The testing procedure of participants was entirely passive, therefore, there was no learning effect between sessions negating the need for any familiarisation session. AKL was determined on

three separate occasions (Trial 1, Trial 2, and Trial 3), following the same protocol on each visit, with each visit being conducted at the same time of day. Laboratory visits were conducted on three consecutive days, but when this preferred option was not feasible for the participants, the tests were conducted as close to one another as possible over a period of 15 days. Testing sessions were conducted in a laboratory with a stable room temperature of 20 to 22 °C. Room temperature and humidity were monitored and registered on every testing session by a digital hygrometer.

Participants were asked to maintain a similar diet (*i.e.*, food intake and its timing, caffeine, tea, and water consumption), levels of physical activity, refrain from strenuous exercise and abstain from alcohol in the 24 hours prior to each laboratory visit. OCP users were instructed to normalise the timing of their pill intake, ensuring it was taken at the same time each day, starting as soon as informed consent had been completed (on average 10 days before testing). Participants were given an OCP diary to record the date and time of their OCP intake (Appendix M). Participants continued tracking and reporting their normalised OCP intake time during the testing period, which lasted an average of 5 days. Compliance with these instructions was confirmed by the participants prior to the start of each trial.

4.2.3 Procedures

4.2.3.1 Anthropometric measurements

Upon arrival to the laboratory participants were asked to be bare feet and wear minimal clothing (*i.e.*, shorts and t-shirt) for height and body mass measurements. Height and body mass were measured and recorded using a stadiometer (Seca, UK) and a digital weighing scale (Seca, UK). Due to the very short timeframe of this reliability study (*i.e.*, over a couple of days), height and body mass were not expected to vary significantly between all three testing sessions therefore height and body mass measurements were only measured at participants' first visit. In addition, participants self-reported their vigorous and moderate physical activity levels from the seven days preceding the first testing session using the International Physical Activity Questionnaire Short Form (IPAQ-SF).

4.2.3.2 Automated arthrometric testing

AKL was assessed, and recorded with an automated knee arthrometer (GNRB Rotab, Genourob – Automated Dynamic Laximetry, France). When participants arrived for testing, they were asked to sit resting comfortably on the GNRB's examination table for 20 min prior to each test session to stabilise their body temperature.

Participants' dominant leg (*i.e.*, preferred leg to kick a ball) was recorded and both knees were tested following an ABBA counterbalancing approach (*e.g.*, 1st session: 1st knee tested = left knee, 2nd knee tested = right knee; 2nd session: 1st knee tested = right knee, 2nd knee tested = left knee etc.) to ensure no bias to the tester or order effect. Participants were then positioned supine on the GNRB's examination table, with the back support set at a 45° angle, the knee flexion set between 25 and 30° – corresponding to the Lachman test position – (Figure 4.1) and their arms resting on the examination table next to their torso. A goniometer was used to ensure both back and knee supports were set at the same angle on each trial. Participants were asked to keep the tested leg fully relaxed to prevent any muscular activation from the hamstrings.

Participants' lower limb was placed on the device in a neutral position, with the knee positioned in the centre of the knee support, the foot resting on the adjustable boot, the heel and midfoot in contact with the footplate of the boot, whilst in a neutral position between internal and external rotation. The apex of the patella and the anterior tibial tubercle were located by palpation and marked with a pen. The knee-cup was initially fastened (*i.e.*, clamping force sensor of the patella) on the patella, without tightening it yet, ensuring that the mark previously done at the apex of the patella is aligned with the line of the 'FRONT' hole. The knee-cup was positioned horizontally and centred on the patella. The foot was then fastened and tightened in the adjustable boot in a firm but comfortable position. Participants' tibial length (in mm, readable on a metered scale below the boot), and tibial rotation angle (in °, assessed by a sensor and readable within the GNRB software [LDA software]) were recorded for retest purposes.

The displacement sensor was positioned on the anterior tibial tubercle skin mark, perpendicular to the tibia. A goniometer was used to ensure that the

displacement sensor was set perpendicular to the tibia on each trial of each leg. Finally, the knee-cup was tightened between a minimum force of 50N, pressure assessed by a sensor and readable within LDA software, and a maximum of individual comfortable force that participants could tolerate for testing. The knee-cup force initially set was recorded for each knee on Trial 1 and reproduced +/- 10N on subsequent trials. The thigh strap was then tightened to optimise the stability of the lower limb during testing. Once the final testing position set the tester reminded the process of the test to the participants and participants were asked to remain relaxed.

The GNRB was programmed to perform five consecutive motorised anterior tibial translations – produced by an automated ramp of pressure on the calf – at 134N, 150N and three pushes at 200N. A displacement transducer, with an accuracy of 0.1mm, recorded the displacement of the anterior tibial tuberosity with respect to the femur. Anterior tibial displacement data, in millimetres, were recorded on a laptop provided by the manufacturer, on which a file of measurement results was created for each participant. Once the first testing set was completed on the first leg, participants' leg was released from the GNRB, and the same measurement protocol was repeated on the contralateral leg. To ensure excellent test repeatability between the first and the second knees' set, the researcher applied the following settings: (i) up to 1cm difference of lower leg length was tolerated between left and right side; (ii) up to 2° difference in the tibial rotation angle was tolerated between left and right side; and (iii) up to 10% difference in the knee-cup pressure, with a minimum force of 50N, was tolerated between both sides. All procedures were repeated in Trial 2 and 3.

ACL compliance/slope, also called ACL elasticity, is the inverse of ACL stiffness (*i.e.,* differential load/differential displacement) (Markolf, Kochan, & Amstutz, 1984). The ACL compliance/slope value represents the slope of the curve during testing between a pressure load of 100N and the maximum pressure load applied by the GNRB device (*i.e.*, 200N in the study herein) (Figure 4.2) (Robert et al., 2009). ACL compliance is the gradient of the curve between two loading forces, using the formula:

$$\text{ACL compliance } (\mu m/N) = (Y2-Y1) / (X2-X1)$$

where Y (displacement) is in μm, X (Force) is in N.

ACL compliance/slope curves and values were acquired using the manufacturer's laptop provided with its dedicated LDA software. Raw data were exported from LDA software as a PDF file without modifications.

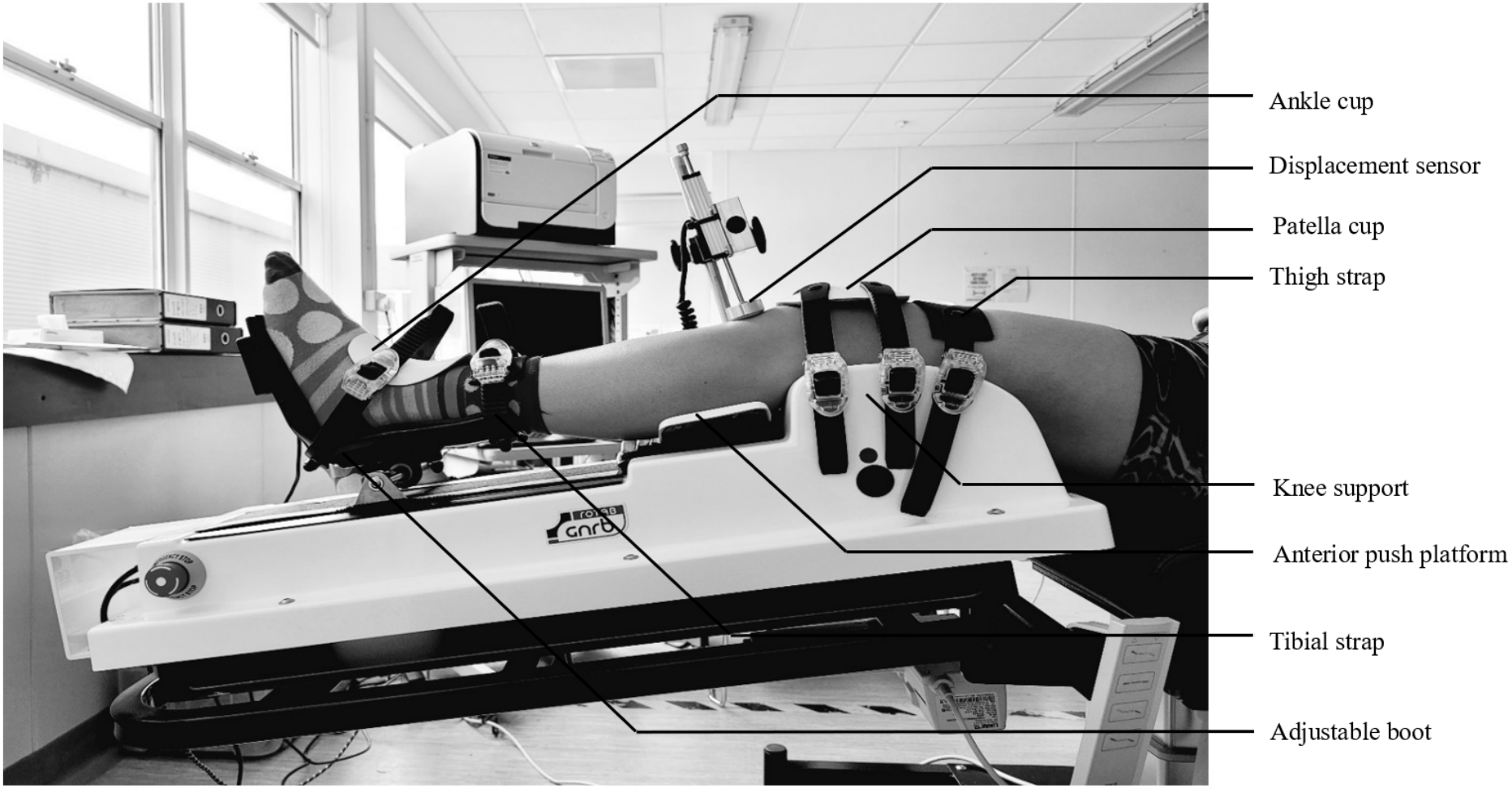

Figure 4.1. Participant positioning for assessments on the GRNB.

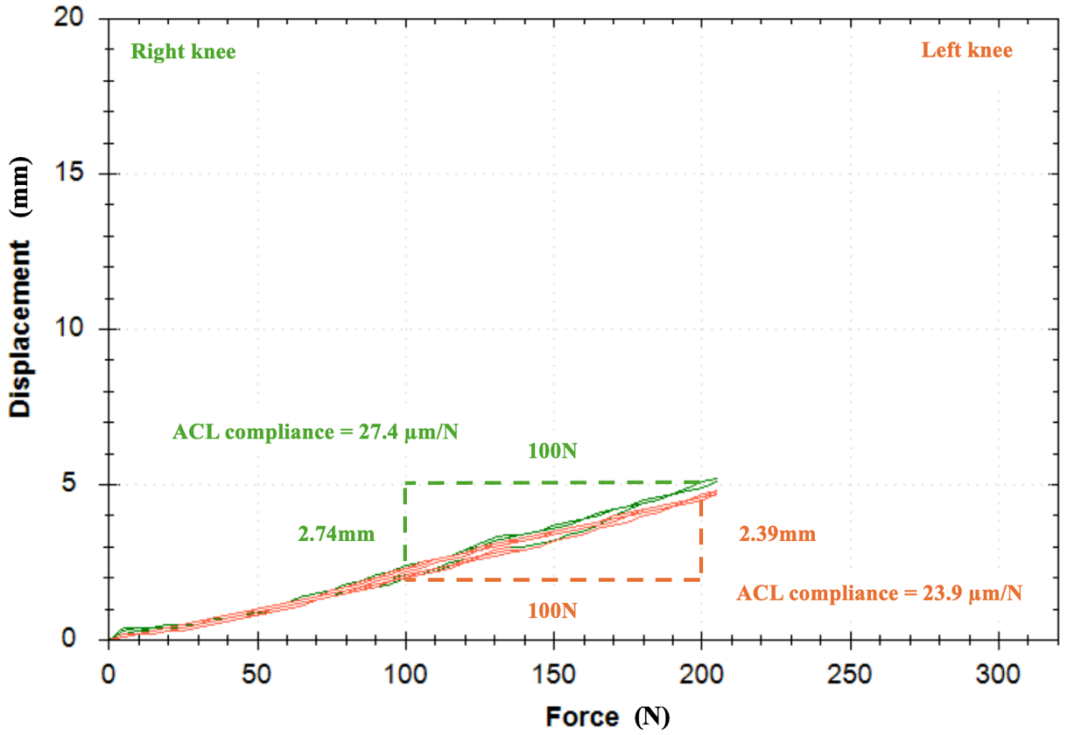

Figure 4.2. A female participant's ACL compliance/slope in both knees, between 100N and 200N of loading force. ACL, anterior cruciate ligament; N, Newton. Green and orange curves correspond to the anterior tibial displacement (in mm) in both knees, right and left, respectively, with incremental loading force (from 0 to 134N, 150N, and 200N).

4.2.4 Statistical analyses

The assumption of normality was verified using the Shapiro-Wilk test and the assumption of sphericity using the Mauchly's test of sphericity. Descriptive data are reported as the mean ± standard deviation (SD) and the level of significance was set at $p < 0.05$. A two-sample t-test assuming equal variances was performed on all variables reported in Table 4.1. A repeated measures analysis of variance (ANOVA) was performed to determine the effect of separate testing occasions/time on anterior knee laxity measurements and anterior cruciate ligament compliance/slope (Table 4.2). Effect size, reported as partial eta squared ($\eta^2_{partial}$), was calculated and reported for comparisons of effect sizes across studies with the same experimental design (Lakens, 2013).

A two-way random-effects model intraclass correlation coefficient (ICC), based on the mean of the three repeated measures, with absolute agreement, and reporting 95% confidence intervals (95%CI), was used alongside the coefficient of variation (CV%) to evaluate relative intra-tester test-retest reliability. Standard deviation/mean *100 was used to calculate the coefficient of variation. An ICC below 0.5 indicates poor reliability, an ICC between 0.5. and 0.75 moderate reliability, an ICC between 0.75 and 0.9 good reliability, and an ICC greater than 0.9 excellent reliability (Koo & Li, 2016).

The standard error of measurement (SEM) was calculated to provide an estimate of the precision of the tests and was calculated as SEM = SD√(1-ICC), where SD is the standard deviation of all AKL measurements taken at a certain force on a lower limb in all participants (Weir, 2005). Estimation of the minimum detectable change (MDC), representing the minimum differences in the measurements of AKL and ACL compliance considered true changes were established using MDC = SEM*1.96*√2 (Weir, 2005) and MDC% = (MDC/mean of all observations) *100 (Webber & Porter, 2010). IBM SPSS Statistics for Macintosh, version 29 (IBM Corp., Armonk, N.Y., USA) software and Excel for Macintosh, version 16.79.1 (Microsoft 365, Microsoft Corp., USA) were used in the statistical analysis.

4.3 Results

4.3.1 Participant characteristics

Eleven participants, including seven males (age 29 ± 4 years; height 1.84 ± 0.1 m; body mass 85.3 ± 7.6 kg) and four females (age 27 ± 2 years; height 1.68 ± 0.0 m; body mass 71.2 ± 6.0 kg) all of whom were combined monophasic OCP users, completed the study without any issues. The females were on a 21/7-day regimen of the following OCPs: Millinette 30/75 (30 µg ethinyloestradiol, 75 µg gestodene); Denille (30 µg ethinyloestradiol, 2 mg dienogest); Levest 150/30 (30 µg ethinyloestradiol, 150 µg levonorgestrel); and Cilique 250/35 (35 µg ethinyloestradiol, 250 µg norgestimate). They had been using this method of contraception for an average of 9 years, with a range of 3 to 12 years. Twenty-two lower limbs were tested across three timepoints. All participants reported their right leg to be their dominant leg.

Female participants height and weight were significantly lower than their male counterparts (p = 0.01). However, BMI and physical activity levels for combined moderate and vigorous activity were similar between males (25.33 ± 1.73 kg/m^2; 2392.86 ± 698.25 MET-min/week) and females (25.26 ± 2.07 kg/m^2; 2600 ± 1033.83 MET-min/week). Table 4.1 presents participants characteristics, AKL at 134N and 200N, ACL compliance/slope at 200N in males and females, and the t-test results for all variables. Among all the tests performed, the side-to-side AKL difference was ≤ 3 mm at 134N, confirming that no knee suffered from a previous total ACL tear (Daniel et al., 1985a; Markolf, Graff-Radford, & Amstutz, 1978; Robert et al., 2009).

4.3.2 Anterior knee laxity

Mean AKL of the right and left knee at both 134N and 200N did not differ significantly between the three testing sessions (Table 4.2).

4.3.3 Sex-specific anterior knee laxity

AKL measurements in right knee at 134N were not significantly different between males (3.96 ± 1.35 mm) and females (4.36 ± 1.56 mm). Equally, AKL measurements in left knee at 134N were not significantly different between males (3.75 ± 1.27 mm) and females (4.04 ± 1.65 mm). However, AKL measurements in right knee at 200N were significantly different between males (5.32 ± 1.12 mm) and females (6.29 ± 1.89 mm), with $p < 0.001$. There was no significant difference between AKL measurements in left knee at 200N in males (5.09 ± 1.06 mm) and females (5.57 ± 1.7 mm). Table 4.1 presents left and right AKL at 134N and 200N in males and females, and the t-test results for these variables.

4.3.4 Anterior cruciate ligament compliance

Mean ACL compliance/slope of the right knee at 200N did not differ significantly across the three testing sessions ($F_{(2, 20)}$ = 0.18, p = 0.84). Equally, the mean ACL compliance/slope of the left knee at 200N did not differ significantly between the three testing sessions ($F_{(2, 20)}$ = 0.41, p = 0.67). Table 4.2 presents results from the repeated measures ANOVA tests performed for left and right ACL compliance/slope at 200N.

4.3.5 Sex-specific anterior cruciate ligament compliance

ACL compliance/slope in right knee at 200N was significantly different between males (24.74 ± 5.06 μm/N) and females (29.61 ± 3.93 μm/N), with $p < 0.001$. However, there was no significant difference between ACL compliance/slope in left knee at 200N in males (24 ± 4.59 μm/N) and females (25.64 ± 3.24 μm/N). Table 4.1 presents left and right ACL compliance/slope at 200N in males and females, and the t-test results for these variables.

Table 4.1. Participants characteristics, anterior knee laxity at 134N and 200N, and anterior cruciate ligament compliance at 200N (N = 11).

	All participants (N = 11)	**Male participants (n = 7; 64%)**	**Female participants, combined monophasic OCP users (n = 4; 36%)**	**t-test *p*-value**
Age mean, in years (SD)	27.9 (3.81)	28.71 (4.46)	26.5 (2.08)	0.38
Height mean, in m (SD)	1.78 (0.1)	1.84 (0.08)	1.68 (0.04)*	**0.01**
Weight mean, in kg (SD)	80.19 (9.8)	85.34 (7.54)	71.2 (6.03)*	**0.01**
BMI mean, in kg/m^2 (SD)	25.31 (1.76)	25.33 (1.73)	25.26 (2.07)	0.95
Physical activity levels, in MET-min/week (SD)	2468.18 (790)	2392.86 (698.25)	2600 (1033.83)	0.35
Anterior knee laxity mean, in mm (SD)				
Right knee, 134N	4.11 (1.42)	3.96 (1.35)	4.36 (1.56)	0.45
Left knee, 134N	3.86 (1.4)	3.75 (1.27)	4.04 (1.65)	0.58
Right knee, 200N	5.67 (1.5)	5.32 (1.12)	6.29 (1.89)*	**<0.001**
Left knee, 200N	5.27 (1.32)	5.09 (1.06)	5.57 (1.7)	0.08
Anterior cruciate ligament compliance/slope mean, in µm/N (SD)				
Right knee, 200N	26.51 (5.19)	24.74 (5.06)	29.61 (3.93)*	**<0.001**
Left knee, 200N	24.59 (4.17)	24 (4.59)	25.64 (3.24)	0.06

Descriptive data are reported as the mean ± standard deviation (SD). BMI, body mass index; MET, metabolic equivalent; n, sample size; N, Newton; OCP, oral contraceptive pill; SD, standard deviation. * indicates a statistically significant *p*-value between female and male participants, with a *p*-value < 0.05 considered significant. Values in bold reflect statistically significant ($p < 0.05$) results.

Table 4.2. Intra-rater reliability for anterior knee laxity measurements and anterior cruciate ligament compliance/slope (N = 11).

	Trial 1 mean (SD)	Trial 2 mean (SD)	Trial 3 mean (SD)	F	*p*-value[a]	$\eta^2_{partial}$	ICC (95% CI)	*p*-value[b]	CV (%)
AKL (mm) at 134N									
Right knee	4.04 (1.31)	4.04 (1.37)	4.25 (1.67)	0.51	0.61	0.05	0.86 (0.67-0.96)	**<0.001**	12.35
Left knee	4.11 (1.57)	3.72 (1.34)	3.75 (1.39)	1.42	0.26	0.13	0.82 (0.59-0.94)	**<0.001**	11.87
AKL (mm) at 200N, average of 3 measurements									
Right knee	5.65 (1.40)	5.58 (1.62)	5.78 (1.60)	0.30	0.75	0.03	0.94 (0.84-0.98)	**<0.001**	9.75
Left knee	5.57 (1.37)	5.19 (1.34)	5.04 (1.32)	3.07	0.07	0.24	0.94 (0.60-0.95)	**<0.001**	9.19
ACL compliance/slope (µm/N) at 200N, average of 3 measurements									
Right knee	26.13 (4.51)	26.62 (5.55)	26.77 (5.91)	0.18	0.84	0.02	0.91 (0.76-0.98)	**<0.001**	8.04
Left knee	24.64 (3.23)	25.17 (4.14)	23.94 (5.23)	0.41	0.67	0.04	0.72 (0.20-0.92)	**0.01**	10.4

ACL, anterior cruciate ligament; AKL, anterior knee laxity; CV, coefficient of variation; ICC, intra-class correlation; CI, confidence interval; $\eta^2_{partial}$, partial eta squared; *p*-value[a], *p*-value of the repeated measures ANOVA test (test of within-subjects effects); *p*-value[b], *p*-value of the F test with true value 0 in ICC calculation using single-rating, absolute agreement, 2-way mixed-effects model. Values in bold reflect statistically significant ($p < 0.05$) results.

4.3.6 Intra-rater reliability

The intra-rater reliability of the GNRB is presented in Table 4.2, Figures 4.3 and 4.4. The ICC values show moderate intra-rater reliability for the left ACL compliance/slope at 200N (ICC_{left} = 0.72 [0.20-0.92]; CV = 10.2%), good intra-rater reliability for right and left AKL at 134N (ICC_{right} = 0.86 [0.67-0.96], CV = 12.35%; ICC_{left} = 0.82 [0.59-0.94], CV = 11.87%), and excellent intra-rater reliability for right and left AKL at 200N (ICC_{right} = 0.94 [0.84-0.98], CV = 9.75%; ICC_{left} = 0.94 [0.60-0.95], CV = 9.19%), as well as for the right ACL compliance/slope at 200N (ICC_{right} = 0.91 [0.76-0.98]; CV = 8.04%)

4.3.7 Standard error of measurement

SEM for mean AKL and mean ACL compliance/slope at 134N and at 200N is presented in Table 4.3. The calculated SEM for mean AKL was greater at a testing force of 134N (SEM_{right_134N} = 0.53 mm [0.81-0.28], SEM_{left_134N} = 0.59 mm [0.90-0.34]) than at a testing force of 200N (SEM_{right_200N} = 0.36 mm [0.60-0.21], SEM_{left_200N} = 0.32 mm [0.83-0.29]).

4.3.8 Minimum detectable change

MDC and %MDC for mean AKL at 134N and 200N, and mean ACL compliance/slope at 200N are presented in Table 4.3. Equally to the calculated SEM, MDC and %MDC values for mean AKL were greater at a testing force of 134N than at a testing force of 200N. MDC and %MDC values at 134N and 200N were ≥ 1.5 mm (36 to 43%) and ≤ 1 mm (17 to 18%) respectively.

Table 4.3. Standard error of measurement and minimum detectable change for anterior knee laxity measurements and anterior cruciate ligament compliance/slope (N = 11).

	SEM (95%CI)	MDC	MDC%
AKL (mm) at 134N			
Right knee	0.53 (0.28-0.81)	1.47	35.82
Left knee	0.59 (0.34-0.90)	1.65	42.73
AKL (mm) at 200N, average of 3 measurements			
Right knee	0.36 (0.21-0.60)	1.01	17.83
Left knee	0.32 (0.29-0.83)	0.89	16.96
ACL compliance/slope (µm/N) at 200N, average of 3 measurements			
Right knee	1.60 (0.76-2.61)	4.44	16.75
Left knee	2.26 (1.21-3.82)	6.26	25.47

ACL, anterior cruciate ligament; AKL, anterior knee laxity; CI, confidence interval; MDC, minimum detectable change (in mm for AKL, and in mm/N for ACL compliance/slope); N, Newton; SD, standard deviation; SEM, standard error of measurement (in mm for AKL, and in mm/N for ACL compliance/slope).

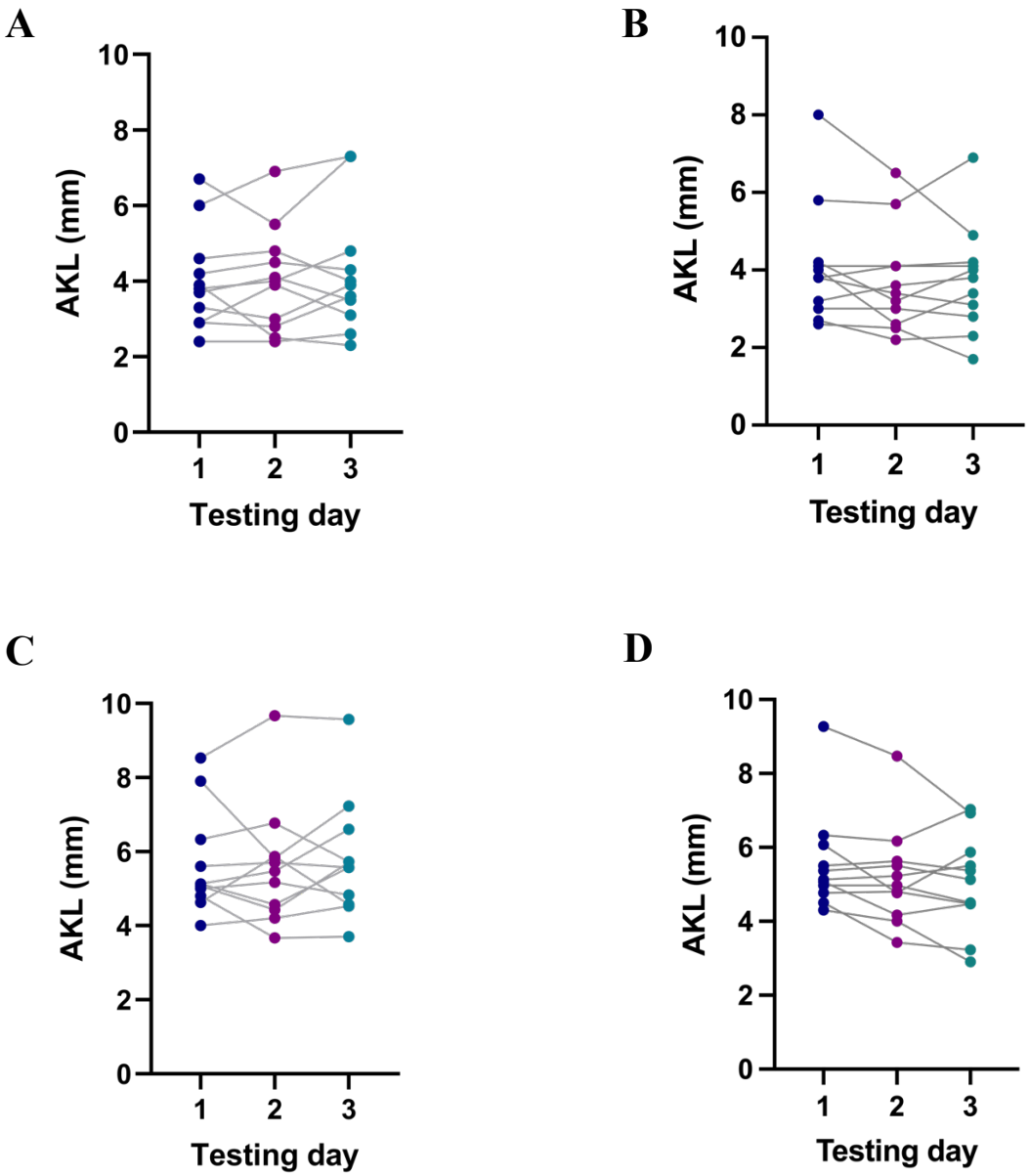

Figure 4.3. Individual data points of repeated measures of right (A) and left (B) AKL at 134N and right (C) and left (D) AKL at 200N across three timepoints (N = 11). AKL, anterior knee laxity; N, Newton.

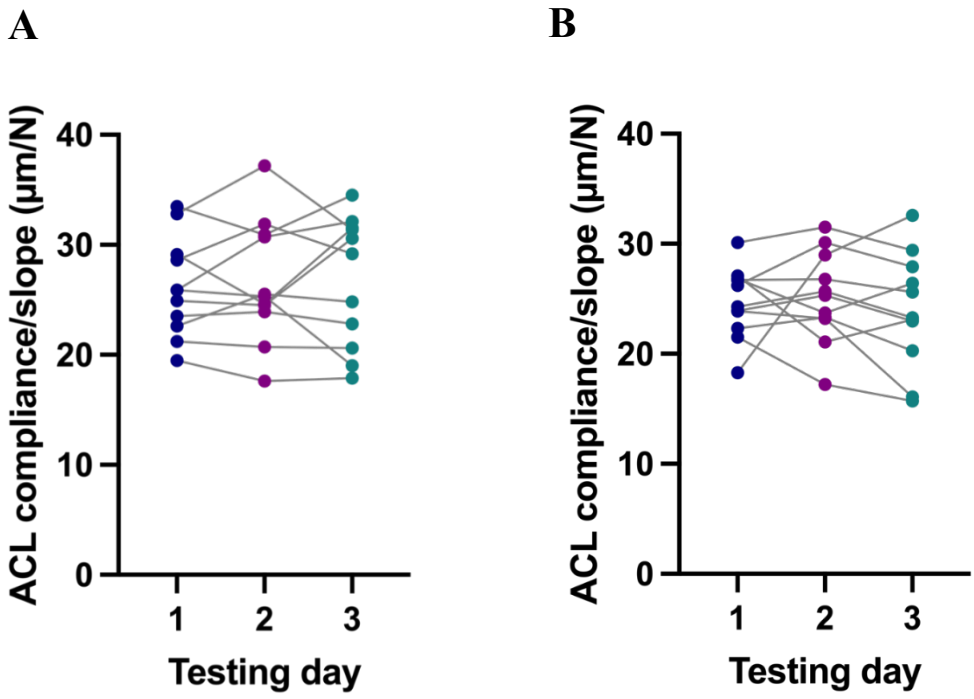

Figure 4.4. Individual data points of repeated measures of right (A) and left (B) ACL compliance/slope at 200N across three timepoints (N = 11). ACL, anterior cruciate ligament; N, Newton.

4.4 Discussion

4.4.1 Overview

The purpose of this study was to determine the intra-rater reliability of the GNRB in quantifying AKL and ACL compliance/slope and exploring its potential utility in clinical settings and sports medicine for evaluating the initial AKL profile among exercisers and athletes, thus assessing baseline anterior knee stability and ACL integrity. Data presented herein indicate that, at both 134N and 200N, the GNRB produced reliable results across participants. These findings align closely with the outcomes of previous intra-rater test-retest reliability studies involving the GNRB at 134N and 200N in healthy knees (Magdič, Dahmane & Vauhnik, 2023; Mouarbes et al., 2018; Smith, Miller, & Laslovich, 2022; Vauhnik et al., 2013). These data will contribute to identifying non-contact ACL injury-prone profiles within female populations.

No statistically significant differences were observed in AKL measurements at 134N and 200N across the three trials. The AKL measurements in the right and left

knees at 134N, conducted across the three trials, demonstrated acceptable CV at 12.35% and 11.87%, respectively, along with good ICC of 0.86 and 0.82, respectively. Similarly, AKL measurements in the right and left knees at 200N exhibited improved CV values at 9.75% and 9.19%, surpassing those at 134N. Additionally, these measurements showed excellent ICC values of 0.94. These results compare favourably with findings from previous intra-rater test-retest reliability studies involving the GNRB at 134N (ICC = 0.41 - 0.85; CV = 22.4% - 28.1%) and at 200N (ICC = 0.45 – 0.85; CV = 20.6%) in healthy knees (Magdič, Dahmane & Vauhnik, 2023; Mouarbes et al., 2018; Smith, Miller, & Laslovich, 2022; Vauhnik et al., 2013).

Equally, no statistically significant differences were observed in ACL compliance/slope values in the right and left knees at 200N between the three trials. ACL compliance/slope in right and left knees at 200N performed across the three trials also had acceptable CV (8.04% and 10.4% respectively), along with good to excellent ICC values of 0.91 and 0.72 respectively. These values compare favourably to previous intra-rater test-retest reliability studies on the GNRB at 200N (ICC = 0.77; CV = 14.25%) in healthy knees (Smith, Miller, & Laslovich, 2022).

In addition, SEM, MDC, and %MDC values were smaller when AKL measurements were performed at 200N rather than at 134N, indicating that more accurate measurements are obtained when a 200N testing load is applied. The SEM and MDC values of this study are, in fact, more favourable than those reported in the only other intra-tester test-retest reliability study with similar indexes (Smith, Miller, & Laslovich, 2022), where these were 135 to 260% greater than the SEM and MDC values presented here.

Overall, the pool of ICC, CV, SEM, MDC, and MDC% values obtained in this study indicates that the GNRB has improved reliability when used at a force of 200N in healthy knees, similarly to those of Robert et al. (2009) who observed a load of 200N to yield good reproducibility of measurements in ACL-injured knees. Similarly, Klouche et al. (2015) concluded that a pressure load of 200N was optimal for diagnosing total ACL tears, with a side-to-side difference threshold set at 1.9 mm, achieving very high sensitivity and specificity.

4.4.2 Clinical implications

AKL measurements enable practitioners to define if a person has sustained an ACL injury by comparing AKL values between both knees, in conjunction with clinical assessment and MRI analysis. The results herein suggest the GNRB is a reliable and suitable tool for more accurate assessment of AKL measurements. As a clinical benchmark, a side-to-side difference in AKL values ≥ 3 mm at 134N (Daniel et al., 1985b; Markolf, Graff-Radford, & Amstutz, 1978; Robert et al., 2009), or ≥ 1.9 mm at 200N (Klouche et al., 2015), indicates a complete ACL tear on the laxer side. Additionally, a side-to-side difference in AKL values ranging from 1.5 to 3 mm at 134N indicates a potential partial ACL tear (Robert et al., 2009). However, when utilised in silo, this side-to-side difference is not always sufficient to reach a clinical conclusion, especially when it is near the cut-off values (Daniel et al., 1985a; Daniel et al., 1985b).

To bolster the AKL measurement, the ACL compliance/slope data offer insight on the structural and mechanical behaviour of the ACL across various loading ranges (Bercovy & Weber, 1995; Maitland et al., 1995; Schmitz & Shultz, 2013). According to Robert et al. (2009), a slope value comprised between 20 and 35µm/N would indicate a healthy knee. Additionally, visual inspection of both AKL curves informs whether they run parallel or divergent at higher loads. If both curves run divergently at higher pressure loads, it suggests that the side-to-side difference in ACL compliance/slope value should be investigated, as it may indicate functional knee instability. A side-to-side difference in ACL compliance/slope ≤ 5 µm/N, between 5 and 10 µm/N, or ≥ 10 µm/N would suggest a low, medium, or high risk of functional knee instability, respectively.

The rich qualitative and quantitative data obtained from automated dynamic laximetry testing with the GNRB provides a comprehensive overview of the ACL integrity and can facilitate early assessment of predictive secondary issues, such as knee osteoarthritis (OA). Chronic knee instability, resulting from unconstrained anterior tibial translation, is a major arthrogenic factor causing knee OA in ACL-injured knees (Louboutin et al., 2009; Simon et al., 2015). Coupled with the presented reliability data, the GNRB emerges as a valuable tool that should be more widely implemented in clinical practice. It offers a more accurate representation of ACL

integrity in both injured and uninjured knees, which is crucial for identifying side-to-side differences. Furthermore, the GNRB plays a significant role in preventing and delaying the onset of OA in ACL-injured patients by detecting knee instability and altered knee kinematics.

4.4.3 Sex differences

In this study, AKL measurements and ACL compliance/slope in the right knee at 200N were significantly different between males and females, with females exhibiting greater values. Although the same measurements in the left knee at 200N did not show a statistically significant difference between sexes, with t-test p-values of 0.08 and 0.06, respectively, females also exhibited greater values than males. However, data were not different between sexes at 134N, but it was observed that both measurements were higher in females than in males.

Several previous studies have measured AKL using various devices and loads, comparing results between adult males and females. Some of these studies accounted for hormonal status and/or reproductive hormone fluctuations among female participants (Beynnon et al., 2005; Pollard et al., 2006; Shultz et al., 2005), while others did not (Huston & Wojtys, 1996; Rosene & Fogarty, 1999; Rozzi et al., 1999; Smith, Miller, & Laslovich, 2022). Nonetheless, all studies consistently demonstrated that females exhibit greater AKL than males, regardless of hormonal status. Although some research did not provide a rationale for these observed differences (Beynnon et al., 2005; Rozzi et al., 1999), most studies attributed this phenomenon to the chronic exposure to ovarian steroid hormones experienced by females during their fertile years (Pollard, Braun, & Hamill, 2006; Rosene & Fogarty, 1999; Shultz et al., 2005; Smith, Miller, & Laslovich, 2022).

A few studies also considered other factors, such as differences in hamstring muscle strength (Hutson & Wojtys, 1996), ACL size, strength and conditioning programmes, and anatomical features (Hutson & Wojtys, 1996; Rosene & Fogarty, 1999). However, after Yu et al. (2001) revealed that short-term and high fluctuations in ovarian steroid hormone levels across the MC could alter ACL collagen synthesis and structure by affecting fibroblast proliferation in ACL cells, subsequent research hypothesised that these hormonal fluctuations are the primary reason for the observed sex difference in AKL in adults. Supporting this hypothesis, studies measuring AKL

in pre-, during, and post-puberty identified young males and females to have similar AKL values before puberty. However, as males mature through puberty, their AKL values decrease, while females maintain higher AKL values during and after menarche onset (Ahmad et al., 2006; Shultz, Nguyen, & Schmitz, 2008).

Despite the female participants in the study herein having a stable reproductive hormonal status due to OCP use, they were previously exposed to regular fluctuations of oestrogen and progesterone from menarche onset until the start of OCP use. This prolonged exposure over several years could be a contributing factor to the current state of their ACL structure. A recent study (Shultz, Morrissey, & Vauhnik, 2024) showed that earlier and greater exposure to oestrogen at a younger age, specifically with an earlier menarche onset at 12 years of age or younger, may lead to some females developing above-average AKL, increasing their risk of ACL injury during their active lives. Earlier menarche onset is associated with greater oestrogen levels post-menarche and in the early years of adulthood, particularly during the follicular phase of the MC (Apter, Reinilä, & Vihko, 1989; Biro et al., 2021). Additionally, the authors observed that individuals with an earlier menarche onset had a higher BMI, which is also linked to greater oestrogen levels (de Ridder et al., 1990; Bhardwaj et al., 2019).

As demonstrated by Lee et al. (2015), greater levels of oestrogen, mimicking the pre-ovulation peak, inhibited LOX activity, altering collagen cross-linking and reducing the stiffness of the human engineered ACL. Therefore, it can be hypothesised that the duration, magnitude, and timing of exposure to oestrogen and progesterone during a female's reproductive lifespan could impact AKL, regardless of the type of hormonal contraception used at the time of measurement. Future studies should explore this further within female populations across different types of hormonal contraception, gathering information about: (i) the age of menarche onset; (ii) the years of exposure to endogenous reproductive hormones without hormonal contraception; and (iii) the number of years of exposure to exogenous reproductive hormones, including their brands, types, and dosage.

The significantly increased difference in AKL and ACL compliance between males and females could be attributed to differences in tensile properties between sexes. Although there are some ACL size variations within and between sexes (Cone, Howe, & Fisher, 2019a), the female ACL is found to be generally smaller than in males

(Anderson et al., 2001; Chandrashekar, Slauterbeck, & Hashemi, 2005 [in cadavers]; Muneta, Takakuda, & Yamamoto, 1997 [in cadavers]), and having poorer mechanical properties (Chandrashekar et al., 2006 [in cadavers]) than males, which might result in an ACL tear happening at lower loads than in males (Chandrashekar et al., 2006 [in cadavers]). In addition, a recent study by Wang et al. (2021) highlighted that after adjusting for weight and sex, a smaller ACL volume was a strong predictor of greater AKL. In a complementary study (Shultz et al., 2022), the same research team found that there was a positive association between the ACL size of the participants and their AKL, either in the dominant or in the non-dominant leg, and suggested that ACL size may, in part, be modifiable by increasing quadriceps volume with training, bearing in mind that a balanced quadriceps:hamstrings strength ratio needs to be maintained to prevent adding further musculoskeletal injury risks. Both of which could be notable findings when building preventative and monitoring practices within the applied setting.

4.4.4 Testing at higher pressure loads

In addition, depending on the population studied, it might be of great interest to perform automated dynamic laxity testing at higher pressure loads to investigate the ACL tissue response in terminal stiffness (Schmitz & Shultz, 2013), with terminal stiffness being defined as ACL stiffness in the last phase of anterior tibial translation restraint during which the ACL is fully engaged (Maitland et al., 1995). As explored by Schmitz and Shultz (2013), greater magnitude of cyclic change of AKL in female responders (*i.e.*, eumenorrheic female participants in which the absolute magnitude of their cyclic changes in AKL across the MC is > 1.75 mm) resulted in decreased ACL stiffness, but only at the loading ranges, between 100N and 130N.

To date, researchers were restricted to using testing loads ≤ 134N due to the maximal load threshold of the testing devices (Deie et al., 2002; Park et al., 2009a, 2009b; Romani et al., 2003; Schmitz & Shultz, 2013; Shagawa et al., 2021). However, increasing testing loads would be of interest to explore ACL tissue response in females identified with a greater magnitude of cyclic changes in AKL across the MC, thus being closer to ACL rupture threshold, reported at 1266 +/- 527N in the ACL of female cadavers (Chandrashekar et al., 2006). The GNRB knee arthrometer used in this study can perform tests with loads up to 250N, provided the patient or participant tolerates

it well. This feature could help explore ACL terminal stiffness under higher pressure loads, providing more relevant information about the potential risk of non-contact ACL injuries in the said population. However, despite the feasibility of testing at 250N, such force is not always well tolerated by the patients or participants, as some have reported pain during the test, both in healthy and ACL-injured knees (Beldame et al., 2012; Klouche et al., 2015; Vauhnik et al., 2013).

4.4.5 Role in non-contact ACL injury prevention and rehabilitation

This study is the first comparing AKL and ACL compliance/slope measurements at 134N and 200N between males and combined monophasic OCP users. Although combined monophasic OCP users have a stable endogenous and exogenous hormonal profile, the results indicate that AKL and ACL compliance/slope measurements are significantly greater in females at 200N, respectively. This finding suggests that factors other than the current reproductive hormonal status of female participants at the time of testing, which did not feature meaningful endogenous or exogenous variations, contribute to the generally increased AKL in females. Such factors include, for example, early and chronic exposure to ovarian steroid hormones during puberty and maturation until the commencement of hormonal contraception, when applicable (Shultz, Morrissey, & Vauhnik, 2024), as well as anatomical differences like a shorter ACL in females (Anderson et al., 2001; Chandrashekar, Slauterbeck, & Hashemi, 2005 [in cadavers]; Muneta, Takakuda, & Yamamoto, 1997 [in cadavers]). A greater AKL in females, regardless of their endogenous or exogenous hormonal status, could have potential clinical implications for non-contact ACL injury prevention in the field.

Implementing sport-specific ACL injury prevention programmes as early as possible, ideally before or at the onset of puberty when AKL is still similar between young males and females, is crucial. During this period, cyclic ovarian steroid fluctuations have not yet impacted ACL structure (Shultz, Morrissey, & Vauhnik, 2024), and high-risk movement patterns have not developed yet (Otsuki et al., 2021). Ensuring that young females continue these programmes during and after puberty is essential (Otsuki et al., 2021; Renström et al., 2008). Consistent maintenance and adherence to these programmes throughout the pre-, during, and post-season is key to achieving proactive results (Renström et al., 2008). Despite the importance, the

blanket implementation of primary injury prevention programmes remains a missing piece in the ACL injury prevention puzzle, especially in elite girls' and women's football (Bandak et al., 2024).

Regular monitoring of ACL integrity in female athletes participating in high-risk sports for non-contact ACL injury is necessary. This involves building AKL profiles by screening uninjured female knees to identify those with potentially higher-than-physiological AKL values (Mouton, Theisen, & Seil, 2016) and monitoring the contralateral knees of females with non-contact ACL injuries, as these display greater AKL than those of uninjured individuals, predisposing them to a higher risk of secondary ACL (Mouton et al., 2015; Uhorchak et al., 2003).

Given the variability in methodologies with regards to the testing devices and the loading force levels used to assess AKL (Beynnon et al., 2005; Carcia et al., 2004; Maruyama et al., 2021; Park et al., 2009a, 2009b) and ACL compliance/slope (Schmitz and Shultz, 2013; Shagawa et al., 2021; Park et al., 2009a, 2009b), and consequently, the increased risk of non-contact ACL injuries (Uhorchak et al., 2003; Vacek et al., 2016; Woodford-Rogers, Cyphert, & Denegar, 1994;), this study reinforces previous research affirming the GNRB knee arthrometer's utility and enhanced reliability (Magdič, Dahmane & Vauhnik, 2023; Mouarbes et al., 2018; Smith, Miller, & Laslovich, 2022; Vauhnik et al., 2013). The device could be consistently used by sports medical teams, orthopaedic surgeons, and physiotherapists for (1) performing baseline clinical assessments of knee stability and ACL integrity contributing to non-contact ACL injury prevention; (2) complementing MRI diagnoses of partial or complete ACL tears; (3) assessing continuously knee stability during in-season monitoring; and (4) monitoring objectively rehabilitation processes. (Cojean et al., 2023; Collette et al., 2012; Gokeler et al., 2017; Jenny & Arndt, 2013; Mouarbes et al., 2018; Nouveau, Robert & Viel, 2017; Robert et al., 2009; Ryu, Na, & Shon, 2018; Saravia et al., 2020; Vauhnik et al., 2013; Vauhnik et al., 2014). Given the increasing annual rise in non-contact ACL injuries (Hootman, Dick, & Agel, 2007; Maniar et al., 2022), particularly amongst females (Chia et al., 2022; Maniar et al., 2022), such a reliable device is needed, alongside clinical assessments, to better understand and assess knee stability profiles among female exercisers and athletes, and therefore play a key role in non-contact ACL injury prevention.

4.4.6 Strengths and limitations

This study is the first to compare AKL and ACL compliance/slope values at 134N and 200N between males and combined monophasic OCP users, who had controlled endogenous and exogenous hormonal statuses. Although minor variability in exogenous hormone concentrations may have occurred across the combined monophasic OCP cycle – due to differences in exogenous hormone half-lives (*i.e.*, ranging from 10 to 24 hours for ethinyl estradiol and 9 to 45 hours for various progestins), as well as slight variations in pill formulations among participants – female participants were tested within the most stable exogenous hormonal environment. Specifically, testing occurred during the pill-taking phase (*i.e.*, days 7 to 21), when OCP users take a consistent daily dose of exogenous ovarian steroid hormones. To further minimise the potential impact of hormonal fluctuations on arthrometric outcomes, the three testing sessions were completed within the shortest possible timeframe, averaging 4.75 days. Additionally, daily pill-taking and session visit times were strictly standardised and consistently replicated across all sessions.

A total of 22 participants was required to match the power calculation computed during the study design phase. However, only 11 participants could complete the study due to a shortened timeframe caused by unforeseen circumstances. Six additional participants were eligible from the initial pool of screened individuals but were unavailable to partake in the study within the restricted timeframe. Although the study herein is underpowered, various complementary reliability indexes were explored (*i.e.*, SEM, MDC, %MDC), all of which returned satisfactory results, demonstrating good to excellent intra-rater reliability of the GNRB. These index results also confirm the relevance of using this device in sports clinical practice to assess the AKL profile of female athletes at different stages of the season, providing partial insights into their knee health, and reviewing the efficiency of already implemented ACL injury prevention programmes.

Finally, the GNRB used in this study was not equipped with a surface EMG (sEMG) activity feedback option, which prevented the control of hamstring muscles activation during testing. Since even slightly early activation of the hamstring muscles affects AKL by restraining anterior tibial translation, and thereby reducing AKL in both uninjured and ACL-injured individuals (Barcellona et al., 2014), it would be

necessary to use knee arthrometers equipped with sEMG activity feedback to obtain accurate AKL values. However, the absence of sEMG activity feedback was considered during testing, and participants were consistently reminded to keep their tested lower limb relaxed before and during each arthrometric testing.

4.5 Conclusion

This study aimed to define the intra-rater test-retest reliability of the GNRB for assessing AKL and ACL compliance/slope in a mixed-sex cohort of males and combined monophasic OCP users, who had controlled endogenous and exogenous hormonal statuses (*i.e.*, participants who do not have chronic ovarian steroid hormone fluctuations). The findings reveal that the GNRB provides good to excellent intra-rater reliability in healthy individuals, with ICC, CV, SEM, MDC, and MDC% values indicating improved reliability when used with a testing load of 200N.

The reliability index results reported in this study support the widespread implementation of the GNRB in clinical practice for the following purposes:

1. Baseline clinical assessment: Qualitative and quantitative evaluation of ACL integrity and functional knee stability in ACL-uninjured individuals who participate in sports with a high risk of ACL injury. This is particularly important for female athletes, especially during pre-adolescence and adolescence, to detect any functional knee instability and to implement customised, sport-specific ACL injury prevention programmes early on;

2. Seasonal surveillance: Pro-active monitoring of ACL integrity and functional knee stability in ACL-uninjured athletes before, during, and after the season. This helps assess the effects of training and competition loads, as well as injury prevention programmes, on these variables, allowing for adjustments in strength and conditioning, training, and/or rehabilitation as needed;

3. Primary diagnosis of ACL injury: Non-invasive diagnosis of acute ACL injuries (partial or total tear) by comparing AKL and ACL compliance/slope in both knees, supplemented with MRI examination;

4. Post-ACL reconstruction monitoring: Continuous non-invasive monitoring of ACL graft integrity and the contralateral knee at multiple points during the rehabilitation process and after the athlete or exerciser has returned to sport;

5. Conservative treatment monitoring: Non-invasive follow-up monitoring of functional knee stability in ACL-injured individuals who have opted for conservative, non-surgical treatment.

Despite the limitations associated with the study being underpowered and the lack of sEMG hamstring muscle activity feedback on the GNRB, this study highlights the significant and near-significant differences in AKL and ACL compliance/slope values between males and combined monophasic OCP users at a loading force of 200N, with values consistently higher in females. Although the endogenous and exogenous reproductive hormone status of the female participants was controlled and stable, the results confirm a notable difference in AKL and ACL compliance/slope, and thus in functional knee stability, between sexes regardless of current reproductive hormone status. Given this observed trend, it is crucial to rapidly and efficiently implement evidence-based, sport-specific ACL injury prevention programmes at pre-adolescence in the exercising female population to help mitigate the constant annual rise of non-contact ACL injuries in females at both amateur and professional levels.

Furthermore, future studies should use the GNRB to investigate:

1. Whether AKL and ACL compliance/slope values are affected by changes in reproductive hormone statuses between the pill-taking phase (*i.e.*, day 7 to day 21 when users take the daily dose of exogenous ovarian steroid hormones) and the peak moment in the hormone-free interval when endogenous ovarian steroid hormones are at their highest levels without interference from exogenous hormone intake (*i.e.*, day 26, 27, and 28 in the hormonal contraceptive cycle) in combined monophasic OCP users;

2. AKL and ACL compliance/slope values in a broader range of hormonal profiles in female athletes and exercisers (*e.g.*, progestin-only HC users with OC, implant, intrauterine system [IUS]), including detailed lists of the brand, type, and dosage of each included HC;

3. ACL terminal stiffness at higher loading forces (> 134N) in females who show great magnitudes of AKL variations across the MC.

Finally, future studies examining the potential effects of endogenous and exogenous reproductive hormones on AKL should include a comprehensive MC and HC history questionnaire. This questionnaire should cover the menarche onset date, the duration spent without using any HC between menarche onset and first HC use (when applicable), and a complete history of HC use, including the timeframe and type of HC used. Collecting this detailed information would provide researchers with valuable insights on the potential impact of endogenous and exogenous reproductive hormones exposure duration on the behaviour of the ACL structure at the time of arthrometric testing.

In conclusion, the systematic use of the GNRB in clinical practice, supported by its repeatedly proven reliability, would greatly enhance non-contact ACL injury prevention programmes, particularly for the exercising female population. Additionally, it would improve ACL injury medical diagnosis, ACL graft healing surveillance, the rehabilitation process, and return-to-play protocols. This chapter underscores the necessity for widespread adoption of the GNRB and demonstrates how such a tool could help stabilise, and potentially reduce the currently high incidence of non-contact ACL injuries in both professional and amateur female athletes.

Chapter 5. Methodological considerations for the assessment of force steadiness

5.1 Introduction

As highlighted in Chapter 1, Section 1.3.4, the incidence of ACL injuries has steadily increased by approximately 10% every year over the past two decades, particularly among females (Chia et al., 2022; Maniar et al., 2022; Weitz, Sillanpää, & Mattila, 2019). Notably, non-contact mechanisms account for over 50% of all ACL injuries (Chia et al., 2022). Despite the development of evidence-based ACL injury prevention programmes, which have been shown to reduce the incidence of non-contact ACL injuries by two-thirds in female athletes (Webster & Hewett, 2018) and mitigate the severity of lower limb injuries (Crossley et al., 2020), the injury rates are still increasing. These established preventative programmes are not widely implemented outside controlled environments such as research studies and clinical trials. Even when adopted, they are often not consistently maintained throughout sports seasons (Bandak et al., 2024, Slauterbeck et al., 2019), which may partly explain their limited impact on curbing the rise in non-contact ACL injuries among females.

These programmes, such as the 11+ (Soligard et al., 2008), HarmoKnee (Kiani et al., 2010), Knäkontroll (Hägglund et al., 2013), and Prep-to-Play (Patterson et al., 2024) are typically integrated into dynamic warm-up routines aimed at enhancing the neuromuscular system. They involve multicomponent neuromuscular training focused on strength, endurance, proprioception, and balance (Grindstaff et al., 2006). However, a comprehensive understanding of all primary ACL injury factors, including genetic, psychosocial, and neuromuscular aspects remains incomplete (Shultz et al., 2019). In order to develop more substantial preventative programmes for non-contact ACL injuries, and given the underlying neuromuscular mechanisms responsible for knee joint control, which, when sub-optimal, contribute to subsequent non-contact ACL injury mechanisms (Griffin et al., 2000: Riemann & Lephart, 2002a; Shultz et al., 2012a; Shultz et al., 2019; Swanik, 2015), it is crucial to assess whether particular elements of neuromuscular training may need further enhancement and/or understanding. Exploring specific aspects of the neuromuscular system could be key to mitigating the risk of non-contact ACL injuries in females.

A key component of the neuromuscular system that can be readily assessed is force output generated during voluntary movement. Increases in muscle force during voluntary contractions are primarily driven by two factors: (i) increased motor unit

firing rate (MUFR); and (ii) increased numbers of MU recruited, making it a key interplay between the nervous and musculoskeletal systems (Enoka & Duchateau, 2017). During a voluntary isometric contraction, force exerted over time is not constant, but instead fluctuates around an average value (Christou, 2010; Enoka et al., 2003). These fluctuations serve as an indicator of force control and are quantified by the neuromuscular force steadiness (FS), also referred to as variability, during submaximal voluntary isometric contractions (Galganski, Fuglevand, & Enoka, 1993). Absolute variability is represented by the standard deviation (SD) of the force within a time series, while normalised variability is captured by the coefficient of variation (CoV) of the force output. In essence, CoV provides a magnitude-based perspective on force steadiness (Pethick, Winter, & Burnley, 2015), making it easier to compare groups with different maximal voluntary contraction (MVC) values (Pethick & Piasecki, 2022).

FS varies based on several factors: (i) the specific characteristics of the muscle (*i.e.*, the number of MU, their recruitment thresholds, and contractile properties [Castronovo et al., 2018; Dideriksen et al., 2012; Watanabe et al., 2013]); (ii) the number of muscles involved and their respective contributions to overall muscle force output (Castronovo et al., 2018; Davis et al., 2020); (iii) the intensity of the contraction (Barry et al., 2007; Jones, Hamilton, & Wolpert, 2002); and (iv) the type of task required to produce the force output (Almuklass et al., 2016; Del Vecchio et al., 2019; Hamilton et al., 2017). FS is a widely used research measure with functional implications, as it is linked to the ability to perform accurate movements during dynamic tasks, thereby indicating the extent of impairment (Christou & Tracy, 2005; Davis et al., 2020; Galganski, Fuglevand, & Enoka, 1993). This makes FS clinically relevant across various populations (Enoka & Farina, 2021; Mear, Gladwell, & Pethick, 2023).

FS also varies across different populations. For instance, FS CoV tends to be greater (*i.e.*, indicating poorer motor control) in elderly individuals compared to young adults (Christou, 2011; Enoka & Farina, 2021; Tracy & Enoka, 2002). Neurological diseases can further alter FS, leading to increased FS CoV in individuals with multiple sclerosis (Davis et al., 2020), Parkinson's disease, or essential tremor (Poon et al., 2011; Sheridan & Flowers, 1990). Additionally, the literature is equivocal on potential

sex-based differences in FS, with some studies showing that females exhibit greater FS CoV than males at low-to-moderate force intensities across most muscle groups (Brown, Edwards, & Jakobi, 2010; Guo et al., 2024; Inglis & Gabriel, 2021; Jakobi, Haynes, & Smart, 2018), while others report no significant differences between sexes in young individuals (Guo et al., 2022), or in elite master athletes at 25% and 40% of MVC (Piasecki et al., 2021). These discrepancies might be due to muscle- and tendon-specific effects, ageing, or the proportion of a contraction used to calculate FS CoV (Guo et al., 2024; Jakobi, Haynes, & Smart, 2018).

Variations in FS might also manifest in sports performance following musculoskeletal injuries, such as ACL injuries. Individuals with ACL injuries may display greater knee extensor force or torque variability during submaximal isometric voluntary contraction tasks compared to uninjured individuals or to their own contralateral healthy knee. This increased FS may be due to the loss of the ACL, which plays a crucial role in motor control and movement quality through its mechanoreceptors (Riemann & Lephart, 2002b; Solomonow, 2006), as well as reduced quadriceps activation following the injury (Hart et al., 2010).

Data from two existing studies on knee extensor torque variability do not fully support this hypothesis (Hollman et al., 2021; Skurvydas et al., 2011). One study had a small sample size (N = 13 [Skurvydas et al., 2011]), and both included participants who were four to eight weeks post-ACL injury without undergoing reconstruction surgery or postoperative rehabilitation. A recent systematic review and meta-analysis (Tayfur et al., 2021) showed there is currently moderate evidence that knee extensor torque variability increases over time, particularly 24+ months post-injury or surgery. Therefore, it is possible that these two studies did not capture knee extensor torque variability due to the short-term status of their participants post-injury.

There is no consensus on the optimal duration of isometric contractions used to calculate FS, which makes it challenging to compare CoV of force values across studies. For instance, Brown, Edwards, and Jakobi (2010) calculated CoV of force over the entire 7.5-second hold phase of each submaximal voluntary isometric contraction. Similarly, Tracy and Enoka (2002) used an 8-second duration from the middle of a 10 to 12-second contraction to calculate CoV of force. In contrast, other studies have focused on the most stable segments, calculating CoV of force over two

seconds (Inglis & Gabriel, 2021) or five seconds (Harrison et al., 2023; Pethick, Winter, & Burnley, 2015; Mear, Gladwell, & Pethick, 2023) of the hold phase.

Given that greater FS can indicate impaired ability to perform precise dynamic movements, using the measure of FS in a sports clinical or applied setting could be valuable for enhancing ACL injury prevention programmes or monitoring postoperative rehabilitation progress of motor recovery after an ACL injury. However, to ensure the accuracy and reproducibility of FS measurements, a better mechanistic understanding of how FS varies with different measurement durations is needed. The purpose of this study was to compare FS CoV calculated from various durations during a submaximal voluntary isometric contraction.

5.2 Methods

5.2.1 Participants

This study involved a mixed-sex cohort of males, and eumenorrheic females who participated in testing sessions during the early follicular phase of the menstrual cycle (MC) – between day 2 and 4 – minimising the potential impact of ovarian steroid hormone fluctuations on the nervous system. Twenty-two participants (11 males and 11 females aged 18 to 39 years) were recruited locally from the community from September 2023 to July 2024 through posters, word of mouth, and email. Before participating, all participants completed an informed consent form, a health and COVID-19 questionnaire, and a menstrual history questionnaire (Appendix N). The study received approval from the Nottingham Trent University Research Ethics Committee (Humans – Invasive, approval ID: 1606166), and participants were free to withdraw at any time.

Participants were eligible if they were between 18 and 40 years old. Female participants were included if they had regular duration MCs (21 to 35 days [Fehring, Schneider, & Raviele, 2006]). They were excluded if they had chronic menstrual irregularities (*e.g.*, amenorrhea, oligomenorrhea, polymenorrhea, or luteal phase deficiency), were using medication known to impact the level of endogenous ovarian steroid hormones, were pregnant or had been pregnant within the past 12 months, were breastfeeding or had breastfed in the past 12 months, had used any type of hormonal contraceptive (HC) or undergone hormone replacement therapy in the past 12 months,

or had experienced irregular periods due to peri-menopause or menopause within the past 12 months.

Participants were excluded if they had any chronic illness or disease, had a body mass index (BMI) below 15 or above 30 kg/m^2 – due to the potential impact on endogenous ovarian steroid hormone levels and the composition of soft tissues, affecting the collection of sufficient and clean neuromuscular data – or any contraindications to the procedures (*e.g.*, lower limb musculoskeletal injury, neurological medication intake). The participants' level of physical activity was assessed using the international physical activity questionnaire (IPAQ) (Craig et al., 2003) (Appendix N), and those who met the criteria for being minimally active (category 2) or health-enhancing physically active (HEPA) (category 3) were included in the study.

5.2.2 Research design

Four experienced examiners (EN, MOH, TI, and JP) conducted all measurements of force on an isometric dynamometer in a single visit. The testing procedure required participants to understand and control specific movements, such as ankle dorsiflexion and knee extension, necessitating a familiarisation session before the experimental visit, conducted between 24 and 36 hours prior to the main experimental trial. Force data, including MVC and FS, also referred to as CoV of force, were recorded during contractions held at 40% of MVC. Participants were instructed to arrive at the laboratory after an overnight fast, refrain from strenuous exercise and alcohol consumption for 24 hours prior to the experimental visit, and avoid caffeinated drinks within 12 hours before and during testing. All testing took place between 7:30 and 9:30am.

5.2.3 Procedures

5.2.3.1 Menstrual cycle assessment

To account for the influence of endogenous ovarian steroid hormones on neuromuscular performance, female participants were assessed during the early follicular phase of their MC (days 2 to 4 after the onset of menstruation) (Ansdell et al., 2019; Piasecki et al., 2023), when both oestrogen and progesterone levels are at their nadirs (Fehring, Schneider, & Raviele, 2006). Female participants tracked their

MC for at least one month before testing and during the testing month, using calendar-based counting and home-based urinary ovulation detection kits, which they began using from day 9 of their menstrual cycle onwards (Boots, Boots UK Limited, England. Ovulation accuracy > 99%; Clearblue advanced digital, SPD Swiss Precision Diagnostics GmbH, Switzerland. Ovulation accuracy > 99%). Participants were instructed to test using the second urine of the day, as per manufacturers guidelines, to take a photograph of the result 10 minutes after testing, and to submit it by email. Testing continued daily until a positive result indicated a rise in luteinising hormone (LH), indicating the occurrence and timing of ovulation (Janse de Jonge, Thompson, & Han, 2019).

5.2.3.2 Anthropometric measurements

After blood collection and breakfast, participants proceeded to the neurophysiology laboratory. They were asked to be barefoot and wear minimal clothing (*i.e.*, shorts and a t-shirt) for height and body mass measurements. Height was measured using a stadiometer in centimetres (Seca, UK) and body mass was recorded using a digital weighing scale in kilograms (Seca, UK).

5.2.3.3 Force recordings

Participants were seated in a custom-built isometric dynamometer with hips flexed at 90°. The knee of the dominant leg – defined as the preferred leg for kicking a ball – was positioned either at 0° for ankle dorsiflexion or 90° for knee extension, with the foot set at a 90° ankle angle for dorsiflexion. During ankle dorsiflexion testing, the participants' leg rested on a custom-built bar in front of them, perpendicular to the chair, ensuring that the tested leg remained aligned with the pelvis in a neutral position. The metatarsals of the tested foot were securely attached to a plate connected to a force transducer using a non-compliant strap (purpose-built calibrated strain gauge, Research Solutions [Alsager] Ltd, Stoke-on-Trent, UK). Participants were instructed to pull their foot and toes towards themselves to engage the dorsiflexors, while refraining from bending their knee or lifting their hip.

During knee extension testing, the lower leg of the tested limb was similarly secured to a plate connected to a force transducer with a non-compliant strap positioned above the medial malleolus (Figure 5.1). Participants were instructed to

push their lower leg away from themselves to activate the knee extensors, while avoiding lifting their hip or grasping the seat of the isometric dynamometer. Additionally, a seat belt was fastened across the pelvis to minimise upper trunk movement during testing.

To record maximal voluntary force, the amplified analogue force signal was recorded at 2kHz *via* a portable amplifier and analogue-to-digital converter (Sessantaquattro+, OT Bioelettronica, Torino, Italy) for simultaneous visualisation in OT+ Biolab software (OT Bioelettronica, Torino, Italy).

All participants completed a standardised warm-up of submaximal isometric contractions, which included three contractions of 10 seconds at 25% of their perceived MVC, three contractions of 10 seconds at 50%, two contractions of 10 seconds at 75%, and one contraction of 5 seconds at 90%. During the warm-up, participants received real-time visual feedback of force traces in Spike2 software (version 10; Cambridge Electronic Design Ltd [CED], Cambridge, UK) displayed on screen in front of them. After completing the warm-up and receiving full instructions, participants were asked to perform an MVC with maximal effort, pushing as hard as possible for three to five seconds. They received real-time visual feedback in OT+ Biolab (Figure 5.2) and verbal encouragement from the researchers, such as "pull, pull, pull" or "push, push, push", depending on the contraction. This procedure was repeated three times with 60 seconds of rest between attempts, with the highest values (in Newtons) being accepted as the MVC. If the difference between the last two recorded MVC values exceeded 5%, a fourth trial was required to ensure accuracy and consistency of the MVC measurements.

After the MVC trials, participants were given at least two minutes to recover before proceeding with the force steadiness task. To quantify force steadiness, a single target line representing a normalised contraction intensity was displayed on screen in OT+ Biolab, which participants were instructed to follow as closely as possible (Figure 5.2). They were guided to perform a series of voluntary isometric trapezoid contractions, consisting of four sustained contractions at 10% and 25% MVC, and two sustained contractions at 40% MVC, in the following sequence: 10%, 25%, 40%, 10%, 25%, 10%, 25%, 40%, 10%, 25%. Each contraction involved gradually increasing the force over five seconds (ascending phase/MU recruitment), maintaining a plateau for

12 seconds (hold phase), and then gradually decreasing over five seconds (descending phase/MU derecruitment) (Figure 5.3). To prevent muscle fatigue, a 30-second rest period was granted between each contraction.

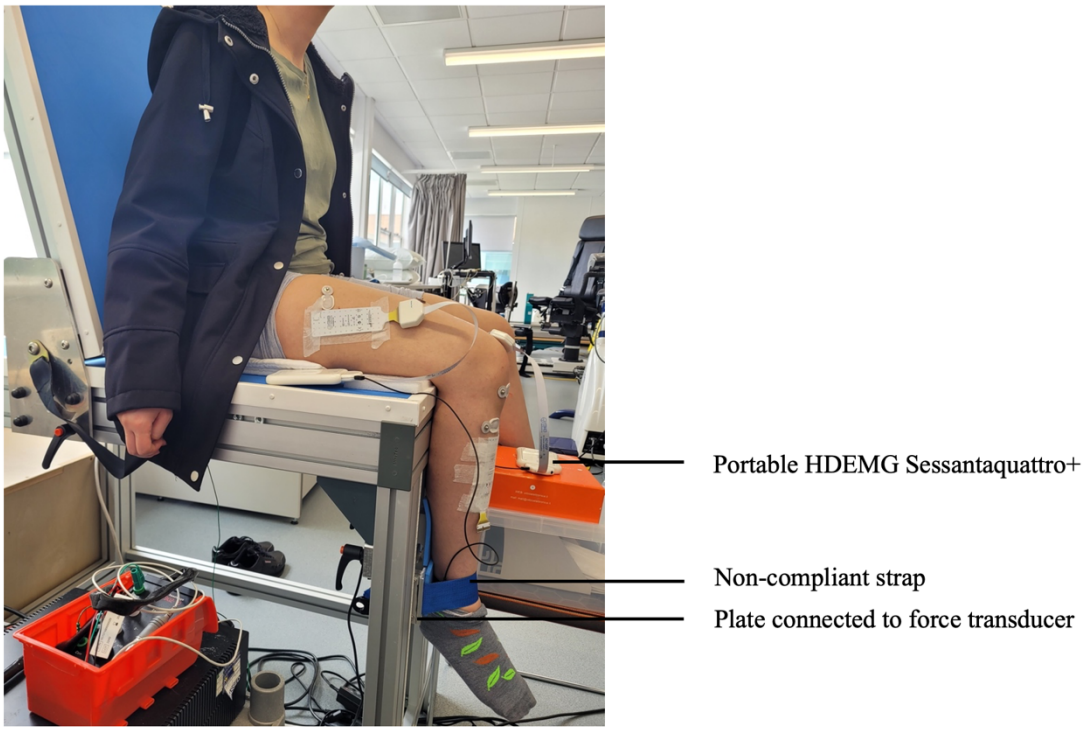

Figure 5.1. Isometric dynamometer set-up for knee extension neuromuscular performance assessment.

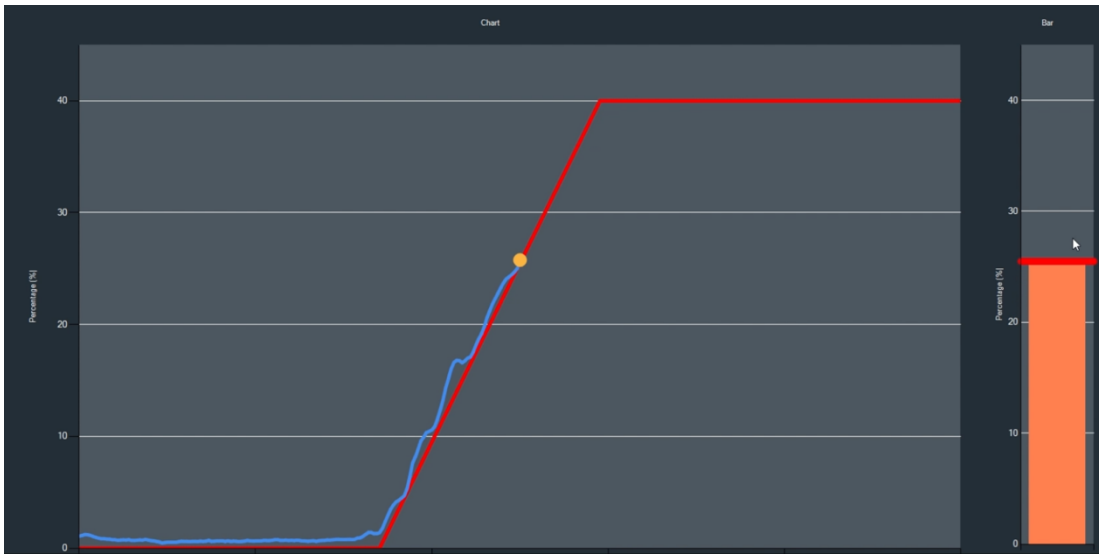

Figure 5.2. Real-time visual feedback of a participant performing a voluntary isometric trapezoid contraction held at 40% MVC in OT+ Biolab. MVC, maximum voluntary contraction; s, second. Y axis: force output's relative percentage, up to 40% here; X axis: time (s); red line: target force; blue line: force trace output performed by the participant; yellow dot: live force output.

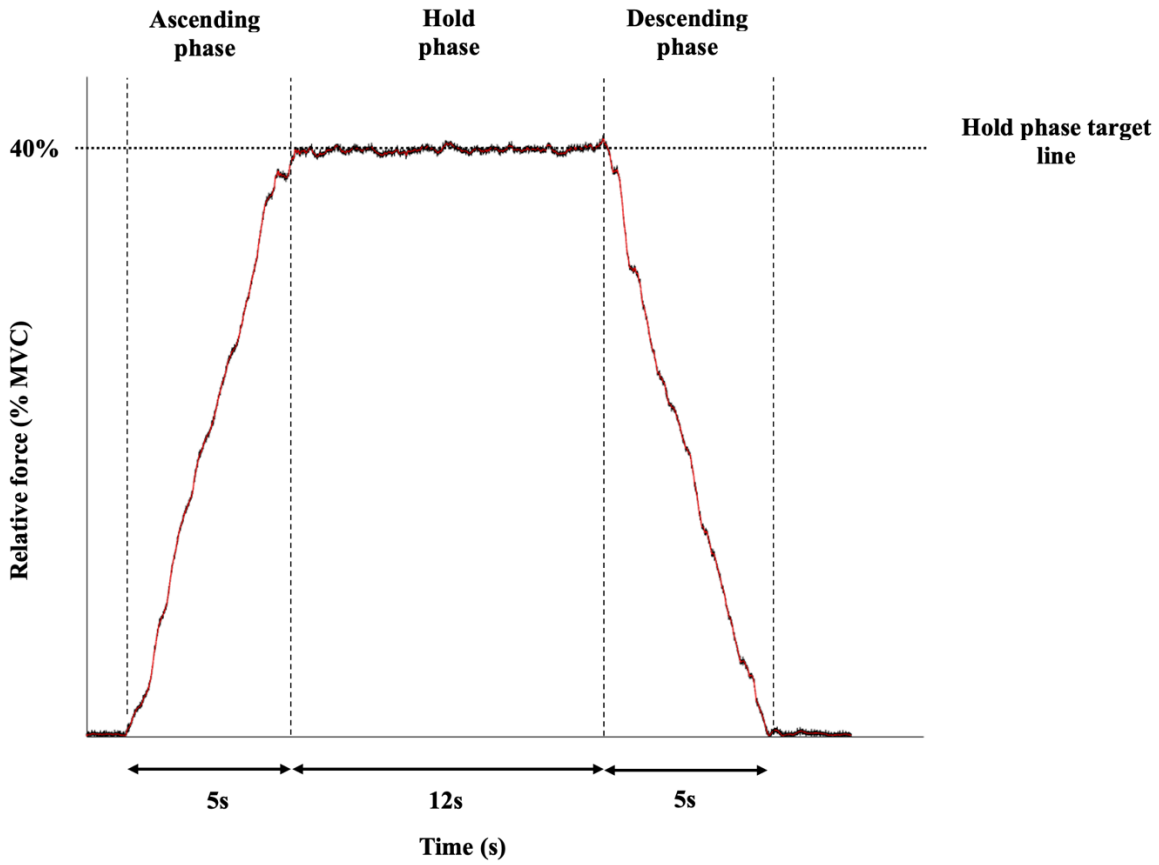

Figure 5.3. Force output during an isometric voluntary contraction held at 40% MVC. MVC, maximum voluntary contraction; s, seconds. Black: raw signal, red: filtered signal.

5.2.3.4 Data analysis

MVC recordings were visualised in OT+ Biolab, and the highest value was selected as the maximal and used to determine isometric voluntary contraction intensity. Acquired force trace data of the second of two isometric voluntary contraction performed at 40% MVC was visualised in OT+ Biolab and selected for analysis. The entire contraction was then exported in MATLAB (MATLAB version: 23.2 [R2023b], MathWorks, Inc., Natick, Massachusetts, USA) for further analysis.

The force trace was filtered using a Butterworth filter with a high cut-off frequency of 20Hz. Specific regions of interest in the force trace were selected, including the ascending, hold, and descending phases (Figure 5.3).

Force steadiness during the 12-second hold phase was quantified using the coefficient of variation of the force output across three durations: the full duration of the hold phase, the five seconds with the lowest CoV, and the two seconds with the lowest CoV (Figures 5.4.A and 5.4.B). The lowest CoV for five and two seconds was automatically calculated across the 12-second force trace *via* a moving window. These CoV were determined using the formula:

$$CoV = [SD/mean] \text{ X } 100$$

where SD represents the force standard deviation, and mean is the mean force, for each of the three durations.

CoV z-scores were calculated to standardise CoV values. These z-scores were determined using the formula:

$$z = (x-\mu)/\sigma$$

where x represents the raw CoV value, μ is the sample mean, and σ is the sample standard deviation, for each of the three durations.

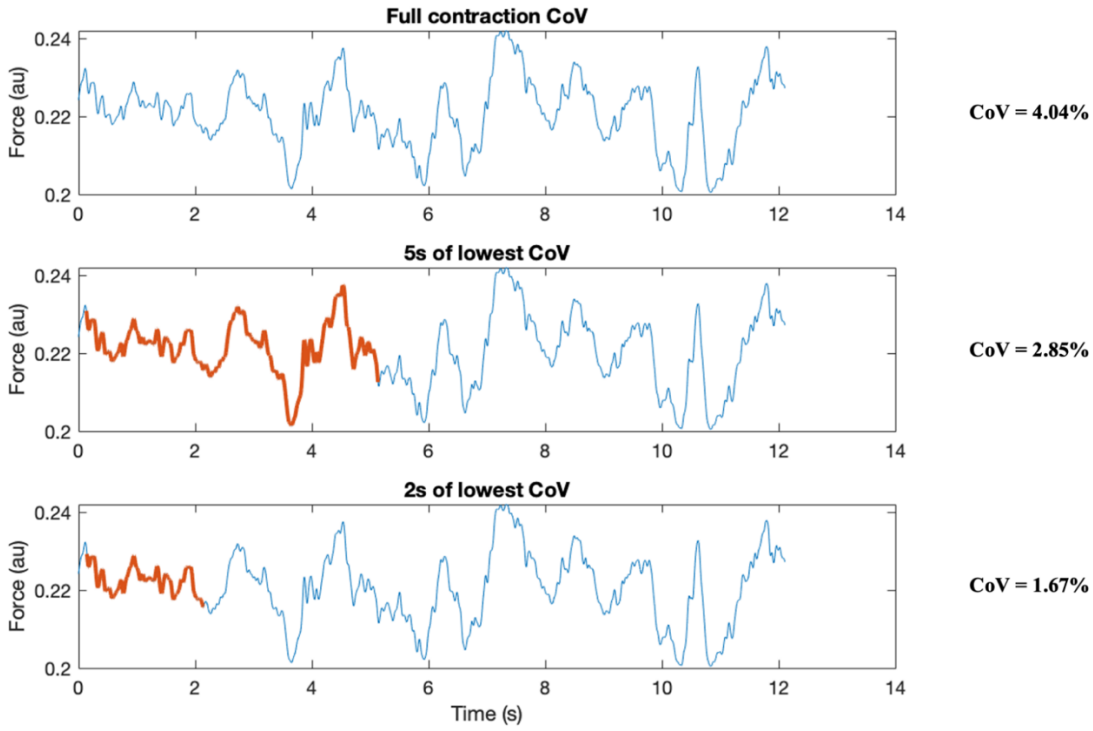

Figure 5.4.A. Force output from the hold phase of an isometric voluntary contraction held at 40% MVC in a participant with a high CoV (less steady), showing the full hold phase CoV (top), 5s of the lowest CoV in red (middle), and 2s of the lowest CoV in red (bottom). au, arbitrary unit; CoV, coefficient of variation; MVC, maximum voluntary contraction; s, seconds.

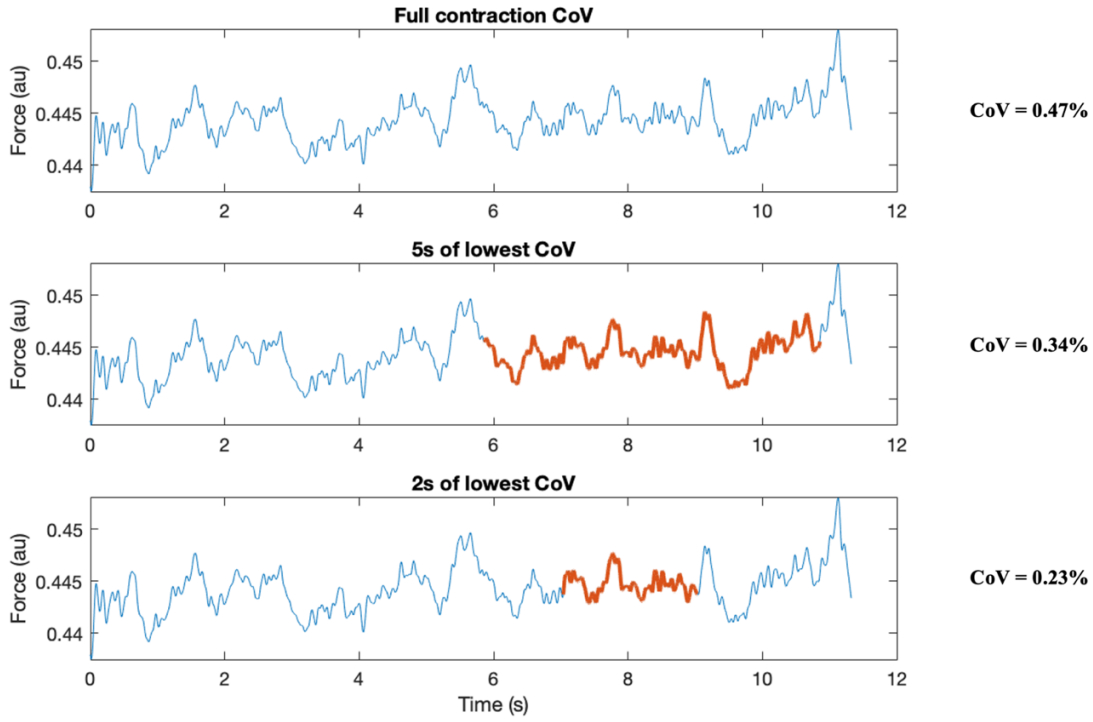

Figure 5.4.B. Force output from the hold phase of an isometric voluntary contraction held at 40% MVC in a participant with a low CoV (more steady), showing the full hold phase CoV (top), 5s of the lowest CoV in red (middle), and 2s of the lowest CoV in red (bottom). au, arbitrary unit; CoV, coefficient of variation; MVC, maximum voluntary contraction; s, seconds.

5.2.4 Statistical analysis

The Shapiro-Wilk test was performed using GraphPad Prism (version 10.3.0) to assess the data distribution. Data were normally distributed; descriptive statistics are presented as the mean ± standard deviation (SD) in Table 5.1, and were conducted using Excel for Macintosh, version 16.79.1 (Microsoft 365, Microsoft Corp., USA).

For the statistical analysis of force steadiness, R Statistical software (version 4.4.0, R Core Team 2023), implemented in RStudio (Version 2024.4.2.764, Integrated Development Environment for R. Posit Software, PBC, Boston, Massachusetts, USA), was used. Linear mixed-effects regression models were generated using the *lme4* package (version 1.1.35.3; Bates et al., 2015) to compare CoV values and standardised CoV z-scores across three durations. The model included contraction duration (*i.e.*, full duration of the hold phase, 5s, and 2s) and movement type as fixed-effects, with participant as a random-effect to account for variability across participants. This was expressed as model <- *lmer(FS ~ ConDur + Movement + (1 | Participant)*.

Results, presented as β coefficient estimates, 95% confidence intervals, and *p*-values, are detailed in sections 5.3.2 and 5.3.3, with statistical significance determined at $p < 0.05$. Visualisation of the statistical outcomes for force output was performed with *ggplot2* package (version 3.5.1; Wickham, 2016).

5.3 Results

5.3.1 Participant characteristics

Force data were sampled from 33 isometric knee extensor and dorsiflexor contractions in 22 adults (11 males, 11 females; age 27 ± 7 years; height 1.73 ± 0.09 m, weight 72.4 ± 11.5 kg, BMI 24.2 ± 2.5 kg/m^2) (Table 5.1).

5.3.2 Force steadiness (CoV)

Force steadiness (CoV) was calculated across the full duration of the hold phase, 5-, and 2-second durations during a 12-second held contraction. Significant differences were found between the full duration (1.67 ± 0.80%) and both the 5-second (1.07 ± 0.59%) and 2-second (0.73 ± 0.36%) durations, using absolute CoV (%) values (all $p < 0.001$). The statistical analysis from the linear mixed-effects model comparing the 10-second to the 5-second duration yielded β = -0.602, 95% CI = -0.742 to -0.461 ($p < 0.001$). Similarly, the comparison between the 10-second and 2-second durations

showed β = -0.945, 95% CI = -1.086 to -0.805 ($p < 0.001$). Individual CoV (%) values for these three durations during the same contraction are depicted in Figure 5.5.

5.3.3 Standardised force steadiness

When force steadiness CoV values were converted into z-scores, they were compared across the three contraction durations – full, five, and two seconds – and no significant differences were found (all p = 1.000). The linear mixed-effects model results for both the full duration *versus* 5-second and the full duration *versus* 2-second comparisons showed β = 0.000, 95% CI = -0.166 to 0.166 (p = 1.000). Individual data points for the standardised force steadiness (CoV z-scores) across the three contraction durations are presented in Figure 5.6.

Table 5.1. Descriptive characteristics of participants showing mean and standard deviation (SD).

N = 22	**Mean (SD)**
Age, years	27 (7)
Height, m	1.73 (0.09)
Weight, kg	72.4 (11.5)
BMI, kg/m^2	24.2 (2.5)

BMI, body mass index; SD, standard deviation.

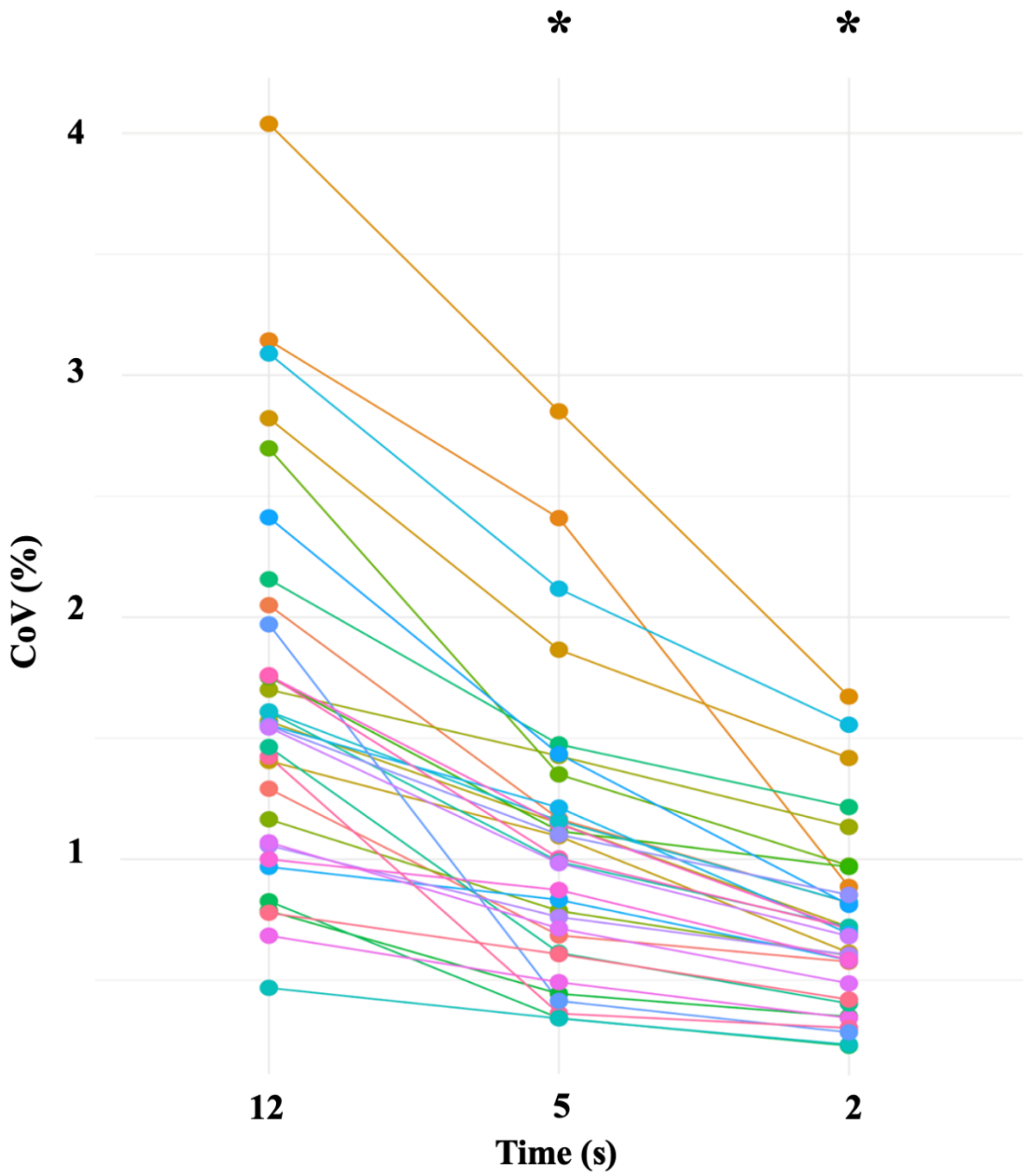

Figure 5.5. Ankle dorsiflexion and knee extension force steadiness assessed at 40% MVC in all participants. Higher CoV values indicate a greater degree of variability (i.e., poorer neuromuscular control) (N = 22). CoV, coefficient of variation; MVC, maximum voluntary contraction; s, seconds. * indicates a statistically significant p-value between the three durations, with a p-value < 0.05 considered significant. The colour code used for each participant's data points is the same between Fig. 5.5 and Fig. 5.6.

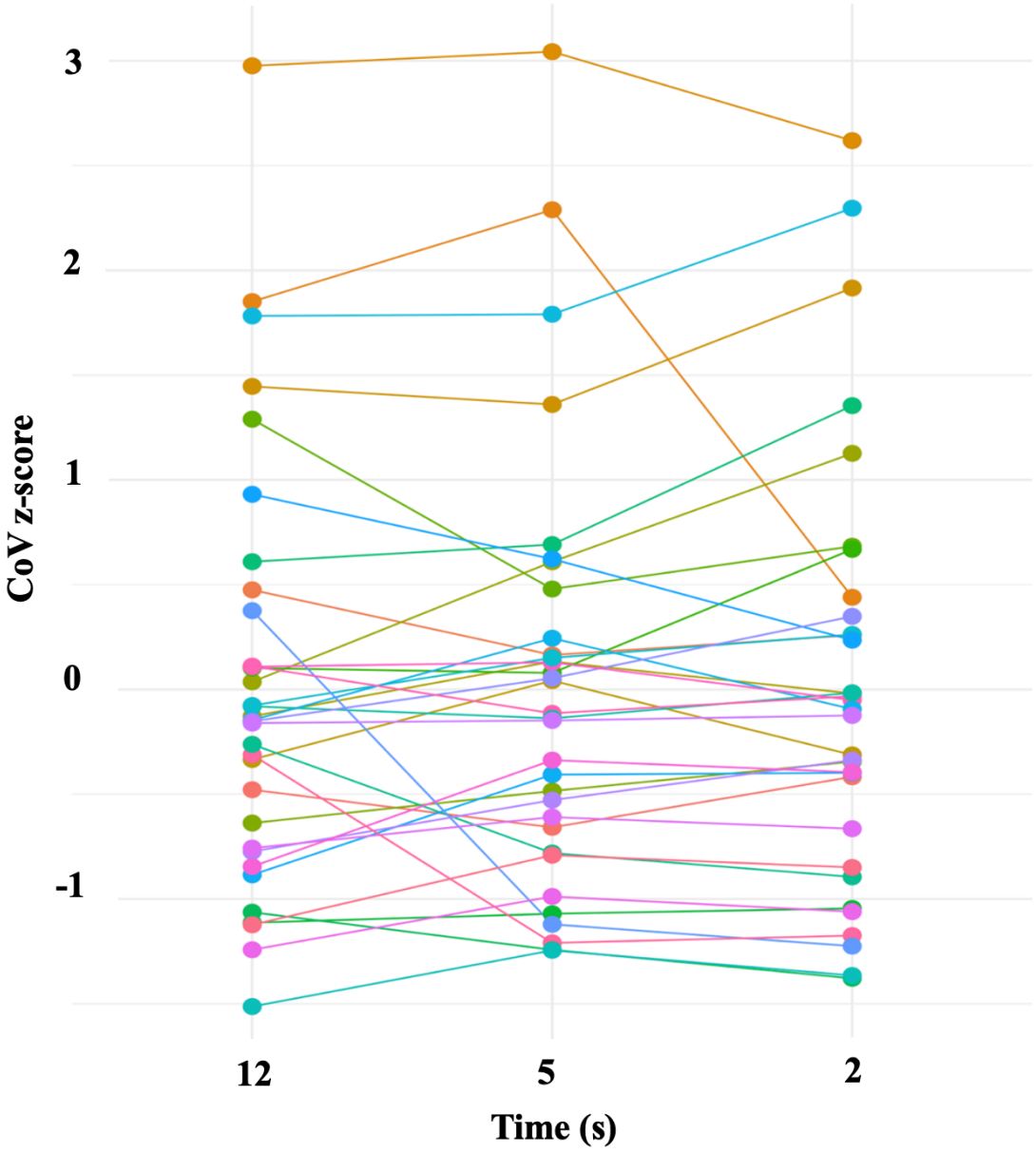

Figure 5.6. The standardised force steadiness (i.e., CoV z-score) values corresponding to those in Figure 5.5 (N = 22). CoV, coefficient of variation; s, seconds.

5.4 Discussion

The study aimed to compare FS CoV values calculated from various durations during a submaximal isometric contraction, along with their corresponding standardised values (*i.e.*, CoV z-scores). The findings revealed significant differences in absolute FS CoV values depending on the duration of the contraction and the quality of the segment selected. Specifically, using a shorter, more stable segment (*i.e.*, the lowest and steadiest CoV) to calculate FS CoV, as done in some previous studies (Harrison et al., 2023; Inglis & Gabriel, 2021; Pethick, Winter, & Burnley, 2015), resulted in lower FS CoV values. Consequently, relying on the most stable segment of a contraction while excluding segments with greater fluctuations might not provide the fullest and most representative FS CoV data for evaluating functional performance.

The significant differences in FS CoV values observed when using different contraction durations highlight a challenge in comparing results from previous studies that have not employed a common time series. This inconsistency complicates the development of clinical ranges for FS CoV across different muscles, tasks, populations, diseases, and musculoskeletal injuries. However, these differences in FS CoV values are mitigated when they are standardised. The study found that standardised FS CoV values calculated from the full contraction duration were consistent with those derived from the 5- and 2-second durations, indicating that FS CoV z-scores remain reliable and comparable across studies, regardless of the duration of the contraction segment.

FS CoV is a valuable measure for assessing neuromuscular control (Galganski, Fuglevand, & Enoka, 1993) and is relevant for activities of daily living, particularly in the elderly population (Feeney, Mani, & Enoka, 2021; Seynnes et al., 2005), as well as for sports-specific skills (Enoka & Farina, 2021; Kouzaki & Shinohara, 2010; Mear, Gladwell, & Pethick, 2023). Monitoring FS CoV may provide important insights into neuromuscular plasticity following skilled training, injury, and rehabilitation. Indeed, studies have shown that FS CoV can be significantly improved through low-intensity training in older adults' knee extensors (Christou, Yang, & Rosengren, 2003; Kobayashi et al., 2014; Tracy & Enoka, 2006).

As discussed earlier in this chapter, knee extensor torque variability has been explored in ACL-injured individuals, with no significant differences observed

compared to uninjured individuals or their contralateral knee (Hollman et al., 2021; Skurvydas et al., 2011). However, differences in the time series analysed for contraction durations between the two studies make direct comparisons difficult. For instance, one study calculated FS from an 8-second segment during a 10-second hold phase (CoV mean values ranging from 3.2 to 4.2%) (Hollman et al., 2021), while the other did not specify the time series used but mentioned that contraction durations ranged from 5 to 15 seconds (CoV mean values ranging from 3.7 to 3.8%) (Skurvydas et al., 2011). Additionally, Skurvydas et al. (2011) assessed FS CoV at 20% of MVC, while Hollman et al. (2021) evaluated FS CoV at various intensities (*i.e.*, 10%, 25%, 35%, and 50% of MVC), further complicating comparisons.

In rehabilitation, steadiness training is not commonly practiced, despite emerging evidence of its potential benefits (Ely et al., 2022). A recent study (De la Fuente et al., 2022) implemented a 7-week isometric steadiness training programme for knee extensors in patients with ACL reconstruction who continued to experience postoperative quadriceps weakness after nine months of rehabilitation. The programme led to improvements in quadriceps strength, self-reported knee stability, and pain outcomes. Given that FS CoV is easy to assess, train, and modify across various populations, incorporating this measure into sports performance and rehabilitation could represent a valuable improvement.

Although FS CoV measure is frequently used in research, methodological variations across studies hinder the ability to compare findings and establish accurate reference ranges for standard FS CoV across different populations. This study highlights the significant differences that can arise when different contraction durations are selected for analysis and recommends caution when interpreting existing literature on FS CoV values. Future studies should consider adopting a common time series for FS CoV analysis or using CoV z-scores to ensure consistency and comparability. Once a consensus is established, future literature can be more objectively analysed. Furthermore, broader populations, such as elite athletes and exercisers, could benefit from FS CoV measure reports, with ACL injury prevention programmes and rehabilitation protocols incorporating FS CoV measures to gain insights into this critical aspect of force control.

5.4.1 Strengths and limitations

This study is the first to investigate the impact of selecting different contraction durations for analysis on FS CoV values and their corresponding z-scores, addressing the lack of consensus on the optimal duration for such comparisons. The findings reveal that varying the contraction duration chosen for analysis significantly alters muscle FS CoV values, complicating comparisons across studies. As the body of literature on muscle FS during submaximal isometric contractions continues to grow, along with the practical applications of this measure, there is a pressing need to standardise research methods to establish reliable reference ranges of FS CoV values for both healthy and clinical populations.

To ensure the female participants were eumenorrheic, MC tracking assessment was conducted for at least one month prior to testing and during the testing period (Janse de Jonge, Thompson, & Han, 2019). Calendar-based counting and home-based urinary ovulation detection kits were used to confirm participants were in the early follicular phase of their MC, a phase where oestrogen and progesterone levels are at their lowest, thereby minimising any potential impact on neuromuscular performance. While endogenous ovarian steroid hormones are thought to influence MU behaviour (Jenz et al., 2023), and potentially affect neuromuscular performance, many previous studies have not accounted for MC/HC history, or status, during testing (Guo et al., 2022; Harrison et al., 2023; Inglis & Gabriel, 2021; Tracy & Enoka, 2002). When HC history was considered, detailed information about the type of OCP used was lacking (Brown, Edwards, & Jakobi, 2010). As highlighted by Harrison et al. (2023), MC assessment should be prioritised in relevant studies, especially since home-based urinary ovulation detection kits are affordable and accessible.

This study included 22 participants, which may seem relatively small. However, as this is exploratory research with no prior studies on which to base a meaningful effect size (Daniel, 2012), the significant differences observed are noteworthy. Despite the sample size, participants represented a single group in which three different contraction durations were analysed, resulting in a total of 33 contractions across two lower limb muscle groups (*i.e.*, 11 knee extensor isometric contractions, and 22 ankle dorsiflexor contractions).

This study focused on trapezoid contractions held at 40% of MVC, simulating the maintenance of a required force – a condition commonly encountered in daily activities (Knol et al., 2019). Trapezoid contractions, which represent an isometric constant force, were selected because they are the most reported type for FS CoV changes in the literature (Enoka et al., 2002). A low-to-moderate contraction intensity of 40% of MVC was chosen, as this mirrors the intensity level involved in more strenuous daily activities, such as climbing stairs or squatting, where quadriceps muscle activity typically ranges between 32% and 60% of MVC (Kern, Semmler, & Enoka, 2001; Tikkanen et al., 2013). Although existing research predominantly explores FS CoV within a 5% to 40% MVC range (Pethick, Taylor, & Harridge, 2022), investigating other contraction types, such as sine wave, and intensity levels would be valuable for confirming or challenging these findings.

Finally, a knee joint angle of 90° was used for knee extensor FS CoV, although previous research has shown that FS varies with knee joint angle (Krishnan, Allen, & Williams, 2011). While this study did not explore this variable, future research aiming to produce knee extension CoV values should consider different knee joint angles. Accounting for knee joint angle in such studies would enhance the translation of research findings to applied settings, such as daily activities or sports, where lower limbs dynamic movement is involved.

5.5 Conclusion

This study demonstrates that shorter contraction durations analysed for FS CoV result in lower values, indicating a steadier force output. However, when FS CoV values are standardised using z-scores, the duration of the time series has minimal effect. Therefore, caution is advised when comparing FS CoV across studies that use different contraction durations. Future research should investigate a broader range of contraction intensities, alternative contraction types, and diverse populations – such as older adults, individuals with neurological diseases, or elite athletes – to gain more comprehensive insights.

Chapter 6. Knee extensor neuromuscular function across the menstrual cycle

6.1 Introduction

6.1.1 Neuromuscular function and non-contact ACL injuries

As discussed in Chapter 1, Section 1.3.4, and Chapter 5, Section 5.1, neuromuscular contributions are among the most significant risk factors for non-contact ACL injuries in females (Griffin et al., 2000: Riemann & Lephart, 2002; Shultz et al., 2012a; Shultz et al., 2019; Swanik, 2015). However, the underlying mechanisms remain only partially understood (Shultz et al., 2019). Neuromuscular function deficits appear to contribute to motor coordination errors, leading to altered movement patterns, and muscle activation strategies, along with altered muscle stiffness during at-risk movements, such as landing from a jump, changing direction, or sudden deceleration. These altered responses seem to increase knee valgus motion, which in turn places greater strain on the ACL, increasing the likelihood of partial or complete rupture. As a result, they are significant contributors to non-contact ACL injuries in females (Alentorn-Geli et al., 2009; Griffin et al., 2000; Hewett, Myer, & Ford, 2004; Hewett et al., 2005).

Complex neuromuscular control mechanisms are essential for optimally adapting to and stabilising the knee joint in high-risk scenarios (DeMont et al., 1999; Johansson, Sjölander, & Sojka, 1991). These control strategies are influenced by sensory inputs, along with central and peripheral motor commands (Wolpert & Bastian, 2021).

A few seminal studies have prospectively investigated the role of the central nervous system (CNS) ahead of non-contact ACL injuries. For example, neurocognitive testing has revealed that young athletes who later sustained non-contact ACL injuries demonstrated poorer reaction time, processing speed, visual memory, and verbal memory compared to their non-injured peers (Swanik et al., 2007). Additionally, a case report by Grindstaff et al. (2008) found that, four hours before a non-contact ACL injury, the participant's maximal knee extensors strength and activation had decreased by 4% and 5.3%, respectively, compared to measurements taken one week prior to the injury (Grindstaff et al., 2008). These findings raise the possibility of a disturbance to the neuromuscular system prior to the onset of non-contact ACL injuries.

Finally, recent research is increasingly focused on interhemispheric brain connectivity as a potential factor contributing to neuromuscular deficits in female athletes. A study by Diekfuss et al. (2019) using functional MRI found that athletes who did not sustain subsequent non-contact ACL injuries exhibited stronger interhemispheric connections between the sensory-motor region and the cerebellum, a key area responsible for balance and coordination. While further research is needed, such findings suggest that enhanced neural communication in these regions might play a protective role in preventing such injuries.

6.1.2 Neuromuscular system

Descending central motor commands converge at spinal motor neurons and interneurons at the spinal cord, where they are modulated via a combination of excitatory and inhibitory feedback to activate motor units (MU) (Côté, Murray, & Knikou, 2018). The MU is the fundamental component of the neuromuscular system, consisting of a motor neuron, its dendrites and axon, and the muscle fibres innervated by the axon through the neuromuscular junction. This structure is responsible for generating and controlling force and movement (Clarac & Barbara, 2011; Duchateau & Enoka, 2011; Liddell & Sherrington, 1925).

The force produced by the neuromuscular system during voluntary maximal and sub-maximal muscle contractions is determined by the number of MUs recruited and the discharge rate of action potentials in each recruited MU (Enoka, 1995; Enoka & Duchateau, 2017). The specific interaction between MU recruitment and discharge rate varies depending on the muscle and the task being performed (De Luca et al., 1982; Fuglevand, Winter, & Patla, 1993).

In the context of neurophysiological ageing, research has shown that MUs undergo various changes with age, including a reduction in number due to MU loss and denervation at the muscle fibre level (McNeil et al., 2005; Rowan et al., 2012; Tomlinson & Irving, 1977). These age-related changes contribute to declines in neuromuscular function, such as poorer motor control and decreased strength (Christou, 2011; Enoka & Farina, 2021; Power, Dalton, & Rice, 2013; Tracy & Enoka, 2002).

6.1.3 Neuromuscular variables

6.1.3.1 Force steadiness

During voluntary sub-maximal steady contractions, the force produced is not constant but fluctuates around an average value. These fluctuations are key in determining an individual's ability to generate the required force for a specific movement (Christou, 2010; Enoka et al., 2003). To achieve optimal strength and movement precision for a given task, the activation signals sent from the CNS to the corresponding muscles must be appropriately regulated (Enoka et al., 2003).

As introduced in Chapter 5, Section 5.1, these force fluctuations are quantified by neuromuscular force steadiness (FS), typically measured by either the standard deviation (SD) or the coefficient of variation (CoV) of the force output. FS can vary based on several factors, such as whether multiple muscles are involved in producing the movement. However, fluctuations in the common modulation of MU firing rates are a primary factor influencing these variations (Enoka & Duchateau, 2017; Enoka & Farina, 2021).

One challenge, as highlighted in the previous chapter, is that FS CoV values reported in the literature are difficult to compare across studies due to their sensitivity to the duration of the analysis window used during isometric contractions. Shorter analysis durations tend to show lower FS CoV values, reflecting steadier force output. As a result, comparisons between studies that use different analysis durations, or fail to report their methodology in detail, are problematic. This methodological inconsistency also hinders the development of clinical benchmarks for FS across various populations or in individuals with neurological disorders.

6.1.3.2 Relative surface electromyography root mean square

In addition to FS, which evaluates one aspect of motor control, maximum voluntary contraction (MVC) and surface electromyography (sEMG) root mean square (RMS) provide complementary insights into other facets of neuromuscular function. While FS and MVC assess motor control and maximal strength, thus evaluating the output of a muscle group or joint torque, sEMG RMS measures muscle activation by analysing the amplitude of the signal, which reflects MU activity and peripheral factors prior to muscle contraction (Bilodeau et al., 2003; Clancy et al.,

2023; De Luca, 1997; Farina et al., 2002; Winter & Yack, 1987). Essentially, sEMG RMS offers a window into the activity of a specific muscle region and its associated MUs.

The amplitude of the sEMG signal correlates to muscle force output (Milner-Brown & Stein, 1975), with increases in MU recruitment and firing rates corresponding to increases in sEMG RMS (De Luca, 1997; Clancy et al., 2023) but can also be influenced by other factors such as superimposition of signals, whereby simultaneous positive and negative waves cancel each other out.

sEMG RMS has been used to assess motor impairments and guide rehabilitation in clinical populations with neurological disorders (Balbinot et al., 2022; Banks et al., 2017; Gagnat, Brændvik, & Roeleveld, 2020). More recently, it has gained popularity in sports and exercise research, where it is used to investigate neuromuscular mechanisms underlying performance or to assess adaptations resulting from specific training protocols (Vigotsky et al., 2018).

However, caution is needed when interpreting sEMG RMS as it provides only partial information about muscle properties. The signal is influenced by both central and peripheral factors, including anatomical variables (*e.g.*, subcutaneous tissue thickness between the active muscle and the electrode) and features of the detection system itself (*e.g.*, electrode shape and size) (De Luca, 1997; Farina, Cescon, & Merletti, 2002; Vigotsky et al., 2018). Normalising sEMG RMS to a standard value, such as peak sEMG during an MVC, helps account for between-participants variability and is commonly used in research (De Luca, 1997).

Relative sEMG RMS (relRMS) provides a non-invasive and accessible method to assess muscle activation. However, it should be considered within the context of other variables and combined with a broader assessment of muscle properties. For more comprehensive insights into MU function and muscle characteristics, additional techniques like intramuscular EMG or high-density sEMG (HDsEMG) MU recordings with MU enhanced decomposition should be used alongside relRMS (Balbinot et al., 2022; Clancy et al., 2023; Vigotsky et al., 2018).

6.1.4 Ovarian steroid hormones and central and peripheral nervous systems

As outlined in Chapter 1, Section 1.4.1, the primary ovarian steroid hormones in females are 17β-oestradiol (*i.e.*, circulating oestrogen) and progesterone. These hormones are classified as neurosteroids due to their capacity to cross the blood-brain barrier, thereby potentially influencing CNS function (Stoffel-Wagner, 2001). Initial studies in animal models, and more recently in humans, have demonstrated that oestrogen has a net excitatory effect on the nervous system, while progesterone exerts an inhibitory effect. These neuromodulatory effects are mediated by the level of inhibitory response from the primary gamma-aminobutyric acid (GABA) receptors (*i.e.*, $GABA_A$ receptors) located in the neuronal cell membrane (Schultz et al., 2009; Smith, 1989; Smith, Woodward, & Chapin, 1989).

In addition to their influence on the CNS, oestrogen and progesterone appear to affect the peripheral nervous system, including muscles fibres (Lowe, Baltgalvis, & Greising, 2010) and musculoskeletal connective tissues, such as the ACL. Studies have found oestrogen and progesterone receptors on human female ACLs harvested during surgeries, suggesting direct hormonal interactions with these tissues (Dragoo et al., 2003; Liu et al., 1996). As noted in Chapter 4, Section 4.1, while human studies have shown conflicting results compared to animal models, they suggest that significant fluctuations in ovarian steroid hormone levels during the MC may alter ACL collagen synthesis and structure. This could occur through changes in fibroblast proliferation and collagen cross-linking, potentially weakening the ACL and increasing the risk of ACL rupture (Yu et al., 2001; Lee et al., 2015).

However, as highlighted in Chapter 3, Section 3.5.5, it was noted that inconsistent classification of the MC timepoints and the lack of standardised methods, such as urinary ovulation detection kits and serum sample analysis, make it difficult to draw any conclusions regarding a specific at-risk time during the MC for non-contact ACL injuries. Similarly, in Chapter 3, section 3.5.2, it was noted that the extent of anterior knee laxity (AKL) variation across the MC remains unclear due to these same methodological issues. Many of the existing studies are of generally low quality, further complicating any definitive conclusions. These findings underscore the need

for stricter and more consistent methodologies when investigating the impact of the MC on musculoskeletal systems in eumenorrheic female participants.

6.1.5 Ovarian steroid hormones and neuromuscular function

Since fluctuations in ovarian steroid hormone can potentially impact both the central and peripheral nervous systems – and as shown by Hewett, Myer, & Ford (2004), neuromuscular control worsens in young females after menarche onset – it is essential to examine neuromuscular function, which integrates both systems. Given the quadriceps muscles' key role contributing to AKL and initial knee stabilisation in dynamic sporting tasks such as landing and countermovement (Beaulieu, Ashton-Miller, & Wojtys, 2023; Maniar et al., 2020; Smeets et al., 2019), the focus herein is on the knee extensors.

Several studies have investigated the effects of ovarian steroid hormone fluctuations on knee extensor force output, though the findings are inconsistent. For example, some research found that knee extensor MVC and/or knee extension isokinetic peak torque were highest during the ovulatory phase (Sarwar, Niclos, & Rutherford, 1996), while others reported peak values in both the ovulatory and mid-luteal phases (Weidauer et al., 2020). In contrast, one study found significantly lower MVC during the mid-luteal phase in naturally menstruating females (Tenan, Hackney, & Griffin, 2016). However, these studies relied solely on calendar-counting method to determine MC phases, which raises questions about whether participants were tested at the correct timepoints, as recommended by methodological standards (Janse de Jonge, Thompson, & Han, 2019).

On the other hand, several studies found no significant changes of knee extensor MVC and/or knee extension isokinetic peak torque across key MC timepoints in naturally menstruating (Dibrezzo, Fort, & Brown, 1988; Kubo et al., 2009) or eumenorrheic females (Ansdell et al., 2019; Hertel et al., 2006; Janse de Jonge et al., 2001; Montgomery & Shultz, 2010; Piasecki et al., 2023; Thompson et al., 2021). Most of these studies employed the gold-standard methodology that included urinary ovulation-detection kits combined with serum sample analysis of circulating oestrogen and progesterone, ensuring that participants were eumenorrheic and tested at the correct MC timepoints without luteal-phase deficiency (Ansdell et al., 2019; Janse de Jonge et al., 2001; Montgomery & Shultz, 2010; Piasecki et al., 2023; Thompson et

al., 2021). However, such studies omit the mechanisms in the production of such force outputs.

Other studies have attempted to explore knee extensor neuromuscular function across the MC in naturally menstruating (Tenan et al., 2013; Tenan, Hackney, & Griffin, 2016) and eumenorrheic females (Ansdell et al.; Piasecki et al., 2023). One study found that MU firing rates in two knee extensor muscles increased significantly during the late luteal phase compared to the early follicular phase (Tenan et al., 2013), while another reported poorer FS during the mid-luteal phase (Tenan, Hackney, & Griffin, 2016). However, the methods of quantifying motor unit firing rate (MUFR) in these papers are questionable, using only the average of the first three observed discharges. Additionally, Piasecki et al. (2023) observed a suppression (approximately 10%) of firing rates in low-threshold MUs in the vastus lateralis (VL) during ovulation and mid-luteal phases, comparatively to menstrual phase, though they found no significant effect of ovarian steroid hormone fluctuations on FS. Ansdell et al. (2019) investigated the CNS and reported greater voluntary activation in the late follicular phase, increased intracortical inhibition and lower fatigability during the mid-luteal phase. Notably, only two studies employed standardised methodologies for tracking and confirming MC timepoints at the time of testing (Ansdell et al., 2019; Piasecki et al., 2023). These studies suggest that while ovarian steroid hormone fluctuations may influence the CNS and some aspects of knee extensor neuromuscular performance, they do not appear to significantly affect knee extensor force output or force control in eumenorrheic females.

Although the current findings do not provide definitive conclusions on the impact or direction of ovarian steroid hormone fluctuations on knee extensor neuromuscular function, they underscore the need for further research. With the growing number of exercising females and the increasing incidence of non-contact ACL injuries, it would be valuable to explore additional variables of knee extensor neuromuscular function and performance across varying intensities. Such research would: (i) contribute to the limited but growing body of knowledge on female neurophysiology (Lulic-Kuryllo & Inglis, 2022) and female sports and exercise science (Cowley et al., 2021); and (ii) provide new insights into knee extensor

neuromuscular function that could inform the literature on non-contact ACL injury prevention and rehabilitation in eumenorrheic females.

This study aimed to evaluate knee extensor neuromuscular performance and activation of the vastus medialis (VM) and VL muscles, while tracking the MC and quantifying circulating ovarian steroid hormone levels using a standardised methodology. Knee extensor neuromuscular performance was assessed through MVC and FS CoV at 40% and 75% of MVC. Muscle activation was measured using relRMS of the VM and VL across three key MC timepoints: early follicular (EF), pre-ovulation (pre-O), and mid-luteal (ML), in healthy, recreationally active eumenorrheic females.

6.2 Methods

6.2.1 Participants

Twenty-two naturally menstruating female participants were initially recruited locally from the community from September 2023 to July 2024 through posters, word of mouth, and email. Before participating, all participants completed an informed consent form, a health and COVID-19 questionnaire, and a menstrual history questionnaire (Appendix N). The study received approval from the Nottingham Trent University Research Ethics Committee (Humans – Invasive, approval ID: 1606166), and participants were free to withdraw at any time.

Participants were eligible if they were between 18 and 40 years old. They were included if they had regular duration MCs (21 to 35 days [Fehring, Schneider, & Raviele, 2006]). They were excluded if they had chronic menstrual irregularities (*e.g.*, amenorrhea, oligomenorrhea, polymenorrhea, or luteal phase deficiency), were using medication known to impact the level of endogenous ovarian steroid hormones, were pregnant or had been pregnant within the past 12 months, were breastfeeding or had breastfed in the past 12 months, had used any type of hormonal contraceptive (HC) or undergone hormone replacement therapy in the past 12 months, or had experienced irregular periods due to peri-menopause or menopause within the past 12 months. Luteal phase deficiency was controlled for and excluded based on retrospective serum analysis, with mid-luteal progesterone serum levels required to exceed the 16 nmol/L (5.03 ng/ml) threshold (Janse de Jonge, Thompson, & Han, 2019).

Participants were excluded if they had any chronic illness or disease, had a body mass index (BMI) below 15 or above 30 kg/m^2 – due to the potential impact on endogenous ovarian steroid hormone levels and the composition of soft tissues, affecting the collection of sufficient and clean neuromuscular data – or any contraindications to the procedures (*e.g.*, lower limb musculoskeletal injury, neurological medication intake). The participants' level of physical activity was assessed using the international physical activity questionnaire (IPAQ) (Craig et al., 2003) (Appendix N), and those who met the criteria for being minimally active (category 2) or health-enhancing physically active (HEPA) (category 3) were included in the study.

Twelve participants were unable to complete the study for a variety of reasons: time constraints (n = 6), inconsistent use of home-based urinary ovulation detection kits (n = 2), relocation (n = 1), anovulatory cycles (n = 1), and MC length outside the range of 21 to 35 days (n = 2). As a result, the final sample consisted of ten eumenorrheic participants, aged 20 to 40 years, who were the same female participants as in Chapter 5, except for one individual who was unable to attend the full testing sessions due to time constraints. Additionally, due to intrinsic or environmental noise affecting HDsEMG signal quality – either during MVC or 40%MVC contraction – data were collected from 9 participants for VM relRMS, and four participants for VL relRMS. Furthermore, due to participant time constraint, FS CoV at 75%MVC data were available for 9 participants.

6.2.2 Research design

All force measurements were conducted by the same examiners mentioned in Chapter 5, Section 5.2.2, using an isometric dynamometer. The testing procedure required participants to understand and control specific movements, such as knee extension, necessitating a familiarisation session before the experimental visit, conducted between 24 and 36 hours prior to the first experimental trial. Force data included MVC, relative RMS, and FS, quantified as CoV of force, during isometric knee extensor contractions held at 40% and 75% of MVC. Participants were instructed to arrive at the laboratory after an overnight fast, refrain from strenuous exercise and alcohol consumption for 24 hours prior to the experimental visit, and avoid caffeinated

drinks within 12 hours before and during testing. All testing took place between 7:30 and 9:30am.

6.2.3 Procedures

6.2.3.1 Menstrual cycle assessment

Participants tracked their MC for at least one month before testing and during the testing month, using calendar-based counting and home-based urinary ovulation detection kits, which they began using from day 9 of their menstrual cycle onwards (Boots, Boots UK Limited, England. Ovulation accuracy > 99%; Clearblue advanced digital, SPD Swiss Precision Diagnostics GmbH, Switzerland. Ovulation accuracy > 99%). Participants were instructed to test using the second urine of the day, as per manufacturers guidelines, to take a photograph of the result 10 minutes after testing, and to submit it by email. Testing continued daily until a positive result indicated a rise in luteinising hormone (LH), indicating the occurrence and timing of ovulation (Janse de Jonge, Thompson, & Han, 2019). This gold-standard methodology ensured that all female participants were eumenorrheic and tested at the correct phase of their MC (Janse de Jonge, Thompson, & Han, 2019).

Testing occurred at three key MC timepoints: the EF phase (day 2 to 4), pre-O phase (24 to 48hours before a surge in luteinising hormone [LH] confirmed by a urinary ovulation detection kit), and ML phase (7 to 9 days following the confirmed LH surge) (Janse de Jonge, Thompson, & Han, 2019; Schaumberg et al., 2017). The pre-O testing window was estimated using the LH surge date from the participant's previously tracked cycle. Participants were asked to attend their pre-O testing session 24 to 48 hours before the anticipated LH surge, calculated based on prior cycle data. The actual occurrence and timing of ovulation were verified retrospectively by participant-reported confirmation of the LH surge following the pre-O visit. These timepoints were chosen to reflect distinct endogenous profiles of oestrogen (E) and progesterone (P), where: (i) both E and P levels were low (*i.e.*, EF); (ii) E was rising to peak while P remained low (*i.e.*, pre-O); and (iii) mid-level E and peak levels of P were present (*i.e.*, ML). The order of the first visit was randomised (Table 6.1). For instance, participants beginning the trials at their pre-O timepoint would wait for the next identifiable MC timepoint (*i.e.*, confirmed ovulation by home-based urinary ovulation detection kit) to schedule their ML testing session. If participants were

unavailable for the next timepoint, testing resumed in the following MC. Consequently, three participants completed all tests within the same MC, six across two MCs, and one across three MCs.

Table 6.1 Number of participants who completed their first, second, and third experimental trial at each MC timepoint (N = 10).

Phase	Session		
	First	Second	Third
EF	3	2	5
Pre-O	4	4	2
ML	3	4	3

EF, early follicular; MC, menstrual cycle; ML, mid-luteal; Pre-O, pre-ovulatory.

6.2.3.2 Serum hormones assessment

Upon arrival at the laboratory, a trained phlebotomist (EN, TI, or JP) collected blood samples (20ml in total) from the antecubital vein using two serum tubes (10ml, BD vacutainer®). The blood was left to clot for 20 minutes, followed by centrifugation at 2000*g at 4°C for 15 minutes. Afterward, the serum was aliquoted into 2ml Eppendorf tubes and stored at -80°C, upon completion of the study.

6.2.3.3 Anthropometric measurements

After blood collection and breakfast, participants proceeded to the neurophysiology laboratory. They were asked to be barefoot and wear minimal clothing (*i.e.*, shorts and a t-shirt) for height and body mass measurements. Height was measured using a stadiometer in centimetres (Seca, UK) and body mass was recorded using a digital weighing scale in kilograms (Seca, UK).

6.2.3.4 High-density surface electromyography (HDsEMG)

HDsEMG signals for calculating relRMS were captured from the VM and VL muscles using 64-electrode matrices (1mm diameter, 8mm interelectrode distance, 13 rows, 5 columns, OT Bioelettronica, Torino, Italy). These matrices, attached to the skin *via* matching adhesive foams (OT Bioelettronica, Torino, Italy) filled with conductive cream (AC Cream, Spes Medica S.r.l., Genoa, Italy), were positioned at

approximately 50° and 20° relative to the line between the superior iliac spine and the medial and lateral patellar borders, respectively (Barbero, Marletti, & Rainoldi, 2012). Placement accuracy and muscle fibre alignment were confirmed using ultrasound (Siemens Acuson P500, Siemens, Munich, Germany). Matrix contours were marked on the skin with a permanent marker to assist with positioning and skin preparation, which involved shaving, abrading with skin sandpaper and gel (3M Red Dot Trace Prep, 3M, St. Paul, Minnesota, USA; NuPrep SkinPrep gel, Weaver and company, Aurora, Colorado, USA) and drying the area.

Once the skin was prepared, the VM and VL matrices were securely adhered, and additional skin tape (3M Micropore surgical tape, 3M, 3M, St. Paul, Minnesota, USA) was applied to ensure they remain in place during testing. A self-adhesive ground electrode (48 X 30mm Ambu Neuroline Ground, Ambu A/S, Ballerup, Denmark) was placed on the patella, while a water-soaked strap electrode (OT Bioelettronica, Torino, Italy) wrapped around the ankle grounded the signal (Figure 6.1). HDsEMG signals were sampled at 2kHz using a portable amplifier and analogue-to-digital converter (Sessantaquattro+, OT Bioelettronica, Torino, Italy) and visualised in OT+ Biolab software (OT Bioelettronica, Torino, Italy) (Figure 6.2).

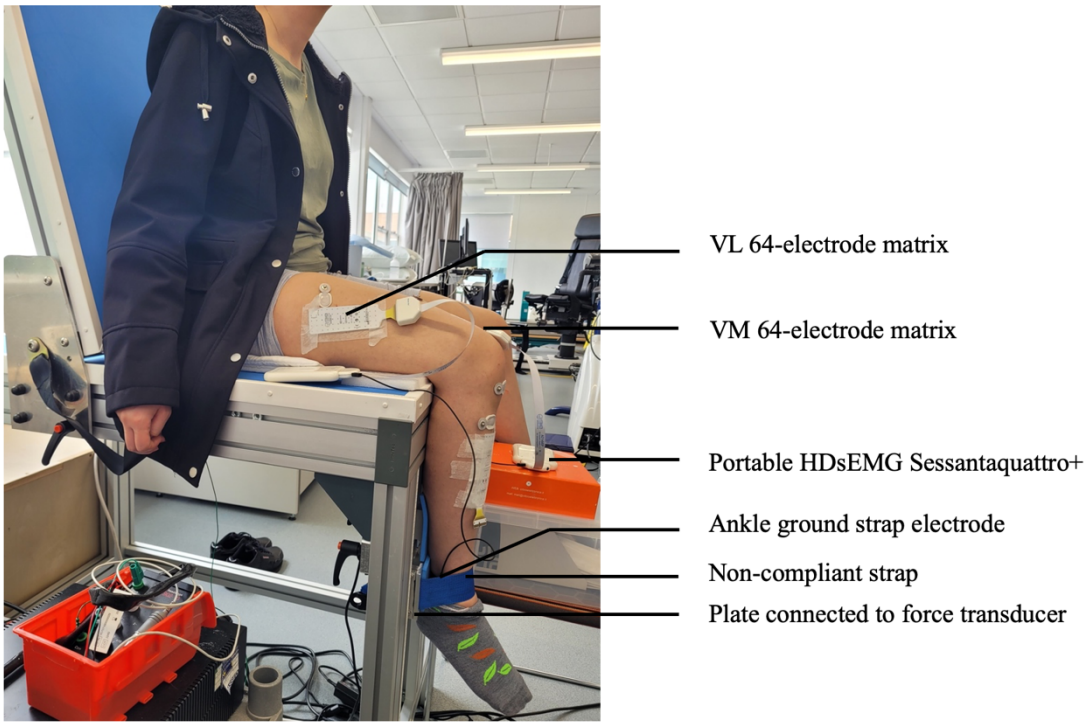

Figure 6.1. Isometric dynamometer set-up for knee extension neuromuscular performance assessment. HDsEMG, high-density surface electromyography; VM, vastus medialis; VL, vastus lateralis.

6.2.3.5 Force recordings

Participants were seated in a custom-built isometric dynamometer, with their hips flexed at 90°. The knee of the dominant leg – defined as the preferred leg for kicking a ball – was also positioned at 90°. The lower leg of the tested limb was secured to a plate connected to a force transducer with a non-compliant strap (purpose-built calibrated strain gauge, Research Solutions [Alsager] Ltd, Stoke-on-Trent, UK) which was positioned above the medial malleolus (Figure 6.1). Participants were instructed to push their lower leg away from themselves to activate the knee extensors, ensuring they did not lift their hip or grasp the seat of the dynamometer. To further minimise upper trunk movement during testing, a seat belt was fastened across the pelvis.

To record maximal voluntary force, the amplified analogue force signal was recorded at 2kHz *via* a portable amplifier and analogue-to-digital converter (Sessantaquattro+, OT Bioelettronica, Torino, Italy) for simultaneous visualisation in OT+ Biolab software (OT Bioelettronica, Torino, Italy).

All participants completed a standardised warm-up of submaximal isometric contractions, which included three contractions of 10 seconds at 25% of their perceived MVC, three contractions of 10 seconds at 50%, two contractions of 10 seconds at 75%, and one contraction of 5 sec at 90%. During the warm-up, participants received real-time visual feedback of force traces in Spike2 software (version 10; Cambridge Electronic Design Ltd [CED], Cambridge, UK) displayed on screen in front of them. After completing the warm-up and receiving full instructions, participants were asked to perform an MVC with maximal effort, pushing as hard as possible for two to five seconds. They received real-time visual feedback in OT+ Biolab (Figure 6.2) and verbal encouragement from the researchers, such as "push, push, push". This procedure was repeated three times with 60 seconds of rest between attempts, with the highest values (in Newtons) being accepted as the MVC. If the difference between the last two recorded MVC values exceeded 5%, a fourth trial was required to ensure accuracy and consistency of the MVC measurements.

After completing the MVC trials, participants were given at least two minutes to recover before proceeding with the FS task. To quantify FS, a single target line representing a normalised contraction intensity was displayed on screen in OT+ Biolab, which participants were instructed to follow as closely as possible (Figure 6.2). They were guided to perform a series of voluntary isometric trapezoid contractions, consisting of four sustained contractions at 10% and 25% MVC, two sustained contractions at 40% of MVC, and two sustained contractions at 75% of MVC, in the following sequence: 10%, 25%, 40%, 10%, 25%, 10%, 25%, 40%, 10%, 25%, 75%, 75%.

Each contraction involved a gradual force increase over five seconds (ascending phase/MU recruitment), a plateau phase of 12 seconds (hold phase), followed by a gradual decrease over five seconds (descending phase/MU derecruitment) (Figure 6.3). Given that contractions at 75% of MVC are more demanding and could induce muscle fatigue if sustained for a long period of time, participants maintained the plateau phase for only five seconds instead of 12. To prevent muscle fatigue, a 30-second rest period was granted between each contraction.

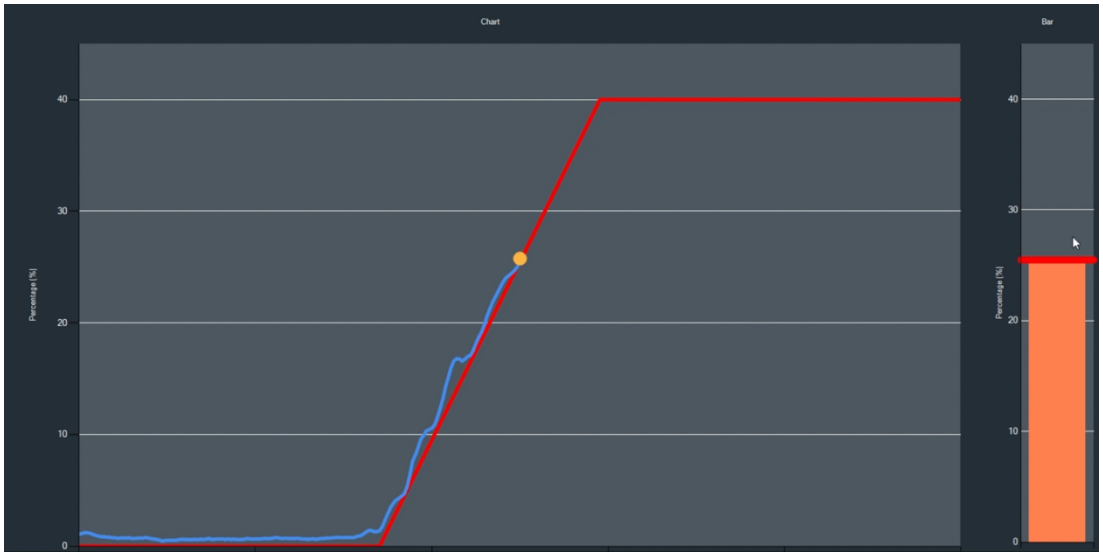

Figure 6.2. Real-time visual feedback of a participant performing a voluntary isometric trapezoid contraction held at 40% MVC in OT+ Biolab. MVC, maximum voluntary contraction; s, second. Red line: target force; blue line: force trace output performed by the participant; yellow dot: live force output.

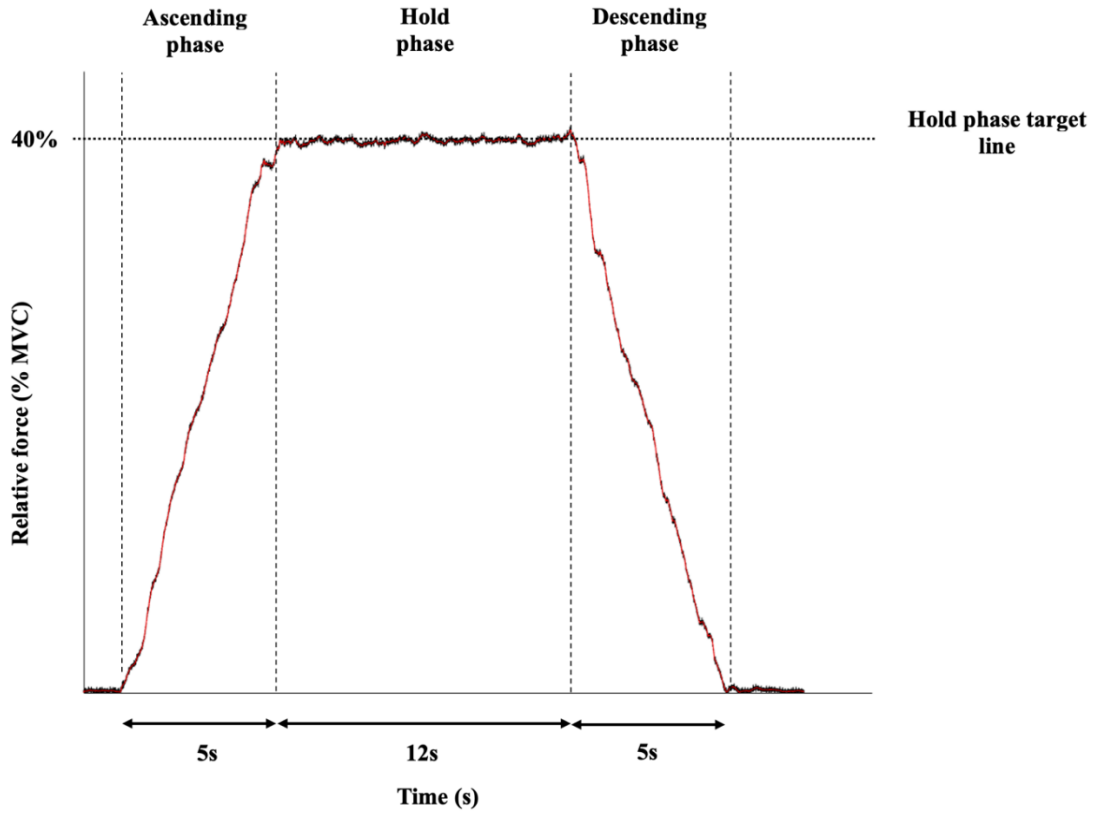

Figure 6.3. Force output during an isometric voluntary contraction held at 40% MVC. MVC, maximum voluntary contraction; s, seconds. Black: raw signal, red: filtered signal.

6.2.3.6 Data analysis

The MC timepoints were confirmed by measuring serum concentrations of 17β-oestradiol and progesterone using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems [for 17β-oestradiol] and Novus Biologicals [for progesterone], Bio-Techne, Minneapolis, MN), in accordance with the manufacturer's guidelines. Each sample was analysed in duplicate, with standard curves generated using six standards for 17β-oestradiol and five standards for progesterone. Hormone concentrations were calculated based on the mean absorbance of the duplicates, interpolated from the standard curve. Absorbance for 17β-oestradiol was measured at 450 nm, with a correction at 590 nm. The minimal detection limits were 4.84 pg/ml for 17β-oestradiol and 0.2 ng/ml for progesterone, with a coefficient of variation (CV) ranging from 5.4% to 8.9% for 17β-oestradiol and < 15% for progesterone. All samples collected confirmed the appropriate MC timepoints.

The progesterone to 17β-oestradiol ratio was calculated to assess whether progesterone dominance occurred during the ML phase. The ratio was determined using the formula:

$$\text{P to E ratio} = (P \times 1000) / E$$

where P is in ng/ml (converted to pg/ml) and E is in pg/ml.

For the force assessment, the highest MVC value was selected, and FS CoV was calculated as described in Chapter 5, Section 5.2.3. For contractions held at 40% of MVC, FS CoV was calculated during the entire 12-second hold phase, while for 75% of MVC contractions, it was calculated during the full 5-second hold phase.

To calculate relRMS, the HDsEMG signals of the highest MVC and the second isometric voluntary contraction held at 40% of MVC for both VM and VL were visualised in OT+ Biolab software (OT Bioelettronica, Torino, Italy), and selected for analysis. The entire contractions were then exported in MATLAB (MATLAB version: 23.2 [R2023b], MathWorks, Inc., Natick, Massachusetts, USA) for further analysis.

The HDsEMG signals (Figures 6.4 and 6.5) were filtered using a Butterworth filter with a low cut-off frequency of 20Hz and a high cut-off frequency of 500Hz. RMS values were calculated using 50 ms time windows across the contraction

duration through a moving window method. The maximum RMS value was obtained by identifying the highest amplitude of samples within a 50 ms time window.

For each HDsEMG signal from the MVC, VM, and VL (at 40% of MVC), the maximum RMS values were calculated. The relRMS value was then calculated as the percentage of the maximum RMS value at 40% of MVC relative to the maximum RMS value at MVC using the formula:

relRMS = (max RMS value 40% MVC / max RMS value MVC) X 100

where relRMS is expressed in percentage (%).

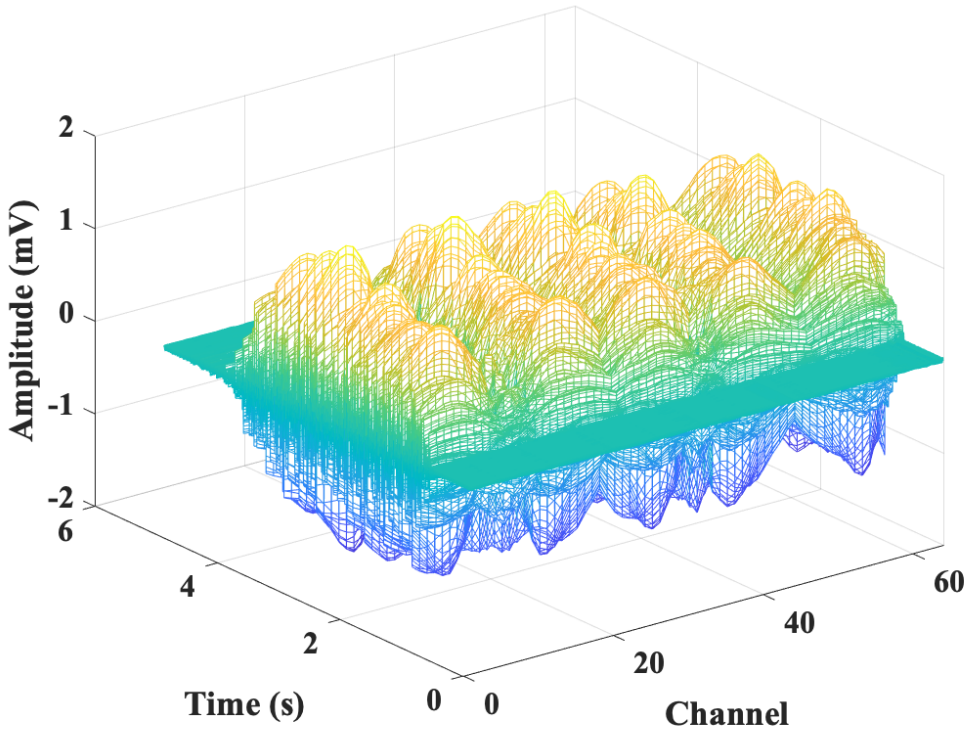

Figure 6.4. 3D HDsEMG signal amplitude of an isometric MVC in VM muscle. 3D, three dimensional; HDsEMG, high-density surface electromyography; mV, millivolts; MVC, maximum voluntary contraction; VM, vastus medialis; s, second.

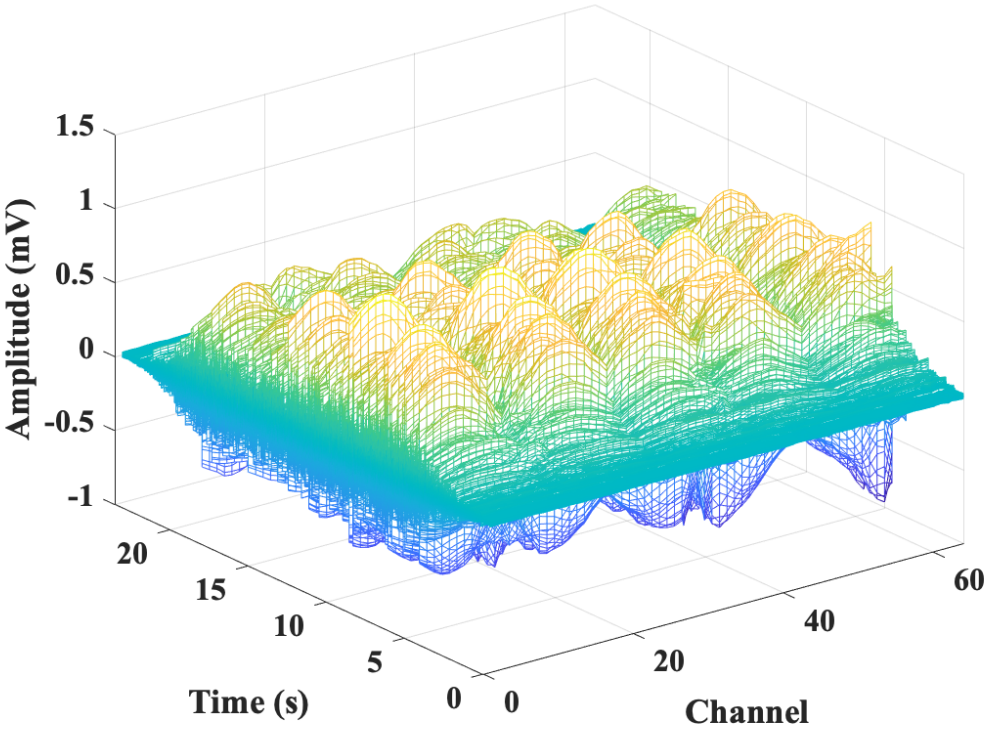

Figure 6.5. 3D HDsEMG signal amplitude of an isometric trapezoid 12-second hold phase contraction at 40% of MVC in VM muscle, from the same participant than in Fig.6.4. 3D, three dimensional; HDsEMG, high-density surface electromyography; mV, millivolts; MVC, maximum voluntary contraction; VM, vastus medialis; s, second.

6.2.4 Statistical analysis

The data distribution was assessed for normality using the Shapiro-Wilk test. For data that followed a normal distribution, descriptive statistics are presented as the mean ± standard deviation (SD). A one-way repeated measures analysis of variance (ANOVA) was conducted on the normally distributed variables to identify differences in 17β-oestradiol serum concentration, MVC, relRMS, and FS CoV at 40% MVC. When the assumption of sphericity was violated, Greenhouse-Geisser corrections were applied, particularly for MVC, progesterone serum concentration, and the progesterone to 17β-oestradiol ratio. In instances where data were missing, such as for progesterone serum concentration and consequently the progesterone to 17β-oestradiol ratio, a mixed effects model was fitted, as implemented in GraphPad Prism 10.

Significant effects were further explored using Tukey's post-hoc multiple comparison test. For data that did not meet normality (*i.e.*, FS CoV at 75% MVC), the non-parametric Friedman test was applied. Estimated mean differences (EMD) are reported for statistically significant results from the repeated measures analysis. Statistical significance was set at $p < 0.05$.

The statistical analyses were performed using Excel for Macintosh, version 16.79.1 (Microsoft 365, Microsoft Corp., USA), SPSS Statistics software (version 29.0.2.0, IBM SPSS Statistics), and GraphPad Prism (version 10.3.0). Visualisations of the statistical outcomes, excluding 17β-oestradiol and progesterone serum concentration, were generated using the *ggplot2* package (version 3.5.1; Wickham, 2016) in R Statistical software (version 4.4.0, R Core Team 2023), implemented in RStudio (Version 2024.4.2.764, Integrated Development Environment for R. Posit Software, PBC, Boston, Massachusetts, USA). Serum concentration data for 17β-oestradiol and progesterone were visualised using GraphPad Prism (version 10.3.0).

6.3 Results

6.3.1 Participant characteristics

Data were collected from the VM and VL muscles of ten eumenorrheic female participants (age 30 ± 8 years; height 1.66 ± 0.05 m, weight 66.6 ± 9.8 kg, BMI 24 ± 2.8 kg/m^2). On average, participants had a MC length of 28 ± 3 days, with the LH surge detected on 14 ± 2 days.

6.3.2 Ovarian steroid hormone concentrations

17β-oestradiol serum concentrations varied across the three MC timepoints ($F_{(2, 14)} = 4.66$, $p = 0.028$). The mean concentration was 159 pg/ml in the EF phase, which increased to 200 pg/ml in the pre-O phase ($p = 0.075$, EMD = 41.77), and reached 208 pg/ml in the ML phase, which was significantly higher than in the EF phase ($p = 0.033$, EMD = 49.48), but not significantly different from the pre-O phase ($p = 0.899$, EMD = 7.714) (Figure 6.6.A; Table 6.2).

Progesterone serum concentrations also showed variation across the three MC timepoints ($F_{(1.073, 10.73)} = 5.598$, $p = 0.036$). The mean concentration was 11.96 ng/ml in the EF phase, which increased slightly to 12.11 ng/ml in the pre-O phase ($p = 0.998$, EMD = 0.149). In the ML phase, the concentration was higher than in the EF phase (*p*

= 0.122, EMD = 28.97) with a mean of 39.27 ng/ml, but did not differ significantly from the pre-O phase (p = 0.170, EMD = 26.49) (Figure 6.6.B; Table 6.2). No participant had progesterone serum levels below 16 nmol/L (*i.e.*, 5.03ng/ml) during MF phase testing (Janse de Jonge, Thompson, & Han, 2019).

The progesterone to 17β-oestradiol ratio also varied across the three MC timepoints ($F_{(1.046, 10.46)}$ = 5.205, p = 0.043). The mean ratio was 85.58 in the EF phase and decreased to 76.41 in the pre-O phase (p = 0.689, EMD = 9.166). The ratio was higher in the ML phase compared to the EF phase (p = 0.198, EMD = 127.9) with a mean of 215.1, and compared to the pre-O phase (p = 0.199, EMD = 130.3) (Figure 6.7, Table 6.2).

6.3.3 Maximum voluntary contraction

MVC mean values did not differ significantly across the three MC timepoints ($F_{(1.246, 11.217)}$ = 0.876, p = 0.393) (Table 6.3) (Figure 6.8).

6.3.4 Normalised relative root mean square

VM RelRMS mean values did not differ significantly across the three MC timepoints ($F_{(2, 16)}$ = 1.209, p = 0.324) (Table 6.3) (Figure 6.9). Equally, VL RelRMS mean values did not differ significantly across the three MC timepoints ($F_{(2, 6)}$ = 0.266, p = 0.775) (Table 6.3) (Figure 6.10).

6.3.5 Force steadiness (CoV)

Knee extensor FS CoV held at 40% of MVC did not differ significantly across the three MC timepoints ($F_{(2, 18)}$ = 0.285, p = 0.756) (Figure 6.11). Equally, knee extensor FS CoV held at 75% of MVC did not differ significantly across the three MC timepoints ($\chi^2(2)$ = 0.222, p = 0.895) (Figure 6.12).

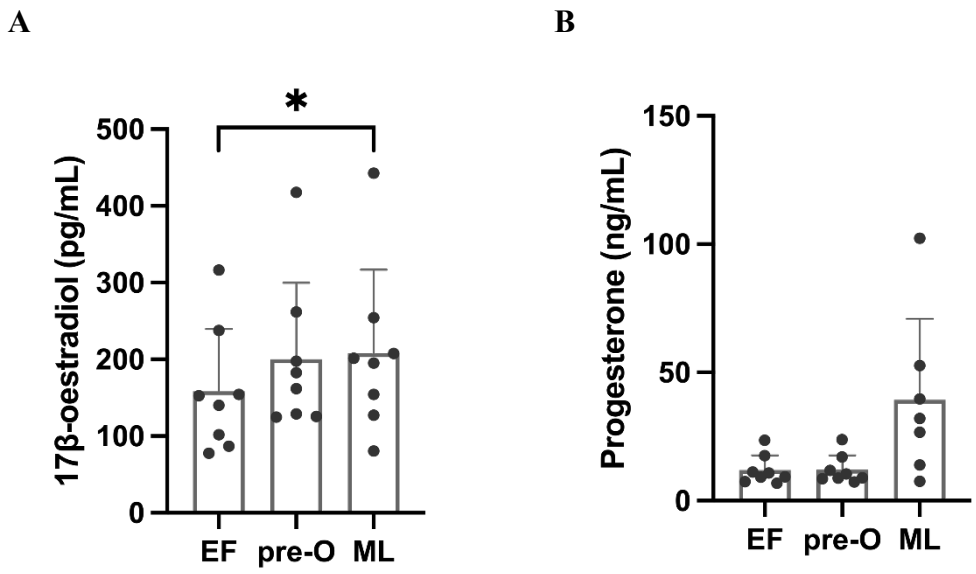

Figure 6.6. Circulating concentrations of **A** 17β-oestradiol and **B** progesterone across three MC timepoints (n = 8). EF, early follicular; pre-O, pre-ovulatory; MC, menstrual cycle; ML, mid-luteal. * indicates $p < 0.05$.

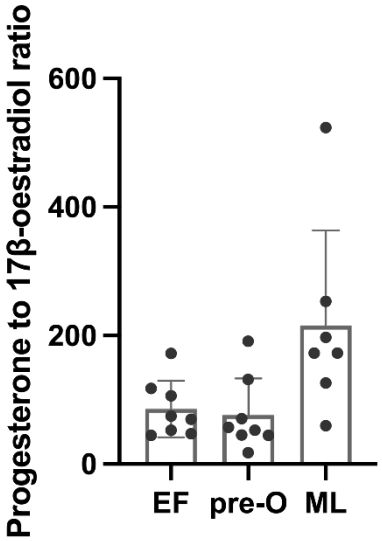

Figure 6.7. Progesterone to 17β-oestradiol ratio across three MC timepoints (n = 8). EF, early follicular; pre-O, pre-ovulatory; MC, menstrual cycle; ML, mid-luteal.

Table 6.2. Circulating concentrations of 17β-oestradiol and progesterone, and progesterone to 17β-oestradiol ratio across three MC timepoints (n = 8).

Variable (mean ± SD)	MC timepoint			p value
	EF	pre-O	ML	
17β-oestradiol (pg/ml)	159 (81)	200 (99)	208 (109)	0.028*
Progesterone (ng/ml)	11.96 (5.74)	12.11 (5.61)	39.27 (31.63)	0.036*
P to E ratio	85.58 (43.99)	76.41 (56.81)	215.1 (148.7)	0.043*

E, 17β-oestradiol; EF, early follicular; MC, menstrual cycle; ML, mid-luteal; P, progesterone; pre-O, pre-ovulatory; SD, standard deviation. * represents overall $p < 0.05$ from one-way repeated measures ANOVA and mixed effects model.

Table 6.3. Knee extensor MVC, VM and VL relRMS, and knee extensor FS CoV at 40% and 75% of MVC across three MC timepoints.

Variable (mean ± SD)	MC timepoint			p value
	EF	pre-O	ML	
MVC (N) (N = 10)	408 (101)	395 (103)	419 (91)	0.393
VM relRMS (%) (n = 9)	58 (18)	49 (16)	55 (10)	0.324
VL relRMS (%) (n = 4)	54 (16)	60 (22)	50 (26)	0.775
FS CoV at 40%MVC (%) (N = 10)	1.55 (0.50)	1.49 (0.45)	1.6 (0.41)	0.756
FS CoV at 75%MVC (%) (n = 9)	2.08 (0.99)	2.07 (1.01)	1.95 (0.72)	0.895

CoV, coefficient of variation; EF, early follicular; FS, force steadiness; MC, menstrual cycle; ML, mid-luteal; MVC, maximum voluntary contraction; N, Newton; P, progesterone; pre-O, pre-ovulatory; SD, standard deviation; VM, vastus medialis; VL, vastus lateralis.

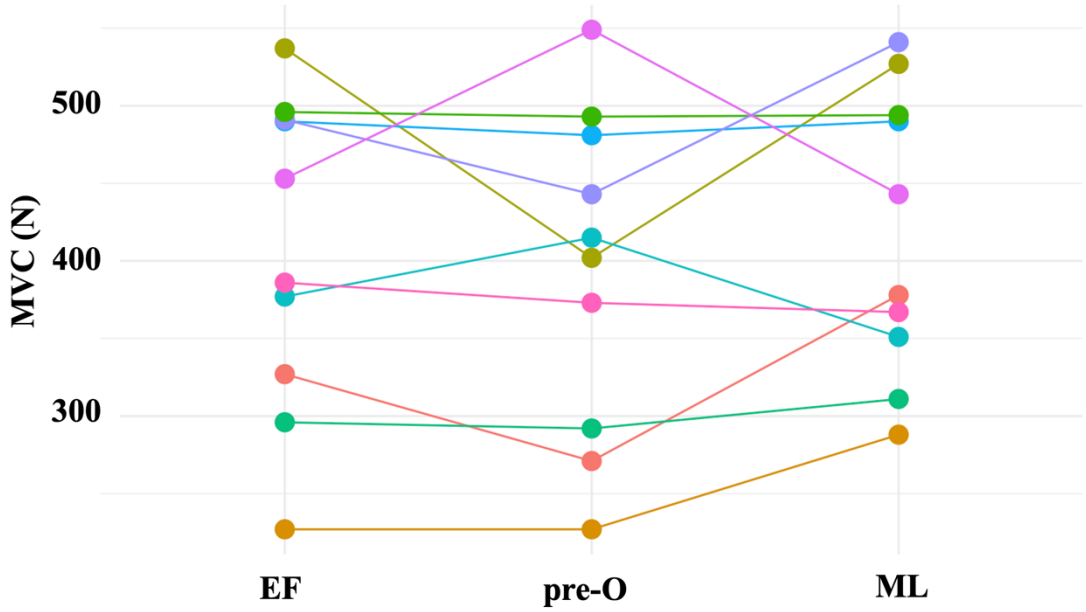

Figure 6.8. Knee extensor MVC across three MC timepoints (N = 10). EF, early follicular; MC, menstrual cycle; ML, mid-luteal; MVC, maximum voluntary contraction; pre-O, pre-ovulatory. The colour code used for each participant's data points is the same between Fig. 6.8, 6.9, 6.10, 6.11, and 6.12.

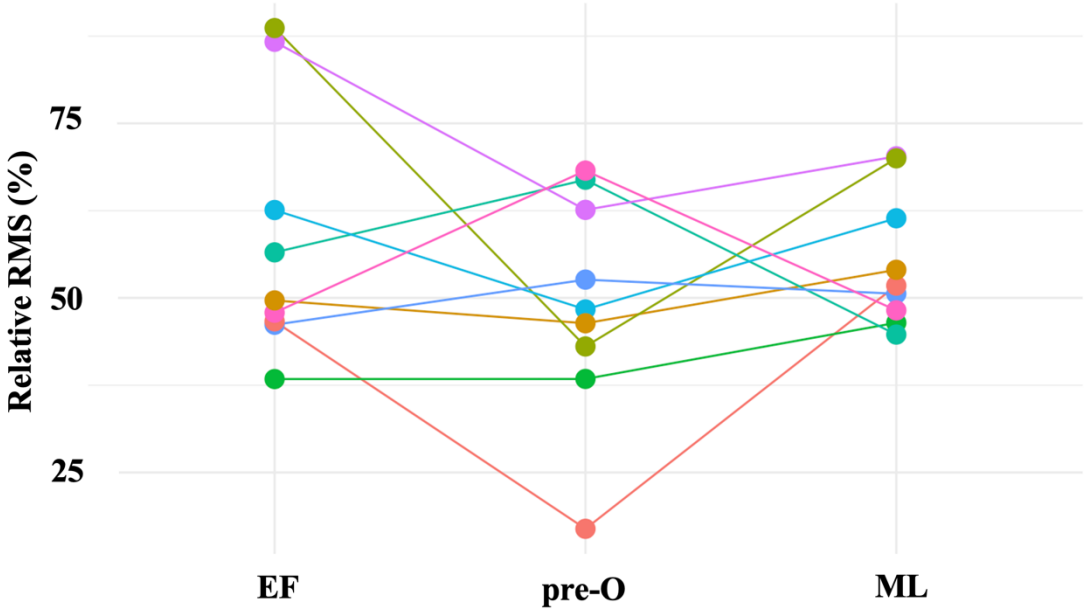

Figure 6.9. VM relRMS across three MC timepoints (n = 9). EF, early follicular; MC, menstrual cycle; ML, mid-luteal; pre-O, pre-ovulatory; relRMS, relative root mean square; VM, vastus medialis.

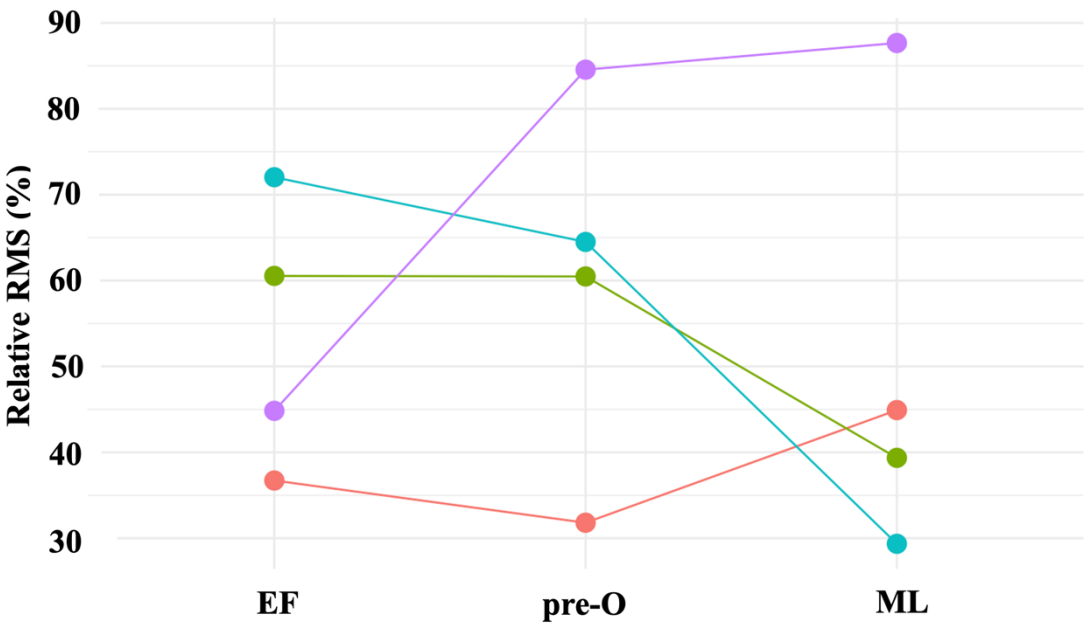

Figure 6.10. VL relRMS across three MC timepoints (n = 4). EF, early follicular; MC, menstrual cycle; ML, mid-luteal; pre-O, pre-ovulatory; relRMS, relative root mean square; VL, vastus lateralis.

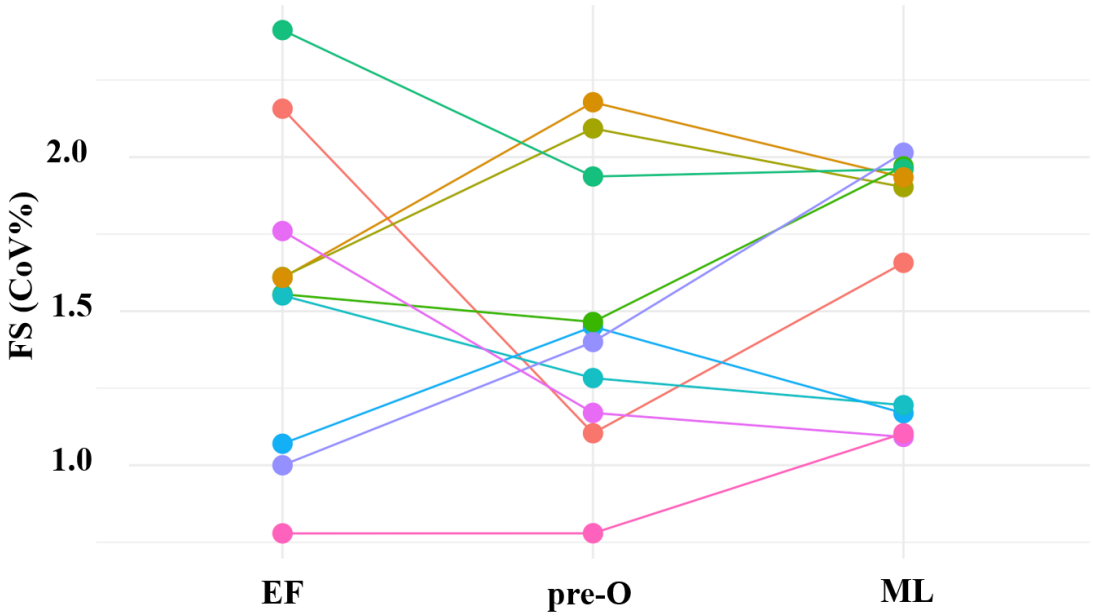

Figure 6.11. Knee extensor FS CoV during a 12-second isometric trapezoid contraction held at 40% of MVC across three MC timepoints (N = 10). CoV, coefficient of variation; EF, early follicular; FS, force steadiness; MC, menstrual cycle; ML, mid-luteal; MVC, maximum voluntary contraction; pre-O, pre-ovulatory.

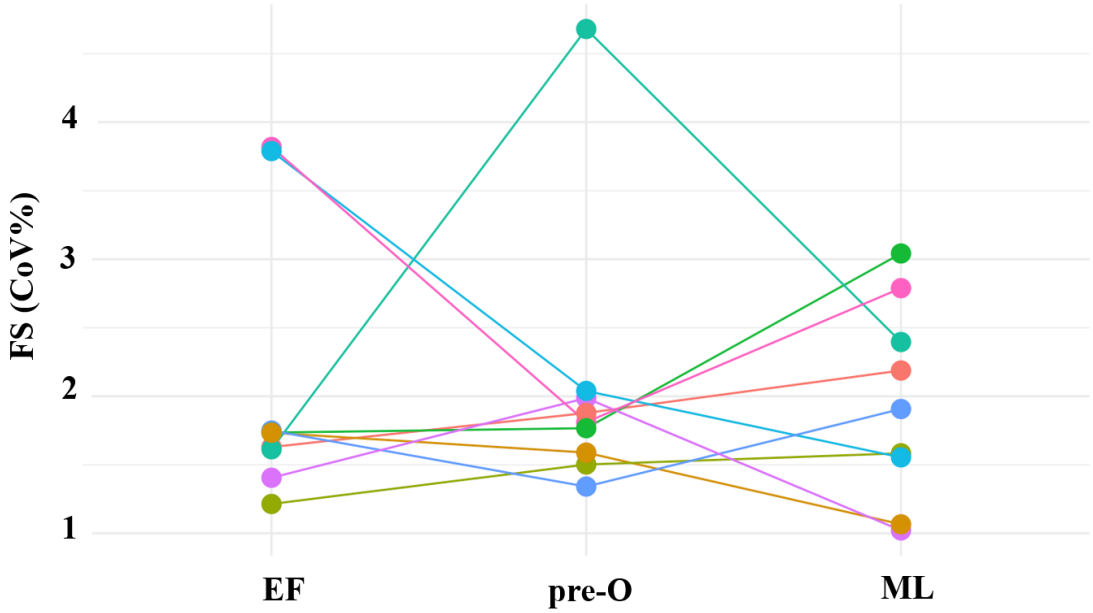

Figure 6.12. Knee extensor FS CoV during a 12-second isometric trapezoid contraction held at 75% of MVC across three MC timepoints (n = 9). CoV, coefficient of variation; EF, early follicular; FS, force steadiness; MC, menstrual cycle; ML, mid-luteal; MVC, maximum voluntary contraction; pre-O, pre-ovulatory.

6.4 Discussion

This study aimed to assess knee extensor neuromuscular function and performance in eumenorrheic females by comparing knee extensor MVC, VM relRMS, VL relRMS, and knee extensor FS CoV at 40% and 75% of MVC across three MC timepoints representing distinct hormonal milieus: early follicular, pre-ovulation, and mid-luteal. The findings showed no significant differences in any of these variables across the MC timepoints, suggesting minimal effect of endogenous ovarian steroid hormone fluctuations on knee extensor maximal force output, VM and VL muscle activation, and knee extensor neuromuscular control at 40% and 75% of MVC. In essence, ovarian steroid hormone fluctuations do not seem to influence the selected knee extensor neuromuscular function and performance variables across MC timepoints in eumenorrheic females.

These findings align with recent studies that, using the same rigorous methodology to identify and verify MC timepoints, found no variation in knee extensor MVC (Ansdell et al., 2019; Janse de Jonge et al., 2001; Montgomery & Shultz, 2010; Piasecki et al., 2023; Thompson et al., 2021) or FS (Piasecki et al., 2023)

across the MC in eumenorrheic females. However, they contrast with studies reporting significant increases in knee extensor MVC during the ovulatory phase (Sarwar, Niclos, & Rutherford, 1996), both ovulatory and mid-luteal phases (Weidauer et al., 2020), or significantly lower MVC in the mid-luteal phase (Tenan, Hackney, & Griffin, 2016) in naturally menstruating females. In addition, the current findings on FS differ from those of Tenan, Hackney and Griffin (2016). While both the current study and Piasecki et al. (2023) assessed knee extensor FS during isometric trapezoidal contractions at various intensities (current study: 40% and 75% of MVC; Piasecki et al., 2023: 10%, 25%, and 40% of MVC), Tenan, Hackney, and Griffin (2016) investigated FS during an endurance task at 25% of MVC. These discrepancies in the results may be due to differences in the standards used to identify and verify MC timepoints and the types of contractions performed during FS assessment, highlighting the need for standardised methods in MC timepoints tracking and assessment to ensure more accurate results.

Although lower-than-expected levels of 17β-oestradiol were observed during the pre-O phase compared to the ML phase, normal 17β-oestradiol levels and patterns were observed at the EF and ML timepoints. One plausible explanation is that pre-O testing was scheduled based on predicted timing from the previously tracked MC, which – upon retrospective verification – accurately captured the intended pre-O window (*i.e.*, 24 to 48 hours before the LH surge) in five out of ten participants. This window is typically characterised by a sharp rise or peak in oestrogen levels. However, in three participants, the LH surge occurred later than predicted, resulting in testing outside the optimal pre-O timeframe. In addition, two participants did not complete daily LH surge testing following their pre-O session, which was their final scheduled visit. To address these limitations, future research should consider implementing multiple pre-O testing sessions based on the previous MC's LH surge to increase the likelihood of capturing the precise hormonal window. While this approach presents logistical challenges related to participant, researcher, and facility availability, it may enhance data accuracy. Furthermore, embedding automated daily reminders for LH surge testing may help minimise participant omission and improve adherence to the testing protocol.

Progesterone levels were higher compared to other studies with similar designs (Ansdell et al., 2019; Piasecki et al., 2023), but consistent with findings from Dam et al. (2022), which examined muscle performance and psychological well-being across the MC in 30 participants. Despite the elevated progesterone levels, the overall pattern across MC timepoints mirrored those of previous studies (Ansdell et al., 2019; Piasecki et al., 2023) and the progesterone to 17β-oestradiol ratio indicated that progesterone was dominant at the ML testing timepoint. In addition, none of the MCs were luteal-phase deficient, as ML progesterone serum levels were consistently above the 16 nmol/L (*i.e.*, 5.03 ng/ml) threshold (Janse de Jonge, Thompson, & Han, 2019).

As reported by Dam et al. (2022), individual hormonal fluctuation profiles varied distinctly between participants, even though average hormone levels fell within the normal range for eumenorrheic individuals. While the authors conducted an analysis of variation based on these individual fluctuations, no significant differences in strength performance were found across the MC.

Data presented in this study do not provide a neurophysiological rationale for recent reports of naturally menstruating female athletes who declare that they observed a negative alteration to their sporting performance at some point during their MC (Armour et al., 2020; Brown, Knight, & Forrest Née Whyte, 2021; Ekenros et al., 2022; Findlay et al., 2020; Jones et al., 2024; Ihalainen et al., 2024; McNamara, Harris, & Minahan, 2022). However, the high variability in the type and magnitude of reported symptoms highlights the need for an individualised approach to managing performance across the MC. Further research on neuromuscular, neurocognitive, nociceptive variables, as well as an individualised approach to training regimes across the MC may help address these concerns in female athletes.

Given the consistency of these findings with high methodological quality studies (Ansdell et al., 2019; Piasecki et al., 2023), which suggest that ovarian steroid hormone fluctuations do not affect knee extensor force output and control, further research should focus on CNS effects and other aspects of knee extensor neuromuscular performance in eumenorrheic females. In addition, a recent study (Ronca et al., 2024) suggests that attentional, anticipatory and spatial cognition fluctuate across the MC in naturally menstruating females, which could be relevant

for reaction time and sensory signals in exercise, particularly in non-contact ACL injury prevention (Riemann & Lephart, 2002; Shultz et al., 2019; Swanik, 2015).

Although this study found no differences in knee extensor force output or neuromuscular control across the MC in eumenorrheic females, further research with larger sample sizes and additional MC testing timepoints is necessary. Future studies should investigate additional neuromuscular factors of knee extensor and flexor neuromuscular performance, such as MU behaviour at various contraction levels and intramuscular EMG, throughout the MC. It would also be valuable to include females with varying hormonal profiles, such as combined monophasic OCP users at different phases of hormone use, intrauterine system (IUS) users at different stages of IUS lifetime, and adolescents with varying maturing ages. This would help determine whether these hormonal statuses influence knee extensor and flexor neuromuscular performance during the MC or with HC use.

6.4.1 Strengths and limitations

This study is the first to investigate multiple factors of knee extensor neuromuscular performance in both the VM and VL muscles across three MC timepoints, using rigorous methods for quantifying circulating serum 17β-oestradiol and progesterone. While no other study has assessed VM or VL relRMS across the MC in eumenorrheic females, it serves as a foundational step in understanding knee extensor muscle activation at the peripheral end of the MU. However, this study was conducted in a highly controlled environment using isometric contractions. Given the growing interest in female neurophysiology and ACL injury prevention, future research should include a wider range of neuromuscular variables and use HDsEMG. To more accurately reflect real-world scenarios where non-contact ACL injuries occur, future studies should also include dynamic tasks, various contraction types, and complementary muscles, such as knee flexors. While HDsEMG has demonstrated promising data quality, it has not yet been optimised for wearable devices, limiting its use in more applied settings (Felici & Del Vecchio, 2020). Nonetheless, it has revealed some significant differences in neuromuscular mechanisms between the sexes (Jenz et al., 2023).

A key strength of this study was the comprehensive approach to MC tracking, which included calendar-based counting, home-based urinary ovulation detection kits,

and serum analyses of circulating 17β-oestradiol and progesterone to confirm participants' eumenorrheic status (Janse de Jonge, Thompson, & Han, 2019). This multi-method strategy ensured that most hormonal timepoints were accurately identified. However, a limitation emerged during the pre-O phase, where 17β-oestradiol levels were lower than expected. While EF and ML hormone profiles aligned with physiological norms, variation in pre-O values likely resulted from the reliance on predicted LH surge timing based on prior cycles. This method was effective for half the participants, whereas others experienced delayed LH surges or omitted post-session LH confirmation. Despite these challenges, the approach proved largely feasible. Future studies may benefit from scheduling multiple pre-O sessions and using automated daily reminders to improve adherence and phase accuracy. Notably, many previous studies did not confirm MC phases via hormonal verification during testing (Dibrezzo, Fort, & Brown, 1988; Kubo et al., 2009; Sarwar, Niclos, & Rutherford, 1996; Tenan et al., 2013; Tenan, Hackney, & Griffin, 2016; Weidauer et al., 2020). As emphasised by Janse de Jonge, Thompson, and Han (2019), higher methodological standards in MC tracking are crucial to clarify inconsistent findings on ovarian hormone effects on exercise performance.

Although the sample size of ten participants may appear small, it is comparable to similar studies (*i.e.*, N = 9 in Piasecki et al., 2023; N = 13 in Ansdell et al., 2019), and recruitment and retention challenges necessitated an extended data collection period adjusting for a moderate retention rate. In addition, as mentioned in Section 6.2.1, intrinsic and environmental noise affected HDsEMG signal quality, and participant time constraint further reduced the available data. As a result, participant samples were reduced by 60% for VL relRMS, 10% for VM relRMS, and 10% for FS CoV at 75%MVC. Finally, seven participants visited the laboratory over multiple MCs due to timetable constraints and randomisation of their first visit, introducing inevitable multi-cycle variability, but the randomisation of the first visit helped mitigate any potential learning effects.

6.5 Conclusion

This study found minimal effects of endogenous ovarian steroid hormone fluctuations on knee extensor maximal strength, VM and VL muscle activation, and knee extensor neuromuscular performance throughout the MC. Neuromuscular

strength and control remained stable despite hormonal fluctuations, suggesting that these fluctuations have little to no impact on the selected variables of knee extensor neuromuscular function in eumenorrheic females. However, due to the small sample size, strong clinical conclusions cannot be drawn. Consistent with most studies, these findings support the inclusion of a broader range of female participants in neuromuscular physiology research, regardless of their MC status. This study contributes valuable neurophysiological data related to knee extensor neuromuscular performance, which is pertinent for non-contact ACL injury risk prevention. Future research should aim for larger participant sample size, add more testing timepoints, especially around pre-ovulation phase, explore additional factors potentially affecting neuromuscular performance, and investigate both the central and peripheral ends of the MU by combining HDsEMG and intramuscular EMG. Including diverse populations with varying hormonal profiles and ages would further enrich understanding of how hormonal variations may affect neuromuscular performance.

Chapter 7. General discussion

7.1 Introduction

Endogenous female ovarian steroid hormone fluctuations may significantly contribute to the risk of non-contact ACL injuries in exercising females and athletes. However, the physiological and neuromuscular effects of these hormones on the ACL, as well as their interrelations, remain a topic of debate (Herzberg et al., 2017; Shultz et al., 2019). This programme of work aimed to: (i) review the existing literature on the potential impact of endogenous and exogenous ovarian steroid hormones on anterior knee laxity (AKL) and non-contact ACL injury rates in females; (ii) assess the intra-rater reliability of an automated knee arthrometer (*i.e.*, the GNRB) for evaluating AKL in a mixed cohort of males and females using combined monophasic oral contraceptive pill (OCP); (iii) evaluate methodological inconsistencies in muscle force steadiness (FS) assessment; and (iv) investigate the impact of ovarian steroid hormone fluctuations across the menstrual (MC) on knee extensor neuromuscular function and performance in eumenorrheic females.

7.2 Key findings

Chapter 3

- Neither endogenous nor exogenous ovarian steroid hormones exerted a significant effect on AKL throughout: (i) the MC in naturally menstruating or eumenorrheic females; or (ii) the hormonal contraceptive (HC) cycle in OCP users, whether in the active or inactive phase.
- No significant difference in AKL was identified between naturally menstruating or eumenorrheic females and OCP users.
- No conclusive evidence was determined regarding the potential effect of endogenous or exogenous ovarian steroid hormones on non-contact ACL injury rates in females.
- A limited number of studies met the predefined inclusion criteria (*i.e.*, N = 9 for AKL, and N = 3 for ACL injuries), and these studies showed high heterogeneity due to variations in sample size, participant inclusion criteria, methodological disparities in exposure assessment, and the devices used for outcome measurement.

- There is a need to improve the quality of the research in this field by adopting methodological standards to accurately identify and verify participants' hormonal status during testing.
- There is a lack of studies examining female populations with menstrual irregularities or users of HC methods other than combined OCPs.

Chapter 4

- The GNRB automated knee arthrometer demonstrated good to excellent intra-rater reliability in healthy individuals, with AKL measurements showing strong intraclass correlation coefficient (ICC) and coefficient of variation (CV) values (ICC_{right} = 0.94 [0.84-0.98], CV = 9.75%; ICC_{left} = 0.94 [0.60-0.95], CV = 9.19%); standard error measurement (SEM) values (SEM_{right_200N} = 0.36 mm [0.60-0.21], SEM_{left_200N} = 0.32 mm [0.83-0.29]), minimum detectable change (MDC) values (≤ 1 mm), and MDC% (17 to 18%), and with ACL compliance/slope also showing solid ICC and CV values (ICC_{right} = 0.91 [0.76-0.98], CV = 8.04%; ICC_{left} = 0.72 [0.20-0.92]; CV = 10.2%), SEM values (SEM_{right_200N} = 1.60 µm/N [0.76-2.61], SEM_{left_200N} = 2.26 µm/N [1.21-3.82]), MDC values (4.4 to 6.3 µm/N), and MDC% (17 to 25.5%).
- AKL and ACL compliance/slope in the right knee were significantly greater in combined monophasic OCP users compared to males ($p < 0.001$) at a 200N loading force. In the left knee, AKL and ACL compliance/slope were not significantly greater in combined monophasic OCP users than males although p values were close to significance at $p = 0.08$, and $p = 0.06$, respectively, at a 200N loading force.

Chapter 5

- Shorter contraction durations selected for FS coefficient of variation (CoV) calculation resulted in significantly lower CoV values (all $p < 0.001$), suggesting steadier muscle force control, which introduces potential bias in interpreting results across the literature.
- When FS CoV values were normalised through conversion to z-scores, contraction duration had no effect on the z-scores.

Chapter 6

- Endogenous ovarian steroid hormone fluctuations had minimal impact on knee extensor maximal strength (p = 0.393), vastus medialis (VM) and vastus lateralis (VL) activation (p = 0.324; p = 0.775, respectively), and knee extensor neuromuscular control at 40% and 75% of MVC (p = 0.756; p = 0.895, respectively) across three MC timepoints in eumenorrheic females.

7.3 Anterior knee laxity and non-contact ACL injury rate

7.3.1 Anterior knee laxity

Chapter 3 presented a meta-analysis of seven studies, revealing no significant variation in AKL across four phases of the MC in naturally menstruating or eumenorrheic females. This analysis used an enhanced classification of MC timepoints from Elliott-Sale et al. (2021). Additionally, 70% of studies excluded from the meta-analysis due to a lack of quantitative measures also reported no significant AKL changes throughout the MC. However, many excluded studies (70%) were categorised as having a high risk of bias in the 'assessment of exposure' domain.

While these results differed from recent meta-analyses, which suggested that AKL is greater during the ovulatory and luteal phases compared to the follicular phase (Moriceau et al., 2022) or exclusively during ovulation (Herzberg et al., 2017), they aligned with the results of Dos'Santos et al. (2023). Notably, all three recent meta-analyses (Dos'Santos et al., 2023; Herzberg et al., 2017; Moriceau et al., 2022), in addition to the current one, highlighted the very low to low quality of studies and evidence due to methodological limitations in the included studies.

A separate meta-analysis of two studies found that AKL did not vary across the HC cycle in combined OCP users. However, one study was rated as high risk of bias in 'participant representativeness' and 'ascertainment of exposure' domains, as it did not provide a comprehensive list of the OCPs used by participants (Burrows & Peters, 2007). Both studies also had a high risk of bias in 'assessment of outcome' domain, which made it difficult to draw reliable conclusions.

Another meta-analysis of two studies showed no significant differences in AKL between non-combined OCP users and naturally menstruating or eumenorrheic

females. Studies on this topic are limited and often fail to provide adequate information about participants' hormonal profiles.

7.3.1 Non-contact ACL injury rate

A synthesis without meta-analysis of two studies found no consensus on which MC phase might increase the risk of non-contact ACL injuries in females. These studies used inconsistent MC phase classifications, making their findings difficult to compare. Both were rated as high risk of bias in the 'ascertainment of exposure' domain, as they relied on self-reported hormonal status without verification.

Additionally, a synthesis of two studies found no difference in non-contact ACL injury rates between naturally menstruating females and OCP users. Despite reaching the same conclusion, both studies were assessed at high risk of bias in the 'ascertainment of exposure' domain due to reliance on self-reported hormonal status.

This systematic review and meta-analysis was the first to apply strict inclusion criteria for MC timepoints classification and exclusively considering primary non-contact ACL injuries, as registered in the PROSPERO database (CRD42021252365, Nédélec et al., 2021) (see Chapter 2). The augmented classification framework (Elliott-Sale et al., 2021) improved the scrutiny of study quality and facilitated cross-study comparisons. This approach also adhered to gold-standard methodology for assessing and verifying hormonal status in female participants (Janse de Jonge, Thompson, & Han, 2019).

A detailed risk of bias assessment was conducted using the Newcastle-Ottawa quality assessment scale for cohort or case-studies, without the star-rating system. This approach helped identify specific domains prone to bias (Ekås et al., 2020; Page et al., 2021b; Wells et al., 2013). Additionally, this systematic review and meta-analysis was the first to examine AKL across the HC cycle in OCP users, highlighting the scarcity of research on other HC types like the progestogen-only pill, contraceptive injection, or IUS, which are frequently used by exercising females and athletes (Baumgartner et al., 2023; Doohan et al., 2023; Martin et al., 2018).

This systematic review and meta-analysis underscored the lack of strong evidence for significant effects of either exogenous or endogenous ovarian steroid

profiles on AKL or non-contact ACL injury rates. This gap is largely due to substantial methodological variability in (i) assessing and verifying participants' hormonal profiles; and (ii) accurately assessing the dependent variables of interest. To address these issues, future studies should use reliable, objective methods to identify and verify hormonal profiles, ensure accurate reporting of these statuses, assess AKL using an automated knee arthrometer with a minimum load of 134N, ideally 200N, and confirm ACL injuries through MRI to reduce methodological inconsistencies.

7.4 Reliability of GNRB knee arthrometer

The hand-held nature of knee arthrometers used in studies reviewed in Chapter 3 has introduced potential biases, such as examiner experience (Ballantyne et al., 1995), gender (Klasan et al., 2019), and hand dominance (Sernert et al., 2007). To address these issues, the GNRB automated knee arthrometer was developed and has demonstrated good reliability in both healthy knees (Magdič, Dahmane & Vauhnik, 2023; Mouarbes et al., 2018; Smith, Miller, & Laslovich, 2022; Vauhnik et al., 2013) and ACL-injured knees (Robert et al., 2009). However, most of these reliability studies did not account for the reproductive hormone status of female participants and lacked sex-specific data analysis.

This study was the first to include combined monophasic OCP users during the pill-taking phase, ensuring their hormonal status was controlled across all three testing sessions. The GNRB demonstrated good to excellent intra-rater reliability in assessing AKL and ACL compliance/slope with a 200N testing load in a mixed-sex cohort of healthy males and combined monophasic OCP users. These results support previous research on the reliability of the device (Magdič, Dahmane & Vauhnik, 2023; Mouarbes et al., 2018; Smith, Miller, & Laslovich, 2022; Vauhnik et al., 2013). In addition, it was observed that right knee AKL and ACL compliance/slope were significantly greater in combined monophasic OCP users compared to males at the 200N load.

This sex difference in AKL and ACL elasticity may be attributed to prior exposure to endogenous hormonal fluctuations from menarche until the onset of OCP use, which could have altered ACL structure and properties (Shultz, Morrissey, & Vauhnik, 2024). It may also stem from differences in ACL size and tensile properties, as females generally have smaller ACLs (Anderson et al., 2001; Chandrashekar,

Slauterbeck, & Hashemi, 2005 [in cadavers]; Muneta, Takakuda, & Yamamoto, 1997 [in cadavers]) with poorer mechanical properties (Chandrashekar et al., 2006 [in cadavers]), making them more prone to greater AKL (Wang et al., 2021) and therefore more susceptible to non-contact ACL injuries.

The observed sex difference in AKL and ACL compliance/slope, further validated by this reliability study, support the broader adoption of the GNRB knee arthrometer in clinical practice to: (i) conduct baseline assessments; (ii) monitor ACL integrity throughout sports seasons; (iii) complement MRI examinations for non-invasive ACL injury diagnosis; and (iv) track progress during rehabilitation.

7.5 Variations in FS calculations

Chapters 2, 3, and 4 considered the potential physiological effects of endogenous and exogenous ovarian steroid profiles and fluctuations on the ACL's connective tissue through AKL assessment. In further developing a multifactorial approach to non-contact ACL injuries in females, it was also important to explore muscle force output, which contributes to executing precise movements. However, methodological inconsistencies exist in the literature on muscle force control (*i.e.*, force steadiness CoV). There is no consensus on the optimal duration of isometric contractions used to calculate FS CoV, making it difficult to compare FS CoV values across studies and to establish clinical standards for different muscles, tasks, populations, diseases, and musculoskeletal injuries.

This study was the first to address this methodological discrepancy. The findings revealed that FS CoV values differ significantly depending on the duration and quality of the contraction segment selected for analysis (Figures 5.4.A and 5.4.B) (all $p < 0.001$). Using shorter, more stable segments with the lowest CoV value, as seen in previous studies (*e.g.*, Harrison et al., 2023; Inglis & Gabriel, 2021; Pethick, Winter, and Burnley, 2015), significantly lower FS CoV values were identified. While this approach might not fully capture the participant's functional performance, these differences were mitigated when raw values were standardised through z-scores conversion (all $p = 1.000$), making FS CoV values reliable and comparable across studies, regardless of the contraction duration.

Implementing rigorous methodological standards for FS CoV calculation and reporting would facilitate objective analysis of future research. Monitoring and training FS CoV could be valuable for various populations, including those with neurological diseases, exercisers, and athletes. Recent findings suggest that such training may benefit neuromuscular control, with potential applications in injury prevention and rehabilitation programmes (De la Fuente et al., 2022; Ely et al., 2022).

7.6 Neuromuscular function

Chapter 6 revealed no significant differences in knee extensor maximal strength, VM and VL muscle activation, or knee extensor neuromuscular control during isometric trapezoidal contractions held at 40% and 75% of MVC across three MC timepoints in healthy, recreationally active eumenorrheic females. These findings suggest that fluctuations in endogenous ovarian steroid hormone do not seem to alter the selected variables of knee extensor neuromuscular function and performance in this population.

The results are consistent with other recent studies that used gold-standard methodology (Janse de Jonge, Thompson, & Han, 2019; Schaumberg et al., 2017) to verify MC timepoints, showing that knee extensor maximal strength remained stable across the MC in eumenorrheic females (Ansdell et al., 2019; Janse de Jonge et al., 2001; Montgomery & Shultz, 2010; Piasecki et al., 2023; Thompson et al., 2021), as does knee extensor FS CoV at 40% of MVC (Piasecki et al., 2023).

While no significant changes were observed in the selected knee extensor muscle force output variables due to hormonal fluctuations, other studies have reported alterations in the central nervous system (CNS) (Ansdell et al., 2019) and motor unit (MU) behaviour (Piasecki et al., 2023) at specific MC timepoints. This current study was the first to investigate multiple factors of knee extensor neuromuscular performance in both the VM and VL muscles across three MC timepoints using gold-standard methodology for MC tracking and quantifying serum levels of 17β-oestradiol and progesterone. It was also the first to assess VM and VL muscle activation using relRMS across the MC in eumenorrheic females, providing new insights into knee extensor neuromuscular function at the peripheral end of the MUs.

These findings underscore the need for further research to expand on current methods by incorporating additional variables and techniques to assess knee extensor neuromuscular function and performance. Increasing sample sizes and including a broader range of MC timepoints across varying contraction intensities in eumenorrheic females would also enhance the robustness and generalisability of findings.

7.7 Limitations

1. In Chapter 3, a secondary screening of the selected reports was conducted during data extraction. This was due to the limited experience of the review researchers who initially performed the selection process. This extra step aimed to prevent the omission of relevant data. In addition, data extraction was performed by a single researcher instead of two, as initially planned in Chapter 2. However, the meticulous and rigorous protocol followed for this systematic review and meta-analysis provided a robust framework, and this minor change was not deemed to significantly impact the results.

2. Chapter 4 noted that the study was underpowered due to a reduced timeframe caused by unforeseen circumstances, which resulted in the exclusion of six eligible participants and the premature conclusion of recruitment. The study ideally required 22 participants, as initially calculated. Despite this limitation, additional reliability indexes such as SEM, MDC, and MDC% were computed to explore intra-rater reliability factors. These indexes were satisfactory and supported the strong ICC and CV values, offering valuable insights for clinical practice.

3. In Chapter 4, the GNRB knee arthrometer used did not include a surface EMG (sEMG) activity feedback option to capture hamstring muscle activation during testing. Early activation of these muscles can affect automated anterior tibial translation and reduce the translation generated by the GNRB's anterior pushes (Barcellona et al., 2014). To mitigate this, participants were repeatedly reminded to keep their tested leg fully relaxed before and during each automated anterior tibial push. Future research should consider using the GNRB knee arthrometer version with sEMG functionality for hamstrings muscle activation.

4. In Chapter 6, while the sample size of ten participants might seem small, it was comparable to similar recent studies that used gold-standard methodology to

identify and verify MC timepoints in eumenorrheic participants (Ansdell et al., 2019; Piasecki et al., 2023). To address potential issues with retention and drop-outs, the study adopted an over-recruitment strategy. However, as detailed in Chapter 6, Section 6.2.1, 12 participants could not complete the study due to various reasons including time constraints (n = 6), inconsistent use of home-based urinary ovulation detection kits (n = 2), relocation (n = 1), anovulatory cycles (n = 1), and MC length outside the 21 to 35 days range (n = 2). With a retention rate of 45%, recruiting and retaining eligible participants, along with verifying the correct MC timepoint, proved challenging within the study period from September 2023 to July 2024. Future research should consider over-recruitment and a longer study timeframe to accommodate moderate retention rates.

5. Chapter 6 noted that a limitation of the study was the lower-than-expected 17β-oestradiol levels during the pre-ovulatory phase, likely due to reliance on predicted LH surge timing from the prior cycle. While this approach correctly identified the intended window in half the participants, it remains a practical method for scheduling in applied research. Future studies may improve phase accuracy by incorporating multiple pre-ovulatory sessions and using automated reminders to support adherence and post-hoc hormonal confirmation.

6. In Chapter 6, it was noted that seven participants visited the laboratory over multiple MCs due to scheduling constraints and randomisation of their initial experimental visit. This introduced multi-cycle intra-variability, but the randomisation of the first visit helped mitigate potential learning effects.

7.8 Future directions

This thesis examined how physiological and neurophysiological systems respond to fluctuations in endogenous ovarian steroid hormone and hormonal contraception status, with a focus on non-contact ACL injury prevention in females. Future research should adhere to higher methodological standards (*i.e.*, the three-step method including MC mapping, urinary ovulation detection kits, and serum sample assessment of 17β-oestradiol and progesterone [Janse de Jonge, Thompson, & Han, 2019; Schaumberg et al., 2017]). Implementing these methods will help accurately assess and verify participants' reproductive hormone status, addressing the current disparities in outcomes observed in existing literature.

Chapter 3 highlighted significant heterogeneity across studies, partly due to discrepancies in assessing and verifying participants' hormonal statuses and the methods used to evaluate key outcomes. Future research should focus on accurately assessing, verifying, and reporting participants' hormonal profiles. Additionally, studies should also use reliable automated knee arthrometers with a minimum load of 134N, ideally 200N, to measure AKL, and use MRI to confirm ACL injuries, providing objective measures that enhances comparability and would facilitate future meta-analyses.

Current research often excludes female athletes with menstrual irregularities, such as amenorrhea and oligomenorrhea, which are common in this population, affecting 20 to 32% of athletes (Nichols et al., 2007; Ravi et al., 2021). These conditions are linked to hormonal imbalances that may increase ACL injury risk by affecting ligament structure and joint stability. This exclusion overlooks a substantial proportion of at-risk athletes, limiting the generalisability and real-world relevance of current findings. Chapter 3 highlighted the lack of studies involving individuals with menstrual irregularities and users of HCs other than OCPs. Future research should expand hormonal profiling to capture a broader range of reproductive hormone statuses, including less commonly studied hormones such as testosterone and relaxin. Both may play key roles, in addition to oestrogen, in modulating knee laxity – testosterone and progesterone are known to regulate relaxin expression, and relaxin has been associated with increased general and knee laxity (Dehghan et al., 2014a, 2014b; Gilmer, Krasta, & Tanaka, 2025; Konopka et al., 2016). Importantly, this expansion must be supported by robust and standardised hormone assessment methods to enable reliable comparisons across studies. Applying such approaches, especially in adolescent athletes, will help uncover underexplored hormonal mechanisms contributing to ACL injury risk and inform more inclusive prevention strategies.

One of the main limitations identified in the systematic review and meta-analysis was the frequent merging of contact and non-contact ACL injuries, which reduces the precision of injury surveillance – especially when examining intrinsic risk factors such as hormonal status. Since hormones are unlikely to influence contact injuries, future research should clearly differentiate injury mechanisms to enable more accurate and meaningful analysis (Bahr et al., 2020). Furthermore, sport-specific and

positional demands (*e.g.*, wingers *versus* central defenders in football) are associated with distinct biomechanical profiles, particularly during high-risk movements such as deceleration, landing, and changes of direction. These movements are frequently involved in defending and tackling actions, which have been identified as common mechanisms for primary non-contact ACL injuries (Brophy et al., 2015; Lucarno et al., 2021). This suggests a potential positional predisposition to injury risk. However, current evidence is limited, especially concerning female athletes, highlighting the need for further position-specific investigations in other high-risk sports. These context-specific factors can influence movement patterns and exposure to risk, making them critical when analysing injury mechanisms. Therefore, studies should report not only the sport but also the athlete's playing position, where relevant. To improve consistency and comparability across studies, the development of standardised reporting guidelines is essential. These should include clear injury classifications, sport and positional context, competition level, and relevant biological factors such as sex and hormonal status – building on existing consensus statements in sports injury surveillance (Bahr et al., 2020). Establishing such standards would enhance the quality of meta-analyses and contribute to more targeted efforts in preventing non-contact ACL injury in female athletes.

Chapter 4 demonstrated that the GNRB automated knee arthrometer was a reliable device for assessing AKL and ACL compliance/slope. This device is well-suited for broader clinical and sports performance applications, supporting optimal knee health strategies for both non-contact ACL injury prevention and rehabilitation, particularly in females who tend to have higher AKL compared to males.

Chapter 5 uncovered methodological inconsistencies in calculating FS CoV, particularly related to contraction duration chosen for the calculations. Future research should investigate a wider range of contraction intensities and include diverse populations to validate or refine these findings. In addition, achieving methodological consensus and enhancing report transparency will facilitate more accurate comparisons of study outcomes.

In Chapter 6, knee extensor neuromuscular function and performance were assessed using a limited set of variables (*i.e.*, MVC, relRMS, and FS at two contraction intensities). Future research should investigate additional neuromuscular variables and

explore both the central and peripheral aspects of MUs using HDsEMG and intramuscular EMG. Moreover, including a broader range of MC timepoints and individuals with varied reproductive hormone profiles and ages will help clarify the role of reproductive hormones in neuromuscular function and performance.

Finally, in future research, it is crucial to consider the whole-body effects of hormonal fluctuations, particularly those associated with the menstrual cycle, on athletic performance and injury risk. Menstrual cycle-related symptoms – such as fatigue, mood changes, and pain – can significantly impact an athlete's physical and psychological well-being, influencing performance and increasing susceptibility to injuries (Hayward et al., 2024). Beyond hormonal factors, other risk elements, such as biomechanics, training loads, and psychological stress, should also be examined, as they can interact with hormonal changes and affect sports-specific movement patterns, neuromuscular control, and recovery. Expanding the scope of research to include these broader risk factors, particularly the impact of menstrual cycle-related symptoms and irregularities, will not only improve the accuracy of non-contact ACL injury risk assessments but also enable the development of more precise, evidence-based strategies. Importantly, these strategies must be tailored to the individual, considering the unique hormonal, physiological, and psychological profiles of female athletes, as each athlete's response to these factors may vary.

7.9 Practical applications

The findings from this thesis provide valuable insights into the role of endogenous and exogenous ovarian steroid hormones on AKL, non-contact ACL injury rates, and knee extensor neuromuscular function. These results have several important practical implications for both clinical and athletic settings. Potential applications based on the key findings of this project and how they could influence both elite and health environments are outlined below.

7.9.1 Use of the GNRB in clinical and athletic environments

The GNRB automated knee arthrometer demonstrated excellent intra-rater reliability in assessing AKL and ACL compliance/slope in healthy individuals, including combined monophasic OCP users. This consistency supports its application as a reliable, non-invasive tool for monitoring knee stability, which can be particularly

useful in clinical setting for both baseline assessments and tracking progress during rehabilitation.

In elite sports environments:

- Pre-season assessments: The GNRB can be used in elite athletes to assess baseline AKL and ACL compliance/slope, providing critical data for coaches and physiotherapists to monitor knee stability and identify athletes at higher risk of non-contact ACL injuries.
- Injury prevention programmes: Continuous monitoring of AKL throughout the sports season can help detect subtle changes in knee stability, which could indicate the early onset of non-contact ACL injury risk. This allows for targeted and individualised intervention strategies, including strengthening and neuromuscular training programmes.
- Rehabilitation: After an ACL injury, the GNRB can be incorporated into rehabilitation programmes to track the progression of knee stability as athletes recover, ensuring that their knees return to optimal functionality before they resume high-level sports-specific activities.

In health and clinical environments:

- Post-surgery rehabilitation: For patients recovering from ACL reconstruction, the GNRB can help assess knee stability and integrity during the rehabilitation process. Accurate monitoring can help clinicians evaluate whether rehabilitation is progressing as expected or if adjustments are needed.

7.9.2 Enhancing neuromuscular training and FS monitoring

The analysis of muscle FS in this thesis underscores the importance of consistent neuromuscular control in non-contact ACL injury prevention. Given the methodological discrepancies identified in the literature, future practice should prioritise standardised techniques for assessing FS to allow for reliable comparisons across patient populations.

In athletic training:

- Neuromuscular training programmes: Training that focuses on improving FS CoV can enhance an athlete's ability to maintain steady muscle force, which is crucial for performing complex, high-intensity movements that require precision and stability. Implementing targeted FS training could reduce the risk of non-contact ACL injuries, particularly in sports involving pivoting and jumping.
- Use of FS in injury prevention: Coaches and strength conditioning professionals could integrate FS assessments into regular training routines to monitor athlete's neuromuscular function. Variations in FS CoV could signal potential deficiencies in neuromuscular control, suggesting earlier interventions.

In rehabilitation:

- Assessing recovery progress: In patients recovering from ACL injuries or surgeries, monitoring FS CoV could serve as an objective and accessible indicator of neuromuscular recovery. As neuromuscular function improves, FS CoV values should decrease, indicating better force control and coordination, which is essential for preventing secondary ACL injuries.

7.9.3 Practical applications in research

The gaps identified in this thesis, including the lack of research on menstrual irregularities and HC users other than OCP users, suggest important areas for future investigation.

- Research on alternative hormonal contraceptive methods: Given the limited research on other forms of hormonal contraception, further studies are needed to understand their effects on AKL and non-contact ACL injury risk.
- Research on menstrual irregularities: Given the limited research on the effects of menstrual irregularities, further studies are needed to understand their impact on AKL, non-contact ACL injury risk and recovery, particularly in relation to hormonal fluctuations and their interaction with other risk factors.

- Longitudinal studies: Conducting long-term, large-scale studies on the impact of hormonal fluctuations and HC use on knee health could provide deeper insights into the role of reproductive hormones in non-contact ACL injury risk and recovery, further informing clinical practice and sports medicine.

The practical applications of this thesis suggest that incorporating standardised testing tools like the GNRB knee arthrometer, integrating FS monitoring into neuromuscular training programmes and broadening hormonal statuses and study timeframes can enhance both clinical care and athletic performance. These strategies could help in better managing non-contact ACL injury risk and improving rehabilitation outcomes. Moreover, the insights gained from this project can inform future studies, leading to more accurate and reliable data in the field of sports medicine and injury prevention, particularly for females, an area where data is currently lacking.

7.10 Conclusions

The literature showed conflicting findings regarding effects of endogenous ovarian steroid hormone fluctuations and hormonal contraception on AKL and non-contact ACL injuries in females. This thesis demonstrated that due to methodological disparities in accurately assessing participants' hormonal status, no definitive conclusions can yet be drawn to support specific strategies for addressing the rising rates of non-contact ACL injuries in females (Chia et al., 2022; Maniar et al., 2022; Weitz, Sillanpää, & Mattila, 2019), despite the availability of non-contact ACL injury prevention programmes. To bridge this gap, the thesis examined additional physiological and neurophysiological factors that may influence non-contact ACL injuries in females. Notably, the research highlighted the reliability of the GNRB automated knee arthrometer, which could be widely adopted in sports and clinical settings to monitor AKL and ACL compliance/slope, thereby aiding in non-contact ACL injury prevention and rehabilitation for females. The study also identified methodological issues in calculating and reporting force steadiness CoV, which, if addressed in future research, could enhance the quality of neuromuscular control assessments and improve understanding of this variable across diverse populations and tasks. Lastly, while evidence suggests that knee extensor maximal strength, VM and VL muscle activation, and knee extensor force steadiness CoV do not vary across

the MC in eumenorrheic females, there may be a complex interplay between ovarian steroid hormones and the neuromuscular system (Piasecki et al., 2023; Jenz et al., 2023). This underscores the need for further investigation at the neuromuscular function's root level – the MU – and calls for high-quality studies to address the current data scarcity in female neurophysiology.

Chapter 8. References

Adachi, N., Nawata, K., Maeta, M., & Kurozawa, Y. (2008). Relationship of the menstrual cycle phase to anterior cruciate ligament injuries in teenaged female athletes. *Archives of orthopaedic and trauma surgery, 128*(5), 473–478.

Agel, J., Bershadsky, B., & Arendt, E. A. (2006). Hormonal therapy: ACL and ankle injury. *Medicine and science in sports and exercise*, *38*(1), 7-12.

Agel, J., Olson, D. E., Dick, R., Arendt, E. A., Marshall, S. W., & Sikka, R. S. (2007). Descriptive epidemiology of collegiate women's basketball injuries: National Collegiate Athletic Association Injury Surveillance System, 1988-1989 through 2003-2004. *Journal of athletic training, 42*(2), 202–210.

Agel, J., Rockwood, T., & Klossner, D. (2016). Collegiate ACL Injury Rates Across 15 Sports: National Collegiate Athletic Association Injury Surveillance System Data Update (2004-2005 Through 2012-2013). *Clinical journal of sport medicine: official journal of the Canadian Academy of Sport Medicine, 26*(6), 518–523.

Ahmad, C. S., Clark, A. M., Heilmann, N., Schoeb, J. S., Gardner, T. R., & Levine, W. N. (2006). Effect of gender and maturity on quadriceps-to-hamstring strength ratio and anterior cruciate ligament laxity. *The American journal of sports medicine, 34*(3), 370-374.

Alahmad, T. A., Kearney, P., & Cahalan, R. (2020). Injury in elite women's soccer: a systematic review. *The Physician and sportsmedicine*, *48*(3), 259-265.

Alentorn-Geli, E., Myer, G. D., Silvers, H. J., Samitier, G., Romero, D., Lázaro-Haro, C., & Cugat, R. (2009). Prevention of non-contact anterior cruciate ligament injuries in soccer players. Part 1: Mechanisms of injury and underlying risk factors. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA*, *17*(7), 705–729.

Almuklass, A. M., Price, R. C., Gould, J. R., & Enoka, R. M. (2016). Force steadiness as a predictor of time to complete a pegboard test of dexterity in young men and women. *Journal of applied physiology (Bethesda, Md. : 1985), 120*(12), 1410–1417.

Amis, A. A., & Dawkins, G. P. (1991). Functional anatomy of the anterior cruciate ligament. Fibre bundle actions related to ligament replacements and injuries. *The Journal of Bone & Joint Surgery British Volume*, *73*(2), 260-267.

Anderson, A. F., Dome, D. C., Gautam, S., Awh, M. H., & Rennirt, G. W. (2001). Correlation of anthropometric measurements, strength, anterior cruciate ligament size, and intercondylar notch characteristics to sex differences in anterior cruciate ligament tear rates. *The American journal of sports medicine, 29*(1), 58–66.

Ansdell, P., Brownstein, C. G., Škarabot, J., Hicks, K. M., Simoes, D. C. M., Thomas, K., Howatson, G., Hunter, S. K., & Goodall, S. (2019). Menstrual cycle-associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic women. *Journal of applied physiology, 126*(6), 1701–1712.

Apter, D., Reinilä, M., & Vihko, R. (1989). Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood. *International journal of cancer, 44*(5), 783–787.

Armour, M., Parry, K. A., Steel, K., & Smith, C. A. (2020). Australian female athlete perceptions of the challenges associated with training and competing when menstrual symptoms are present. *International Journal of Sports Science & Coaching, 15*(3), 316-323.

Arnoczky, S. P. (1983). Anatomy of the anterior cruciate ligament. *Clinical Orthopaedics and Related Research*, *172*, 19-25.

Bahr, R., & Krosshaug, T. (2005). Understanding injury mechanisms: a key component of preventing injuries in sport. *British journal of sports medicine*, *39*(6), 324-329.

Bahr, R., Clarsen, B., Derman, W., Dvorak, J., Emery, C. A., Finch, C. F., Hägglund, M., Junge, A., Kemp, S., Khan, K. M., Marshall, S. W., Meeuwisse, W., Mountjoy, M., Orchard, J. W., Pluim, B., Quarrie, K. L., Reider, B., Schwellnus, M., Soligard, T., Stokes, K. A., … Chamari, K. (2020).

International Olympic Committee consensus statement: methods for recording and reporting of epidemiological data on injury and illness in sport 2020 (including STROBE Extension for Sport Injury and Illness Surveillance (STROBE-SIIS)). *British journal of sports medicine, 54*(7), 372–389.

Bakos, O., Lundkvist, Ö., Wide, L., & Bergh, T. (1994). Ultrasonographical and hormonal description of the normal ovulatory menstrual cycle. *Acta obstetricia et gynecologica Scandinavica*, *73*(10), 790-796.

Balbinot, G., Joner Wiest, M., Li, G., Pakosh, M., Cesar Furlan, J., Kalsi-Ryan, S., & Zariffa, J. (2022). The use of surface EMG in neurorehabilitation following traumatic spinal cord injury: A scoping review. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, *138*, 61–73.

Ballantyne, B. T., French, A. K., Heimsoth, S. L., Kachingwe, A. F., Lee, J. B., & Soderberg, G. L. (1995). Influence of examiner experience and gender on interrater reliability of KT-1000 arthrometer measurements. *Physical therapy, 75*(10), 898–906.

Balshem, H., Helfand, M., Schünemann, H. J., Oxman, A. D., Kunz, R., Brozek, J., Vist, G. E., Falck-Ytter, Y., Meerpohl, J., Norris, S., & Guyatt, G. H. (2011). GRADE guidelines: 3. Rating the quality of evidence. *Journal of clinical epidemiology, 64*(4), 401–406.

Bandak, E., Zebis, M., Alkjaer, T., Nielsen, S. M., Bennike, S., & Thorborg, K. (2024). Injury Prevention Training in Girls' and Women's Elite Football in Denmark: A Survey of Practices and Perceptions. *Scandinavian journal of medicine & science in sports, 34*(7), e14696.

Banks, C. L., Huang, H. J., Little, V. L., & Patten, C. (2017). Electromyography Exposes Heterogeneity in Muscle Co-Contraction following Stroke. *Frontiers in neurology*, *8*, 699.

Barbero M., Merletti R., Rainoldi A. (2012). Lower limb. In: Barbero M., Merletti R., Rainoldi A. (Eds), *Atlas of Muscle Innervation Zones: Understanding Surface*

Electromyography and Its Applications (pp. 131–132). Milan (Italy); New York: Springer.

Barber-Westin, S., & Noyes, F. R. (2020). One in 5 Athletes Sustain Reinjury Upon Return to High-Risk Sports After ACL Reconstruction: A Systematic Review in 1239 Athletes Younger Than 20 Years. *Sports health*, *12*(6), 587–597.

Barcellona, M. G., Morrissey, M. C., Milligan, P., & Amis, A. A. (2014). The effect of thigh muscle activity on anterior knee laxity in the uninjured and anterior cruciate ligament-injured knee. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA, 22*(11), 2821–2829.

Barry, B. K., Pascoe, M. A., Jesunathadas, M., & Enoka, R. M. (2007). Rate coding is compressed but variability is unaltered for motor units in a hand muscle of old adults. *Journal of neurophysiology, 97*(5), 3206–3218.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1–48.

Baumgartner, S., Bitterlich, N., Geboltsberger, S., Neuenschwander, M., Matter, S., & Stute, P. (2023). Contraception, female cycle disorders and injuries in Swiss female elite athletes-a cross sectional study. *Frontiers in physiology*, *14*, 1232656

Beaulieu, M. L., Ashton-Miller, J. A., & Wojtys, E. M. (2023). Loading mechanisms of the anterior cruciate ligament. *Sports biomechanics*, *22*(1), 1–29.

Beck, N. A., Lawrence, J. T. R., Nordin, J. D., DeFor, T. A., & Tompkins, M. (2017). ACL tears in school-aged children and adolescents over 20 years. *Pediatrics, 139*(3).

Belanger, M. J., Moore, D. C., Crisco, J. J., 3rd, Fadale, P. D., Hulstyn, M. J., & Ehrlich, M. G. (2004). Knee laxity does not vary with the menstrual cycle, before or after exercise. *The American journal of sports medicine*, *32*(5), 1150–1157.

Beldame, J., Mouchel, S., Bertiaux, S., Adam, J. M., Mouilhade, F., Roussignol, X., & Dujardin, F. (2012). Anterior knee laxity measurement: comparison of passive stress radiographs Telos(®) and "Lerat", and GNRB(®) arthrometer. *Orthopaedics & traumatology, surgery & research : OTSR, 98*(7), 744–750.

Bennell, K., White, S., & Crossley, K. (1999). The oral contraceptive pill: a revolution for sportswomen?. *British journal of sports medicine*, *33*(4), 231-238.

Bercovy, M., & Weber, E. (1995). Evaluation de la laxité, de la rigidité et de la compliance du genou normal et pathologique. Application à la courbe de survie des ligamentoplasties [Evaluation of laxity, rigidity and compliance of the normal and pathological knee. Application to survival curves of ligamentoplasties]. *Revue de chirurgie orthopedique et reparatrice de l'appareil moteur, 81*(2), 114–127.

Beynnon, B. D., Bernstein, I. M., Belisle, A., Brattbakk, B., Devanny, P., Risinger, R., & Durant, D. (2005). The effect of estradiol and progesterone on knee and ankle joint laxity. *The American journal of sports medicine, 33*(9), 1298-1304.

Beynnon, B. D., Fleming, B. C., Labovitch, R., & Parsons, B. (2002). Chronic anterior cruciate ligament deficiency is associated with increased anterior translation of the tibia during the transition from non-weightbearing to weightbearing. *Journal of orthopaedic research*, *20*(2), 332-337.

Beynnon B.D., Johnson R.J. (1994). Relevant biomechanics. In: DeLee J.C., Drez D., (Eds.), *Orthopaedic sports medicine* (pp. 1113–1133). Philadelpia: PA:WB Saunders.

Beynnon, B. D., Johnson, R. J., Braun, S., Sargent, M., Bernstein, I. M., Skelly, J. M., & Vacek, P. M. (2006). The relationship between menstrual cycle phase and anterior cruciate ligament injury: a case-control study of recreational alpine skiers. *The American journal of sports medicine*, *34*(5), 757-764.

Beynnon, B. D., Johnson, R. J., Fleming, B. C., Peura, G. D., Renstrom, P. A., Nichols, C. E., & Pope, M. H. (1997). The effect of functional knee bracing on the

anterior cruciate ligament in the weightbearing and nonweightbearing knee. *The American journal of sports medicine*, *25*(3), 353-359.

Bhardwaj, P., Au, C. C., Benito-Martin, A., Ladumor, H., Oshchepkova, S., Moges, R., & Brown, K. A. (2019). Estrogens and breast cancer: Mechanisms involved in obesity-related development, growth and progression. *The Journal of steroid biochemistry and molecular biology, 189*, 161–170.

Bilodeau, M., Schindler-Ivens, S., Williams, D. M., Chandran, R., & Sharma, S. S. (2003). EMG frequency content changes with increasing force and during fatigue in the quadriceps femoris muscle of men and women. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, *13*(1), 83–92.

Biro, F. M., Huang, B., Wasserman, H., Gordon, C. M., & Pinney, S. M. (2021). Pubertal Growth, IGF-1, and Windows of Susceptibility: Puberty and Future Breast Cancer Risk. *The Journal of adolescent health : official publication of the Society for Adolescent Medicine, 68*(3), 517–522.

Boden, B. P., Dean, G. S., Feagin, J. A., & Garrett, W. E. (2000). Mechanisms of anterior cruciate ligament injury. *Orthopedics*, *23*(6), 573-578.

Bolling, C., Van Mechelen, W., Pasman, H. R., & Verhagen, E. (2018). Context matters: revisiting the first step of the 'sequence of prevention'of sports injuries. *Sports medicine*, *48*(10), 2227-2234.

Bram, J. T., Magee, L. C., Mehta, N. N., Patel, N. M., & Ganley, T. J. (2021). Anterior cruciate ligament injury incidence in adolescent athletes: a systematic review and meta-analysis. *The American journal of sports medicine*, *49*(7), 1962-1972.

Brophy, R. H., Stepan, J. G., Silvers, H. J., & Mandelbaum, B. R. (2015). Defending Puts the Anterior Cruciate Ligament at Risk During Soccer: A Gender-Based Analysis. *Sports health, 7*(3), 244–249.

Brown, N., Knight, C. J., & Forrest Née Whyte, L. J. (2021). Elite female athletes' experiences and perceptions of the menstrual cycle on training and sport

performance. *Scandinavian journal of medicine & science in sports*, *31*(1), 52–69.

Brown, R. E., Edwards, D. L., & Jakobi, J. M. (2010). Sex differences in force steadiness in three positions of the forearm. *European journal of applied physiology, 110*(6), 1251–1257.

Bruder, A. M., Culvenor, A. G., King, M. G., Haberfield, M., Roughead, E. A., Mastwyk, J., Kemp, J. L., Ferraz Pazzinatto, M., West, T. J., Coburn, S. L., Cowan, S. M., Ezzat, A. M., To, L., Chilman, K., Couch, J. L., Whittaker, J. L., & Crossley, K. M. (2023). Let's talk about sex (and gender) after ACL injury: a systematic review and meta-analysis of self-reported activity and knee-related outcomes. *British journal of sports medicine*, *57*(10), 602–610.

Bruinvels, G., Goldsmith, E., Blagrove, R., Simpkin, A., Lewis, N., Morton, K., Suppiah, A., Rogers, J. P., Ackerman, K. E., Newell, J., & Pedlar, C. (2021). Prevalence and frequency of menstrual cycle symptoms are associated with availability to train and compete: a study of 6812 exercising women recruited using the Strava exercise app. *British journal of sports medicine*, *55*(8), 438–443.

Burrows, M., & Peters, C. E. (2007). The influence of oral contraceptives on athletic performance in female athletes. *Sports medicine, 37*, 557-574.

Butler, D. L., Noyes, F. R., & Grood, E. S. (1980). Ligamentous restraints to anterior-posterior drawer in the human knee. A biomechanical study. *JBJS*, *62*(2), 259-270.

Carcia, C. R., Shultz, S. J., Granata, K. P., Gansneder, B. M., & Perrin, D. H. (2004). Knee ligament behavior following a controlled loading protocol does not differ by menstrual cycle day. *Clinical biomechanics, 19*(10), 1048-1054.

Casey, E., Hameed, F., & Dhaher, Y. Y. (2014). The muscle stretch reflex throughout the menstrual cycle. *Medicine and science in sports and exercise*, *46*(3), 600.

Castronovo, A. M., Mrachacz-Kersting, N., Stevenson, A. J. T., Holobar, A., Enoka, R. M., & Farina, D. (2018). Decrease in force steadiness with aging is

associated with increased power of the common but not independent input to motor neurons. *Journal of neurophysiology, 120*(4), 1616–1624.

Cea-Soriano, L., García Rodríguez, L. A., Machlitt, A., & Wallander, M. A. (2014). Use of prescription contraceptive methods in the UK general population: a primary care study. BJOG: *An International Journal of Obstetrics & Gynaecology, 121*(1), 53-61.

Chandrashekar, N., Mansouri, H., Slauterbeck, J., & Hashemi, J. (2006). Sex-based differences in the tensile properties of the human anterior cruciate ligament. *Journal of biomechanics, 39*(16), 2943–2950.

Chandrashekar, N., Slauterbeck, J., & Hashemi, J. (2005). Sex-based differences in the anthropometric characteristics of the anterior cruciate ligament and its relation to intercondylar notch geometry: a cadaveric study. *The American journal of sports medicine*, *33*(10), 1492-1498.

Chia, L., De Oliveira Silva, D., Whalan, M., McKay, M. J., Sullivan, J., Fuller, C. W., & Pappas, E. (2022). Non-contact anterior cruciate ligament injury epidemiology in team-ball sports: a systematic review with meta-analysis by sex, age, sport, participation level, and exposure type. *Sports medicine*, *52*(10), 2447-2467.

Chidi-Ogbolu, N., & Baar, K. (2019). Effect of Estrogen on Musculoskeletal Performance and Injury Risk. *Frontiers in physiology, 9*, 1834.

Christou E. A. (2011). Aging and variability of voluntary contractions. *Exercise and sport sciences reviews, 39*(2), 77–84.

Christou E. A. (2010). Motor output variability. In: Kompoliti K. & Verhagen Metman L. (Eds.), *Encyclopedia of Movement Disorders* (pp. 202-204). Oxford (UK): Academic Press.

Christou E. A., Tracy B. L. (2005). Aging and motor output variability. In: Davids K., Bennett S., Newell K. (Eds), *Movement System Variability* (pp. 199–215). Champaign (IL): Human Kinetics.

Christou, E. A., Yang, Y., & Rosengren, K. S. (2003). Taiji training improves knee extensor strength and force control in older adults. *The journals of gerontology. Series A, Biological sciences and medical sciences, 58*(8), 763–766.

Clancy, E. A., Morin, E. L., Hajian, G., & Merletti, R. (2023). Tutorial. Surface electromyogram (sEMG) amplitude estimation: Best practices. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, *72*, 102807.

Clarac, F., & Barbara, J. G. (2011). The emergence of the "motoneuron concept": From the early 19th C to the beginning of the 20th C. Brain research, 1409, 23–41.

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences* (2nd ed.). Routledge.

Cojean, T., Batailler, C., Robert, H., & Cheze, L. (2023). GNRB® laximeter with magnetic resonance imaging in clinical practice for complete and partial anterior cruciate ligament tears detection: A prospective diagnostic study with arthroscopic validation on 214 patients. *The Knee, 42*, 373–381.

Collette, M., Courville, J., Forton, M., & Gagnière, B. (2012). Objective evaluation of anterior knee laxity; comparison of the KT-1000 and GNRB® arthrometers. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA, 20*(11), 2233–2238.

Colombet, P., Robinson, J., Christel, P., Franceschi, J. P., Djian, P., Bellier, G., & Sbihi, A. (2006). Morphology of anterior cruciate ligament attachments for anatomic reconstruction: a cadaveric dissection and radiographic study. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, *22*(9), 984-992.

Cone, S. G., Howe, D., & Fisher, M. B. (2019a). Size and Shape of the Human Anterior Cruciate Ligament and the Impact of Sex and Skeletal Growth: A Systematic Review. *JBJS reviews, 7*(6), e8.

Cone, S. G., Lambeth, E. P., Ru, H., Fordham, L. A., Piedrahita, J. A., Spang, J. T., & Fisher, M. B. (2019b). Biomechanical function and size of the anteromedial and posterolateral bundles of the ACL change differently with skeletal growth in the pig model. *Clinical Orthopaedics and Related Research*, *477*(9), 2161.

Cooperman, J. M., Riddle, D. L., & Rothstein, J. M. (1990). Reliability and validity of judgments of the integrity of the anterior cruciate ligament of the knee using the Lachman's test. *Physical therapy, 70*(4), 225–233.

Costello, J. T., Bieuzen, F., & Bleakley, C. M. (2014). Where are all the female participants in sports and exercise medicine research?. *European journal of sport science*, *14*(8), 847-851.

Côté, M. P., Murray, L. M., & Knikou, M. (2018). Spinal Control of Locomotion: Individual Neurons, Their Circuits and Functions. *Frontiers in physiology*, *9*, 784.

Cowley, E. S., Olenick, A. A., McNulty, K. L., & Ross, E. Z. (2021). "Invisible sportswomen": the sex data gap in sport and exercise science research. *Women in Sport and Physical Activity Journal*, *29*(2), 146-151.

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F., & Oja, P. (2003). International physical activity questionnaire: 12-country reliability and validity. *Medicine and science in sports and exercise, 35*(8), 1381–1395.

Crossley, K. M., Patterson, B. E., Culvenor, A. G., Bruder, A. M., Mosler, A. B., & Mentiplay, B. F. (2020). Making football safer for women: a systematic review and meta-analysis of injury prevention programmes in 11 773 female football (soccer) players. *British journal of sports medicine, 54*(18), 1089–1098.

Dam, T. V., Dalgaard, L. B., Sevdalis, V., Bibby, B. M., Janse DE Jonge, X., Gravholt, C. H., & Hansen, M. (2022). Muscle Performance during the Menstrual Cycle Correlates with Psychological Well-Being, but Not Fluctuations in Sex Hormones. *Medicine and science in sports and exercise*, *54*(10), 1678–1689.

Daniel, D. M., Malcom, L. L., Losse, G., Stone, M. L., Sachs, R., & Burks, R. (1985a). Instrumented measurement of anterior laxity of the knee. *The Journal of bone and joint surgery. American volume, 67*(5), 720–726.

Daniel, D. M., Stone, M. L., Sachs, R., & Malcom, L. (1985b). Instrumented measurement of anterior knee laxity in patients with acute anterior cruciate ligament disruption. *The American journal of sports medicine, 13*(6), 401–407.

Daniel, J. (2012). Choosing the size of the sample. In *Sampling Essentials: Practical Guidelines for Making Sampling Choices* (pp. 236-253). SAGE Publications, Inc.

Davis, L. A., Alenazy, M. S., Almuklass, A. M., Feeney, D. F., Vieira, T., Botter, A., & Enoka, R. M. (2020). Force control during submaximal isometric contractions is associated with walking performance in persons with multiple sclerosis. *Journal of neurophysiology, 123*(6), 2191–2200.

De la Fuente, C., Stoelben, K. J. V., Silvestre, R., Yañez, R., Cheyre, J., Guadagnin, E. C., & Carpes, F. P. (2022). Steadiness training improves the quadriceps strength and self-reported outcomes in persistent quadriceps weakness following nine months of anterior cruciate ligament reconstruction and failed conventional physiotherapy. *Clinical biomechanics (Bristol, Avon)*, *92*, 105585.

De Luca, C. J. (1997). The Use of Surface Electromyography in Biomechanics. *Journal of Applied Biomechanics*, *13*(2), 135-163.

De Luca, C. J., LeFever, R. S., McCue, M. P., & Xenakis, A. P. (1982). Behaviour of human motor units in different muscles during linearly varying contractions. *The Journal of physiology*, *329*, 113–128.

de Ridder, C. M., Bruning, P. F., Zonderland, M. L., Thijssen, J. H., Bonfrer, J. M., Blankenstein, M. A., Huisveld, I. A., & Erich, W. B. (1990). Body fat mass, body fat distribution, and plasma hormones in early puberty in females. *The Journal of clinical endocrinology and metabolism, 70*(4), 888–893.

De Souza, M. J., Toombs, R. J., Scheid, J. L., O'Donnell, E., West, S. L., & Williams, N. I. (2010). High prevalence of subtle and severe menstrual disturbances in exercising women: confirmation using daily hormone measures. *Human reproduction*, *25*(2), 491-503.

Deeks, J. J., Higgins, J. P. T., Altman, D. G. (2021). Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (Eds), *Cochrane Handbook for Systematic Reviews of Interventions* (version 6.4). Cochrane

DeFroda, S. F., Bokshan, S. L., Worobey, S., Ready, L., Daniels, A. H., & Owens, B. D. (2019). Oral contraceptives provide protection against anterior cruciate ligament tears: a national database study of 165,748 female patients. *The Physician and sportsmedicine*, *47*(4), 416-420.

Dehghan, F., Muniandy, S., Yusof, A., & Salleh, N. (2014a). Sex-steroid regulation of relaxin receptor isoforms (RXFP1 & RXFP2) expression in the patellar tendon and lateral collateral ligament of female WKY rats. *International journal of medical sciences, 11*(2), 180–191.

Dehghan, F., Muniandy, S., Yusof, A., & Salleh, N. (2014b). Testosterone reduces knee passive range of motion and expression of relaxin receptor isoforms via 5α-dihydrotestosterone and androgen receptor binding. *International journal of molecular sciences, 15*(3), 4619–4634.

Deie, M., Sakamaki, Y., Sumen, Y., Urabe, Y., & Ikuta, Y. (2002). Anterior knee laxity in young women varies with their menstrual cycle. *International orthopaedics, 26*(3), 154–156.

Dejour, H., & Bonnin, M. (1994). Tibial translation after anterior cruciate ligament rupture. Two radiological tests compared. *The Journal of Bone & Joint Surgery British Volume*, *76*(5), 745-749.

Del Vecchio, A., Germer, C. M., Elias, L. A., Fu, Q., Fine, J., Santello, M., & Farina, D. (2019). The human central nervous system transmits common synaptic

inputs to distinct motor neuron pools during non-synergistic digit actions. *The Journal of physiology, 597*(24), 5935–5948.

DeMont, R. G., Lephart, S. M., Giraldo, J. L., Swanik, C. B., & Fu, F. H. (1999). Muscle preactivity of anterior cruciate ligament-deficient and -reconstructed females during functional activities. *Journal of athletic training*, *34*(2), 115–120.

Dibrezzo, R., Fort, I. L., & Brown, B. (1988). Dynamic strength and work variations during three stages of the menstrual cycle. *The Journal of orthopaedic and sports physical therapy*, *10*(4), 113–116.

Dideriksen, J. L., Negro, F., Enoka, R. M., & Farina, D. (2012). Motor unit recruitment strategies and muscle properties determine the influence of synaptic noise on force steadiness. *Journal of neurophysiology, 107*(12), 3357–3369.

Diekfuss, J. A., Grooms, D. R., Yuan, W., Dudley, J., Barber Foss, K. D., Thomas, S., Ellis, J. D., Schneider, D. K., Leach, J., Bonnette, S., & Myer, G. D. (2019). Does brain functional connectivity contribute to musculoskeletal injury? A preliminary prospective analysis of a neural biomarker of ACL injury risk. *Journal of science and medicine in sport*, *22*(2), 169–174.

DiStefano, L. J., Dann, C. L., Chang, C. J., Putukian, M., Pierpoint, L. A., Currie, D. W., Knowles, S. B., Wasserman, E. B., Dompier, T. P., Comstock, R. D., Marshall, S. W., & Kerr, Z. Y. (2018). The First Decade of Web-Based Sports Injury Surveillance: Descriptive Epidemiology of Injuries in US High School Girls' Soccer (2005-2006 Through 2013-2014) and National Collegiate Athletic Association Women's Soccer (2004-2005 Through 2013-2014). *Journal of athletic training, 53*(9), 880–892.

Doohan, M. A., King, N., White, M. J., & Stewart, I. B. (2023). Trends in menstrual cycle symptoms, physical activity avoidance, and hormonal contraceptive use in a general population of adult women. *Sexual & Reproductive Healthcare*, *36*, 100853.

Dos'Santos, T., Stebbings, G. K., Morse, C., Shashidharan, M., Daniels, K. A. J., & Sanderson, A. (2023). Effects of the menstrual cycle phase on anterior cruciate ligament neuromuscular and biomechanical injury risk surrogates in eumenorrheic and naturally menstruating women: A systematic review. *PloS one*, *18*(1), e0280800.

Dragoo, J. L., Lee, R. S., Benhaim, P., Finerman, G. A., & Hame, S. L. (2003). Relaxin receptors in the human female anterior cruciate ligament. *The American journal of sports medicine, 31*(4), 577–584.

Drew, M. K., & Finch, C. F. (2016). The relationship between training load and injury, illness and soreness: a systematic and literature review. *Sports medicine*, *46*, 861-883.

Duchateau, J., & Enoka, R. M. (2011). Human motor unit recordings: origins and insight into the integrated motor system. *Brain research*, *1409*, 42–61.

Duthon, V. B., Barea, C., Abrassart, S., Fasel, J. H., Fritschy, D., & Menetrey, J. (2006). Anatomy of the anterior cruciate ligament. *Knee surgery, sports traumatology, arthroscopy*, *14*, 204-213.

Eiling, E., Bryant, A. L., Petersen, W., Murphy, A., & Hohmann, E. (2007). Effects of menstrual-cycle hormone fluctuations on musculotendinous stiffness and knee joint laxity. *Knee surgery, sports traumatology, arthroscopy*, *15*, 126-132.

Ekås, G. R., Ardern, C. L., Grindem, H., & Engebretsen, L. (2020). Evidence too weak to guide surgical treatment decisions for anterior cruciate ligament injury: a systematic review of the risk of new meniscal tears after anterior cruciate ligament injury. *British journal of sports medicine, 54*(9), 520–527.

Ekenros, L., von Rosen, P., Solli, G. S., Sandbakk, Ø., Holmberg, H. C., Hirschberg, A. L., & Fridén, C. (2022). Perceived impact of the menstrual cycle and hormonal contraceptives on physical exercise and performance in 1,086 athletes from 57 sports. *Frontiers in physiology*, *13*, 954760.

Elliott-Sale, K. J., McNulty, K. L., Ansdell, P., Goodall, S., Hicks, K. M., Thomas, K., Swinton, P. A., & Dolan, E. (2020). The Effects of Oral Contraceptives on Exercise Performance in Women: A Systematic Review and Meta-analysis. *Sports medicine (Auckland, N.Z.), 50*(10), 1785–1812.

Elliott-Sale, K. J., Minahan, C. L., de Jonge, X. A. K. J., Ackerman, K. E., Sipilä, S., Constantini, N. W., Lebrun, C. M., & Hackney, A. C. (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 medicine (Auckland, N.Z.)*, *51*(5), 843–861.

Ely, I. A., Jones, E. J., Inns, T. B., Dooley, S., Miller, S. B. J., Stashuk, D. W., Atherton, P. J., Phillips, B. E., & Piasecki, M. (2022). Training-induced improvements in knee extensor force accuracy are associated with reduced vastus lateralis motor unit firing variability. *Experimental physiology, 107*(9), 1061–1070.

Engebretsen, L., Soligard, T., Steffen, K., Alonso, J. M., Aubry, M., Budgett, R., Dvorak, J., Jegathesan, M., Meeuwisse, W. H., Mountjoy, M., Palmer-Green, D., Vanhegan, I., & Renström, P. A. (2013). Sports injuries and illnesses during the London Summer Olympic Games 2012. *British journal of sports medicine*, *47*(7), 407–414.

Enoka R. M. (1995). Morphological features and activation patterns of motor units. *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society, 12*(6), 538–559.

Enoka, R. M., & Duchateau, J. (2017). Rate Coding and the Control of Muscle Force. *Cold Spring Harbor perspectives in medicine, 7*(10), a029702.

Enoka, R. M., & Farina, D. (2021). Force Steadiness: From Motor Units to Voluntary Actions. *Physiology, 36*(2), 114–130.

Enoka, R. M., Christou, E. A., Hunter, S. K., Kornatz, K. W., Semmler, J. G., Taylor, A. M., & Tracy, B. L. (2003). Mechanisms that contribute to differences in motor performance between young and old adults. *Journal of*

electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology, 13(1), 1–12.

Falciglia, F., Guzzanti, V., Ciommo, V. D., & Poggiaroni, A. (2009). Physiological knee laxity during pubertal growth. *Bulletin of the NYU hospital for joint diseases, 67*(4).

Farina, D., Cescon, C., & Merletti, R. (2002). Influence of anatomical, physical, and detection-system parameters on surface EMG. *Biological cybernetics*, *86*(6), 445–456.

Farina, D., Madeleine, P., Graven-Nielsen, T., Merletti, R., & Arendt-Nielsen, L. (2002). Standardising surface electromyogram recordings for assessment of activity and fatigue in the human upper trapezius muscle. *European journal of applied physiology*, *86*(6), 469–478.

Feeney, D. F., Mani, D., & Enoka, R. M. (2018). Variability in common synaptic input to motor neurons modulates both force steadiness and pegboard time in young and older adults. *The Journal of physiology*, *596*(16), 3793–3806. https://doi.org/10.1113/JP275658

Fehring, R. J., Schneider, M., & Raviele, K. (2006). Variability in the phases of the menstrual cycle. *Journal of obstetric, gynecologic, and neonatal nursing : JOGNN, 35*(3), 376–384.

Felici, F., & Del Vecchio, A. (2020). Surface Electromyography: What Limits Its Use in Exercise and Sport Physiology?. *Frontiers in neurology*, *11*, 578504.

Fernandez-Castilla B., Declercq L., Jamshidi L., Beretvas N., Onghena P., & Van den Noortgate W. (2019). Detecting selection bias in meta-analyses with multiple outcomes: a simulation study. *The Journal of Experimental Education, 89*(1), 125–144.

Findlay, R. J., Macrae, E. H. R., Whyte, I. Y., Easton, C., & Forrest Née Whyte, L. J. (2020). How the menstrual cycle and menstruation affect sporting performance: experiences and perceptions of elite female rugby players. *British journal of sports medicine*, *54*(18), 1108–1113.

Fisher, R., McLellan, C., Sinclair, W., & Minahan, C. (2015). Oral contraception and the menstrual cycle in exercise science and sports medicine research–Should it be considered?: The role of physical activity in oral contraceptive use in Australian women. *Journal of Science and Medicine in Sport*, *19*, e31.

Fu, R., Gartlehner, G., Grant, M., Shamliyan, T., Sedrakyan, A., Wilt, T. J., Griffith, L., Oremus, M., Raina, P., Ismaila, A., Santaguida, P., Lau, J., & Trikalinos, T. A. (2011). Conducting quantitative synthesis when comparing medical interventions: AHRQ and the Effective Health Care Program. *Journal of clinical epidemiology, 64*(11), 1187–1197.

Fuglevand, A. J., Winter, D. A., & Patla, A. E. (1993). Models of recruitment and rate coding organization in motor-unit pools. *Journal of neurophysiology*, *70*(6), 2470–2488.

Gagnat, Y., Brændvik, S. M., & Roeleveld, K. (2020). Surface Electromyography Normalization Affects the Interpretation of Muscle Activity and Coactivation in Children With Cerebral Palsy During Walking. *Frontiers in neurology*, *11*, 202.

Galganski, M. E., Fuglevand, A. J., & Enoka, R. M. (1993). Reduced control of motor output in a human hand muscle of elderly subjects during submaximal contractions. *Journal of neurophysiology, 69*(6), 2108–2115.

Gardner, E. J., Noyes, F. R., Jetter, A. W., Grood, E. S., Harms, S. P., & Levy, M. S. (2015). Effect of anteromedial and posterolateral anterior cruciate ligament bundles on resisting medial and lateral tibiofemoral compartment subluxations. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, *31*(5), 901-910.

Giffin, J. R., Vogrin, T. M., Zantop, T., Woo, S. L., & Harner, C. D. (2004). Effects of increasing tibial slope on the biomechanics of the knee. *The American journal of sports medicine*, *32*(2), 376-382.

Gilmer, G., Crasta, N., & Tanaka, M. J. (2025). The Effect of Sex Hormones on Joint Ligament Properties: A Systematic Review and Meta-analysis. *The American journal of sports medicine*, 3635465241310145. Advance online publication.

Girgis, F. G., Marshall, J. L., & Monajem, A. (1975). The cruciate ligaments of the knee joint. Anatomical, functional and experimental analysis. *Clinical orthopaedics and related research*, (106), 216–231.

Gokeler, A., Dingenen, B., Mouton, C., & Seil, R. (2017). Clinical course and recommendations for patients after anterior cruciate ligament injury and subsequent reconstruction: A narrative review. *EFORT open reviews, 2*(10), 410–420.

Griffin, L. Y., Agel, J., Albohm, M. J., Arendt, E. A., Dick, R. W., Garrett, W. E., Garrick, J. G., Hewett, T. E., Huston, L., Ireland, M. L., Johnson, R. J., Kibler, W. B., Lephart, S., Lewis, J. L., Lindenfeld, T. N., Mandelbaum, B. R., Marchak, P., Teitz, C. C., & Wojtys, E. M. (2000). Noncontact anterior cruciate ligament injuries: risk factors and prevention strategies. *The Journal of the American Academy of Orthopaedic Surgeons, 8*(3), 141–150.

Griffin, L. Y., Albohm, M. J., Arendt, E. A., Bahr, R., Beynnon, B. D., Demaio, M., Dick, R. W., Engebretsen, L., Garrett, W. E., Jr, Hannafin, J. A., Hewett, T. E., Huston, L. J., Ireland, M. L., Johnson, R. J., Lephart, S., Mandelbaum, B. R., Mann, B. J., Marks, P. H., Marshall, S. W., Myklebust, G., … Yu, B. (2006). Understanding and preventing noncontact anterior cruciate ligament injuries: a review of the Hunt Valley II meeting, January 2005. *The American journal of sports medicine, 34*(9), 1512–1532.

Grindstaff, T. L., Hammill, R. R., Tuzson, A. E., & Hertel, J. (2006). Neuromuscular control training programs and noncontact anterior cruciate ligament injury rates in female athletes: a numbers-needed-to-treat analysis. *Journal of athletic training, 41*(4), 450–456.

Grindstaff, T. L., Jackson, K. R., Garrison, J. C., Diduch, D. R., & Ingersoll, C. D. (2008). Decreased quadriceps activation measured hours prior to a noncontact

anterior cruciate ligament tear. *The Journal of orthopaedic and sports physical therapy*, *38*(8), 508–516.

Guo, Y., Jones, E. J., Inns, T. B., Ely, I. A., Stashuk, D. W., Wilkinson, D. J., Smith, K., Piasecki, J., Phillips, B. E., Atherton, P. J., & Piasecki, M. (2022). Neuromuscular recruitment strategies of the vastus lateralis according to sex. *Acta physiologica (Oxford, England)*, *235*(2), e13803.

Guo, Y., Jones, E. J., Smart, T. F., Altheyab, A., Gamage, N., Stashuk, D. W., Piasecki, J., Phillips, B. E., Atherton, P. J., & Piasecki, M. (2024). Sex disparities of human neuromuscular decline in older humans. *The Journal of physiology*, 10.1113/JP285653. Advance online publication.

Guyatt, G., Oxman, A. D., Akl, E. A., Kunz, R., Vist, G., Brozek, J., Norris, S., Falck-Ytter, Y., Glasziou, P., DeBeer, H., Jaeschke, R., Rind, D., Meerpohl, J., Dahm, P., & Schünemann, H. J. (2011). GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *Journal of clinical epidemiology, 64*(4), 383–394.

Hägglund, M., Atroshi, I., Wagner, P., & Waldén, M. (2013). Superior compliance with a neuromuscular training programme is associated with fewer ACL injuries and fewer acute knee injuries in female adolescent football players: secondary analysis of an RCT. *British journal of sports medicine, 47*(15), 974–979.

Hägglund, M., Waldén, M., & Ekstrand, J. (2009). Injuries among male and female elite football players. *Scandinavian journal of medicine & science in sports*, *19*(6), 819-827.

Hamilton, L. D., Thomas, E., Almuklass, A. M., & Enoka, R. M. (2017). A framework for identifying the adaptations responsible for differences in pegboard times between middle-aged and older adults. *Experimental gerontology, 97*, 9–16.

Hamlet, W. P., Liu, S. H., Panossian, V., & Finerman, G. A. (1997). Primary immunolocalization of androgen target cells in the human anterior cruciate ligament. *Journal of orthopaedic research*, *15*(5), 657-663.

Harrison, S., Clark, N. C., Ansdell, P., & Pethick, J. (2023). Sex differences in knee extensor torque control. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, *72*, 102806.

Hart, D. A., & Achari, Y. (2010). Alterations to cell metabolism in connective tissues of the knee after ovariohysterectomy in a rabbit model: are there implications for the postmenopausal athlete?. *British journal of sports medicine*, *44*(12), 867-871.

Hart, J. M., Pietrosimone, B., Hertel, J., & Ingersoll, C. D. (2010). Quadriceps activation following knee injuries: a systematic review. *Journal of athletic training, 45*(1), 87–97.

Hayward, E., Akam, L., Hunter, D., & Mastana, S. (2024). Role of the Menstrual Cycle on Performance and Injury Risk: A Survey of Female Professional Rugby Players in the United Kingdom. *International journal of environmental research and public health, 21*(2), 150.

Hecht, S. S., & Arendt, E. (2014). Training the female athlete. *Handbook of sports medicine and science: The female athlete*, 1-8.

Heikura, I. A., Uusitalo, A. L., Stellingwerff, T., Bergland, D., Mero, A. A., & Burke, L. M. (2018). Low energy availability is difficult to assess but outcomes have large impact on bone injury rates in elite distance athletes. *International journal of sport nutrition and exercise metabolism*, *28*(4), 403-411.

Heitz, N. A., Eisenman, P. A., Beck, C. L., & Walker, J. A. (1999). Hormonal changes throughout the menstrual cycle and increased anterior cruciate ligament laxity in females. *Journal of athletic training*, *34*(2), 144.

Hertel, J., Williams, N. I., Olmsted-Kramer, L. C., Leidy, H. J., & Putukian, M. (2006). Neuromuscular performance and knee laxity do not change across the menstrual cycle in female athletes. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA*, *14*(9), 817–822.

Herzberg, S. D., Motu'apuaka, M. L., Lambert, W., Fu, R., Brady, J., & Guise, J. M. (2017). The effect of menstrual cycle and contraceptives on ACL injuries and laxity: a systematic review and meta-analysis. *Orthopaedic journal of sports medicine*, *5*(7), 2325967117718781.

Hewett, T. E., Myer, G. D., & Ford, K. R. (2004). Decrease in neuromuscular control about the knee with maturation in female athletes. *The Journal of bone and joint surgery. American volume*, *86*(8), 1601–1608.

Hewett, T. E., Myer, G. D., Ford, K. R., Heidt, R. S., Jr, Colosimo, A. J., McLean, S. G., van den Bogert, A. J., Paterno, M. V., & Succop, P. (2005). Biomechanical measures of neuromuscular control and valgus loading of the knee predict anterior cruciate ligament injury risk in female athletes: a prospective study. *The American journal of sports medicine*, *33*(4), 492–501.

Hewett, T. E., Myer, G. D., Ford, K. R., Paterno, M. V., & Quatman, C. E. (2016). Mechanisms, prediction, and prevention of ACL injuries: Cut risk with three sharpened and validated tools. *Journal of Orthopaedic Research*, *34*(11), 1843-1855.

Hicks-Little, C. A., Thatcher, J. R., Hauth, J. M., Goldfuss, A. J., & Cordova, M. L. (2007). Menstrual cycle stage and oral contraceptive effects on anterior tibial displacement in collegiate female athletes. *Journal of sports medicine and physical fitness*, *47*(2), 255.

Higgins, J. P., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ (Clinical research ed.)*, *327*(7414), 557–560.

Highgenboten, C. L., Jackson, A. W., Jansson, K. A., & Meske, N. B. (1992). KT-1000 arthrometer: conscious and unconscious test results using 15, 20, and 30 pounds of force. *The American journal of sports medicine*, *20*(4), 450-454.

Hollman, J. H., Nagai, T., Bates, N. A., McPherson, A. L., & Schilaty, N. D. (2021). Diminished neuromuscular system adaptability following anterior cruciate

ligament injury: Examination of knee muscle force variability and complexity. *Clinical biomechanics (Bristol, Avon), 90*, 105513.

Hong, I. S., Pierpoint, L. A., Hellwinkel, J. E., Berk, A. N., Salandra, J. M., Meade, J. D., Piasecki, D. P., Fleischli, J. E., Ahmad, C. S., Trofa, D. P., & Saltzman, B. M. (2023). Clinical Outcomes After ACL Reconstruction in Soccer (Football, Futbol) Players: A Systematic Review and Meta-Analysis. *Sports health*, *15*(6), 788–804.

Hootman, J. M., Dick, R., & Agel, J. (2007). Epidemiology of collegiate injuries for 15 sports: summary and recommendations for injury prevention initiatives. *Journal of athletic training, 42*(2), 311–319.

Houghton, E.J., Pieper, L.P., & Smith, M.M. (2022). *Women in the 2022 Olympic and Paralympic Games: An Analysis of Participation, Leadership, and Media Coverage*. Women's Sports Foundation.

Howe, D., Cone, S. G., Piedrahita, J. A., Collins, B., Fordham, L. A., Griffith, E. H., Spang, J. T., & Fisher, M. B. (2022). Sex-specific biomechanics and morphology of the anterior cruciate ligament during skeletal growth in a porcine model. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*, *40*(8), 1853–1864.

Huston, L. J., & Wojtys, E. M. (1996). Neuromuscular performance characteristics in elite female athletes. *The American journal of sports medicine, 24*(4), 427–436.

Ihalainen, J. K., Takalo, S., Mjøsund, K., Solli, G. S., Valtonen, M., Kokkonen, M., Hackney, A. C., & Mikkonen, R. S. (2024). Self-Reported Performance and Hormonal-Cycle-Related Symptoms in Competitive Female Athletes. *Women in Sport and Physical Activity Journal*, *32*(1), wspaj.2023-0102.

Inglis, J. G., & Gabriel, D. A. (2021). Sex differences in the modulation of the motor unit discharge rate leads to reduced force steadiness. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme, 46*(9), 1065–1072.

International Olympic Committee Injury and Illness Epidemiology Consensus Group, Bahr, R., Clarsen, B., Derman, W., Dvorak, J., Emery, C. A., ... & Chamari, K. (2020). International Olympic Committee consensus statement: methods for recording and reporting of epidemiological data on injury and illness in sports 2020 (including the STROBE extension for sports injury and illness surveillance (STROBE-SIIS)). *Orthopaedic journal of sports medicine*, *8*(2), 2325967120902908.

International Olympic Committee. (2024, August 17). *Factsheet women in the olympic movement.* In: *Gender equality through time: at the olympic Games*. https://stillmed.olympics.com/media/Documents/Olympic-Movement/Factsheets/Women-in-the-Olympic-Movement.pdf

International Paralympic Committee. (2024, September 21). *Paris 2024: Record number of delegations and females to compete*. https://www.paralympic.org/paris-2024/news/paris-2024-record-number-delegations-and-females-compete

Jakobi, J. M., Haynes, E. M. K., & Smart, R. R. (2018). Is there sufficient evidence to explain the cause of sexually dimorphic behaviour in force steadiness?. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme, 43*(11), 1207–1214.

Janse de Jonge, X. A., Boot, C. R., Thom, J. M., Ruell, P. A., & Thompson, M. W. (2001). The influence of menstrual cycle phase on skeletal muscle contractile characteristics in humans. *The Journal of physiology*, *530*(Pt 1), 161–166.

Janse de Jonge, X. A., Thompson, B., & Han, A. (2019). Methodological Recommendations for Menstrual Cycle Research in Sports and Exercise. *Medicine and science in sports and exercise, 51*(12), 2610–2617.

Jenny, J. Y., Arndt, J., & Computer Assisted Orthopaedic Surgery-France (2013). Anterior knee laxity measurement using stress radiographs and the GNRB(®) system versus intraoperative navigation. *Orthopaedics & traumatology, surgery & research : OTSR, 99*(6 Suppl), S297–S300.

Jenz, S. T., Beauchamp, J. A., Gomes, M. M., Negro, F., Heckman, C. J., & Pearcey, G. E. P. (2023). Estimates of persistent inward currents in lower limb motoneurons are larger in females than in males. *Journal of neurophysiology*, *129*(6), 1322–1333.

Johansson, H., Sjölander, P., & Sojka, P. (1991). A sensory role for the cruciate ligaments. *Clinical orthopaedics and related research*, (268), 161–178.

Jones, B. P., L'Heveder, A., Bishop, C., Kasaven, L., Saso, S., Davies, S., Chakraverty, R., Brown, J., & Pollock, N. (2024). Menstrual cycles and the impact upon performance in elite British track and field athletes: a longitudinal study. *Frontiers in sports and active living*, *6*, 1296189.

Jones, K. E., Hamilton, A. F., & Wolpert, D. M. (2002). Sources of signal-dependent noise during isometric force production. *Journal of neurophysiology, 88*(3), 1533–1544.

Kagan, H. M., & Li, W. (2003). Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. *Journal of cellular biochemistry, 88*(4), 660–672.

Karageanes, S. J., Blackburn, K., & Vangelos, Z. A. (2000). The association of the menstrual cycle with the laxity of the anterior cruciate ligament in adolescent female athletes. *Clinical Journal of Sport Medicine*, *10*(3), 162-168.

Kern, D. S., Semmler, J. G., & Enoka, R. M. (2001). Long-term activity in upper- and lower-limb muscles of humans. *Journal of applied physiology (Bethesda, Md. : 1985)*, *91*(5), 2224–2232.

Khowailed, I. A., Petrofsky, J., Lohman, E., Daher, N., & Mohamed, O. (2015). 17β-estradiol induced effects on anterior cruciate ligament laxness and neuromuscular activation patterns in female runners. *Journal of Women's Health*, *24*(8), 670-680.

Kiani, A., Hellquist, E., Ahlqvist, K., Gedeborg, R., Michaëlsson, K., & Byberg, L. (2010). Prevention of soccer-related knee injuries in teenaged girls. *Archives of internal medicine, 170*(1), 43–49.

Kim, S., & Joo, K. W. (2007). Electrolyte and Acid-base disturbances associated with non-steroidal anti-inflammatory drugs. *Electrolyte & blood pressure: E & BP, 5*(2), 116–125.

Klasan, A., Putnis, S. E., Kandhari, V., Oshima, T., Fritsch, B. A., & Parker, D. A. (2020). Healthy knee KT1000 measurements of anterior tibial translation have significant variation. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA, 28*(7), 2177–2183.

Klouche, S., Lefevre, N., Cascua, S., Herman, S., Gerometta, A., & Bohu, Y. (2015). Diagnostic value of the GNRB ® in relation to pressure load for complete ACL tears: A prospective case-control study of 118 subjects. *Orthopaedics & traumatology, surgery & research : OTSR, 101*(3), 297–300.

Knol, H., Huys, R., Temprado, J. J., & Sleimen-Malkoun, R. (2019). Performance, complexity and dynamics of force maintenance and modulation in young and older adults. *PloS one*, *14*(12), e0225925.

Kobayashi, H., Koyama, Y., Enoka, R. M., & Suzuki, S. (2014). A unique form of light-load training improves steadiness and performance on some functional tasks in older adults. *Scandinavian journal of medicine & science in sports, 24*(1), 98–110.

Konopka, J. A., DeBaun, M. R., Chang, W., & Dragoo, J. L. (2016). The Intracellular Effect of Relaxin on Female Anterior Cruciate Ligament Cells. *The American journal of sports medicine, 44*(9), 2384–2392.

Konopka, J. A., Hsue, L. J., & Dragoo, J. L. (2019). Effect of oral contraceptives on soft tissue injury risk, soft tissue laxity, and muscle strength: a systematic review of the literature. *Orthopaedic journal of sports medicine*, *7*(3), 2325967119831061.

Kouzaki, M., & Shinohara, M. (2010). Steadiness in plantar flexor muscles and its relation to postural sway in young and elderly adults. *Muscle & nerve, 42*(1), 78–87.

Kramer, L. C., Denegar, C. R., Buckley, W. E., & Hertel, J. (2007). Factors associated with anterior cruciate ligament injury: history in female athletes. *The Journal of sports medicine and physical fitness*, *47*(4), 446–454.

Krishnan, C., Allen, E. J., & Williams, G. N. (2011). Effect of knee position on quadriceps muscle force steadiness and activation strategies. *Muscle & nerve*, *43*(4), 563–573.

Kruschke, J. K., & Liddell, T. M. (2018). The Bayesian New Statistics: Hypothesis testing, estimation, meta-analysis, and power analysis from a Bayesian perspective. *Psychonomic bulletin & review, 25*(1), 178–206.

Kubo, K., Miyamoto, M., Tanaka, S., Maki, A., Tsunoda, N., & Kanehisa, H. (2009). Muscle and tendon properties during menstrual cycle. *International journal of sports medicine*, *30*(2), 139–143.

Lakens D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in psychology, 4*, 863.

Landram, M. J., & Halligan, M. K. (2020). Continuous moderate intensity versus discontinuous high intensity treadmill running on anterior cruciate ligament laxity and hamstrings flexibility in eumenorrheic women. *The Journal of the Canadian Chiropractic Association*, *64*(3), 227–236.

Larruskain, J., Lekue, J. A., Diaz, N., Odriozola, A., & Gil, S. M. (2018). A comparison of injuries in elite male and female football players: A five-season prospective study. *Scandinavian journal of medicine & science in sports, 28*(1), 237-245.

Lebrun, C. M. (1994). The effect of the phase of the menstrual cycle and the birth control pill on athletic performance. *Clinics in sports medicine*, *13*(2), 419-441.

Lee, C. A., Lee-Barthel, A., Marquino, L., Sandoval, N., Marcotte, G. R., & Baar, K. (2015). Estrogen inhibits lysyl oxidase and decreases mechanical function in

engineered ligaments. *Journal of applied physiology (Bethesda, Md.: 1985), 118*(10), 1250–1257.

Lee, C. Y., Smith, C. L., Zhang, X., Hsu, H. C., Wang, D. Y., & Luo, Z. P. (2004). Tensile forces attenuate estrogen-stimulated collagen synthesis in the ACL. *Biochemical and biophysical research communications*, *317*(4), 1221-1225.

Lee, H., Petrofsky, J. S., Daher, N., Berk, L., & Laymon, M. (2014). Differences in anterior cruciate ligament elasticity and force for knee flexion in women: oral contraceptive users versus non-oral contraceptive users. *European journal of applied physiology*, *114*, 285-294.

Lee, H., Petrofsky, J. S., Daher, N., Berk, L., Laymon, M., & Khowailed, I. A. (2013). Anterior cruciate ligament elasticity and force for flexion during the menstrual cycle. *Medical science monitor: international medical journal of experimental and clinical research, 19*, 1080–1088.

Lefevre, N., Bohu, Y., Klouche, S., Lecocq, J., & Herman, S. (2013). Anterior cruciate ligament tear during the menstrual cycle in female recreational skiers. *Orthopaedics & Traumatology: Surgery & Research*, *99*(5), 571-575.

Liddell, E. G. T., & Sherrington, C. S. (1925). Recruitment and some other features of reflex inhibition. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character, 97*(686), 488–518.

Lipps, D. B., Oh, Y. K., Ashton-Miller, J. A., & Wojtys, E. M. (2012). Morphologic characteristics help explain the gender difference in peak anterior cruciate ligament strain during a simulated pivot landing. *The American journal of sports medicine*, *40*(1), 32-40.

Liu, K. A., & Mager, N. A. (2016). Women's involvement in clinical trials: historical perspective and future implications. *Pharmacy practice*, *14*(1), 708.

Liu, S. H., Al-Shaikh, R. A., Panossian, V., Finerman, G. A., & Lane, J. M. (1997). Estrogen affects the cellular metabolism of the anterior cruciate ligament: a

potential explanation for female athletic injury. *The American journal of sports medicine, 25*(5), 704-709.

Liu, S. H., al-Shaikh, R., Panossian, V., Yang, R. S., Nelson, S. D., Soleiman, N., Finerman, G. A., & Lane, J. M. (1996). Primary immunolocalization of estrogen and progesterone target cells in the human anterior cruciate ligament. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*, *14*(4), 526–533.

Louboutin, H., Debarge, R., Richou, J., Selmi, T. A., Donell, S. T., Neyret, P., & Dubrana, F. (2009). Osteoarthritis in patients with anterior cruciate ligament rupture: a review of risk factors. *The Knee, 16*(4), 239–244.

Lowe, D. A., Baltgalvis, K. A., & Greising, S. M. (2010). Mechanisms behind estrogen's beneficial effect on muscle strength in females. *Exercise and sport sciences reviews*, *38*(2), 61–67.

Lucarno, S., Zago, M., Buckthorpe, M., Grassi, A., Tosarelli, F., Smith, R., & Della Villa, F. (2021). Systematic Video Analysis of Anterior Cruciate Ligament Injuries in Professional Female Soccer Players. *The American journal of sports medicine, 49*(7), 1794–1802.

Lulic-Kuryllo, T., & Inglis, J. G. (2022). Sex differences in motor unit behaviour: A review. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, *66*, 102689.

MacMahon, C., & Parrington, L. (2017). Not All Athletes Are Equal, But Don't Call Me an Exerciser: Response to Araujo and Scharhag. *Scandinavian journal of medicine & science in sports, 27*(8), 904–906.

MacNutt, M. J., De Souza, M. J., Tomczak, S. E., Homer, J. L., & Sheel, A. W. (2012). Resting and exercise ventilatory chemosensitivity across the menstrual cycle. *Journal of Applied Physiology*, *112*(5), 737-747.

Magdič, M., Dahmane, R. G., & Vauhnik, R. (2023). Intra-rater reliability of the knee arthrometer GNRB® for measuring knee anterior laxity in healthy, active subjects. *Journal of orthopaedics, 39*, 7–10.

Maitland, M. E., Bell, G. D., Mohtadi, N. G., & Herzog, W. (1995). Quantitative analysis of anterior cruciate ligament instability. *Clinical biomechanics (Bristol, Avon), 10*(2), 93–97.

Maniar, N., Schache, A. G., Pizzolato, C., & Opar, D. A. (2020). Muscle contributions to tibiofemoral shear forces and valgus and rotational joint moments during single leg drop landing. *Scandinavian journal of medicine & science in sports*, *30*(9), 1664–1674.

Maniar, N., Verhagen, E., Bryant, A. L., & Opar, D. A. (2022). Trends in Australian knee injury rates: An epidemiological analysis of 228,344 knee injuries over 20 years. *The Lancet regional health. Western Pacific, 21*, 100409.

Markolf, K. L., Graff-Radford, A., & Amstutz, H. C. (1978). In vivo knee stability. A quantitative assessment using an instrumented clinical testing apparatus. *The Journal of bone and joint surgery. American volume*, *60*(5), 664–674.

Markolf, K. L., Kochan, A., & Amstutz, H. C. (1984). Measurement of knee stiffness and laxity in patients with documented absence of the anterior cruciate ligament. *The Journal of bone and joint surgery. American volume, 66*(2), 242–252.

Maron, B. J., Thompson, P. D., Ackerman, M. J., Balady, G., Berger, S., Cohen, D., Dimeff, R., Douglas, P. S., Glover, D. W., Hutter, A. M., Jr, Krauss, M. D., Maron, M. S., Mitten, M. J., Roberts, W. O., Puffer, J. C., & American Heart Association Council on Nutrition, Physical Activity, and Metabolism (2007). Recommendations and considerations related to preparticipation screening for cardiovascular abnormalities in competitive athletes: 2007 update: a scientific statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Foundation. *Circulation, 115*(12), 1643-455.

Martin, D., Sale, C., Cooper, S. B., & Elliott-Sale, K. J. (2018). Period prevalence and perceived side effects of hormonal contraceptive use and the menstrual cycle in elite athletes. *International journal of sports physiology and performance*, *13*(7), 926-932.

Martin, D., Timmins, K., Cowie, C., Alty, J., Mehta, R., Tang, A., & Varley, I. (2021). Injury Incidence Across the Menstrual Cycle in International Footballers. *Frontiers in sports and active living*, *3*, 616999.

Martínez-Fortuny, N., Alonso-Calvete, A., Da Cuña-Carrera, I., & Abalo-Núñez, R. (2023). Menstrual cycle and sport injuries: a systematic review. *International Journal of Environmental Research and Public Health*, *20*(4), 3264.

Maruyama, S., Yamazaki, T., Sato, Y., Suzuki, Y., Shimizu, S., Ikezu, M., Kaneko, F., Matsuzawa, K., Hirabayashi, R., & Edama, M. (2021). Relationship Between Anterior Knee Laxity and General Joint Laxity During the Menstrual Cycle. *Orthopaedic journal of sports medicine*, *9*(3), 2325967121993045.

Matsumoto, H., Suda, Y., Otani, T., Niki, Y., Seedhom, B. B., & Fujikawa, K. (2001). Roles of the anterior cruciate ligament and the medial collateral ligament in preventing valgus instability. *Journal of orthopaedic science*, *6*(1), 28-32.

McGowan, J., Sampson, M., Salzwedel, D. M., Cogo, E., Foerster, V., & Lefebvre, C. (2016). PRESS Peer Review of Electronic Search Strategies: 2015 Guideline Statement. *Journal of clinical epidemiology, 75*, 40–46.

McKay, A. K. A., Stellingwerff, T., Smith, E. S., Martin, D. T., Mujika, I., Goosey-Tolfrey, V. L., Sheppard, J., & Burke, L. M. (2022). Defining Training and Performance Caliber: A Participant Classification Framework. *International journal of sports physiology and performance, 17*(2), 317–331.

McLean, S. G., Lucey, S. M., Rohrer, S., & Brandon, C. (2010). Knee joint anatomy predicts high-risk in vivo dynamic landing knee biomechanics. *Clinical biomechanics*, *25*(8), 781-788.

McLean, S. G., Oh, Y. K., Palmer, M. L., Lucey, S. M., Lucarelli, D. G., Ashton-Miller, J. A., & Wojtys, E. M. (2011). The relationship between anterior tibial acceleration, tibial slope, and ACL strain during a simulated jump landing task. *The Journal of bone and joint surgery. American volume*, *93*(14), 1310.

McNamara, A., Harris, R., & Minahan, C. (2022). 'That time of the month' … for the biggest event of your career! Perception of menstrual cycle on performance of

Australian athletes training for the 2020 Olympic and Paralympic Games. *BMJ open sport & exercise medicine*, *8*(2), e001300.

McNeil, C. J., Doherty, T. J., Stashuk, D. W., & Rice, C. L. (2005). Motor unit number estimates in the tibialis anterior muscle of young, old, and very old men. *Muscle & nerve, 31*(4), 461–467.

McNulty, K. L., Elliott-Sale, K. J., Dolan, E., Swinton, P. A., Ansdell, P., Goodall, S., Thomas, K., & Hicks, K. M. (2020). The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis. *Sports medicine (Auckland, N.Z.)*, *50*(10), 1813–1827.

Mear, E., Gladwell, V., & Pethick, J. (2023). Knee extensor force control as a predictor of dynamic balance in healthy adults. *Gait & posture, 100*, 230–235.

Meeuwisse, W. H. (1994). Assessing causation in sport injury: a multifactorial model. *Clinical Journal of Sport Medicine*, *4*(3), 166-170.

Meeuwisse, W. H., Tyreman, H., Hagel, B., & Emery, C. (2007). A dynamic model of etiology in sport injury: the recursive nature of risk and causation. *Clinical journal of sport medicine*, *17*(3), 215-219.

Melmed, S., Koenig, R. J., Clifford, J., Auchus, R. J., Goldfine, A.B. (2019). *Williams Textbook of Endocrinology* (14th ed.). Elsevier.

Mihm, M., Gangooly, S., & Muttukrishna, S. (2011). The normal menstrual cycle in women. *Animal reproduction science*, *124*(3-4), 229-236.

Milner-Brown, H. S., & Stein, R. B. (1975). The relation between the surface electromyogram and muscular force. *The Journal of physiology*, *246*(3), 549–569.

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L. A., & PRISMA-P Group (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic reviews, 4*(1), 1.

Möller-Nielsen, J., & Hammar, M. (1989). Women's soccer injuries in relation to the menstrual cycle and oral contraceptive use. *Medicine and science in sports and exercise*, *21*(2), 126-129.

Montalvo, A. M., Schneider, D. K., Webster, K. E., Yut, L., Galloway, M. T., Heidt Jr, R. S., ... & Myer, G. D. (2019). Anterior cruciate ligament injury risk in sport: a systematic review and meta-analysis of injury incidence by sex and sport classification. *Journal of athletic training*, *54*(5), 472-482.

Montgomery, M. M., & Shultz, S. J. (2010). Isometric knee-extension and knee-flexion torque production during early follicular and postovulatory phases in recreationally active women. *Journal of athletic training*, *45*(6), 586–593.

Moriceau, J., Fevre, A., Domínguez-Balmaseda, D., González-de-la-Flor, Á., Simón-Areces, J., & García-Pérez-de-Sevilla, G. (2022). The Influence of the Menstrual Cycle and Oral Contraceptives on Knee Laxity or Anterior Cruciate Ligament Injury Risk: A Systematic Review. *Applied Sciences*, *12*(24), 12627.

Morris, S. B. (2008). Estimating Effect Sizes From Pretest-Posttest-Control Group Designs. *Organizational Research Methods, 11*(2), 364–386.

Mouarbes, D., Cavaignac, E., Chiron, P., Bérard, E., & Murgier, J. (2018). Evaluation of reproducibility of robotic knee testing device (GNRB) on 60 healthy knees. *Journal of orthopaedics, 15*(1), 94–98.

Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., Meyer, N., Sherman, R., Steffen, K., Budgett, R., & Ljungqvist, A. (2014). The IOC consensus statement: beyond the Female Athlete Triad—Relative Energy Deficiency in Sport (RED-S). *British journal of sports medicine*, *48*(7), 491–497.

Mouton, C., Theisen, D., & Seil, R. (2016). Objective measurements of static anterior and rotational knee laxity. *Current reviews in musculoskeletal medicine, 9*(2), 139–147.

Mouton, C., Theisen, D., Meyer, T., Agostinis, H., Nührenbörger, C., Pape, D., & Seil, R. (2015). Noninjured Knees of Patients With Noncontact ACL Injuries

Display Higher Average Anterior and Internal Rotational Knee Laxity Compared With Healthy Knees of a Noninjured Population. The American journal of sports medicine, 43(8), 1918–1923.

Muneta, T., Takakuda, K., & Yamamoto, H. (1997). Intercondylar notch width and its relation to the configuration and cross-sectional area of the anterior cruciate ligament. A cadaveric knee study. *The American journal of sports medicine, 25*(1), 69–72.

Murphy, D. F., Connolly, D. A. J., & Beynnon, B. D. (2003). Risk factors for lower extremity injury: a review of the literature. *British journal of sports medicine*, *37*(1), 13-29.

Myer, G. D., Ford, K. R., Paterno, M. V., Nick, T. G., & Hewett, T. E. (2008). The effects of generalized joint laxity on risk of anterior cruciate ligament injury in young female athletes. *The American journal of sports medicine*, *36*(6), 1073-1080.

Myklebust, G., Maehlum, S., Holm, I., & Bahr, R. (1998). A prospective cohort study of anterior cruciate ligament injuries in elite Norwegian team handball. *Scandinavian journal of medicine & science in sports*, *8*(3), 149-153.

Nédélec, E., Foli, E., Shultz, S. J., Swinton, P. A., Dolan, E., Enright, K., Piasecki, J., Matthews, J. J., Sale, C., & Elliott-Sale, K. J. (2021). Effect of menstrual cycle phase, menstrual irregularities and hormonal contraceptive use on anterior knee laxity and non-contact anterior cruciate ligament injury occurrence in women: a protocol for a systematic review and meta-analysis. *BMJ open sport & exercise medicine*, *7*(4), e001170.

Neuman, P., Kostogiannis, I., Fridén, T., Roos, H., Dahlberg, L. E., & Englund, M. (2012). Knee laxity after complete anterior cruciate ligament tear: a prospective study over 15 years. *Scandinavian journal of medicine & science in sports, 22*(2), 156–163.

Nichols, J. F., Rauh, M. J., Barrack, M. T., Barkai, H. S., & Pernick, Y. (2007). Disordered eating and menstrual irregularity in high school athletes in lean-build and nonlean-build sports. *International journal of sport nutrition and exercise metabolism, 17*(4), 364–377.

Nilstad, A., Andersen, T. E., Bahr, R., Holme, I., & Steffen, K. (2014). Risk factors for lower extremity injuries in elite female soccer players. *The American journal of sports medicine*, *42*(4), 940-948.

Nouveau, S., Robert, H., & Viel T. (2017). ACL Grafts Compliance During Time: Influence of Early Sollicitations on the Final Stiffness of the Graft after Surgery. *Journal of Orthopedic Research & Physiotherapy 3*: 035.

Noyes, F. R., & Barber-Westin, S. (2018). The ACL: Anatomy, biomechanics, mechanisms of injury, and the gender disparity. *ACL injuries in the female athlete: Causes, impacts, and conditioning programs*, 3-32.

Olsen, O. E., Myklebust, G., Engebretsen, L., & Bahr, R. (2004). Injury mechanisms for anterior cruciate ligament injuries in team handball: a systematic video analysis. *The American journal of sports medicine, 32*(4), 1002–1012. https://doi.org/10.1177/0363546503261724

Otsuki, R., Benoit, D., Hirose, N., & Fukubayashi, T. (2021). Effects of an Injury Prevention Program on Anterior Cruciate Ligament Injury Risk Factors in Adolescent Females at Different Stages of Maturation. *Journal of sports science & medicine, 20*(2), 365–372.

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., McGuinness, L. A., … Moher, D. (2021a). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ (Clinical research ed.)*, *372*, n71.

Page, M. J., Moher, D., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville,

J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., McGuinness, L. A., … McKenzie, J. E. (2021b). PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ (Clinical research ed.), 372*, n160.

Parekh, A., Fadiran, E. O., Uhl, K., & Throckmorton, D. C. (2011). Adverse effects in women: implications for drug development and regulatory policies. *Expert review of clinical pharmacology*, *4*(4), 453-466.

Park, S. K., Stefanyshyn, D. J., Loitz-Ramage, B., Hart, D. A., & Ronsky, J. L. (2009a). Changing hormone levels during the menstrual cycle affect knee laxity and stiffness in healthy female subjects. *The American journal of sports medicine, 37*(3), 588-598.

Park, S. K., Stefanyshyn, D. J., Ramage, B., Hart, D. A., & Ronsky, J. L. (2009b). Relationship between knee joint laxity and knee joint mechanics during the menstrual cycle. *British journal of sports medicine, 43*(3), 174-179.

Parsons, J. L., Coen, S. E., & Bekker, S. (2021). Anterior cruciate ligament injury: towards a gendered environmental approach. *British journal of sports medicine*, *55*(17), 984-990.

Patterson, B. E., Crossley, K. M., Haberfield, M. J., Mosler, A. B., Cowan, S. M., Lawrence, J., Rath, D., Livingstone, N., Barton, C. J., Bruder, A. M., & Donaldson, A. (2024). Injury prevention for women and girls playing Australian Football: programme cocreation, dissemination and early adopter coach feedback. *BMJ open sport & exercise medicine, 10*(1), e001711.

Pethick, J., & Piasecki, M. (2022). Alterations in Muscle Force Control With Aging: Is There a Modulatory Effect of Lifelong Physical Activity?. *Frontiers in sports and active living*, *4*, 817770.

Pethick, J., Taylor, M. J. D., & Harridge, S. D. R. (2022). Aging and skeletal muscle force control: Current perspectives and future directions. *Scandinavian journal of medicine & science in sports*, *32*(10), 1430–1443.

Pethick, J., Winter, S. L., & Burnley, M. (2015). Fatigue reduces the complexity of knee extensor torque fluctuations during maximal and submaximal intermittent isometric contractions in man. *The Journal of physiology, 593*(8), 2085–2096.

Piasecki, J., Guo, Y., Jones, E. J., Phillips, B. E., Stashuk, D. W., Atherton, P. J., & Piasecki, M. (2023). Menstrual Cycle Associated Alteration of Vastus Lateralis Motor Unit Function. *Sports medicine - open, 9*(1), 97.

Piasecki, J., Inns, T. B., Bass, J. J., Scott, R., Stashuk, D. W., Phillips, B. E., Atherton, P. J., & Piasecki, M. (2021). Influence of sex on the age-related adaptations of neuromuscular function and motor unit properties in elite masters athletes. *The Journal of physiology, 599*(1), 193–205.

Pitchers, G., & Elliott-Sale, K. (2019). Considerations for coaches training female athletes. *Professional Strength & Conditioning*, *55*, 19-30.

Pollard, C. D., Braun, B., & Hamill, J. (2006). Influence of gender, estrogen and exercise on anterior knee laxity. *Clinical Biomechanics, 21*(10), 1060-1066.

Poon, C., Robichaud, J. A., Corcos, D. M., Goldman, J. G., & Vaillancourt, D. E. (2011). Combined measures of movement and force variability distinguish Parkinson's disease from essential tremor. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology, 122*(11), 2268–2275.

Power, G. A., Dalton, B. H., & Rice, C. L. (2013). Human neuromuscular structure and function in old age: A brief review. *Journal of sport and health science*, *2*(4), 215–226.

Prior, J. C., Naess, M., Langhammer, A., & Forsmo, S. (2015). Ovulation Prevalence in Women with Spontaneous Normal-Length Menstrual Cycles - A Population-Based Cohort from HUNT3, Norway. *PloS one*, *10*(8), e0134473.

Rahr-Wagner, L., Thillemann, T. M., Mehnert, F., Pedersen, A. B., & Lind, M. (2014). Is the use of oral contraceptives associated with operatively treated anterior cruciate ligament injury? A case-control study from the Danish Knee Ligament

Reconstruction Registry. *The American journal of sports medicine*, *42*(12), 2897-2905.

Ravi, S., Ihalainen, J. K., Taipale-Mikkonen, R. S., Kujala, U. M., Waller, B., Mierlahti, L., Lehto, J., & Valtonen, M. (2021). Self-Reported Restrictive Eating, Eating Disorders, Menstrual Dysfunction, and Injuries in Athletes Competing at Different Levels and Sports. *Nutrients, 13*(9), 3275.

Rechichi, C., Dawson, B., & Goodman, C. (2008). Oral contraceptive phase has no effect on endurance test. *International journal of sports medicine, 29*(4), 277–281.

Reed, B. G., & Carr, B. R. (2018). *The Normal Menstrual Cycle and the Control of Ovulation*. Endotext.

Renström, P., Ljungqvist, A., Arendt, E., Beynnon, B., Fukubayashi, T., Garrett, W., Georgoulis, T., Hewett, T. E., Johnson, R., Krosshaug, T., Mandelbaum, B., Micheli, L., Myklebust, G., Roos, E., Roos, H., Schamasch, P., Shultz, S., Werner, S., Wojtys, E., & Engebretsen, L. (2008). Non-contact ACL injuries in female athletes: an International Olympic Committee current concepts statement. *British journal of sports medicine, 42*(6), 394–412.

Rethlefsen, M. L., Kirtley, S., Waffenschmidt, S., Ayala, A. P., Moher, D., Page, M. J., Koffel, J. B., & PRISMA-S Group (2021). PRISMA-S: an extension to the PRISMA Statement for Reporting Literature Searches in Systematic Reviews. *Systematic reviews, 10*(1), 39.

Riemann, B. L., & Lephart, S. M. (2002a). The Sensorimotor System, Part II: The Role of Proprioception in Motor Control and Functional Joint Stability. *Journal of athletic training, 37*(1), 80–84.

Riemann, B. L., & Lephart, S. M. (2002b). The sensorimotor system, part I: the physiologic basis of functional joint stability. *Journal of athletic training, 37*(1), 71–79.

Robert, H., Nouveau, S., Gageot, S., & Gagnière, B. (2009). A new knee arthrometer, the GNRB: experience in ACL complete and partial tears. *Orthopaedics & traumatology, surgery & research : OTSR, 95*(3), 171–176.

Rodriguez, L. A., Casey, E., Crossley, E., Williams, N., & Dhaher, Y. Y. (2024). The hormonal profile in women using combined monophasic oral contraceptive pills varies across the pill cycle: a temporal analysis of serum endogenous and exogenous hormones using liquid chromatography with tandem mass spectroscopy. *American journal of physiology. Endocrinology and metabolism*, *327*(1), E121–E133.

Romani, W., Patrie, J., Curl, L. A., & Flaws, J. A. (2003). The correlations between estradiol, estrone, estriol, progesterone, and sex hormone-binding globulin and anterior cruciate ligament stiffness in healthy, active females. *Journal of women's health (2002), 12*(3), 287–298.

Ronca, F., Blodgett, J. M., Bruinvels, G., Lowery, M., Raviraj, M., Sandhar, G., Symeonides, N., Jones, C., Loosemore, M., & Burgess, P. W. (2024). Attentional, anticipatory and spatial cognition fluctuate throughout the menstrual cycle: Potential implications for female sport. *Neuropsychologia*, 108909. Advance online publication.

Rosene, J. M., & Fogarty, T. D. (1999). Anterior tibial translation in collegiate athletes with normal anterior cruciate ligament integrity. *Journal of athletic training, 34*(2), 93–98.

Rowan, S. L., Rygiel, K., Purves-Smith, F. M., Solbak, N. M., Turnbull, D. M., & Hepple, R. T. (2012). Denervation causes fiber atrophy and myosin heavy chain co-expression in senescent skeletal muscle. *PloS one*, *7*(1), e29082.

Rozzi, S. L., Lephart, S. M., Gear, W. S., & Fu, F. H. (1999). Knee joint laxity and neuromuscular characteristics of male and female soccer and basketball players. *The American journal of sports medicine, 27*(3), 312–319.

Ruedl, G., Ploner, P., Linortner, I., Schranz, A., Fink, C., Patterson, C.S., Nachbauer, W., & Burtscher, M. (2011). Interaction of Potential Intrinsic and Extrinsic

Risk Factors in ACL Injured Recreational Female Skiers. *International Journal of sports Medicine, 32*, 618 - 622.

Ruedl, G., Ploner, P., Linortner, I., Schranz, A., Fink, C., Sommersacher, R., Pocecco, E., Nachbauer, W., & Burtscher, M. (2009). Are oral contraceptive use and menstrual cycle phase related to anterior cruciate ligament injury risk in female recreational skiers?. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA*, *17*(9), 1065–1069.

Ryu, S. M., Na, H. D., & Shon, O. J. (2018). Diagnostic Tools for Acute Anterior Cruciate Ligament Injury: GNRB, Lachman Test, and Telos. *Knee surgery & related research, 30*(2), 121–127.

Saravia, A., Cabrera, S., Molina, C. R., Pacheco, L., & Muñoz, G. (2020). Validity of the Genourob arthrometer in the evaluation of total thickness tears of anterior cruciate ligament. *Journal of orthopaedics, 22*, 203–206.

Sarwar, R., Niclos, B. B., & Rutherford, O. M. (1996). Changes in muscle strength, relaxation rate and fatiguability during the human menstrual cycle. *The Journal of physiology*, *493*(Pt 1), 267–272.

Sawilowsky, S.S. (2009). New Effect Size Rules of Thumb. *Journal of Modern Applied Statistical Methods, 8*, 26.

Scerpella, T. A., Stayer, T. J., & Makhuli, B. Z. (2005). Ligamentous laxity and non-contact anterior cruciate ligament tears: a gender-based comparison. *Orthopedics*, *28*(7), 656-660.

Schaumberg, M. A., Emmerton, L. M., Jenkins, D. G., Burton, N. W., Janse de Jonge, X. A., & Skinner, T. L. (2018). Use of oral contraceptives to manipulate menstruation in young, physically active women. *International journal of sports physiology and performance*, *13*(1), 82-87.

Schaumberg, M. A., Jenkins, D. G., de Jonge, X. A. J., Emmerton, L. M., & Skinner, T. L. (2017). Three-step method for menstrual and oral contraceptive cycle verification. *Journal of Science and Medicine in Sport*, *20*(11), 965-969.

Schiebinger, L. (2003). Women's health and clinical trials. *The Journal of clinical investigation*, *112*(7), 973-977.

Schmitz, R. J., & Shultz, S. J. (2013). Anterior knee stiffness changes in laxity "responders" versus "nonresponders" across the menstrual cycle. *Journal of athletic training, 48*(1), 39–46.

Schultz, K. N., von Esenwein, S. A., Hu, M., Bennett, A. L., Kennedy, R. T., Musatov, S., Toran-Allerand, C. D., Kaplitt, M. G., Young, L. J., & Becker, J. B. (2009). Viral vector-mediated overexpression of estrogen receptor-alpha in striatum enhances the estradiol-induced motor activity in female rats and estradiol-modulated GABA release. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(6), 1897–1903.

Seneviratne, A., Attia, E., Williams, R. J., Rodeo, S. A., & Hannafin, J. A. (2004). The effect of estrogen on ovine anterior cruciate ligament fibroblasts: cell proliferation and collagen synthesis. *The American journal of sports medicine, 32*(7), 1613–1618.

Sernert, N., Helmers, J., Kartus, C., Ejerhed, L., & Kartus, J. (2007). Knee-laxity measurements examined by a left-hand- and a right-hand-dominant physiotherapist, in patients with anterior cruciate ligament injuries and healthy controls. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA, 15*(10), 1181–1186.

Seynnes, O., Hue, O. A., Garrandes, F., Colson, S. S., Bernard, P. L., Legros, P., & Fiatarone Singh, M. A. (2005). Force steadiness in the lower extremities as an independent predictor of functional performance in older women. *Journal of aging and physical activity*, *13*(4), 395–408.

Shagawa, M., Maruyama, S., Sekine, C., Yokota, H., Hirabayashi, R., Hirata, A., Yokoyama, M., & Edama, M. (2021). Comparison of anterior knee laxity, stiffness, genu recurvatum, and general joint laxity in the late follicular phase and the ovulatory phase of the menstrual cycle. *BMC musculoskeletal disorders, 22*(1), 886.

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L. A., & PRISMA-P Group (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ (Clinical research ed.)*, *350*, g7647.

Sheridan, M. R., & Flowers, K. A. (1990). Movement variability and bradykinesia in Parkinson's disease. *Brain : a journal of neurology, 113 (Pt 4)*, 1149–1161.

Shultz, S. J., Kirk, S. E., Johnson, M. L., Sander, T. C., & Perrin, D. H. (2004). Relationship between sex hormones and anterior knee laxity across the menstrual cycle. *Medicine and science in sports and exercise*, *36*(7).

Shultz, S. J., Levine, B. J., Nguyen, A. D., Kim, H., Montgomery, M. M., & Perrin, D. H. (2010). A comparison of cyclic variations in anterior knee laxity, genu recurvatum, and general joint laxity across the menstrual cycle. *Journal of orthopaedic research*, *28*(11), 1411-1417.

Shultz, S. J., Morrissey, M. C., & Vauhnik, R. (2024). Anterior knee laxity is greater in athletic females who attain menarche at a younger age. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA, 32*(4), 889–895.

Shultz, S. J., Nguyen, A. D., & Schmitz, R. J. (2008). Differences in lower extremity anatomical and postural characteristics in males and females between maturation groups. *Journal of orthopaedic & sports physical therapy*, *38*(3), 137-149.

Shultz, S. J., Sander, T. C., Kirk, S. E., & Perrin, D. H. (2005). Sex differences in knee joint laxity change across the female menstrual cycle. *The Journal of sports medicine and physical fitness, 45*(4), 594–603.

Shultz, S. J., Schmitz, R. J., & Beynnon, B. D. (2011). Variations in varus/valgus and internal/external rotational knee laxity and stiffness across the menstrual cycle. *Journal of Orthopaedic Research*, *29*(3), 318-325.

Shultz, S. J., Schmitz, R. J., Benjaminse, A., Chaudhari, A. M., Collins, M., & Padua, D. A. (2012a). ACL Research Retreat VI: an update on ACL injury risk and prevention. *Journal of athletic training*, *47*(5), 591–603.

Shultz, S. J., Schmitz, R. J., Benjaminse, A., Collins, M., Ford, K., & Kulas, A. S. (2015). ACL Research retreat VII: an update on anterior cruciate ligament injury risk factor identification, screening, and prevention: March 19–21, 2015; Greensboro, NC. *Journal of athletic training*, *50*(10), 1076-1093.

Shultz, S. J., Schmitz, R. J., Cameron, K. L., Ford, K. R., Grooms, D. R., Lepley, L. K., Myer, G. D., & Pietrosimone, B. (2019). Anterior Cruciate Ligament Research Retreat VIII Summary Statement: An Update on Injury Risk Identification and Prevention Across the Anterior Cruciate Ligament Injury Continuum, March 14-16, 2019, Greensboro, NC. *Journal of athletic training, 54*(9), 970–984.

Shultz, S. J., Schmitz, R. J., Cone, J. R., Copple, T. J., Montgomery, M. M., Pye, M. L., & Tritsch, A. J. (2013). Multiplanar knee laxity increases during a 90-min intermittent exercise protocol. *Med Sci Sports Exerc*, *45*(8), 1553-1561.

Shultz, S. J., Schmitz, R. J., Kulas, A. S., Labban, J. D., & Wang, H. M. (2022). Quadriceps muscle volume positively contributes to ACL volume. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society, 40*(1), 268–276.

Shultz, S. J., Wideman, L., Montgomery, M. M., Beasley, K. N., & Nindl, B. C. (2012b). Changes in serum collagen markers, IGF-I, and Knee joint laxity across the menstrual cycle. *Journal of Orthopaedic Research*, *30*(9), 1405-1412.

Sigward, S. M., Pollard, C. D., & Powers, C. M. (2012). The influence of sex and maturation on landing biomechanics: implications for anterior cruciate ligament injury. *Scandinavian journal of medicine & science in sports*, *22*(4), 502-509.

Simon, D., Mascarenhas, R., Saltzman, B. M., Rollins, M., Bach, B. R., Jr, & MacDonald, P. (2015). The Relationship between Anterior Cruciate Ligament Injury and Osteoarthritis of the Knee. *Advances in orthopedics, 2015*, 928301.

Skelley, N. W., Castile, R. M., Cannon, P. C., Weber, C. I., Brophy, R. H., & Lake, S. P. (2016). Regional variation in the mechanical and microstructural properties of the human anterior cruciate ligament. *The American journal of sports medicine*, *44*(11), 2892-2899.

Skurvydas, A., Masiulis, N., Gudas, R., Dargevičiūtė, G., Parulytė, D., Trumpickas, V., & Kalesinskas, J. R. (2011). Extension and flexion torque variability in ACL deficiency. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA, 19*(8), 1307–1313.

Slauterbeck, J. R., Choquette, R., Tourville, T. W., Krug, M., Mandelbaum, B. R., Vacek, P., & Beynnon, B. D. (2019). Implementation of the FIFA 11+ Injury Prevention Program by High School Athletic Teams Did Not Reduce Lower Extremity Injuries: A Cluster Randomized Controlled Trial. *The American journal of sports medicine, 47*(12), 2844–2852.

Smeets, A., Malfait, B., Dingenen, B., Robinson, M. A., Vanrenterghem, J., Peers, K., Nijs, S., Vereecken, S., Staes, F., & Verschueren, S. (2019). Is knee neuromuscular activity related to anterior cruciate ligament injury risk? A pilot study. *The Knee*, *26*(1), 40–51.

Smith S. S. (1989). Progesterone enhances inhibitory responses of cerebellar Purkinje cells mediated by the GABAA receptor subtype. *Brain research bulletin, 23*(4-5), 317–322.

Smith, K., Miller, N., & Laslovich, S. (2022). The Reliability of the GNRB® Knee Arthrometer in Measuring ACL Stiffness and Laxity: Implications for Clinical Use and Clinical Trial Design. *International journal of sports physical therapy, 17*(6), 1016–1025.

Smith, S. S., Woodward, D. J., & Chapin, J. K. (1989). Sex steroids modulate motor-correlated increases in cerebellar discharge. *Brain research, 476*(2), 307–316.

Soligard, T., Myklebust, G., Steffen, K., Holme, I., Silvers, H., Bizzini, M., Junge, A., Dvorak, J., Bahr, R., & Andersen, T. E. (2008). Comprehensive warm-up

programme to prevent injuries in young female footballers: cluster randomised controlled trial. *BMJ (Clinical research ed.), 337*, a2469.

Soligard, T., Schwellnus, M., Alonso, J. M., Bahr, R., Clarsen, B., Dijkstra, H. P., ... & Engebretsen, L. (2016). How much is too much?(Part 1) International Olympic Committee consensus statement on load in sport and risk of injury. *British journal of sports medicine*, *50*(17), 1030-1041.

Solomonow M. (2006). Sensory-motor control of ligaments and associated neuromuscular disorders. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology, 16*(6), 549–567.

Stoffel-Wagner B. (2001). Neurosteroid metabolism in the human brain. *European journal of endocrinology, 145*(6), 669–679.

Stracciolini, A., Stein, C. J., Zurakowski, D., Meehan III, W. P., Myer, G. D., & Micheli, L. J. (2015). Anterior cruciate ligament injuries in pediatric athletes presenting to sports medicine clinic: a comparison of males and females through growth and development. *Sports health*, *7*(2), 130-136.

Swanik C. B. (2015). Brains and Sprains: The Brain's Role in Noncontact Anterior Cruciate Ligament Injuries. *Journal of athletic training, 50*(10), 1100–1102.

Swanik, C. B., Covassin, T., Stearne, D. J., & Schatz, P. (2007). The relationship between neurocognitive function and noncontact anterior cruciate ligament injuries. *The American journal of sports medicine*, *35*(6), 943–948.

Tayfur, B., Charuphongsa, C., Morrissey, D., & Miller, S. C. (2021). Neuromuscular Function of the Knee Joint Following Knee Injuries: Does It Ever Get Back to Normal? A Systematic Review with Meta-Analyses. *Sports medicine (Auckland, N.Z.), 51*(2), 321–338.

Tenan, M. S., Hackney, A. C., & Griffin, L. (2016). Maximal force and tremor changes across the menstrual cycle. *European journal of applied physiology*, *116*(1), 153–160.

Tenan, M. S., Peng, Y. L., Hackney, A. C., & Griffin, L. (2013). Menstrual cycle mediates vastus medialis and vastus medialis oblique muscle activity. *Medicine and science in sports and exercise*, *45*(11), 2151–2157.

The Football Association. (2020). *The game plan for growth review: participation*. In: *The journey to double participation*. https://www.thefa.com/-/media/thefacom-new/files/womens/2019-20/the-gameplan-for-growth-review---participation.ashx

The UK National Ligament Registry. (2022). *The National Ligament Registry 2022 Annual Report.* https://www.uknlr.co.uk/pdf/annual-report-2022.pdf

Thompson, B. M., Drover, K. B., Stellmaker, R. J., Sculley, D. V., & Janse de Jonge, X. A. K. (2021). The Effect of the Menstrual Cycle and Oral Contraceptive Cycle on Muscle Performance and Perceptual Measures. *International journal of environmental research and public health*, *18*(20), 10565.

Tikkanen, O., Haakana, P., Pesola, A. J., Häkkinen, K., Rantalainen, T., Havu, M., Pullinen, T., & Finni, T. (2013). Muscle activity and inactivity periods during normal daily life. *PloS one*, *8*(1), e52228.

Tomlinson, B. E., & Irving, D. (1977). The numbers of limb motor neurons in the human lumbosacral cord throughout life. *Journal of the neurological sciences, 34*(2), 213–219.

Torg, J. S., Conrad, W., & Kalen, V. (1976). Clinical diagnosis of anterior cruciate ligament instability in the athlete. *The American journal of sports medicine, 4*(2), 84–93.

Tracy, B. L., & Enoka, R. M. (2002). Older adults are less steady during submaximal isometric contractions with the knee extensor muscles. *Journal of applied physiology (Bethesda, Md. : 1985), 92*(3), 1004–1012.

Tracy, B. L., & Enoka, R. M. (2006). Steadiness training with light loads in the knee extensors of elderly adults. *Medicine and science in sports and exercise*, *38*(4), 735–745.

Turner, H. M. I., & Bernard, R. M. (2006). Calculating and synthesizing effect sizes. *Contemporary issues in communication science and disorders*, *33*(Spring), 42-55.

Uhorchak, J. M., Scoville, C. R., Williams, G. N., Arciero, R. A., St Pierre, P., & Taylor, D. C. (2003). Risk factors associated with noncontact injury of the anterior cruciate ligament: a prospective four-year evaluation of 859 West Point cadets. *The American journal of sports medicine*, *31*(6), 831–842

Vacek, P. M., Slauterbeck, J. R., Tourville, T. W., Sturnick, D. R., Holterman, L. A., Smith, H. C., Shultz, S. J., Johnson, R. J., Tourville, K. J., & Beynnon, B. D. (2016). Multivariate Analysis of the Risk Factors for First-Time Noncontact ACL Injury in High School and College Athletes: A Prospective Cohort Study With a Nested, Matched Case-Control Analysis. *The American journal of sports medicine*, *44*(6), 1492–1501.

Van Lunen, B. L., Roberts, J., Branch, J. D., & Dowling, E. A. (2003). Association of Menstrual-Cycle Hormone Changes with Anterior Cruciate Ligament Laxity Measurements. *Journal of athletic training, 38*(4), 298–303.

Vauhnik, R., Morrissey, M. C., Perme, M. P., Sevsek, F., & Rugelj, D. (2014). Inter-rater reliability of the GNRB® knee arthrometer. *The Knee, 21*(2), 541–543.

Vauhnik, R., Perme, M. P., Barcellona, M. G., Rugelj, D., Morrissey, M. C., & Sevsek, F. (2013). Robotic knee laxity testing: reliability and normative data. *The Knee, 20*(4), 250–255.

Vigotsky, A. D., Halperin, I., Lehman, G. J., Trajano, G. S., & Vieira, T. M. (2018). Interpreting Signal Amplitudes in Surface Electromyography Studies in Sport and Rehabilitation Sciences. *Frontiers in physiology*, *8*, 985.

Wang, H. M., Shultz, S. J., Ross, S. E., Henson, R. A., Perrin, D. H., & Schmitz, R. J. (2021). Relationship of Anterior Cruciate Ligament Volume and T2* Relaxation Time to Anterior Knee Laxity. *Orthopaedic journal of sports medicine, 9*(2), 2325967120979986.

Watanabe, R. N., Magalhães, F. H., Elias, L. A., Chaud, V. M., Mello, E. M., & Kohn, A. F. (2013). Influences of premotoneuronal command statistics on the scaling of motor output variability during isometric plantar flexion. *Journal of neurophysiology, 110*(11), 2592–2606.

Webber, S. C., & Porter, M. M. (2010). Reliability of ankle isometric, isotonic, and isokinetic strength and power testing in older women. *Physical therapy, 90*(8), 1165–1175.

Webster, K. E., & Hewett, T. E. (2018). Meta-analysis of meta-analyses of anterior cruciate ligament injury reduction training programs. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society, 36*(10), 2696–2708.

Weidauer, L., Zwart, M. B., Clapper, J., Albert, J., Vukovich, M., & Specker, B. (2020). Neuromuscular performance changes throughout the menstrual cycle in physically active females. *Journal of musculoskeletal & neuronal interactions*, *20*(3), 314–324.

Weir J. P. (2005). Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. *Journal of strength and conditioning research, 19*(1), 231–240.

Weitz, F. K., Sillanpää, P. J., & Mattila, V. M. (2020). The incidence of paediatric ACL injury is increasing in Finland. *Knee Surgery, Sports Traumatology, Arthroscopy*, *28*, 363-368.

Wells, G. A., Shea, B., Higgins, J. P., Sterne, J., Tugwell, P., & Reeves, B. C. (2013). Checklists of methodological issues for review authors to consider when including non-randomized studies in systematic reviews. *Research synthesis methods, 4*(1), 63–77.

Whalan, M., Lovell, R., & Sampson, J. A. (2020). Do Niggles Matter? Increased injury risk following physical complaints in football (soccer). *Science and Medicine in Football*, *4*(3), 216-224.

Wickham H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (2nd ed.). Springer-Verlag New York.

Winter, D. A., & Yack, H. J. (1987). EMG profiles during normal human walking: stride-to-stride and inter-subject variability. *Electroencephalography and clinical neurophysiology*, *67*(5), 402–411.

Wojtys, E. M., Huston, L. J., Boynton, M. D., Spindler, K. P., & Lindenfeld, T. N. (2002). The effect of the menstrual cycle on anterior cruciate ligament injuries in women as determined by hormone levels. *The American journal of sports medicine*, *30*(2), 182-188.

Wojtys, E. M., Huston, L. J., Lindenfeld, T. N., Hewett, T. E., & Greenfield, M. L. V. (1998). Association between the menstrual cycle and anterior cruciate ligament injuries in female athletes. *The American journal of sports medicine*, *26*(5), 614-619.

Wolpert, D.M., & Bastian, A.J. (2021). Principles of sensorimotor control. In Kandel E.R., & Koester J.D., & Mack S.H., & Siegelbaum S.A.(Eds.), *Principles of Neural Science* (6th ed.). McGraw Hill.

Woo, S. L. Y., Hollis, J. M., Adams, D. J., Lyon, R. M., & Takai, S. (1991). Tensile properties of the human femur-anterior cruciate ligament-tibia complex: the effects of specimen age and orientation. *The American journal of sports medicine*, *19*(3), 217-225.

Woodford-Rogers, B., Cyphert, L., & Denegar, C. R. (1994). Risk factors for anterior cruciate ligament injury in high school and college athletes. *Journal of athletic training*, *29*(4), 343.

Yu, W. D., Liu, S. H., Hatch, J. D., Panossian, V., & Finerman, G. A. (1999). Effect of estrogen on cellular metabolism of the human anterior cruciate ligament. *Clinical orthopaedics and related research*, (366), 229–238.

Yu, W. D., Panossian, V., Hatch, J. D., Liu, S. H., & Finerman, G. A. (2001). Combined effects of estrogen and progesterone on the anterior cruciate ligament. *Clinical orthopaedics and related research*, *383*, 268–281.

Zazulak, B. T., Hewett, T. E., Reeves, N. P., Goldberg, B., & Cholewicki, J. (2007). Deficits in neuromuscular control of the trunk predict knee injury risk: a prospective biomechanical-epidemiologic study. *The American journal of sports medicine, 35*(7), 1123–1130.

Zumwalt, M. (2018). Musculoskeletal injury and the exercising female. In Forsyth, J. & Roberts C.M. (Eds), *The Exercising Female: Science and Its Application*, (p. 142). Routledge.

Chapter 9. Appendices

Appendix A: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 Checklist used in Chapter 2 and 3

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	41
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	N/A
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	42 - 43
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	43 - 44
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	44 - 45
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	45
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	45 - 46
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	46 - 47
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	47 - 48
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	47 - 48
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	47 - 48
Study risk of bias	11	Specify the methods used to assess risk of bias in the included studies, including details of the	48 - 49

Section and Topic	Item #	Checklist item	Location where item is reported
assessment		tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	49
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	49
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	49
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	49
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	49
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	49
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	N/A
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	N/A
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	49 - 50
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	50
Study characteristics	17	Cite each included study and present its characteristics.	51 - 52
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	52 - 58

Section and Topic	Item #	Checklist item	Location where item is reported
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	59 - 63
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	59 - 63
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	59 - 63
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	59 - 63
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	N/A
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	64 - 68
	23b	Discuss any limitations of the evidence included in the review.	68 - 69
	23c	Discuss any limitations of the review processes used.	69 - 70
	23d	Discuss implications of the results for practice, policy, and future research.	70 - 72
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	27, 30, 31, 44
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	27, 30, 31, 44
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	70
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	N/A

Section and Topic	Item #	Checklist item	Location where item is reported
Competing interests	26	Declare any competing interests of review authors.	N/A
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	N/A

From: Page et al. (2021a).

Appendix B: Draft search strategy for PubMed Central database used in Chapter 2 and 3

Search Builder

1. "menstrual cycle"[All Fields] OR hormon*[All Fields] OR follicular[All Fields] OR luteal[All Fields] OR progesterone[All Fields] OR ?estrogen[All Fields] OR progestin*[All Fields] OR relaxin[All Fields] OR "birth control"[All Fields] OR contracepti*[All Fields]
2. {anterior cruciate ligament}[MeSH Major Topic]
3. laxity[All Fields] OR rupture[All Fields] OR injury[All Fields] OR strain[All Fields] OR sprain[All Fields] OR tear*[All Fields] OR stiff*[All Fields] OR strength[All Fields]
4. #2 AND #3
5. #1 AND #4

Filters or Limiters: None applied

Search conducted: April 30, 2021

Paper identified: 225

Overview	
Full Search	("menstrual cycle"[All Fields] OR "hormon*"[All Fields] OR "follicular"[All Fields] OR "luteal"[All Fields] OR ("progesterone"[MeSH Terms] OR "progesterone"[All Fields] OR "progesteron"[All Fields] OR "progesterones"[All Fields] OR "progesterone s"[All Fields] OR "progesteronic"[All Fields] OR "progesterons"[All Fields]) OR ("estrogen s"[All Fields] OR "estrogene"[All Fields] OR "estrogenes"[All Fields] OR "estrogenic"[All Fields] OR "estrogenically"[All Fields] OR "estrogenicities"[All Fields] OR "estrogenicity"[All Fields] OR "estrogenization"[All Fields] OR "estrogenized"[All Fields] OR "oestrogen"[All

	Fields] OR "estrogens"[Pharmacological Action] OR "estrogens"[MeSH Terms] OR "estrogens"[All Fields] OR "estrogen"[All Fields] OR "oestrogen s"[All Fields] OR "oestrogenic"[All Fields] OR "oestrogenically"[All Fields] OR "oestrogenicity"[All Fields] OR "oestrogenization"[All Fields] OR "oestrogens"[All Fields]) OR "progestin*"[All Fields] OR ("relaxin"[MeSH Terms] OR "relaxin"[All Fields] OR "relaxin s"[All Fields] OR "relaxins"[All Fields]) OR "birth control"[All Fields] OR "contracepti*"[All Fields]) AND (("anterior cruciate ligament"[MeSH Terms] OR ("anterior"[All Fields] AND "cruciate"[All Fields] AND "ligament"[All Fields]) OR "anterior cruciate ligament"[All Fields]) AND ("laxities"[All Fields] OR "laxity"[All Fields] OR ("ruptur"[All Fields] OR "rupture"[MeSH Terms] OR "rupture"[All Fields] OR "ruptured"[All Fields] OR "ruptures"[All Fields] OR "rupturing"[All Fields]) OR ("injurie"[All Fields] OR "injuried"[All Fields] OR "injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "injurious"[All Fields] OR "injury s"[All Fields] OR "injuryed"[All Fields] OR "injurys"[All Fields] OR "injury"[All Fields]) OR ("sprains and strains"[MeSH Terms] OR ("sprains"[All Fields] AND "strains"[All Fields]) OR "sprains and strains"[All Fields] OR "strain"[All Fields] OR "strains"[All Fields] OR "strain s"[All

	Fields]) OR ("sprained"[All Fields] OR "spraining"[All Fields] OR "sprains and strains"[MeSH Terms] OR ("sprains"[All Fields] AND "strains"[All Fields]) OR "sprains and strains"[All Fields] OR "sprain"[All Fields] OR "sprains"[All Fields]) OR "tear*"[All Fields] OR "stiff*"[All Fields] OR ("strength"[All Fields] OR "strengths"[All Fields]))
Breakdown	
Intervention/Exposure	"menstrual cycle"[All Fields] OR hormon*[All Fields] OR follicular[All Fields] OR luteal[All Fields] OR progesterone[All Fields] OR ?estrogen[All Fields] OR progestin*[All Fields] OR relaxin[All Fields] OR "birth control"[All Fields] OR contracepti*[All Fields]
Outcome Part 1	{anterior cruciate ligament}[MeSH Major Topic]
Outcome Part 2	laxity[All Fields] OR rupture[All Fields] OR injury[All Fields] OR strain[All Fields] OR sprain[All Fields] OR tear*[All Fields] OR stiff*[All Fields] OR strength[All Fields]

Appendix C: The Newcastle Ottawa Quality Assessment Scale (NOS) for Case–Control or Cohort Studies used in Chapter 3

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?

a) yes, with independent validation

b) yes, eg record linkage or based on self-reports

c) no description

2) Representativeness of the cases

a) consecutive or obviously representative series of cases

b) potential for selection biases or not stated

3) Selection of Controls

a) community controls □

b) hospital controls

c) no description

4) Definition of Controls

a) no history of disease (endpoint)

b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis

a) study controls for _____ (Select the most important factor.)

b) study controls for any additional factor (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure

a) secure record (eg surgical records)

b) structured interview where blind to case/control status

c) interview not blinded to case/control status

d) written self-report or medical record only

e) no description

2) Same method of ascertainment for cases and controls

a) yes

b) no

3) Non-Response rate

a) same rate for both groups

b) non respondents described

c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average _____ (describe) in the community

b) somewhat representative of the average _____ in the community

c) selected group of users eg nurses, volunteers

d) no description of the derivation of the cohort

2) Selection of the non-exposed cohort

a) drawn from the same community as the exposed cohort

b) drawn from a different source

c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure

a) secure record (eg surgical records)

b) structured interview

c) written self-report

d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes

b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for _____ (select the most important factor) □

b) study controls for any additional factor (This criterion could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome

a) independent blind assessment

b) record linkage

c) self-report

d) no description

2) Was follow-up long enough for outcomes to occur

a) yes (select an adequate follow up period for outcome of interest)

b) no

3) Adequacy of follow up of cohorts

a) complete follow up - all subjects accounted for

b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost)

c) follow up rate < ____% (select an adequate %) and no description of those lost

d) no statement

Appendix D: The Newcastle Ottawa Quality Assessment Scale (NOS) for Cohort AKL studies used in Chapter 3

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE: COHORT STUDIES

CODING MANUAL FOR AKL STUDIES

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average non-contact ACL-injured female athlete/exerciser* in the community

Included from 2 or more institutions (e.g., sport clubs)

b) somewhat representative of the average non-contact ACL-injured female athlete/exerciser in the community

Included from 1 or 2 institutions

c) selected group of users (e.g., nurses, volunteers)

d) no description of the derivation of the cohort

*Characteristics average non-contact ACL-injured population: young, physically active.

2) Selection of the non-exposed cohort

a) drawn from the same community as the exposed cohort

b) drawn from a different source

c) no description of the derivation of the non-exposed cohort

na no comparison group

3) Ascertainment of exposure (i.e., exposure affecting the endogenous ovarian hormone status of the participants; that is, menstrual cycle and associated disturbances, and hormonal contraceptives)

a) secure record (e.g., institutional record supported with use of ovulation test kit and blood sample analysis)

b) structured interview (i.e., self-report of menstrual history/hormonal contraceptive use tracking via app/notebook + basal body temperature tracking + urine/saliva sample analysis)

c) written self-report (i.e., self-report of menstrual cycle/hormonal contraceptive use, with app or notebook)

d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes (i.e., no previous ACL or knee injury on either knee, and no known co-existing medical conditions affecting the connective tissue)

b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for endogenous ovarian hormone status (e.g., menstrual cycle phase/timepoints, hormonal contraceptive use)

b) study controls for pre-testing physical activity level

0) no comparison group

Outcome

1) Assessment of outcome (i.e., anterior knee laxity)

a) assessment with arthrometer at a defined force (operator blinded to the hormonal status of the participant)

b) assessment with arthrometer at a defined force (operator not blinded to the hormonal status of the participant)

c) assessment with clinical manual examination (i.e., Lachman test or anterior knee laxity assessment at maximum manual force)

d) no description

2) Was follow-up long enough for outcomes to occur *(follow-up time)*

a) yes (i.e., follow-up done over one whole menstrual cycle or hormonal contraceptive cycle)

b) no

3) Adequacy of follow up of cohorts *(follow-up rate)*

a) complete follow up - all subjects accounted for

b) subjects lost to follow up unlikely to introduce bias - small number lost - > 80% follow up, or description provided of those lost

c) follow up rate < 80% and no description of those lost

d) no statement (retrospective study, authors do not report number of eligible participants)

Dichotomization low versus high risk of bias

S1 low risk = a,b

S2 na= not applicable, low risk = a

S3 low risk = a

S4 low risk = a

C1 na= not applicable, low risk = a

O1 low risk = a

O2 low risk = a

O3 low risk = a,b

Appendix E: The Newcastle Ottawa Quality Assessment Scale (NOS) for Case-control and Cohort ACL injuries studies used in Chapter 3

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE: CASE-CONTROL STUDIES

CODING MANUAL FOR ACL INJURY STUDIES

Selection

1) Is the case definition adequate?

a) yes, with independent validation (e.g., primary non-contact ACL injury determined by MRI/by medical hospital records)

b) yes, e.g., record linkage or based on self-reports with no reference to primary record

c) no description

2) Representativeness of the cases

a) consecutive or obviously representative series of cases [i.e., all eligible cases with outcome of interest over a defined period of time, all cases in a defined catchment area, all cases in a defined hospital or clinic, group of hospitals, health maintenance organisation, or an appropriate sample of those cases (e.g., random sample)]

b) potential for selection biases (i.e., not satisfying requirements in part a)) or not stated

3) Selection of Controls

This item assesses whether the control series used in the study is derived from the same population as the cases and essentially would have been cases had the outcome been present.

a) community controls (i.e., same community as cases and would be cases if had outcome)

b) hospital controls, within same community as cases (i.e., not another city) but derived from a hospitalised population

c) no description

4) Definition of Controls

a) no history of disease (i.e., primary non-contact ACL injury on either knee)

b) no description of source, no mention of history of outcome

Comparability

1) Comparability of cases and controls on the basis of the design or analysis

a) study controls for endogenous ovarian hormone status (e.g., menstrual cycle phase/timepoints, hormonal contraceptive use)

b) study controls for pre-injury physical activity level

Exposure

1) Ascertainment of exposure (i.e., exposure affecting the endogenous ovarian hormone status of the participants; that is, menstrual cycle and associated disturbances, and hormonal contraceptive use)

a) secure record (e.g., institutional or surgical record supported with use of ovulation kit and blood sample analysis)

b) structured interview where blind to case/control status (i.e., self-report of menstrual history/hormonal contraceptive use tracking via app/notebook + basal body temperature tracking + urine/saliva sample analysis)

c) interview not blinded to case/control status (i.e., self-report of menstrual history/hormonal contraceptive use tracking via app/notebook + basal body temperature tracking + urine/saliva sample analysis)

d) written self-report or medical record only (i.e., self-report of menstrual cycle/hormonal contraceptive use, with app or notebook)

e) no description

2) Same method of ascertainment for cases and controls

a) yes

b) no

3) Non-Response rate (dropouts)

a) same rate for both groups

b) non respondents described

c) rate different and no designation (description)

Dichotomization low versus high risk of bias

S1 low risk = a

S2 low risk = a

S3 low risk = a

S4 low risk = a

C1 low risk = a

E1 low risk = a, b

E2 low risk = a

E3 low risk = a

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE: COHORT STUDIES

CODING MANUAL FOR ACL INJURY STUDIES

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average non-contact ACL-injured female athlete/exerciser* in the community

Included from 2 or more institutions (e.g., sport clubs)

b) somewhat representative of the average non-contact ACL-injured female athlete/exerciser in the community

Included from 1 or 2 institutions

c) selected group of users (e.g., nurses, volunteers)

Selected group of participants from 1 or several institutions

d) no description of the derivation of the cohort

*Characteristics average non-contact ACL-injured population: young, physically active.

2) Selection of the non-exposed cohort

a) drawn from the same community as the exposed cohort

b) drawn from a different source

c) no description of the derivation of the non-exposed cohort
na no comparison group

3) Ascertainment of exposure (i.e., exposure affecting the endogenous ovarian hormone status of the participants; that is, menstrual cycle and associated disturbances, and hormonal contraceptive use)

a) secure record (e.g., institutional or surgical record supported with use of ovulation kit and blood sample analysis)

b) structured interview (i.e., self-report of menstrual history/hormonal contraceptive use tracking via app/notebook + basal body temperature tracking + urine/saliva sample analysis)

c) written self-report (i.e., self-report of menstrual cycle/hormonal contraceptive use, with app or notebook)

d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes (no previous ACL or knee injury on either knee)

b) no (previous ACL or knee injury on either knee)

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for endogenous ovarian hormone status (e.g., menstrual cycle phase/timepoints, hormonal contraceptive use)

b) study controls for pre-injury physical activity level

0) no comparison group

Outcome

1) Assessment of outcome (i.e., primary non-contact ACL injury)

a) independent assessment with MRI

b) assessment with clinical examination (i.e., Lachman test), but not by an independent practitioner (i.e., doctor/surgeon)

c) self-report

d) no description

2) Was follow-up long enough for outcomes to occur (*follow-up time*)

a) yes (i.e., the participant was observed/followed for a minimum of 1 year of practice/training/competition)

b) no

3) Adequacy of follow up of cohorts (*follow-up rate*)

a) complete follow up - all subjects accounted for

b) subjects lost to follow up unlikely to introduce bias - small number lost - > 80% follow up, or description provided of those lost

c) follow up rate < 80% and no description of those lost

d) no statement (retrospective study, authors do not report number of eligible participants)

Dichotomization low versus high risk of bias

S1 low risk = a,b

S2 na= not applicable, low risk = a

S3 low risk = a

S4 low risk = a

C1 na= not applicable, low risk = a

O1 low risk = a

O2 low risk = a

O3 low risk = a,b

Appendix F: Table of AKL studies included in the systematic review and meta-analysis used in Chapter 3

Author and date	Aim	Population (participant health, menstrual health, training status and sample size)	MC or HC phases tested according to our classification and HC type when applicable	Methods of identifying and verifying MC or HC phase	Outcome measure(s)	Testing device and force level used	Study findings
Belanger et al., 2004	To investigate the combined effects of the MC and exercise on the ACL.	Healthy, naturally menstruating female athletes (n=18)	MC: unable to determine the three phases according to our classification	Charts of BBT + self-reported MC phase	AKL	KT-2000, at 134N	ACL laxity is not significantly different during the follicular, ovulatory, and luteal phases of the MC
Beynnon et al., 2005	To measure serum oestradiol and progesterone levels and laxity of the ankle and knee joints across a MC in women and in a group of men who were	Healthy, naturally menstruating female athletes (n=17)	MC: phases 1, 2, and 4	MC characteristics tracked < 2 months prior to testing with calendar-based tracking + corroboration achieved by	Antero-posterior knee laxity	KT-1000, anterior load at 130N, posterior load at 90N	Antero-posterior laxity does not change across the MC

	tested at similar timepoints			confirmation of ovulation (use of urinary ovulation detection test) and serum oestradiol and progesterone analysis			
Carcia et al., 2004	To evaluate the effects of a controlled anterior tibiofemoral load on passive anterior tibiofemoral displacement and stiffness in a group of healthy females across selected points of the MC	Healthy, eumenorrheic exercising women (n=20)	MC: phases 1 and 3	MC characteristics tracked < 2 months prior to testing with calendar-based tracking + corroboration achieved by confirmation of ovulation (use of urinary ovulation detection test) and serum oestradiol, progesterone, and	AKL	KT-2000, at 134N	Neither AKL nor ACL stiffness were affected by day of the MC

				testosterone analysis			
Eiling et al., 2007	To examine changes in lower limb musculotendinous stiffness and AKL over the course of the MC and investigate the interaction of warm-up on musculotendinous stiffness	Healthy, naturally menstruating female teenagers (n=11)	MC: phase 1 and unable to determine the three other phases according to our classification	MC characteristics tracked for 3 months prior to testing and 3 months following testing period with calendar-based tracking + count back strategy to calculate all testing dates	AKL	KT-2000, at 134N	There is no significant effect of the MC on AKL
Heitz et al., 1999	To determine whether women experience significant differences in ACL laxity in conjunction with oestrogen and progesterone	Healthy, naturally menstruating exercising women (n=7)	MC: phase 1 and unable to determine the two other phases according to our classification	Self-reported MC phase + oestrogen and progesterone serum sample analysis done on day 1, 10, 11, 12, 13, 20, 21, 22, and 23	AKL	KT-2000, at 133N	ACL laxity is significantly greater in follicular and luteal phases when compared to the menstrual phase

	during a normal 28- to 30-day MC						
Hertel et al., 2006	To assess changes in neuromuscular control, *i.e.*, measures of hamstring and quadriceps strength, knee joint position sense, postural control, and knee joint laxity at three points across the MC	Healthy, naturally menstruating collegiate athletes (n=14)	MC: Unable to determine the three phases according to our classification	MC characteristics tracked 1 month prior to testing with calendar-based tracking + corroboration achieved by confirmation of ovulation (use of urinary ovulation detection test) in the month prior to testing. Daily oestrogen and progesterone urinary sample analysis in testing month	AKL	KT-1000, at 133N	Neuromuscular control and AKL do not change substantially across the MC despite varying oestrogen and progesterone levels

Hicks-Little et al., 2007	To determine if there is a difference in the anterior displacement of the tibia in female athletes at 3 different stages of the MC: follicular, ovulation and luteal	Healthy, naturally menstruating female athletes (n=53; MC, n=28; HC, n=25)	MC: phase 1 and unable to determine the two other phases according to our classification HC: HC type not assessed/not reported, testing timepoints on day 1, 13, and 23	MC: self-reported MC phase HC: self-reported HC use, no further information about HC type, brand, formulation, or dosage	AKL	KT-1000, at maximal force level	AKL is significantly greater in ovulation and luteal phases in comparison to the follicular phase. AKL is also significantly greater in HC users when compared to non-HC users.
Karageanes et al., 2000	To identify a significant change in the ACL laxity in the competitive adolescent female athlete throughout the	Healthy, naturally menstruating adolescent female athletes (n=26)	MC: unable to determine the three phases according to our classification	Menstrual history questionnaire + MC calendar-tracking during the testing period (MC phases	AKL	KT-1000, at 89N	AKL did not significantly change across the MC

	difference phases of the MC			calculated a posteriori once calendars submitted at the end of the study)			
Khowailed et al., 2015	To investigate the effects of 17b-oestradiol across MC phases on lower extremity neuromuscular control patterns and AKL during running	Healthy, eumenorrheic recreational female runners (n=11)	MC: phases 1 and 3	MC characteristics tracked < 2 months prior to testing with calendar-based tracking + corroboration achieved by confirmation of ovulation (use of urinary ovulation detection test) and serum oestradiol analysis	AKL	KT-2000, at 133N	There was significant difference in AKL between the follicular phase (phase 1) and the ovulatory phase (phase 3) of the MC. Greatest AKL was found during ovulation (phase 3) and the least AKL was found during follicular phase (phase 1)
Landram and	To determine whether continuous moderate	Healthy, naturally menstruating exercising	MC: unable to determine the two phases	MC characteristics tracked < 2 months prior	APKL	KT-2000, at 90 and 120N	APKL was significantly greater in luteal phase compared to follicular

Halligan, 2020	intensity versus discontinuous high intensity treadmill running has an influence on APKL and hamstrings flexibility in eumenorrheic women across luteal and follicular phases	women (n=10)	according to our classification	to testing with calendar-based tracking + daily BBT check for identification of MC phases and salivary oestrogen analysis			phase at both 90 and 120N
Lee et al., 2013, 2014	To investigate the differences in AKL, force to flex the knee, and knee flexion–extension hysteresis between OCP users and non-OCP users. To investigate these changes, two different knee temperatures were measured,	Healthy, naturally menstruating active women, or OCP users (n=18; MC, n=10; OCP, n=8).	MC: phase 1 OCP: active and inactive phases, no information about the testing timepoints within the OCP phases	MC: self-reported MC phase + serum oestradiol analysis OCP: self-reported OCP phase + serum oestradiol analysis	AKL	KT-2000, at 133N	AKL was significantly lower in OCP users compared to non-OCP users at both temperatures across the MC or HC cycle. AKL did not fluctuate across the HC cycle in OCP users at both temperatures. AKL was significantly greater at ovulation and luteal phases compared to menstruation phase, and at ovulation phase

	i.e., at 22 and 38°C		OCP used: combined low dose (<50 µg-ethinyl estradiol) OCP. No mention of mono, bi, or triphasic type of OCP				compared to follicular phase at 22°C in non-OCP users. AKL was also significantly greater at ovulation phase compared to the follicular phase at 38°C in non-OCP users.
Maruyama et al., 2021	To examine the relationship of AKL, stiffness, and general joint laxity with respect to the MC	Healthy, naturally menstruating exercising women (n=15)	MC: phase 1 and unable to determine the three other phases according to our classification	MC characteristics tracked < 2 months prior to testing with calendar-based tracking + BBT tracking + corroboration achieved by confirmation of ovulation (use of urinary ovulation detection test)	AKL	KS Measure, at 44, 89, and 133N	AKL, stiffness, and general joint laxity did not vary significantly across the MC. In the genu recurvatum group, AKL at 89 and 133 N was significantly higher in the ovulation phase than in the early follicular phase.

Park et al., 2009a, 2009b	To determine whether changing hormone levels influence AKL and stiffness of the non-contractile knee joint structures using a new analysis technique, and whether subsets of women exist who demonstrate (responders) or do not demonstrate (non-responders) changes in AKL in response to circulating hormone levels throughout their MC	Healthy, eumenorrheic exercising women (n=26)	MC: phases 1, 3, and 4	MC characteristics tracked ≥ 2 months prior to testing with calendar-based tracking + corroboration achieved by confirmation of ovulation (use of urinary ovulation detection test) and serum oestradiol and progesterone analysis	AKL	KT-2000, at 89N and manual maximum force	Significantly greater AKL was recorded during ovulation (phase 3) compared to the luteal phase (phase 4) at 89N, and significantly greater AKL was recorded during ovulation (phase 3) compared to the follicular phase (phase 1) at maximal manual force.
Pollard, Braun, and	To investigate the collective effects of gender,	Healthy, naturally menstruating	MC: phase 1 and unable to determine the	Self-reported MC characteristics	AKL	KT-1000, at 89N	Women had greater AKL than men across the MC. AKL did not

Hamill, 2006	oestrogen and exercise on AKL in active men and women	exercising women (n=12)	two other phases according to our classification	+ confirmation of ovulation with use of urinary ovulation detection test + oestrogen serum sample analysis			significantly very across the MC in female participants.
Shagawa et al., 2021	To examine changes in AKL, stiffness, genu recurvatum, and general joint laxity during the late follicular phase and ovulation phase of the MC	Healthy, naturally menstruating exercising women (n=15)	MC: unable to determine the two phases according to our classification	MC characteristics tracked < 2 months prior to testing with calendar-based tracking + daily BBT + corroboration achieved by confirmation of ovulation (use of urinary ovulation detection test) and salivary oestrogen analysis	AKL	KS Measure, at 44, 89, 133N	AKL and ACL stiffness did not significantly vary between the late follicular and ovulatory phases of the MC. Genu recurvatum and general joint laxity were significantly greater in ovulatory phase than in late follicular phase

Shultz et al., 2005	To compare men and normal-menstruating women on serum hormone levels, AKL and linear stiffness at multiple days across the female MC.	Healthy, normally menstruating exercising women (n=22)	MC: phases 1 and 2	MC characteristics tracked < 2 months prior to testing with calendar-based tracking + corroboration achieved by confirmation of ovulation (use of urinary ovulation detection test) and serum oestradiol, progesterone, and testosterone analysis	AKL	KT-2000, at 46N, 89N, and 133N	AKL was greater on day 5 near ovulation (phase 2) compared to day 3 of menses (phase 1), and days 1-3 of the early luteal phase compared to all days of menses (phase 1) and day 1 near ovulation
Shultz et al., 2010	To examine the change in magnitude of AKL, genu recurvatum, and general joint laxity across the	Healthy, naturally menstruating with ovulatory cycles, exercising	MC: phases 1 and 4	MC characteristics tracked < 2 months prior to testing with calendar-based tracking +	AKL	KT-2000, at 133N	The magnitude of variations of cyclic changes in AKL across the MC is quite variable between women. The largest mean difference for AKL was between

	early follicular and early luteal phases of the MC over two cycles, and to examine the similarity in these cyclic variations between the two cycles and between the three laxity variables	women (n=66)		corroboration achieved by confirmation of ovulation (use of urinary ovulation detection test) + outcome measures repeated in a second cycle			day 5 and day 8 of the early luteal phase. At the individual level, mean absolute cyclic changes were more apparent, with minimum, maximum, and delta values being consistent from month to month
Shultz et al., 2012b	To determine if normal physiological variations in hormone concentrations across the MC are of sufficient magnitude to stimulate changes in collagen metabolism as measured by serum collagen markers and	Healthy, naturally menstruating with ovulatory cycles exercising women, or combined OCP users (n=20; MC, n=10; OCP, n=10).	OCP: active and inactive phases. Measurements were taken on days 1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 15, 16, 17, 18, 19 (active phase), 22, 23, 24, 25, and 26 (inactive	OCP: self-reported OCP phase + serum oestradiol, progesterone, and testosterone analysis	AKL	KT-2000, at 133N	AKL was stable across the HC cycle in OCP users

	mediators. To examine whether cyclic changes in serum CICP, ICTP, and IGF-I were associated with cyclic changes in AKL within each group	MC participants were the same than in Shultz et al., 2005, so their data were not extracted from this study to avoid duplicate.	phase) of the HC cycle. OCP: list of type, brand, and dosage of OCPs used in the study available, majority of combined triphasic OCPs and some combined monophasic OCPs				
Van Lunen et al., 2003	To examine the possible associations between AKL and concentrations of total estrogens, estradiol, progesterone, LH, FSH, and testosterone	Healthy, eumenorrheic exercising women (n=12)	MC: phases 1 and 3	MC characteristics tracked < 2 months prior to testing with calendar-based tracking + corroboration achieved by confirmation of ovulation	AKL	KT-2000, at 133N, and Staubli and Jakob's protocol for radiographic	No effects of MC phases on AKL as measured by the KT-2000 and radiographs

during the 3 phases of the MC and to compare AKL using the KT-2000 arthrometer and Staubli and Jakob's protocol for radiographic comparisons	(use of urinary ovulation detection test) and serum oestrogen, oestradiol, progesterone, testosterone, LH, and FSH analysis	comparison s at 133N

AKL, anterior knee laxity; APKL, antero-posterior knee laxity; BBT, basal body temperature; HC, hormonal contraceptive; MC, menstrual cycle; OCP, oral contraceptive pill; Phase 1, indicated by the onset of bleeding until day 5, oestrogen and progesterone levels are low; Phase 2, occurs in the 14–26 h prior to ovulation and the LH surge, oestrogen higher than during phase 1, 3 and 4 and progesterone higher than during phase 1, but lower than 6.36 nmol·L^{-1}/2ng/mL; Phase 3, indicated by a positive urinary ovulation kit and lasts 24–36 h, oestrogen higher than phase 1 but lower than phase 2 and 4 and progesterone higher than phase 1 but lower than 6.4 nmol·L^{-1}/2.01ng/mL; Phase 4, + 7 days after ovulation has been confirmed, oestrogen higher than phase 1 and 3 but lower than phase 2 and progesterone > 16 nmol·L^{-1}/5.03 ng/mL. Studies also included for quantitative analysis (meta-analysis) are marked in bold letters.

Appendix G: Table of ACL injury studies included in the systematic review in Chapter 3

Author and date	Aim	Population (participant health, menstrual health, training status and sample size)	MC or HC phases tested according to our classification and HC type when applicable	Methods of identifying and verifying MC or HC phase	Methods used to determine and verify ACL injury	Outcome measure(s)	Study findings
Adachi et al., 2008	To determine if non-contact ACL injuries occurred randomly or correlate with a specific MC phase in teenaged female athletes, the highest-risk individuals, and then to determine if	ACL injured, naturally menstruating teenaged female athletes (n=18)	MC: unable to determine the three MC phases	Self-reported through MC history and HC use history questionnaire	Medical diagnosis with MRI	ACL injury MC: 13 ACL injuries (72%) occurred at phase 2 (ovulatory: days 10–14) of the MC, while 3 ACL injuries (17%) occurred at phase 3	There is a significant rate of non-contact ACL injuries in teenage female athletes during the ovulatory phase of the MC

	premenstrual and menstrual dysfunctions influenced non-contact ACL injuries.					(luteal: days 15 to end of cycle), and 2 ACL injuries (11%) occurred at phase 1 (follicular: days 1–9)	
Agel et al., 2006	To determine the rate of non-contact ankle and ACL injuries in basketball and soccer for female athletes. To determine if there is a protective effect from the use of OCPs.	Healthy and ACL injured female basketball or soccer athletes, or OCP users with same profile (total cohort, n=3150; MC, n=2026; ACL injured MC, n=29; OCP, n= 1024; ACL injured OCP, n=16)	MC: unable to determine the MC phase OCP: unable to determine the HC cycle timepoint OCP: mix of monophasic and triphasic OCPs used, no further details about	MC: self-reported prior period recall and prospective next period date, without verification OCP: self-reported OCP use	ACL injury determined either by the certified athletic trainer or a team physician. No mention of verification by MRI	ACL injury MC: 29 individuals (1.4%) were injured across the study timeframe OCP: 16 individuals (1.6%) were injured across the	There was no significant difference in ACL injury rate in OCP users and non-OCP users

			brand, formulation, or dosage			study timeframe	
Ruedl et al., 2009, 2011	To investigate a possible protective effect of OCP use against ACL injuries in recreational skiers and to compare the frequencies of non-contact ACL injuries in the preovulatory phase with that in the postovulatory phase of the MC in recreational skiers.	Healthy (controls) and ACL injured naturally menstruating female recreational alpine skiers or OCP users with same profile (ACL injured n=93; MC, n=61; OCP, n=32; and healthy controls n=93; MC, n=60; OCP, n=33)	MC: unable to determine the two phases according to our classification OCP: unable to determine an OCP phase or timepoint OCP: more than one brand/type of OCP per group of participants; no further	MC and OCP: MC history and HC use questionnaire and self-reported MC phase/OCP use, without verification	Medical diagnosis with MRI Note: the ACL injured sample was composed of a mix of primary (90.6%) and secondary (9.4%) non-contact ACL injuries	ACL injury MC: 35 individuals (57.4%) were injured in their preovulatory phase, whilst 26 individuals (42.6%) were injured in their postovulatory phase. OCP: 18 individuals (56%) were injured in	OC use did not show a protective effect against non-contact ACL injuries. Increased risk of non-contact ACL injury in preovulatory phase

	details about brand, type, formulation, or dosage	their preovulatory phase, whilst 14 individuals (44%) were injured in their postovulatory phase Note: unable to interpret what corresponded to pre and postovulatory phases in OCP users in this study

ACL, anterior cruciate ligament; HC, hormonal contraceptive; MC, menstrual cycle; OCP, oral contraceptive pill.

Appendix H: Participant information sheet used in Chapter 4

Please read this document and keep it for your records. The researcher will also go through this with you in the first online meeting.

PARTICIPANT INFORMATION SHEET

Study title: GNRB knee arthrometer reliability study in men, combined monophasic oral contraceptive pill users, combined hormonal contraceptive users, postmenopausal women, and hormone replacement therapy users.

Researchers: Ms Elisa Nédélec, Prof Kirsty Elliott-Sale, Prof Craig Sale, Dr Jessica Piasecki, and Dr Kevin Enright

Invitation:

We invite you to take part in this research study at Nottingham Trent University. Before you decide to do so, it is important that you understand why this study is being done and what we will ask you to do. Please take the time to read the following information carefully. Please contact our researcher (Elisa Nédélec) by email if there is anything that you do not understand or if you would like to have more information about this study: elisa.nedelec2019@my.ntu.ac.uk.

Thank you for considering taking part in this research.

What is the purpose of the study?

We would like to see if an automated machine that measures the elasticity of a large ligament (bands that attach bones together) in the knee (*i.e., the anterior cruciate ligament – which connects the thigh bone to the shin bone and helps to keep the knee stable*) will give similar values when we repeat the test three times. This will give us an idea of how reliable the measurement is, which is important to allow us to know, in future studies, whether our results are due to something we have done (i.e., our intervention) or whether this is just due to normal variations in the measurement.

We will analyse the values that the automated machine (*i.e., GNRB knee arthrometer*) measured on each participant, and we will conclude if we can use this machine in our next study. In this next study we will look to understand whether variations of sex hormones will affect the elasticity of the anterior cruciate ligament in women using the oral contraceptive pill. We are interested in this because it has been identified as a possible risk factor for anterior cruciate ligament injury.

Why have you been chosen?

- You are 18 years-old or older.

AND:

- You are a cisgender man; or
- You are a cisgender woman, and you use a combined monophasic oral contraceptive pill (*i.e., a contraceptive pill that has the same quantity of oestrogen and progesterone for 21 days, followed by 7 days of placebo pills, or 7 pill-free days*) for more than 3 months; or
- You are a cisgender woman, and you use another form of combined hormonal contraceptive: either a transdermal patch or a vaginal ring, for more than 3 months; or
- You are a cisgender woman, and you already went through menopause. You are currently in post menopause (*i.e., you did not have any period/menstrual bleed for at least 12 months in a row*); or
- You are a cisgender woman, and you take a form of combined hormone replacement therapy (HRT) (*i.e., either you take oestrogen and progesterone every day without a break, or you take oestrogen only for several days and oestrogen + progesterone for 14 days at the end of your hormonal replacement therapy cycle*) for more than 6 months.

AND:

- You are physically active with at least 150 to 300 minutes of moderate-intensity activity a week. Moderate-intensity activity is usually rated a 5 or 6 on a rating scale of perceived exertion scale of 0 to 10. In other words, you produce a moderate effort when you practice this physical activity. Additionally, you do muscle-strengthening activities 2 or more days a week; or
- You are physically active with at least 75 to 150 minutes of vigorous-intensity activity a week. Vigorous-intensity activity is usually rated a 7 or 8 on a rating scale of perceived exertion scale of 0 to 10. In other words, you produce an intense effort when you practice this physical activity. Additionally, you do muscle-strengthening activities 2 or more days a week; or
- You train regularly ~ 3 times per week, you identify with a specific sport, you train with the purpose to compete, and you compete at the local/regional level.

AND:

- You never had an anterior cruciate ligament (ACL) injury or any other knee joint injury or surgery.

If you meet these criteria, you are eligible to take part in this research study.

If you are a woman and you are eligible to take part in this study, we will ask you for more details about your menstrual history, which type of contraception you use or, which type of hormone replacement therapy you take, if applicable.

Do you have to take part?

It is entirely up to you to decide whether you take part or not. Your participation is entirely voluntary. If you decide to do so, we will ask you to attend a short online meeting for 10 to 15 minutes with our researcher (Elisa Nédélec) to sign the Informed Consent form. During this meeting, you will be asked to allow our researcher to record a screenshot of your signature on the Informed Consent form. You will also have to send the signed Informed Consent form by email to our researcher (Elisa Nédélec) afterwards. However, you can still change your mind and stop participating in this study before the analysis of the data once all data have been collected.

What would taking part involve?

You will have to fill in a few questionnaires [*i.e., an Informed Consent form, a health screen questionnaire, a COVID-19 Symptom questionnaire, and a menstrual history questionnaire (only for women)*] which will take you approximately 10 minutes. Please feel free to contact our researcher (Elisa Nédélec) by email at the following email address if there is anything that you do not understand in the questionnaires: elisa.nedelec2019@my.ntu.ac.uk.

You will send the completed questionnaires by email to our researcher (Elisa Nédélec). Once our researcher has analysed your answers, she will get back to you and you will be scheduled for 3 visits to the Nottingham Trent University's research laboratory (Clifton Campus), ideally on 3 consecutive days, or on days as close as possible to each other, within 1 or 2 weeks. Each visit will last 30 to 45 minutes and will take place at the same time of day as the first visit (*i.e., if you had your first testing session between 8:00 AM and 9:00 AM, you will be tested between 8:00 AM and 9:00 AM on the following sessions*).

If you are a combined monophasic oral contraceptive pill user, you will be scheduled to visit the research laboratory between day 7 and day 21 of your pill-taking days. You will also be asked to normalise the time you take your pill (*i.e., our researcher will not ask you to change the time you usually take your pill but to take it every day at the same time*).

If you are a cyclical combined hormone replacement therapy user, you will be scheduled to visit the research laboratory in the phase when you take both oestrogen and progesterone (*i.e., between day 7 and day 14 after addition of progesterone to your daily dose of oestrogen*).

You will be asked to meet the following requirements before each research laboratory visit:

- No strenuous exercise to take place in the 24 hours before a testing session; and
- No alcohol consumption in the 24 hours before a testing session; and
- No food supplements (*e.g., whey, vitamins, collagen*) to be absorbed in the 24 hours prior to a testing session; and

- To maintain similar dietary (*i.e., food intake and its timing, caffeine, tea, and water consumption*) and physical activity levels during the trial period.

Once all study requirements are met, your visit to the research laboratory will be organised as follows:

- You will be asked to wear comfortable clothing on your upper body parts, and short to mid-length shorts;
- Your height and weight will be measured at the beginning of the first visit. This is to register some of your personal characteristics and to calculate your body mass index (BMI);
- You will rest comfortably in a room at 20/22°C for 20 min to stabilise your body temperature;
- You will have your anterior knee laxity (*i.e., the elasticity of the anterior cruciate ligament*) tested and measured by the automated machine (*i.e., GNRB knee arthrometer*) on both knees. To do so, you will lie on an examination table where our researcher (Elisa Nédélec) will place the automated machine on your leg. Once you are ready, the automated machine will gently push your calf forward 5 times at different forces. Testing will be passive and painless.

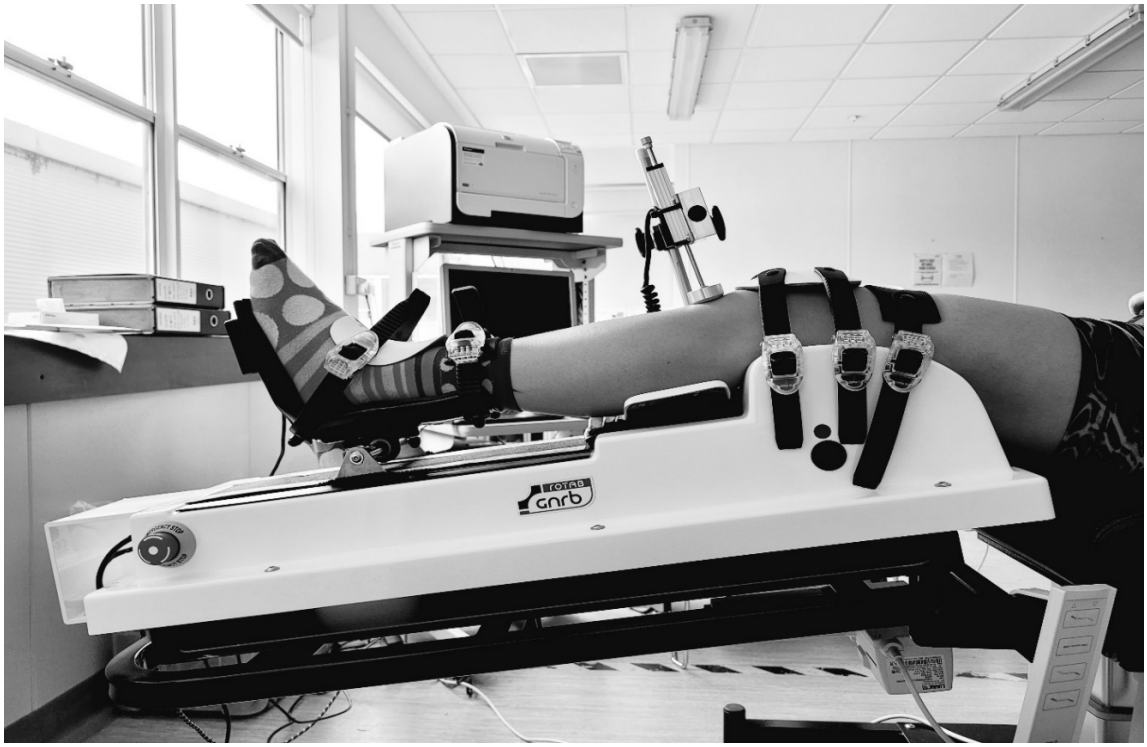

Figure 1. Participant positioning for tests on the GRNB knee arthrometer (automated machine) at the Nottingham Trent University's research laboratory (Clifton Campus)

What are the benefits of taking part in the study?

There are no advantages or rewards if you participate in our study. However, this study will allow us to understand if we can get the same results time after time under the

same conditions from the automated machine that tests the elasticity of the anterior cruciate ligament.

What are the possible disadvantages and risks of taking part?

Participating in the research is not anticipated to disadvantage you nor will you experience any discomfort or psychological harm beyond the experience of everyday life.

Important information:

- The only purpose of our study is to test how reliable the GNRB knee arthrometer is when we repeat the same set of ACL laxity measures on the same individuals on several occasions. We will collect some personal information (such as your name, your signature, your email address, your date of birth, some medical information, and your ACL elasticity variations). Your participation is confidential; neither information about you, your family, your team nor your club will be disclosed to anyone other than the research team (Elisa Nédélec, Kirsty Elliott-Sale, Craig Sale, Jessica Piasecki and Kevin Enright). Please note that confidentiality will be maintained as far as it is possible, unless anything in your responses makes us worried that someone might be in danger of harm, we might have to inform relevant agencies of this. If this were the case, we would inform you of any decisions that might limit your confidentiality.
- The information that you give to us, including your personal data, the information you provide about your medical profile and the results of your laboratory testing, will be stored in secure folders on Nottingham Trent University servers. We will also protect your information by removing any information that could identify you from your data, and then saving your responses under a code. This code is a unique identifier that will allow us to re-identify you should you wish to withdraw from the study.
- You can withdraw from the study, without providing a reason, up until all data have been collected (which we anticipate to occur around March 2023. You can do this by sending an email to our principal investigator (elisa.nedelec2019@my.ntu.ac.uk) or to any researcher from our research team (K.Elliott-Sale@mmu.ac.uk, C.Sale@mmu.ac.uk, jessica.piasecki@ntu.ac.uk, K.J.Enright@ljmu.ac.uk). In your email, please mention your full name for us to know which corresponding answers to delete. If you wish to withdraw from our study, within the timeframe mentioned above, we will automatically delete all answers and personal information collected from you.
- Once all data have been collected, we will delete all your personal data, including the file containing the unique identifiers (codes) of all participants to anonymise the data. Therefore, you will not be able to ask for the deletion of your data since it will no longer be possible for the research team to link you to the information that you have provided.

- Anonymised information collected during the study will be archived and publicly available for ten years on a data repository called Zenodo. This will allow anyone else (including researchers, businesses, governments, charities, and the general public) to use the anonymised data for any purpose that they wish, providing they credit the University and research team as the original creators. You will not be identifiable from these data and future research in this area will further benefit from the reuse of these data.
- The results of the research project will be published and available in Elisa Nédélec's doctoral thesis and might appear in a scientific journal article for specialist doctors and scientists to read. Your name will never be included in any such publications.
- The study has been approved by Nottingham Trent University's Human Invasive Ethical Committee, with the following ID number: 1595733.

What to do if you have any questions?

If you have a concern and/or a question about any aspects of this study, you can speak to us. We will do our best to answer your questions.

- Ms Elisa Nédélec (Principal Investigator)
  PhD Candidate
  Musculoskeletal Physiology Research Group
  Sport, Health & Performance Enhancement (SHAPE) Centre
  Erasmus Darwin Building, Nottingham Trent University, Clifton Lane
  Nottingham, NG11 8NS
  Email: elisa.nedelec2019@my.ntu.ac.uk

Or

- Professor Kirsty Elliott-Sale (Project Supervisor)
  Email: K.Elliott-Sale@mmu.ac.uk

- Professor Craig Sale
  C.Sale@mmu.ac.uk

- Dr Jessica Piasecki
  jessica.piasecki@ntu.ac.uk

- Dr Kevin Enright
  K.J.Enright@ljmu.ac.uk

Next step:

If you are willing to participate in our study, please contact our Principal Investigator (Elisa Nédélec) by email: elisa.nedelec2019@my.ntu.ac.uk.

We are looking forward to meeting you!

Thank you for reading this and for your participation.

Appendix I: Participant consent form used in Chapter 4

PARTICIPANT CONSENT FORM

Title of Project: GNRB knee arthrometer reliability study in men, combined monophasic oral contraceptive pill users, combined hormonal contraceptive users, postmenopausal women, and hormone replacement therapy users

Name of Researchers: Elisa Nédélec, Professor Kirsty Elliott-Sale, Professor Craig Sale, Dr Jessica Piasecki, and Dr Kevin Enright

1. I agree to take part as a participant in the above study.
2. I understand from the participant information sheet, which I have read in full, and from my exchange(s) with Elisa Nédélec that this will involve me visiting the laboratory on 3 separate occasions for approximately 30minutes within 2 weeks. I will need to have an online meeting with Elisa Nédélec to sign this Informed Consent form and allow Elisa Nédélec to take a screenshot of my screen when I sign the Informed Consent form. I will need to send back this completed Informed Consent form, the health screen questionnaire, and the menstrual history questionnaire (if applicable) to Elisa Nédélec by email. I will need to get the Anterior Cruciate Ligament laxity of both knees tested by an automated device.
3. I confirm that I have read the information sheet provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered to my satisfaction.
4. I undertake to abide by University regulations and the advice of researchers regarding safety.
5. I understand that my participation is voluntary and that I am free to withdraw until all data have been collected (which we anticipate to occur around March 2023), without giving any reason.
6. I understand that any personal information about myself will be treated in confidence and will not be shared with anyone who is not part of the research team.
7. I understand that anonymised data, that will not identify me, will be publicly available for future reuse, for example, if other academic researchers work on the same topic.
8. I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information I have provided through my participation in this study, in the form of health screen, questionnaires, blood samples and test data will be handled in accordance with this policy will be handled in accordance with this policy.
9. I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research.

As described in the Participant Information Sheet, you have the right to drop out from the study until all data have been collected (which we anticipate to occur around March 2023). If you wish to do so and if we need to contact you after you have sent us your answers, we ask you to provide your full name, your date of birth and your email.

First Name and Family
Name:……
What is your date of birth?
(dd/mm/yyyy): ……..
Please provide your email
address:……

Participant signature: Date:

Primary Researcher signature: Date:

Appendix J: Health screen questionnaire adapted for Chapter 4

Name or Number .....……

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present,** do you have any health problem for which you are:

(a) on medication, prescribed or otherwise Yes ☐ No ☐

(b) attending your general practitioner Yes ☐ No ☐

(c) on a hospital waiting list Yes ☐ No ☐

2. **In the past two years,** have you had any illness or injury which require you to:

(a) consult your GP Yes ☐ No ☐

(b) attend a hospital outpatient department Yes ☐ No ☐

(c) be admitted to hospital Yes ☐ No ☐

3. **Have you ever** had any of the following?

(a) Convulsions/epilepsy Yes ☐ No ☐

(b) Problems with bones or joints Yes ☐ No ☐

(c) Ear / hearing problems Yes ☐ No ☐

(d) Thyroid problems Yes ☐ No ☐

(e) Allergy to nuts, alcohol etc. Yes ☐ No ☐

(f) Any problems affecting your nose e.g. recurrent nose bleeds Yes ☐ No ☐

COVID19

4. Do you think you have had COVID-19? Yes ☐ No ☐

5. If YES, was this confirmed via a swab test? Yes ☐ No ☐

6. If YES, was this confirmed via an anti-body test? Yes ☐ No ☐

7. If YES, were you hospitalised with COVID-19? Yes ☐ No ☐

8. Please state the dates over which you had COVID-19 symptoms:

FROM…….TO……

NB: Please note that in the 7-day period prior to any visit to the University to undertake a trial in a research study or to visit a University research facility YOU WILL NEED TO COMPLETE a COVID-19 symptom questionnaire. Please DO NOT come to the University if you have not completed this questionnaire and the member of research staff supervising the research study has not confirmed you should attend.

Women only

9. Are you pregnant, trying to become pregnant, breastfeeding, or have you breastfed in the last 12 months? Yes ☐ No ☐

If you have answered YES to any question, please describe briefly if you wish (e.g., to confirm problem was/is short-lived, insignificant, or well controlled).

...……
……
……
……
……
……
……
……
……
……
……
……
……
……
……

PHYSICAL ACTIVITY LEVEL

1. Do you do at least 150 to 300 min moderate-intensity activity [*i.e., usually rated a 5 or 6 on a rating scale of perceived exertion scale of 0–10*], or 75–150 min of vigorous-intensity activity [*i.e., usually rated a 7 or 8 on a rating scale of perceived exertion scale of 0–10*] a week, plus muscle-strengthening activities 2 or more days a week?

Yes ☐ No ☐

2. Do you train regularly ~3 times per week, identify with a specific sport, and train with a purpose to compete?

Yes ☐ No ☐

3. Do you compete at the local/regional level? Yes ☐ No ☐

4. Do you compete at the national level? Yes ☐ No ☐

Sex, gender identity, and preferred pronoun(s):

Please answer the following questions about sex, gender identity, and preferred pronoun(s). The answers to these questions are relevant to the study:

1. What is your current gender identity? (Check all that apply.)

☐Male

☐Female

☐Transgender female / trans woman (or Male-to-Female (MTF) transgender, transsexual, or on the trans female spectrum)

☐Transgender male / trans man (or Female-to-Male (FTM) transgender, transsexual, or on the trans male spectrum)

☐Non-binary, genderqueer, or genderfluid

☐Gender identity not listed:……

☐Prefer not to reply

2. What is your sex assigned/reported at birth?

☐Male

☐Female

☐Intersex

☐Not Listed:…….

☐Prefer not to reply

3. What pronouns would you like the lead investigator (i.e., Elisa Nédélec, she/her/hers) to use when referring to you?

☐He/him/his

☐She/her/hers

☐They/them/theirs

☐Other (please specify):……

Appendix K: COVID-19 Symptom questionnaire used in Chapter 4

COVID-19 Symptom Questionnaire

1. Study Title: Assessing the reliability of the GNRB knee arthrometer

2. Participant's name or number: ……

3. Date: ……..

4. Do you have:

   A high temperature / fever Yes ☐ No ☐

   A sore throat Yes ☐ No ☐

   A new continuous cough* Yes ☐ No ☐

   Loss of, or change in, taste or smell Yes ☐ No ☐

*A new continuous cough means coughing for longer than an hour, or three or more coughing episodes in 24 hours.

5. Have you, or anyone you share a house with, been in close contact with anyone with a suspected of confirmed case of COVID-19 in the last two weeks?

   Yes ☐ No ☐

6. Have you travelled to a 'high risk' region for COVID-19 in the last two weeks?

   Yes ☐ No ☐

7. Please confirm that ALL of the questions 4 – 6 have been answered "NO" and that there are no reasons why you should not participate in the research study:

   Yes – I can confirm that all of my responses to questions 4 – 6 were "NO" ☐

   No – I answered "Yes" to some or all of the questions 4 – 6 above ☐

Appendix L: Adapted Menstrual History Questionnaire used in Chapter 4

(Adapted from the Menstrual History Questionnaire designed by Prof. Kirsty J. Elliott-Sale)

Section A: Current hormonal contraceptive use

1. Have you ever had a period [*i.e., a menstrual bleed*]? YES ☐ NO ☐

***If you answered no and you are not a hormonal contraceptive user, you do not need to complete any other questions**

2. Do you use a **non-hormonal** intrauterine device (copper-based coil)? YES ☐ NO ☐

***If you answered yes you do not need to complete any other questions**

3. Which type of hormonal contraception do you currently use (please tick your answer)?

☐ oral contraceptive ☐ implant ☐ injection ☐ intrauterine device/coil

☐ vaginal ring ☐ contraceptive (transdermal) patch

4. What is the brand of your hormonal contraception [*e.g., for oral contraceptives: Mycrogynon, Rigevidon, Cilest, Yasmin, Dianette; for implant: Nexplanon, Norplant; for injection: Depo Provera; for intrauterine device/coil: Mirena, Kyleena; for vaginal ring: NuvaRing; for contraceptive patch: Evra*]?

_____

5. How long (years or months) have you used this method of contraception? _____

6. If you use an **oral contraceptive**, do you have your 7 pill free days (or placebo pills)? Please tick the answer that applies to you:

☐ ALWAYS ☐ MOSTLY ☐ SOMETIMES ☐ RARELY ☐ NEVER

7. If you use an **oral contraceptive**, what date did you begin taking your current pack of pills?

[*e.g., 12/08/22*]

_____

8. If you use a **vaginal ring**, do you have your 7 vaginal ring free days? Please tick the answer that applies to you:

☐ ALWAYS ☐ MOSTLY ☐ SOMETIMES ☐ RARELY ☐ NEVER

9. If you use a **vaginal ring**, what date did you insert your current vaginal ring?

[*e.g., 12/08/22*] _____

10. If you use a **transdermal contraceptive patch**, do you have your 7 patch free days? Please tick the answer that applies to you:

☐ ALWAYS ☐ MOSTLY ☐ SOMETIMES ☐ RARELY ☐ NEVER

11. If you use a **transdermal contraceptive patch**, in which week are you? Please tick the answer that applies to you:

☐ WEEK 1 ☐ WEEK 2 ☐ WEEK 3 ☐ WEEK 4 (NO-PATCH-WEEK)

12. If you use a **transdermal contraceptive patch**, what date did you apply your current patch on your skin?

[*e.g., 12/08/22*] _____

*Please do **NOT** complete SECTION B and SECTION C*

Section B: Current hormone replacement therapy use

(Remember do NOT answer this section, if you have answered section A)

1. Do you currently take hormone replacement therapy (HRT)? YES ☐
NO ☐

***If you answered no and you are not a hormonal contraceptive user or postmenopausal, you do not need to complete any other questions**

2. Which type of hormone replacement therapy (HRT) do you currently take? Please tick the answer that applies to you:

☐ COMBINED HRT (with oestrogen and progesterone) ☐ OESTROGEN-ONLY HRT

3. In which way do you take your hormone replacement therapy? Please tick the answer that applies to you:

☐ Monthly cyclical/sequential HRT (oestrogen taken every day and progesterone added for 10 to 14 days at the end of the month of therapy)

☐ Three-monthly cyclical/sequential HRT (oestrogen taken every day and progesterone added for 14 days every 13 weeks of therapy)

☐ Continuous combined HRT (oestrogen and progesterone taken every day without a break)

☐ Continuous oestrogen-only HRT (only oestrogen taken every day without a break)

4. How do you currently take your hormone replacement therapy? Please tick the answer(s) that applies/apply to you:

☐HRT patch ☐ HRT gel ☐ HRT spray ☐ HRT tablet

☐vaginal oestrogen ☐ HRT implant

5. What is the brand of your hormone replacement therapy [*e.g., for combined HRT: Femoston, Kliovance, Clinorette, Elleste-Duet, Evorel Sequi, Kliofem, Novofem, Trisequens; for oestrogen-only HRT: Estradot, Lenzetto, Elleste-Solo, Evorel, FemSeven, Progynova, Sandrena, Oestrogel*]?

_____

6. How long (years or months) have you used this type of hormone replacement therapy?

_____

7. If you are a **combined HRT user**, what date did you begin taking your current HRT?

[*e.g., 12/08/2022*] _____

Section C: Postmenopausal women

(Remember do NOT answer this section, if you have answered section A)

1. Have you ever had a period [*i.e., a menstrual bleed*]? YES ☐ NO ☐

***If you answered no and you are not a hormonal contraceptive user, you do not need to complete any other questions**

2. Are you postmenopausal [*i.e., you already had menstrual cycles with periods for years, you went through menopause, and you did not have a menstrual bleed for more than 12 months in a row*]?

YES ☐ NO ☐

3. When did you have your last period [*i.e., menstrual bleed*]?

[*e.g., 01/01/2019*] _____

4. Do you currently use hormone replacement therapy (HRT)? YES ☐ NO ☐

***If you answered yes, please complete section B too**

THANK YOU for completing this questionnaire. Please remember that you can contact Elisa Nédélec (elisa.nedelec2019@my.ntu.ac.uk) if you have any questions regarding the completion of this questionnaire and/or your participation in this research study.

Appendix M: Hormonal contraceptive diary used in Chapter 4

Month:...

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
REFERENCE OF THE PILL																															
TIME																															

Month:...

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
REFERENCE OF THE PILL																															
TIME																															

Month:...

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
REFERENCE OF THE PILL																															
TIME																															

Reference of the pill:
P1= First day of a new pack of pills, P2= Second day of the pill's pack etc. (Maximum of 21)
NO1= First day with a placebo pill, or with no pill, NO2= Second day with a placebo pill, or with no pill etc. (Maximum of 7)

Time:
Example: 7AM, 1PM, 10PM etc.

Appendix N: Participant information sheet, consent form, health screen, Covid screen, IPAQ, menstrual history, previous hormones history, pregnancy history used in Chapters 5 and 6

Start of Block: Participant Information Sheet

PARTICIPANT INFORMATION SHEET

Invitation and brief summary: The aim of this research project is to establish the neuromuscular differences between biological sexes across the adult lifespan. The project will provide missing detail that will help explain the neuromuscular mechanisms underpinning the contributing reasons why females tend to live longer in ill health comparatively to men. Such answers will then contribute to better understanding the biological mechanisms that contribute to the different ageing between men and women, allowing more targeted/specific interventions that will lead to a greater time spent in good health through older age, for both sexes.

What is involved?

To understand the differences between men and women in more detail we wish to perform a number of neuromuscular assessments with different people who are of different ages, therefore capturing a number of stages of the life span. Specifically, we wish to align these changes with the transition through menopause for women, as this is a pivotal time in the female lifespan where changes in hormones levels are experienced. As researchers we believe this dramatic change may contribute to the poor health in older life that faces many females today. Therefore, we wish to recruit 15 women from one of the 4 groups listed below, with 15 men of an equivalent age.

1. The pre-menopause group will report regular duration menstrual cycles (21-34 days) and no hormonal contraceptive use (including copper coil) for the 12 months prior to testing. Approximately ages 18-40 years.

2. The perimenopause group without hormone therapy (HT) will be women aged ≥40 years and report menstrual disturbance (e.g. irregular cycle duration, flow, and vasomotor symptoms), as per the UK National Institute for Health and Care Excellence (NICE) guidelines for menopause diagnosis and management (NICE, 2019). Approximately aged 40-60 years.

3. The post-menopausal group will report 12 consecutive months without

menstruation. Self-reported menstrual information will be obtained via a screening questionnaire. Approximately 60 years and older (maximum age limit of 85 years old)

4. The post-menopausal group with hormone therapy will report 12 consecutive months without menstruation prior to the use of Hormone Therapy. Self-reported menstrual information will be obtained via a screening questionnaire. These women will use a combined hormone therapy (oestrogen + progestin). Approximately 60 years and older (maximum age limit of 85 years old).

What would taking part involve?

Taking part in the research study will require attendance for approximately 4 hours in one of our designated laboratories with one of the named researchers. Testing will begin approximately 9am and will start with a blood sample being taken. This will be used to quantify the level of sex hormones (oestrogen and progesterone for women, testosterone and DHEA for men). Then we will take an ultrasound image of some of your leg muscles. This uses sound waves to create a clear image of the size of your muscles. Then we will carry out the neuromuscular assessments. This will detail how the nerves within your body send signals to muscles and how quickly and/or efficiently they do so. This will be conducted using two methods: transcranial magnetics stimulation (TMS) and intramuscular electromyography (iEMG). TMS is performed by holding a device over a point on your head, which then sends signals to your muscles via the central nervous system. The different signals produced give the researcher information at how well the central nervous system is performing. There is noise associated with the device, but it is not harmful in any way and does not require you to do anything other than sit fairly still. iEMG uses a range of surface electrodes and an electrode pin. This is a small pin that is inserted into the muscle, similar to an acupuncture needle, and measures the electrical signals in that muscle. We will ask you to here perform a number of contractions and small movements that give us information on how well the peripheral nervous system is performing. There are some electrical stimulation pulses that are used in this method to first identify where the majority of your nerves for each muscle are within the muscle, we also use electrical stimulation to identify the highest electrical pulse your muscle can produce. These aspects are a little uncomfortable but not painful and last around 3 minutes at most. The assessments described in this section may sound a little technical, but you should not worry about this, these assessments are routinely performed in our labs.

Our investigators are friendly and very experienced, they will explain everything to you at the time, answer any questions that you may have and will only proceed when you are happy to do so AND YOU ARE ABSOLUTELY FREE TO STOP AT ANY POINT SHOULD YOU WISH TO DO SO. The TMS and iEMG can be an unusual sensation but most people find it acceptable, and you will be given plenty of time to get used to it. We

will always keep the intensity of the stimulation within limits that you are happy with.

All your details will be kept confidential and accessible only by the research team. All your experimental data will be kept with an associated code so is non-identifiable with yourself. We only retain your contact details up to your experimental day, after which this detail will be destroyed. If you do decide to withdraw at any point all your data will be destroyed.

What are the possible benefits of taking part?

There are no direct benefits to taking part other than you will be contributing to an important area of research that will ultimately lead to improvements in health of older individuals, particularly older women.

What are the possible disadvantages and risks of taking part?

You may experience some discomfort when having some of the procedures conducted. The blood sample may cause some discomfort when inserting the needle, as could the pin electrode that will be used in the intramuscular electromyography. Such discomfort is very short lived and temporary. There will be constant communication between the participant and the researcher to minimise any discomfort. There is a very small risk of seizure associated with the TMS method, however this is a very small risk. Our researchers are highly trained in this method and always follow the correct procedures when conducting such methods. We will have the required emergency protocols in place, and we will take every precautionary procedure prior to the sample to ensure you are as comfortable as possible. The researchers are all skilled and trained, regularly using all these techniques and it is very unlikely any issues will arise as a result of the testing procedures.

If at any time you do not wish to proceed with the testing, you are free to withdraw. If at any point you decide to withdraw from the study your data will be destroyed.

Contacts: If you have any further queries regarding the project, please do not hesitate to get in touch with Jessica Piasecki: Jessica.piasecki@ntu.ac.uk or on 07813296063

Further supporting information

What will happen to my data?

We will be using information from you to undertake this study. Research is a task that we perform in the public interest. Nottingham Trent University is the data controller. This means that we, as Nottingham Trent University researchers, are responsible for looking

after your information and using it properly. We will use the minimum personally identifiable information possible. We will keep identifiable information about you until your experimental testing day has been completed, your consent forms will be kept for 10 years after the study has concluded. The coded data will be kept separate, again for 10 years after the study. All research data and any research documents with personal information, such as consent forms, are stored securely at Nottingham Trent University.

What will happen if I don't want to carry on with the study?

If you withdraw from the study, unless you state otherwise, any blood or tissue samples which have been collected whilst you have been in the study will be used for research as detailed in this participant information sheet. You are free to request that your blood or tissue samples are destroyed at any time during or after the study.

What will happen to the results of this study? What happens at the end of the study?

Some of the research being undertaken will also contribute to the fulfilment of an academic publication. Please be assured no data will be identifiable to you at any point or by any other means through the publication.

What if something goes wrong?

If you have any problems during the study or would like to discuss the study, you can contact any of the research investigators. We will have the required emergency protocols in place, and we will take every precautionary procedure prior to the sample to ensure you are as comfortable as possible. The researchers are all skilled and trained, regularly using all these techniques and it is very unlikely any issues will arise as a result of the testing procedures. There is a very small risk of seizure associated with the TMS method, however this is a very small risk. Our researchers are highly trained in this method and always follow the correct procedures when conducting such methods.

If at any time you do not wish to proceed with the testing, you are free to withdraw. If you have any queries or concerns, please do not hesitate to get in touch.

○ Please initial here to indicate you have read and understood the information sheet

_____

End of Block: Participant Information Sheet

Start of Block: General consent

Addressing the female health-lifespan paradox: differences between biological sexes within neuromuscular ageing

Please initial the boxes as applicable

○ 1) I agree to partake as a participant in the above study. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected

_____

○ 2) I understand from the participant information sheet, which I have read in full, and from my discussion(s) with [Jessica Piasecki] that this will involve me [giving a blood sample, having an ultrasound scan and then undergoing neuromuscular assessments via TMS and iEMG

_____

○ 3) It has also been explained to me by [Jessica Piasecki] that the risks and side effects that may result from my participation are as follows: [some discomfort and possible bruising with the venepuncture and some short term discomfort and noises associated with the neuromuscular assesments]

_____

○ 4) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction.

_____

○ 5) I undertake to abide by University regulations and the advice of researchers regarding safety. _____

○ 6) I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material,

my identity will be kept anonymous.

_____

○ 7) Consent for storage and use in possible future research projects:

_____

○ I agree that the sample (s) I have given, and the information gathered about me can be stored by (Jessica Piasecki) at NTU for possible use in future projects of a similar nature. I understand that some of these projects may be carried out by researchers other than (Jessica Piasecki) who ran the first project, including researchers working for commercial companies.

_____

○ 8) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research.

_____

To complete the consent please type your name and date (dd/mm/yyyy)

_____

Please provide a unique, 4 character code for this information. Please use a range of grammar, punctuation, numerical and letters.

_____

End of Block: General consent

Start of Block: Health Screen

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:

	Yes	No
On medication prescribed or otherwise	○	○
Attending your general practitioner	○	○
On a hospital waiting list	○	○

2. In the past two years, have you had any illness which require you to:

	Yes	No
consult your GP	○	○
attend a hospital outpatient department	○	○
be admitted to hospital	○	○

3. Have you ever had any of the following?

	Yes	No
Convulsions/epilepsy	○	○
Asthma	○	○
Eczema	○	○
Diabetes	○	○
A blood disorder	○	○
Head Injury	○	○
Digestive Problems	○	○
Heart Problems	○	○
Problems with bones or joints	○	○
Disturbance of balance/coordination	○	○
Numbness in hands or feet	○	○
Disturbance of vision	○	○
Ear/hearing probems	○	○
Thyroid problems	○	○
Kidney or liver problems	○	○
Allergy to nuts, alcohol etc	○	○

Any problems affecting your nose e.g. recurrent nose bleeds	○	○
Any nasal fracture or deviated septum	○	○

4. Has any, otherwise healthy, member of your family under the age of 50 died suddenly during or soon after exercise?

- ○ Yes
- ○ No

5. Are there any reasons why blood sampling may be difficult?

- ○ Yes
- ○ No

6. Have you had a blood sample taken previously?

- ○ Yes
- ○ No

7. Have you had a cold, flu or any flu like symptoms in the last month?

- ○ Yes
- ○ No

Women only. If not applicable please leave blank
Are you pregnant, trying to become pregnant or breastfeeding?

- ○ Yes
- ○ No

If YES to any question, please describe briefly if you wish (e.g. to confirm problem was/is short-lived, insignificant or well controlled.)

_____

_____

_____

_____

_____

End of Block: Health Screen

Start of Block: Covid Screen

Do you think you have had Covid-19?

- ○ Yes
- ○ No

If YES was this confirmed via swab test?

- ○ Yes
- ○ No

If YES was this was this confirmed via an anti-body test?

- ○ Yes
- ○ No

State the dates from which you had covid symptoms (if in the previous 6 months. FROM and TO)

_____

End of Block: Covid Screen

Start of Block: Covid Symptom Questionnaire

Do you have any of the following:

* A new, continuous cough means coughing for longer than an hour, or three or more

coughing episodes in 24 hours.

	Yes	No
A high temperature/fever	○	○
A sore throat	○	○
A new continuous cough*	○	○
A loss, or change, in taste or smell	○	○

Have you, or anyone you share a house with, been in close contact with anyone with a suspected or confirmed case of COVID-19 in the last two weeks?

- ○ Yes
- ○ No

Have you travelled to a ‘high-risk’ region for COVID-19 in the last two weeks?

- ○ Yes
- ○ No

Please confirm that ALL of the questions 4-6 have been answered “NO” and that there are no reasons why you should not participate in the research study:

- ○ Yes – I can confirm that all of my responses to questions 4-6 above were “NO”
- ○ No – I answered “Yes” to some or all of the questions 4-6 above.

Please confirm the date the questionnaire has been completed (dd/mm/yyyy)

_____

Please confirm the date you are expected in the lab (dd/mm/yyyy)

_____

End of Block: Covid Symptom Questionnaire

Start of Block: Introductory Questions

To ensure anonymity, but allow us to identify your responses if you choose to withdraw, please enter an identification code based on the following format:

- Last two letters of your surname
- First two letters of your first name
- Month and Year of your birth (MMYY)

For example: Smith John born 02/1970 = THJO0270

_____

Please enter your age (years).

_____

What is your highest education level?

- ○ Primary
- ○ Secondary (GCSE, O-Level, A-Level)
- ○ Tertiary (College, University degree or Higher)
- ○ None
- ○ Other (please specify)

_____

What is your marital status?

- ○ Married
- ○ Single
- ○ Cohabiting
- ○ Separated
- ○ Divorced
- ○ Widowed
- ○ Other (please specify)

Are you retired?

- ○ Yes
- ○ No
- ○ Other (please specify)

_____

Do you exercise regularly?

- ○ Yes
- ○ No

How many hours per week do you spend exercising?

_____

What is your main form of exercising? For example, walking, biking, running.

_____

Have you experienced any of the following long term health conditions?

- [ ] Diabetes
- [ ] Chronic Obstructive Pulmonary Disease (COPD)
- [ ] High Blood Pressure
- [ ] Depression
- [ ] Osteoarthritis
- [ ] Asthma
- [ ] Anxiety/Nerves
- [ ] Stroke
- [ ] Heart Disease
- [ ] Pain
- [ ] Memory Problems
- [ ] Chronic Kidney Disease
- [ ] Obstructive Sleep Apnoea
- [ ] Other (please specify)

_____

- [ ] None of the above

In general would you say your health is

- ○ Excellent
- ○ Very Good
- ○ Good
- ○ Fair
- ○ Poor

These questions relate to activities you did over the last week whilst sitting or lying down. Don't count the time you spent in bed. Please select each one and then complete the text entry.

For each of the activities only count the time when this was your main activity.

For example if you are watching television and doing a crossword, count it as television time or crossword time but not as both.
Please enter your answer in Hours: Minutes:

During the last week, how much time in total did you spend sitting or lying down and...

☐ Watching TV or videos/DVDs

_____

☐ Using the computer/internet

_____

☐ Reading _____

☐ Socialising with friends/family

_____

☐ Driving or riding in a car, or time spent on public transport

_____

☐ Doing hobbies e.g. crafts or crosswords

_____

☐ Doing any other activities

_____

End of Block: Introductory Questions

Start of Block: INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

The following questions are aiming to capture your current levels of physical activity.

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person.

Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport. Think

about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

☐ Days per week

_____

☐ No (skip the next question before proceeding with the remaining questions)

How much time did you usually spend doing vigorous physical activities on one of those days?

○ Hours per day _____

○ Minutes per day _____

○ Don't know/not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include

walking.

○ Number of days per week

_____

○ No moderate physical activities (if so skip the next question and the proceed with the remaining questions)

How much time did you usually spend doing moderate physical activities on one of those days?

○ Hours _____

○ Minutes _____

○ Don't know/not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure

During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

○ Number of days per week

_____

○ No walking (skip the following question and then continue with the remaining question)

How much time did you usually spend walking on one of those days?

- ○ Hours per day _____
- ○ Minutes per day _____
- ○ Don't know not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During the last 7 days, how much time did you spend sitting on a week day?

- ○ Hours per day _____
- ○ Minutes per day _____
- ○ Don't know/ not sure

End of Block: INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

Start of Block: Menstrual Screening

Please write your name below

_____

What is your date of birth (dd/mm/yyyy)?

_____

Do you suffer from any medical conditions? If yes please list.

- ○ Yes _____
- ○ No

Are you currently taking any medications? If yes please list

- ○ Yes _____
- ○ No

How many hours a week do you take part in physical activity? (E.g. walking, jogging, team sports, going to the gym)

_____

End of Block: Menstrual Screening

Start of Block: Menstrual History

At what age was your first period?

_____

Have you had any periods in the last 3 months?

- ○ Yes (if yes skip the next question then continue)
- ○ No (answer the next question then move to previous hormone use section)

If you have NOT had periods in the last 3 months, what was the reason for not having periods?

- ○ Taking hormones continuously (e.g. the Pill, injections, Mirena, HRT)
- ○ Pregnant/breastfeeding
- ○ Unsure
- ○ Other (please describe)

_____

Approximately how many periods have you had over the last 12 months?

_____

When was your last period?

- ○ 3-6 months ago
- ○ 7-12 months ago
- ○ Over 12 months ago

If you have had periods in the last 3 months, please answer the following questions about your recent periods:

Were your periods in the last 3 months natural or hormone-induced (e.g. on the Pill, injections, Mirena or HRT)?

- ○ Natural
- ○ Hormone induced

When was the last day of your menstrual period?

- ○ dd/mm/yyyy _____
- ○ Unsure

Were your periods in the last 3 months regular?

- ○ extremely regular (period starts 1-2 days before or after it is expected)
- ○ very regular (period starts 3-4 days before or after it is expected)
- ○ regular (period starts 5-7 days before or after it is expected)
- ○ somewhat irregular (period starts 8-20 days before or after it is expected)
- ○ irregular (period starts more than 20 days before or after it is expected)

How many days of bleeding did you usually have each period in the last 3 months? (Not counting discharge/spotting for which you need a panty liner only)

- ○ Number of days _____
- ○ Too irregular to say

In the last 3 months, how many days were there between the first day of one period and the first day of the next on average? (Not including spotting)

- ○ <24 days
- ○ 24-31 days
- ○ 32-38 days
- ○ 39-50 days
- ○ 51+ days
- ○ Too irregular to estimate

End of Block: Menstrual History

Start of Block: Previous Hormone Use

Please list below all hormones you have ever used for any reason (acne, bad cramping, irregular periods, birth control, fertility treatments). For each hormone used, please indicate what type of hormone it was using the number indicated for the categories below. Please also tell us the age you first used each hormone and the total time used. If you cannot remember the name of the hormone you used, please write "unknown" in the first column. 1=Combined birth control pill (e.g. Marvelon, Yasmin, Microgynon) 2=Progestin only birth control pill ("mini-pill", e.g. Cerazette, Micronor) 3=Unsure of which type of oral birth control pill 4=Progestin injection/shot (e.g. Depo provera) 5=Transdermals: patches (e.g. OrthoEvra, Climara), dots (Vivelle dot) 6=Vaginal ring (NuvaRing) 7=Progesterone containing coil/IUD (Mirena) 8=Hormonal implant (Implanon/Nexplanon) 9=Oral progestins to regulate the cycle (e.g. medroxyprogesterone acetate [Provera], dydrogesterone [Duphaston], dienogest [Visanne], Norethisterone) 10=GnRH agonist injection/shot (e.g. leuprolilide (leuproline) acetate [Prostap], goserelin [Zoladex]) 11=Norethindrone acetate (Aygestin) 12=Danazol (please specify if used

vaginally or orally) 13=Hormone replacement therapy (e.g. Premarin, Provera) 14=Other 15=Don't know what type of hormone

	Name of Hormone	Type of Hormone (selected from list above)	Age started (years old)	Total time used (years and months)	If hormone was an injection when was last injection (dd/mm/yyyy)
Hormone 1					
Hormone 2					
Hormone 3					
Hormone 4					
Hormone 5					
Hormone 6					
Hormone 7					
Hormone 8					

What are/were your reasons for using hormones? (Check all that apply).

- [ ] Birth control/pregnancy prevention
- [ ] Pelvic pain or pain with periods
- [ ] Irregular periods
- [ ] Heavy periods
- [ ] Acne
- [ ] Polycystic Ovarian Syndrome (PCOS)
- [ ] Ovarian Cyst
- [ ] Other (please specify)

_____

End of Block: Previous Hormone Use

Start of Block: Pregnancy History

If you have previously been, or currently are pregnant, please fill in the following table:

	1st pregnancy	2nd Pregnancy	3rd Pregnancy	4th Pregnancy	5th Pregnancy
How old were you at the start of the pregnancy? (please write in age in years and months)					
What fertility treatment was used, if any, for this pregnancy? (Please type yes in the box to those that apply to each pregnancy) Natural conception: no fertility treatment					
Fertility drugs by pills to stimulate ovulation (clomid, clomiphene)					
Intrauterine insemination (IUI)					

In vitro fertilization (IVF/ICSI)					
What was the outcome of this birth? (please type yes in the box to those that apply to each pregnancy) Single live birth					
Twins or Triplets					
Miscarriage					
Still birth					
Termination (abortion)					
Tubal or pregnancy outside the uterus					
Molar					

Currently Pregnant					
Did you have any of the following complications related to pregnancy or breast feeding? (Please type yes to those that apply to each pregnancy) Gestational diabetes					
Pregnancy-related high blood pressure					
Pre-eclampsia/toxemia of pregnancy					
Mastitis/breast infection					
HELLP syndrome					

Hyperemis gravidarum					
Post-natal depression anxiety					
Other (please specify)					